Decoding *Wolffia*: A Predictive Model for Duckweed Growth and Nutrient Uptake for Resource Recovery from Wastewater in Tropical Countries

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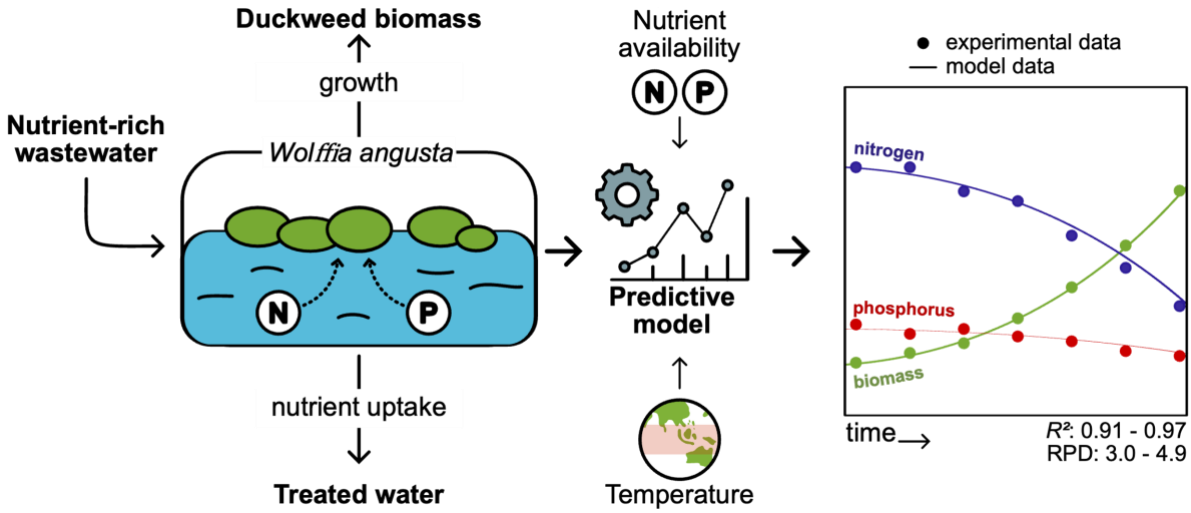
Graphical Abstract



Highlights

- A first kinetic model for *Wolffia*'s growth and nutrient uptake was developed
- The model integrates temperature and nutrient dynamics into duckweed growth
- The model accounts for in-planta nutrients reserves contributing to growth under low nutrient conditions
- The model accurately predicts *Wolffia* biomass production and nutrient removal under simulated tropical conditions

Abstract

Duckweed-based wastewater treatment systems have attracted increasing attention for sustainable water pollution control, yet predictive tools to optimise their operation under real-world conditions remain limited. Here, we develop and validate a temperature- and nutrient-dependent kinetic model for the duckweed species *Wolffia angusta*, integrating nutrient limitation (Monod model), temperature dependence (Arrhenius model) and internal nutrient reserve dynamics. The model was parametrised and validated through laboratory-scale batch experiments (20 - 30°C), in small cylindrical plastic vessels, under varying phosphorus and nitrogen supplies, including nutrient-limiting scenarios. Temperature predominantly drives biomass production, with optimal growth observed at 25 – 30ºC (maximum relative growth rate of 0.427 d^{-1}), while nutrient availability more strongly governs biological nutrient uptake and removal efficiency. Maximum nutrient removal rates reached 6.85 mg N L^{-1} d^{-1} and 1.85 mg P L^{-1} d^{-1} under nutrient-replete conditions. Biomass accumulation increases at the higher end of optimum temperature values, but specific nitrogen uptake rates decrease, indicating metabolic constraints. Model calibration markedly improved predictive accuracy (R^2 > 0.9; *RPD* > 2), even under nutrient-limited conditions. The model was applied to simulate a duckweed-based pond systems to polish the effluent from a hybrid anaerobic baffled reactor (HyABR) processing domestic wastewater under tropical conditions (Indonesia). Beyond predicting effluent nutrient concentrations, the model was used as a process design tool to optimise system configuration to meet local effluent discharge consents. Simulations showed that increasing the number of duckweed ponds in series reduces total hydraulic retention time and total land area while lowering per-pond productivity, with an optimal configuration of two to three ponds balancing treatment performance and resource

recovery. This work advances the mechanistic understanding of *Wolffia*'s growth and nutrient uptake, and delivers a practical, transferable modelling framework for design and optimisation of duckweed-based nutrient control units for decentralised wastewater treatment, particularly in tropical regions. The approach represents a significant step towards integrating nature-based systems into circular, sustainable water management strategies.

Keywords

Duckweed, Growth kinetics, Nutrient uptake, Wastewater treatment, *Wolffia angusta*

1. Introduction

Increasing demand for sustainable and decentralised wastewater treatment has drawn the attention to nature-based systems that remove pollutants while enabling resource recovery. The discharge of excess nitrogen and phosphorus into water sources remains a major factor contributing to water pollution and hence, controlling nitrogen and phosphorus in wastewater remains an urgent priority to prevent environmental an public health impacts such as: eutrophication, hypoxia, degradation of aquatic ecosystems, biodiversity loss, poor water quality and presence of cyanotoxins; this is particularly critical in regions where wastewater and faecal sludge is partially treated or unsafely managed [1]. This challenge is especially critical in tropical regions (i.e., South America, Sub-Saharan Africa, South Asia and Southeast Asia), where decentralised and onsite sanitation systems are widely used, but lead to the discharge of nutrient-rich effluents to aquatic ecosystems due to their limited treatment capacity [2,3].

Conventional ammonium and phosphorus control technologies are effective at large, centralised wastewater treatment works (WWTWs), but they are often expensive to build and operate, energy-intensive, highly dependent on upstream and downstream infrastructure, and hence, poorly suited to small and decentralised wastewater treatment systems [1, 4]. Globally, access to wastewater treatment and safe faecal sludge management remains unequal, with lower coverage in low- and low-middle-income countries (~8 – 28%) than in high-income countries (~70%), with a bigger growing gap on effective nutrient control [5]. Even in high-income countries such as the United Kingdom (UK), where chemical precipitation and enhanced biological processes have contributed to the reduction of inorganic phosphorus in effluent loads from WWTWs [6, 7], seasonal eutrophication is still a matter of concern particularly in catchments served by small treatment works, as they are now facing increasingly stringent phosphorus discharge limits, sometimes below 0.5 mg PO_4-P L^{-1}. In addition, improvements in ammoniacal nitrogen removal have been accompanied by high nitrate fluxes in receiving waters due to nitrification processes [8]. Overall, recent data from the UK shows that treated effluents from WWTWs can still increase phosphate and nitrate concentrations in receiving waters, despite current discharge consents [9].

In low- and middle-income countries, onsite sanitation and decentralised sewer-based systems remain the predominant solutions for faecal sludge and wastewater management [10]. In Indonesia, for example, only 0.8% of the population has access to sewer-based sanitation, while 86.6% rely on onsite sanitation systems (e.g., latrines, septic tanks and anaerobic baffled reactors – ABRs) [10, 11]. In fact, the use of ABRs in Indonesia have been widely implemented under programmes such as Sanitation by Communities (SANIMAS), but most of those treatment systems still discharge effluents that fail to meet regulatory standards (Ministry of Environment and

105 Forestry of Indonesia, Regulation No. P.68/2016) and contribute only marginally to
106 nutrient control targets.

107 Technological advancements for achieving ammoniacal nitrogen and phosphorus
108 discharge limits have largely focused on intensive processes suited to large,
109 centralised WWTWs. Their applicability to small and decentralised systems remains
110 limited, both in industrialised and developing countries. There is therefore a need for
111 low-cost polishing systems that can be coupled to existing treatment facilities and
112 improve nitrogen and phosphorus control and recovery without requiring complex
113 operation.

114 In this context, floating aquatic plants such as duckweeds (DW) have emerged as
115 promising alternatives to conventional nutrient control due to their rapid growth, low
116 operation and maintenance requirements, and exceptional nutrient uptake capacity to
117 promote nutrient recovery and reuse [12, 13]. In nature, these plants thrive in shallow
118 water bodies, capturing nutrients directly from the water column while producing
119 valuable biomass. Among nature-based technologies, duckweed pond systems stand
120 out for their simplicity, scalability and potential for integration into decentralised
121 treatment setups [14, 15]. Duckweed ponds can also serve as polishing stages for
122 conventional wastewater treatment, effectively removing residual nitrogen and
123 phosphorus and improving effluent quality before discharge or reuse [16].
124
125 Duckweed ponds for wastewater treatment have already been implemented at full
126 scale in several regions, including pilot and industrial applications in municipal lagoons
127 and agro-industrial effluent treatment, demonstrating their feasibility beyond laboratory
128 and pilot studies, but performance is dependent on weather conditions [17]. Research

on duckweed systems has expanded rapidly, exploring the effects of environmental and biological factors on growth and nutrient removal. Biotic factors, such as species variability and acclimation, cultivation density and the role of root-associated microbiota have been well documented [18 – 28]. Similarly, extensive work has evaluated abiotic parameters, including temperature, light, salinity and nutrient concentrations [29 – 36]. These studies show that nutrient removal in duckweed systems results from interacting mechanisms, including direct plant uptake, microbial transformation, sedimentation, and nutrient retention in harvested biomass. However, the relative importance of these pathways in engineered systems strongly depends on wastewater composition, hydraulic conditions, temperature, plant density, and harvesting frequency. This variability makes the performance in duckweed pond system difficult to predict across sites and operating conditions.

While valuable, much of this work has focused on individual variables in isolation. Few studies have integrated research findings into optimised culture conditions using pre-treated effluent characteristics [37] or into predictive tools or practical models for the design and operation of wastewater treatment systems [12, 30]. Existing models for nature-based treatment systems, including duckweed ponds and constructed wetlands, rely on empirical data, first-order kinetics, surface loading rates, operational conditions, or Monod-type formulations for predicting biomass growth. These approaches are useful for describing observed biomass productions and pollutant removal trends, but they often simplify the interactions between temperature, nutrient availability, biomass growth and harvesting, internal nutrient storage, and nutrient uptake. As a result, their transferability across species, climates, wastewater types, and operating conditions remains limited.

A critical gap remains in understanding how multiple environmental conditions interact to influence duckweed performance, especially in dynamic wastewater environments. Environmental conditions in general, and temperature in particular, are key but often used as oversimplified drivers, while nutrient availability is typically represented using simplified saturation-based formulations [37]. In practice, environmental conditions and nutrient availability are rarely integrated in a mechanistic framework that resolves their effects on kinetic parameters such as maximum specific growth rate and nutrient affinity. This limits the predictive capability of existing models and restricts the transition of duckweed-based treatment from empirical assessment to design-based engineering.

Within the duckweed family, *Wolffia* species offer exceptional potential for wastewater treatment and biomass valorisation. Native to many tropical and subtropical regions [38], *Wolffia* thrives under warm conditions and is well-suited for deployment in regions most affected by nutrient pollution. It is also the world's smallest flowering plant and one of the fastest growing, with an ability to double its biomass in under 48 hours under optimal conditions [39]. Beyond phytoremediation, *Wolffia* is a rich source of plant-based protein, positioning it as a promising organism for integrated systems that couple wastewater treatment and nutrient recovery with biomass valorisation for food or feed production. Its nutritional profile, rich in essential amino acids, vitamins (including B12) and antioxidants, has supported its traditional consumption in parts of Southeast Asia for centuries [40,41], and more recently to its approval as a novel food in the European Commission (Regulation EU 2025/153). These unique traits make

178 *Wolffia* a superior species over others from the Lemnaceae family for developing
179 sustainable nature-based wastewater treatment solutions in tropical regions.
180
181 Despite its considerable potential, the ecological and operational performance of
182 *Wolffia* under variable environmental conditions remains poorly understood [37],
183 limiting the development of predictive kinetic models for process design and
184 optimisation. Consequently, most existing applications report empirical performance
185 data rather than design-based criteria, restricting their deployment in engineered
186 wastewater systems.
187
188 Recent studies have shown that *Wolffia* growth is sensitive to temperature variability,
189 with elevated temperatures and heatwave-related fluctuations negatively affecting
190 growth dynamics under tropical conditions [42]. This sensitivity further highlights the
191 need for mechanistic predictive tools capable of coupling growth and nutrient uptake
192 dynamics, particularly under increasingly temperature fluctuations associated with
193 climate variability. *Wolffia*-based pond systems nevertheless have strong potential to
194 polish pre-treated wastewater effluents and meet nutrient control targets, particularly
195 in tropical countries where warm temperatures can sustain year-round biomass
196 productivity and overall nutrient removal performance.
197
198 Existing duckweed models have described growth and nutrient removal using
199 empirical, first-order, Monod-type, Droop-type, temperature-dependent, integrated
200 kinetic, and data-driven approaches [12, 29, 30, 32, 35, 43]. However, these models
201 have mostly been developed for duckweed genera such as *Lemna*, *Spirodela*, and
202 *Landoltia*, or for duckweed systems in general, rather than for *Wolffia angusta.*

Therefore, species-specific models that link temperature, nitrogen and phosphorus availability, biomass growth, and nutrient depletion in *W. angusta* remain limited, particularly for wastewater treatment design under tropical conditions.

This study seeks to bridge this gap by investigating the growth and nutrient uptake behaviour of *Wolffia angusta*. The scientific need for this work lies in the absence of mechanistic models linking environmental drivers (temperature and nutrients) to kinetic parameters and system-scale performance. We hypothesise that (i) *Wolffia* growth and nutrient uptake can be described using a temperature- and nutrient-dependent kinetic framework, and (ii) such a framework can be used to predict and optimise duckweed-based wastewater treatment performance under real operational conditions. We developed and validated a simple yet effective predictive model that reflects the key dynamics of biomass production and nutrient uptake and removal under simulated tropical climate conditions. The model incorporates temperature and nutrient availability within a simplified kinetic framework. By accounting for critical factors, such as the role of internal (*in-planta*) nutrient reserves, it enhances prediction accuracy, even when external nutrient levels are low. In addition to experimental validation, the model was applied to evaluate the performance of a *Wolffia*-based system for polishing the effluent from an anaerobic baffled reactors treating domestic wastewater under realistic operational conditions in Indonesia. The main objective of this study was therefore to develop a transferable predictive, robust and design-oriented modelling framework for Wolffia-based wastewater treatment systems, linking kinetics to engineering design parameters.

2. Materials and Methods

2.1. Plant material

A clone of the duckweed species *Wolffia angusta* (strain 8878), native to Southeast Asia and originally isolated from Malaysia, was obtained from the Landolt Duckweed Culture Collection (ETH Zurich, Switzerland) in 2021. This clone was maintained under sterile (axenic) conditions in the lab at the University of Leeds (UK) for approximately six months prior to experimentation. Stock cultures were kept in plastic containers containing Hoagland's growth solution in a Sanyo MLR-351H growth chamber at a constant temperature of 20°C, with an 8-hour light cycle and a light intensity of 150 µmol m^{-2} s^{-1}. The growth solution was replaced every two weeks, and any dead plants on the surface or bottom of the containers were removed monthly.

2.2. Culture medium

Hoagland's growth media (HM) was selected for this study because it contains nitrate as the sole nitrogen source, allowing for a more accurate representation of the targeted nitrogen specie typically found in effluents from small and decentralised wastewater treatment systems, where nitrification processes often convert ammonium into nitrate. HM was prepared from six autoclaved stock solutions containing all the required macro- and micro-nutrients for optimal duckweed growth, as described by Paterson et al. [44]. The final concentrations of nutrients in media are as follows: 543 mM $Ca(NO_3)_2*4H_2O$, 1 mM $MgSO_4*7H_2O$, 0.5 mM KH_2PO_4, 2.5 mM KNO_3, 23.1 µM H_3BO_3, 0.19 µM $NaMoO_4*2H_2O$, 4.6 µM $MnSO_4*5H_2O$, 0.13 µM $ZnSO_4*7H_2O$, 0.18 µM $CuSO_4*5H_2O$, 35.8 µM $FeCl_3*6H_2O$, 103 µM EDTA.

For all experiments, unless stated otherwise, the initial pH of the culture medium was adjusted to 7 using 1M NaOH, without the addition of any buffer. In experiments involving varying inorganic phosphorus levels, the volume of the KH_2PO_4 stock solution was adjusted to achieve the desired phosphorus concentration. Any resultant potassium deficiency was compensated by adding 1M KCl to maintain nutrient balance. Similarly, when modifying the total nitrogen concentration in the medium, the volumes of the $Ca(NO_3)_2$ and KNO_3 stock solutions were proportionally adjusted. Any imbalances in potassium and calcium levels due to these adjustments were corrected by adding 1M KCl and 1M $CaCl_2$, respectively.

2.3. Experimental setup

The first part of the experimental setup involved pre-cultivation. Healthy plants from the stock culture were selected, rinsed with deionised water, and pre-cultivated under specific conditions required for each experiment. Pre-cultures were maintained for a week in 1-litre plastic containers with full Hoagland's media, replenished every two days. Before each experiment, the pre-cultivated fronds were thoroughly washed with deionised water and placed in a separate container with phosphorus-deficient HM. These fronds underwent phosphorus starvation for at least five days to maximise phosphorus uptake response during the experiments [44]. Potassium deficiency was corrected by adding KCl.

Cultivation experiments were conducted inside a Fitotron® growth chamber (model SG066 CHX-F) with controlled temperature, light intensity and photoperiod, using static vessels. Each experimental unit consisted of a cylindrical plastic pot with a total volume of 150 mL (internal diameter of 6.25 cm, and height of 5 cm) filled with 100 mL

of sterile growth media. All experiments were performed under batch, non-sterile conditions, without mixing, aeration, recirculation or continuous inflow/outflow. Light was provided by fluorescent tubes at 150 µmol photons m^{-2} s^{-1}, in 12-hour light cycles. The pots were placed inside a cardboard box to minimise lateral light incidence and reduce algae proliferation. No online sensors were installed in the pots; environmental conditions were controlled by the growth chamber (light intensity and air temperature), while experimental measurements were obtained by scheduling a survey campaign to collect grab samples for analytical testing over the duration of each experiment (i.e., 8 days). Experiments were conducted in triplicate using an initial mass of 100 mg of fresh duckweed per pot.

2.3.1. Temperature-dependent growth experiments

W. angusta was cultivated at three different air temperatures (20, 25 and 30°C) using HM with no nutrient limitations (100 mg NO_3-N L^{-1} and 15 mg PO_4-P L^{-1}). Pre-culture containers were maintained at each set temperature to acclimate the plants. Experiments lasted eight days, with water samples taken every second day for nutrient characterisation, together with top-view photographs for calculating biomass growth using changes in surface area coverage. The 8-day duration was selected to capture growth and nutrient uptake before surface saturation was reached as in extended cultivation periods, duckweed plants approached full pot coverage, and this can introduce confounding effects associated with self-shading, reduced light availability, and space limitation. Experimental pots were replenished daily with HM lacking nitrogen and phosphorus to mitigate evapotranspiration. The resulting optimum temperature for nutrient uptake (25°C) was used for following nutrient controlled experiments.

2.3.2. Phosphorus and nitrogen uptake and removal experiments

Nutrient-dependent growth kinetics were assessed independently for phosphorus and nitrogen in short-term batch cultures by varying initial nutrient concentrations. The selected concentration ranges were chosen to characterise *W. angusta* responses under nutrient-limiting and nutrient-replete conditions for kinetic parameterisation within boundary conditions representing the selected application (hybrid ABR effluents) , rather than to reproduce the full range of nutrient concentrations expected in untreated or partially treated domestic wastewater. Phosphate experiments were conducted at 0.2, 2.0, 4.0, 6.0 and 8.0 mg PO_4-P L^{-1}, while nitrogen experiments were carried out at 0.5, 5.0, 10.0, 15.0 and 20.0 mg NO_3-N L^{-1}. These ranges allow estimation of nutrient-dependent growth and removal parameters, including half-saturation constants and maximum removal rates, while covering concentrations relevant to polishing-stage effluents from primary or secondary treatment systems.

At the start of each experiment series, healthy plants were inoculated into plastic pots containing 100 mL of modified Hoagland´s medium – i.e., with set N and P concentrations. For phosphorus experiments, the initial nitrogen concentration was kept constant at 100 mg NO_3-N L^{-1}, and for nitrogen experiments, the initial phosphorus concentration was kept constant at 15 mg PO_4-P L^{-1}, to ensure single-nutrient limitation. The medium was supplemented with KCl and $CaCl_2$ as needed to keep similar ionic strength across all experiments. Experimental pots were topped up daily with Hoagland's medium lacking either phosphorus or nitrogen to counter evapotranspiration and to keep single-nutrient limitation conditions. Experiments were

conducted at an air temperature of 25°C for eight days, with samples taken every two days.

2.3.3. Model validation experiments

To validate kinetic model and associated constants, *W. angusta* was cultivated under variable temperature and nutrient conditions. Air temperature ranged from 20 to 30°C, and phosphorus and nitrogen concentrations were adjusted to levels typically found in nutrient-rich treated wastewater effluents (0.2–4.0 mg P L^{-1}, 0.5–5 mg N L^{-1}) [45, 46 – 48]. Plants were grown in 150 mL plastic pots with an initial inoculum of 100 mg fresh biomass. Light was provided at 150 µmol photons m^{-2} s^{-1} in 12-hour light cycles. Over five days, biomass growth and nutrient removal from the medium were monitored.

2.4. Analytical methods

2.4.1. Duckweed growth monitoring

The growth of *Wolffia angusta* was assessed by measuring changes in the total frond area over time using image analysis. Overhead photographs of the cultivation pots were taken at a fixed height and analysed using Fiji (ImageJ, version 1.54g; Java 1.8.0_345, 64-bit), a free and open-source image processing software.

2.4.2. Analysis of nutrients in media

Water samples were collected and filtered using 0.45 µm syringe filters (Fisher Scientific, UK). Samples were diluted with distilled water when necessary. Phosphate concentrations were determined using the standard vanadate-molybdate colorimetric method [49]. Nitrate concentrations were measured using Hach® test kits (NO_3-N, LCK339), which have a detection range of 0.23 to 13.5 mg NO_3-N L^{-1}.

2.4.3. Data analysis

All the data were analysed using analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine statistically significant differences at $p = 0.05$. Model fitting and statistical analysis were conducted using Minitab software.

2.5. Model development and accuracy assessment

Building on experimental data, we developed and validated a kinetic model to describe the interconnected dynamics of duckweed growth and nutrient removal. A Monod-type formulation was selected to represent nutrient-limited growth, as duckweed biomass production and uptake rates exhibit saturating responses to external nitrogen and phosphorus concentrations, consistent with substrate-limited biological systems. In this study, the Monod-type formulation was not used to represent steady-state reactor operation, but rather to describe the dependence of duckweed growth and nutrient uptake on external nutrient availability during batch cultivation. The model combines classical first-order Monod kinetics with temperature-dependent functions and yield coefficients that are adjusted according to nutrient availability. Although internal nutrient reserves were not directly modelled, their influence was indirectly captured in the calibrated model. Experiments were allowed to continue even after the depletion of external nutrients, reflecting Duckweed's known ability to metabolise and remobilise their internal phosphorus and nitrogen reserves [44].

Light intensity and photoperiod, though recognised as influential factors in duckweed growth and nutrient uptake, were excluded as model variables. This decision aligns well with the model's intended application in tropical regions, where day length is

relatively consistent all year-round at about 12 hours and ambient light levels are typically in excess of the minimum required to support optimal growth – i.e., sunlight intensity in the tropics ranges from 460 to 6,000 µmol photons m^{-2} s^{-1} during the day [50]. Under these conditions, variations in light intensity and photoperiod are less likely to limit growth or significantly impact nutrient dynamics compared to temperature and nutrient availability.

Model output prediction intervals were calculated at the 95% confidence level, using the standard error of predicted values derived from the linear regression model based on the *t*-distribution of the data. Model performance, both pre- and post-calibration, was assessed using three key accuracy metrics: the coefficient of determination (R^2), root mean square error (*RMSE*), and residual predictive deviation (*RPD*). A well-performing model is typically characterised by a high R^2 value (>0.9), a low *RMSE*, and an *RPD* exceeding 2.5.

3. Results and Discussion

3.1. Temperature-dependent growth and nutrient removal in *Wolffia angusta* cultures

Duckweed growth, expressed as the relative increase in total frond area with respect to the cultivation pot area (fronds cover percentage), at three different temperatures is presented in Figure 1A. The results indicate that duckweed grew at all tested temperatures without a lag phase, thanks to pre-culture conditions, while significant differences in growth rates were observed across tested temperatures. At 20°C, plants exhibited the slowest growth, achieving only 45% surface coverage by the end of the 8-day experiment. In contrast, plants cultivated at 25°C and 30°C displayed

substantially higher growth, reaching full surface coverage by days 7 and 6, respectively. Once plants covered 100% of the surface, fronds began overlapping, making further growth quantification via image analysis impossible. This represents saturation of the coverage-based growth metric rather than a decline or stabilisation in biological growth. The relative growth rate (*RGR*) of *Wolffia* was determined by fitting an exponential growth model to data collected before plants reached full surface area coverage. A statistically significant effect of temperature on *RGR* was found (Table 1). Specifically, increasing the temperature within the tested range enhanced the growth rate of *Wolffia angusta*, with *RGR* values rising from 0.197 day^{-1} at 20°C to 0.427 day^{-1} at 30°C.

Nutrient removal from the growth medium was closely linked to temperature as well. Figure 1B illustrates phosphorus depletion, which occurred more rapidly as temperature increased. By the end of the experiment, phosphorus was nearly undetectable in media from pots maintained at 25°C and 30°C, while approximately 37% of the initial phosphorus supply remained in pots at 20°C. Nitrogen removal followed a similar trend (Figure 1C), with final nitrogen removal efficiencies of 29%, 52%, and 67% at 20°C, 25°C, and 30°C, respectively.

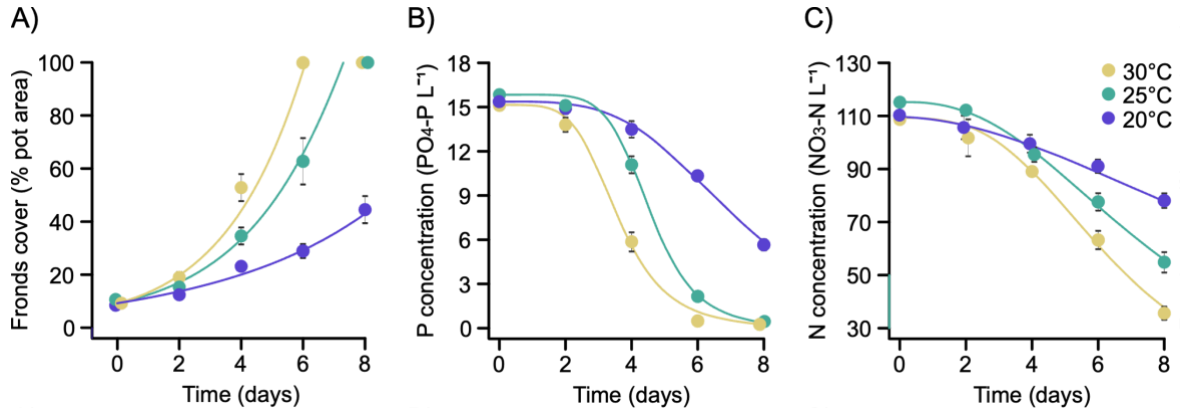


Figure 1. Influence of temperature on *Wolffia angusta* growth and nutrient removal. (A) Increase in duckweed fronds cover. (B) Remaining orthophosphate concentration in media, expressed as mg PO_4-P L^{-1}. (C) Remaining nitrate concentration, expressed as mg NO_3-N L^{-1}. The plants were grown at 20, 25, and 30°C in triplicates in standard Hoagland's media over 8 days. Error bars correspond to the standard deviation.

Table 1. Temperature-dependent rates of growth and nutrient removal in duckweed. The table summarises the relative growth rate and nutrient removal rates and specific nutrient uptake (phosphorus and nitrogen) of *Wolffia angusta* at three different temperatures. Values are presented as means with standard errors in parentheses. Statistical significance between mean values (p = 0.05, ANOVA + Tukey HSD test) at tested temperatures is indicated by different letters (a, b and c).

Temperature	20°C	25°C	30°C
Relative growth rate, d^{-1}	0.197 (0.014) [a]	0.337 (0.010) [b]	0.427 (0.018) [c]
Max P removal rate, mg P L^{-1} d^{-1}	2.08 (0.25) [a]	5.65 (0.57) [b]	5.11 (0.88) [b]
Specific P uptake, g P m^{-2} DW	0.74 (0.04) [a]	0.79 (0.06) [a]	0.75 (0.06) [a]
Max N removal rate, mg N L^{-1} d^{-1}	5.92 (1.16) [a]	9.21 (1.16) [b]	13.29 (1.98) [c]
Specific N uptake, g P m^{-2} DW	2.62 (0.33) [a]	2.18 (0.20) [b]	1.63 (0.20) [c]

Analysis of the maximum nutrient removal rates revealed two distinct trends (Table 1). For phosphorus, the maximum phosphorus removal rate (PPR_{max}) was lowest at 20°C (2.08 mg P L^{-1} d^{-1}) and increased by a factor of 2.7 at 25°C, reaching a plateau. Temperatures above 25°C did not further enhance the phosphorus removal rate. In contrast, the maximum nitrogen removal rate (NRR_{max}) showed a positive correlation with increasing temperature, rising from 5.92 mg N L^{-1} d^{-1} at 20°C to 13.29 mg N L^{-1} d^{-1} at 30°C.

Regarding specific nutrient uptake, temperature changes did not significantly affect specific phosphorus uptake, which remained consistent at an average of 0.77g of P per m^2 of duckweed cover across all tested temperatures. In contrast, temperature appeared to have an inverse effect on specific nitrogen uptake, decreasing from 2.62g of N per m^2 of duckweed cover at 20°C to 1.63 g N m^{-2} at 30°C (Table 1).

These responses indicate that temperature influenced total nutrient removal and biomass production differently. The increase in total nutrient removal at elevated temperatures was mainly associated with greater biomass accumulation, rather than enhanced per-unit biomass metabolic activity. This interpretation is supported by the increase in *RGR* with temperature, while specific P uptake remained unaffected, and specific N uptake decreased with increments in biomass production.

Although visual inspection of Figure 1B suggests faster phosphorus depletion at 30°C, variability among replicates and uncertainty associated with model fitting likely reduced the statistical separation between the 25°C and 30°C treatments. In addition, phosphorus concentrations approached near-complete depletion under both conditions during the experimental period, limiting detectable differences in the estimated maximum removal rates. It should also be considered that the *W. angusta* culture had been maintained under laboratory conditions at 20°C for approximately six months prior to experimentation. Therefore, plants transferred to 25°C and 30°C treatments were exposed to a relatively abrupt increase in temperature, which may have influenced short-term physiological and metabolic responses during the experiment.

Plants grown at 25°C and 30°C also achieved complete surface coverage rapidly, resulting in frond overlap during the later stages of cultivation. Under these conditions, increased competition for space and light may have affected both growth and nutrient uptake by reducing light penetration to lower fronds and altering resource allocation within the population [12, 51]. Although warm tropical conditions may support year-

round duckweed productivity, the increasing frequency of heatwaves could expose shallow ponds to temperatures exceeding the optimal range evaluated in this study (20–30°C). Under such conditions, growth and nutrient removal may be constrained by heat stress, rapid surface saturation, increased evapotranspiration, and intensified competition for light and nutrients [20, 42].

It should also be noted that the reported temperatures refer to controlled ambient air temperatures within a laboratory growth chamber, maintained within ±1°C of the target setpoints. Consequently, our experiments represent relatively stable thermal conditions and do not account for the short-term diurnal fluctuations or elevated seasonal temperatures outside the tested range (20–30°C) that may occur under outdoor operating conditions. Furthermore, the experiments do not capture the extreme temperatures that may become increasingly common under future climate scenarios.

It is worth noting that the mean air temperature of the coldest month is commonly used as the temperature design criterion for outdoor wastewater pond systems [52], owing to the difficulty of obtaining in-pond wastewater temperature data prior to system commissioning. Nevertheless, further work is needed to extend the model developed in this study to account for projected changes in mean air temperature, as well as the increasing frequency and intensity of extreme temperature events in tropical regions due to climate change.

Our results corroborate previous studies indicating that duckweed growth and nutrient removal are temperature-dependent processes, primarily mediated through changes

485 in biomass production. For instance, Wedge and Burris [53] reported an optimal
486 photosynthetic temperature range of 30–35°C for duckweeds, aligning with our
487 observation that *W. angusta* exhibited maximum relative growth rate at 30°C. Similarly,
488 Lasfar et al. [32] suggested an optimal growth temperature range of 23–28°C,
489 reinforcing the idea that duckweed growth efficiency declines when temperatures
490 deviate from this range. The observed disparity in optimal temperature conditions
491 among different duckweed species and isolates highlights the necessity for species-
492 specific studies to refine environmental conditions for maximal growth and nutrient
493 removal [23, 37]. While temperature response data exist for several duckweed genera,
494 published information specifically for *Wolffia* remains comparatively limited, despite
495 indications that isolates within the genus may exhibit distinct thermal optima affecting
496 growth and nutrient uptake [42].
497
498 The low Q10 temperature coefficient for nitrogen uptake (0.62) suggests a reduction
499 in apparent nitrogen assimilation as temperature rises [54], which may reflect changes
500 in metabolic processes, altered transport activity, and density-dependent limitations in
501 nitrogen availability per frond at higher biomass densities. This decline could be due
502 to morphological changes, altered transport protein activity, or temperature-driven
503 shifts in cellular mechanisms, as observed in terrestrial plant species [55, 56]. In
504 *Spirodela polyrhiza*, the reduction in specific nitrogen uptake at higher temperatures
505 is likely due to decreased protein synthesis, with resources being redirected towards
506 the production of heat shock proteins that require less nitrogen [57]. Meanwhile, stable
507 phosphorus uptake is linked to activated metabolic pathways for phosphorus and
508 carbon storage, which require significant ATP and extracellular phosphorus uptake
509 [57].

3.2. Impact of nutrient supply on *Wolffia angusta* growth and nutrient removal

Two separate experiments were conducted to investigate how phosphorus and nitrogen supplies affect both nutrient removal and the growth of *Wolffia angusta*. In each experiment, the concentration of only one nutrient was varied, while the other nutrient was replenished daily. This approach minimised the influence of other nutrient limitations on duckweed growth, as analyses of growth and nutrient uptake were restricted to the pre-saturation phase prior to full surface coverage. In the phosphorus experiment, media concentrations ranged from 0.2 to 8 mg PO_4-P L^{-1}. Phosphorus in media reached its minimum value more rapidly when the initial supply was lower (Figure 2A).

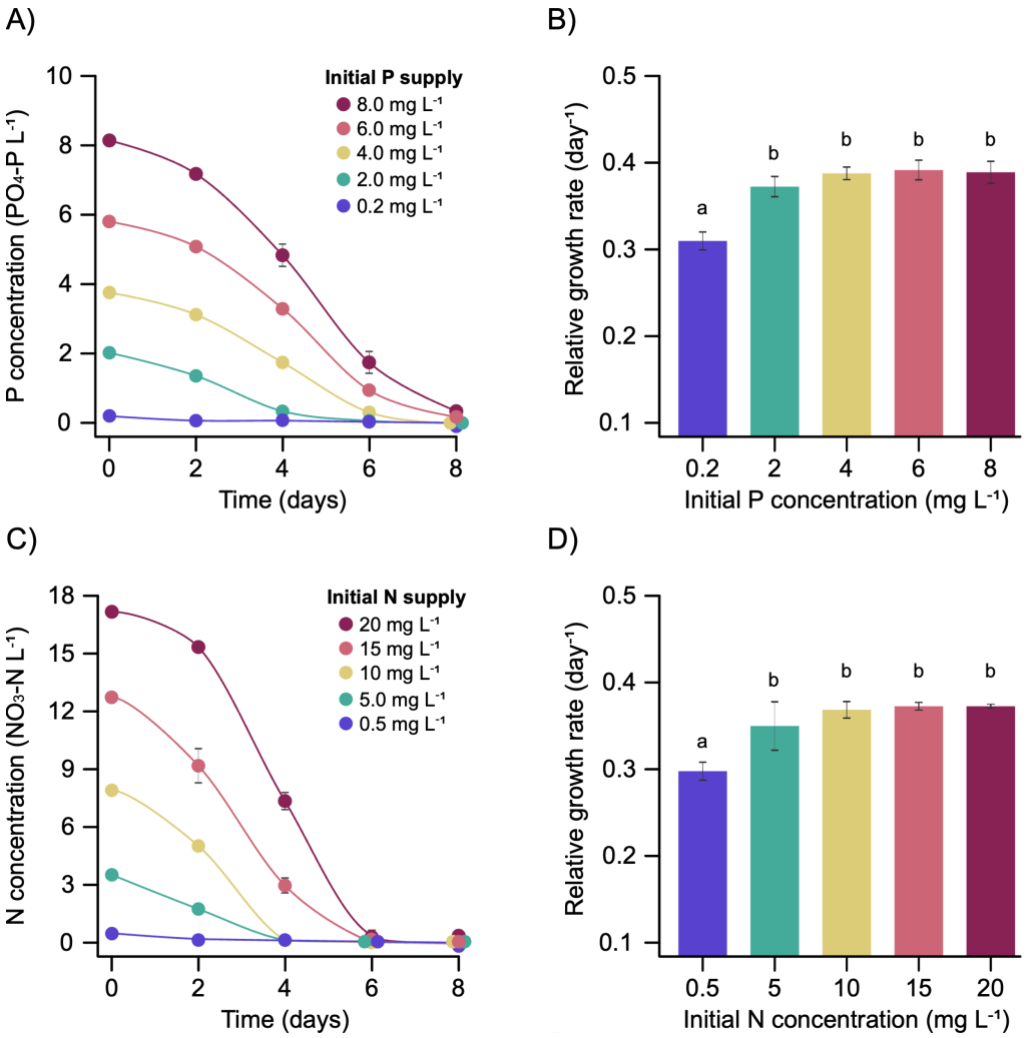


Figure 2. Nutrient removal and growth response of *Wolffia angusta* under different phosphorus and nitrogen supplies. (A) Change in orthophosphate concentration, expressed as mg PO_4-P L^{-1}, and (B) relative growth rate of plants cultivated at 25°C with varying initial phosphorus concentrations while maintaining a constant nitrogen concentration of 100 mg NO_3-N L^{-1}. (C) Change in nitrate concentration, expressed as mg NO_3-N L^{-1}, and (D) relative growth rate of plants cultivated at 25°C with varying initial nitrogen concentrations while keeping phosphorus concentration constant at 15 mg PO_4-P L^{-1}. Error bars correspond to the standard error of three experimental replicates. Bars with the same letter (a or b) are not statistically different from each other (p = 0.05, ANOVA + Tukey HSD test).

Over an 8-day period, complete removal of phosphorus was achieved only in the pots with an initial supply of 0.2 mg P L^{-1}; in all other treatments, removal ranged between 96% and 98%. *W. angusta* exhibited robust growth at all phosphorus concentrations except at 0.2 mg PO_4-P L^{-1} (Figure 2B). Under this low-phosphorus condition, duckweed fronds began to bleach and settle at the bottom of the pots from day 6

530 onwards. By the end of the experiment, duckweed in these pots covered only 36% of
531 the water surface, compared with complete coverage in the other treatments. Apart
532 from the lowest phosphorus treatment, the *RGR* of duckweed was not influenced by
533 the initial phosphorus concentration ($p < 0.05$, ANOVA with Tukey HSD test),
534 maintaining an average value of 0.35 day^{-1}; at 0.2 mg PO_4-P L^{-1}, the *RGR* decreased
535 by 26%.
536
537 Similarly, the nitrogen experiment involved initial nitrate concentrations ranging from
538 0.5 to 20 mg NO_3-N L^{-1}. In all cases, duckweed removed the available nitrogen by the
539 end of the experiment (Figure 2C). When the initial nitrogen concentration was below
540 15 mg NO_3-N L^{-1}, complete removal was achieved by day 4; at higher concentrations,
541 full removal occurred by day 6. With respect to growth, a low initial nitrogen
542 concentration (0.5 mg NO_3-N L^{-1}) resulted in many fronds settling at the bottom from
543 day 4, yielding a final frond cover of only 24%. At 5 mg NO_3-N L^{-1}, fronds began to
544 settle from day 6, although the final cover increased to 43%. At higher nitrogen
545 concentrations, the duckweed completely covered the pot surfaces. The RGR showed
546 a similar pattern to that observed in the phosphorus experiment. At 0.5 mg NO_3-N L^{-1},
547 the *RGR* was 0.25 day^{-1}, but at higher nitrogen concentrations the *RGR* increased and
548 stabilised at an average of 0.34 day^{-1} (Figure 2D, $p < 0.05$, ANOVA with Tukey HSD
549 test).
550
551 In parallel with these growth responses, nutrient removal rates showed clear
552 dependencies on the initial nutrient supply (Table 2). The maximum phosphorus
553 removal rate increased from 0.73 to 1.85 mg PO_4-P L^{-1} d^{-1} as the initial phosphorus
554 concentration rose from 2 to 8 mg PO_4-P L^{-1}. For nitrogen, the maximum removal rate

555 was 1.70 mg NO_3-N L^{-1} d^{-1} at the lowest supply of nitrogen, peaking at 6.85 mg NO_3-
556 N L^{-1} d^{-1} at the highest. Moreover, the specific uptake of both nutrients was directly
557 correlated with their initial concentrations. A 4-fold rise in the initial supply of
558 phosphorus and nitrogen resulted in increases of 79% and 68% in their respective
559 specific uptakes.

Table 2. Nutrient removal and uptake rates of duckweed at different nutrient concentrations. The table presents the nutrient removal and specific uptake rates of phosphorus and nitrogen by *Wolffia angusta* at varying initial concentrations of these nutrients in the growth medium. Duckweed was cultivated under controlled conditions at a constant temperature of 25°C and a daily light integral of 6.5 mol m^{-2}. Values are reported as means with standard errors in parentheses (n = 3). Statistical significance (p = 0.05, ANOVA + Tukey HSD test) between temperatures is indicated by different letters (a, b and c).

P supply (mg P L^{-1})	**2.0**	**4.0**	**6.0**	**8.0**
Max P removal rate mg P L^{-1} d^{-1}	0.73 (0.03) [a]	0.94 (0.19) [a, b]	1.44 (0.27) [b, c]	1.85 (0.33) [c]
Specific P uptake g P m^{-2} DW	0.19 (0.02) [a]	0.25 (0.01) [a, b]	0.30 (0.02) [b, c]	0.34 (0.04) [c]
Initial N:P (w:w)	50:1	25:1	17:1	13:1
N supply (mg N L^{-1})	**5.0**	**10**	**15**	**20**
Max N removal rate mg N L^{-1} d^{-1}	1.70 (0.33) [a]	4.67 (0.65) [b]	4.59 (0.75) [b, c]	6.85 (0.33) [d]
Specific N uptake g P m^{-2} DW	0.81 (0.21) [a]	1.00 (0.06) [a, b]	1.20 (0.09) [b, c]	1.36 (0.10) [c]
Initial N:P (w:w)	0.3:1	0.7:1	1.0:1	1.3:1

560

561 The observed trends in nutrient removal are consistent with earlier reports for
562 *Landoltia punctata*, where the highest phosphorus and nitrogen removal rates
563 occurred at the highest initial nutrient supplies [58]. However, *Wolffia angusta* showed
564 lower removal rates under our experimental conditions, with nitrogen and phosphorus
565 removal rates approximately 3.4-fold and 1.67-fold lower, respectively, likely reflecting
566 a combination of species-specific physiological differences, as well as methodological
567 differences between studies (i.e., including the use of lower nutrient concentrations,
568 the choice of nitrate instead of ammonium as the nitrogen source, and cultivation

conditions). Similar species-specific responses have been documented [23, 59, 60], highlighting that nutrient removal efficiency depends on both the inherent traits of the species and the chemical form of the nutrients. Notably, removal rates in *W. angusta* increased steadily with each increment in nutrient supply, indicating that saturation of nutrient uptake was not reached [37].

Growth responses further illustrate these dynamics (Figures 2B and 2D). *Wolffia angusta* growth was adversely affected only when the initial phosphorus concentration dropped below 2 mg P L^{-1}, a threshold similar to that observed in *Lemna minor* at 27°C [32]. The ability to thrive at low phosphorus levels depends on species-specific traits, the plant's internal phosphorus reserves, and prior environmental acclimation [44, 61]. Even at minimal phosphorus, sustained growth suggests that *W. angusta* mobilises internal reserves to support its metabolism – i.e., in this case, due to pre-culture conditions.

For nitrogen, growth limitation became evident when nitrate levels fell below 10 mg N L^{-1}, aligning with previous findings in *Lemna minor* [32]. This greater sensitivity to nitrogen likely reflects the higher nitrogen content in dry duckweed biomass (N:P = 15:1) and its essential role in protein synthesis, chlorophyll production and reproduction [62]. Moreover, although plant growth was not significantly affected by changes in nutrient concentrations above 0.2 mg P L^{-1} and 0.5 mg N L^{-1}, nutrient removal rates continued to increase. This pattern suggests that nutrient availability beyond a minimum threshold enhances biological uptake per unit biomass until nutrient transport systems reach saturation.

Research indicates that duckweeds reallocate nitrogen to support biomass growth and protein synthesis [18] and accumulate phosphorus as a safeguard against future shortages [63]. These insights are particularly relevant for wastewater treatment systems, especially those handling domestic wastewater, where phosphorus is often low and nitrogen is predominantly present as ammonium in raw influents (or after anaerobic pre-treatment), or as nitrate after aerobic pre-treatment [64]. Recognising the threshold concentrations limiting duckweed growth can inform operational strategies, such as optimising hydraulic residence times and adjusting biomass harvesting schedules, to maintain robust duckweed growth and enhance nutrient removal and uptake efficiency.

3.3. Determination of batch kinetics coefficients

For the formulation of the batch kinetics, several assumptions were made: the cultivation pots are small enough to minimize vertical stratification of nutrient concentrations; duckweed growth is related to the concentrations of nitrogen and phosphorus in the medium, as water and other nutrients are replenished during the test to avoid non-target limitations; no interference from other microorganisms occurs (sterile conditions); temperature remains constant over the course of the experiments; light is not a limiting factor (daily light integral between 5 - 20 mol m^{-2} [37]; water evaporation is negligible; environmental CO_2 is the sole carbon source; and there are no mass transfer limitations affecting nutrient diffusion and transport. While these conditions minimised external constraints, increasing biomass over time may still introduce density-dependent effects and local resource gradients; therefore, results are being interpreted within this dynamic growth context.

Under nutrient-rich conditions, the biomass growth rate can be described by an exponential growth model.

$$r_X = \frac{dX}{dt} = \mu * X \quad (1)$$

Here, r_X (or dX/dt), represents the plant growth rate (expressed as the increase in the pot's surface coverage in $cm^2\ cm^{-2}\ d^{-1}$); μ is the relative growth rate (d^{-1}), and X denotes the plants' surface cover in comparison with the full surface area at any given time ($cm^2\ cm^{-2}$).

To capture the influence of external nutrient concentrations on growth, the Monod's kinetic model [65] is applied:

$$\mu = \mu_{max} * \frac{P}{K_P+P} * \frac{N}{K_N+N} \quad (2)$$

In Equation 2, μ_{max} is the maximum relative growth rate, while P and N are the phosphorus (mg PO_4-P L^{-1}) and nitrogen (mg NO_3-N L^{-1}) concentrations in the medium, respectively. The constants K_P and K_N represent the substrate half-saturation constants – i.e., the nutrient concentrations at which the growth rate is half of μ_{max}.

Under conditions of nutrient excess and constant light, μ reaches μ_{max}, which can be further described as a function of temperature using an Arrhenius-type equation:

$$\mu_{max} = A_1 * exp^{\left(\frac{-E_1}{R*T}\right)} \quad (3)$$

Here, A_1 is a constant (day^{-1}), E_1 is the activation energy (J mol^{-1}), *R* is the universal gas constant (J $mol^{-1}\ K^{-1}$), and *T* is the absolute temperature (K).

The removal of phosphorus and nitrogen from the medium is directly mediated by plant growth. Therefore, the rates of substrate utilization are given by:

642 (4) $$r_P = \frac{r_X}{Y_{XP}}$$

643 (5) $$r_N = \frac{r_X}{Y_{XN}}$$

644 In these equations, r_P and r_N (mg substrate L^{-1} d^{-1}) are the rates of phosphorus and
645 nitrogen removal, and Y_{XP} and Y_{XN} (cm^2 per mg substrate) are the biomass yield
646 coefficients for each nutrient. The inverses of these coefficients represent the specific
647 uptake rates.
648
649 It is important to note that the biomass yield for each nutrient is not constant during
650 treatment but varies with the residual nutrient concentration in the cultivation medium
651 (as previously shown in Table 2). This variation was modelled using a logistic function
652 to describe yields at high nutrient concentrations (denoted as ${Y_{XP}}^{min}$ and ${Y_{XN}}^{min}$) and
653 the maximum yields observed in the absence of external nutrient supply (${Y_{XP}}^{max}$ and
654 ${Y_{XN}}^{max}$). The constants K'_P and K'_N determine the rate of change of the yield.
655 Additionally, a modified Arrhenius equation was used to adjust these parameters for
656 temperature changes relative to values obtained at 25°C:

657 (6) $$Y_{XP} = \frac{K'_P * {Y_{XP}}^{max} + P}{K'_P + (1/{Y_{XP}}^{min}) * P}$$

658 (7) $$Y_{XN} = \frac{K'_N * {Y_{XN}}^{max} + N}{K'_N + (1/{Y_{XN}}^{min}) * N}$$

659 (8) $$Value_T = Value_{25°C} * \theta^{(T-25)}$$

660
661 The kinetic parameters presented in Equations 1-8 were determined from
662 experimental data, and the compiled values are summarized in Table 3. The maximum
663 relative growth rate of *Wolffia angusta* at 25°C aligns with values reported for other
664 *Wolffia* species [23]. Although half-saturation values for phosphorus and nitrogen in
665 *W. angusta* have not been previously reported, the estimated K_P and K_N values are

significantly higher than those reported for *Lemna minor* (1.89 µg P L^{-1}, 0.02 mg N L^{-1}) and *Spirodela polyrhiza* (0.87 mg P L^{-1}, 0.02 mg N L^{-1}) (Kufel et al., 2012). The elevated half-saturation constants indicate that *W. angusta* requires higher nutrient concentrations to reach half of its maximum growth rate, which explain similar growth rate results obtained from this work for a wider range of N:P ratios (0.7:1 < N:P < 25:1). Consequently, while it may grow robustly under nutrient-rich conditions, it could be less competitive in nutrient-poor environments compared to other duckweed species.

Table 3. Kinetic constants for the growth model of *Wolffia angusta*

Parameter	Description	Value (SD)	Unit
$\mu_{max}{}^{25°C}$	Maximum RGR at 25°C	0.30 (0.01)	day^{-1}
$Ln(A_1)$	N-log Arrhenius pre-exponential factor	18.59 (3.90)	day^{-1}
E_1	Growth activation energy	48.97 (9.68)	kJ mol^{-1}
K_P	Half saturation constant for phosphorus	0.048 (0.012)	mg P L^{-1}
K_N	Half saturation constant for nitrogen	0.244 (0.030)	mg N L^{-1}
$Y_{XP}{}^{max}_{25°C}$	Maximum biomass-to-phosphorus yield at 25°C	113.6 (37.1)	cm^2 mg^{-1}
$Y_{XP}{}^{min}_{25°C}$	Minimum biomass-to-phosphorus yield at 25°C	18.9 (2.52)	cm^2 mg^{-1}
$K'_{P}{}_{25°C}$	Biomass-to-phosphorus yield rate constant	0.055 (0.014)	mg^2 L^{-1} cm^{-2}
$Y_{XN}{}^{max}_{25°C}$	Maximum biomass-to-nitrogen yield at 25°C	24.7 (6.6)	cm^2 mg^{-1}
$Y_{XN}{}^{min}_{25°C}$	Minimum biomass-to-nitrogen yield at 25°C	4.94 (0.48)	cm^2 mg^{-1}
$K'_{P}{}_{25°C}$	Biomass-to-phosphorus yield rate constant	0.566 (0.356)	mg^2 L^{-1} cm^{-2}
θ	Temperature correction factor	1.054 (0.012)	-

Nutrient removal rates and kinetic coefficients reported here are based on changes in soluble nitrate and phosphate concentrations in the culture medium. Therefore, they describe liquid-phase nutrient depletion rather than a complete nitrogen and phosphorus mass balance. Since it was not possible to quantify N and P contents in harvested duckweed biomass, the fraction of removed nutrients directly incorporated into *Wolffia* biomass could not be quantified. For that reason, other possible pathways,

including microbial transformation, adsorption, precipitation, or retention in non-harvested biomass or settled material, cannot be entirely dismissed. Consequently, the model should be interpreted as predicting nutrient depletion from the medium, not the full distribution of nitrogen and phosphorus within the experimental system. Future work should therefore quantify nutrient content in harvested biomass to complete the system mass balance and validate nutrient partitioning.

3.4. Batch model validation and prediction

In a batch duckweed cultivation system, biomass accumulation and nutrient removal are dynamically interlinked and hence, our model couples an exponential growth formulation (1) with substrate utilization (4 and (5), to capture this relationship. In axenic cultures, nutrient uptake is directly proportional to biomass growth [44], meaning that as the duckweed surface coverage increases, phosphorus and nitrogen are removed from the medium in fixed proportions defined by their yield coefficients. Furthermore, by incorporating temperature effects on the maximum growth rate via an Arrhenius-type adjustment (3), the model becomes adaptable to temperature-dependent growth responses within the experimentally evaluated range.

The model was calibrated using experimental data from duckweed grown under simulated tropical conditions with varying temperature, (see Section 2.3.3) where phosphorus and nitrogen supplies were varied within ranges typically found in nutrient-rich wastewaters (Figure 3).

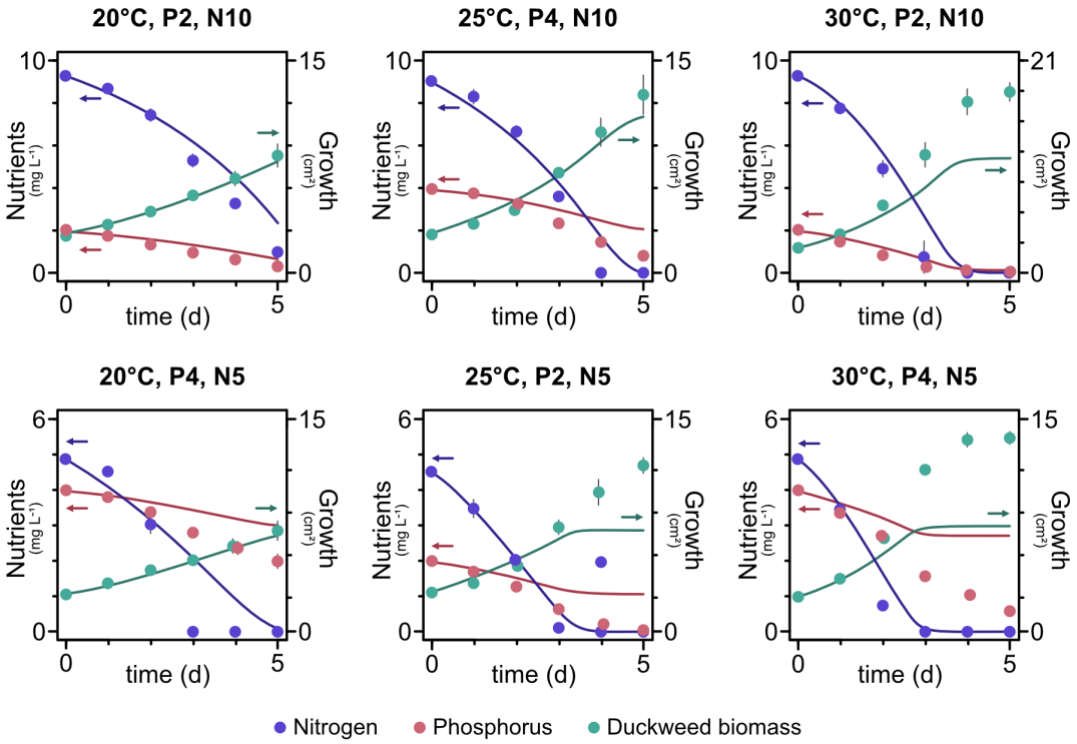


Figure 3. Experimental and modelled outcomes for the cultivation of *W. angusta* at varying culture conditions. Dots represent experimental data while continuous lines predict values from the kinetic model. Data for nitrogen and phosphorus concentration should be read against the left axis while duckweed growth data (expressed as increased in total fronds area) should be read against the right axis, as marked by the arrows. Culture conditions for each experiment are shown at the top of each graph. The initial concentration of phosphorus (P) and nitrogen (N) is represented as the number next to the letters, in mg PO_4-P L^{-1} and mg NO_3-N L^{-1}, respectively.

703

704 Experiments that maintained sufficient nutrient availability throughout, such as those
705 at 20°C with 2 mg PO_4-P L^{-1} and 10 mg NO_3-N L^{-1}, were better reproduced by the
706 model ($R^2 > 0.787$), whereas experiments in which one or both nutrients were absent
707 or completely depleted (e.g., at 30°C) over the course of the experiment resulted in
708 poorer model performance ($R^2 \approx 0.478$). These discrepancies can be attributed to the
709 limitations of the traditional Monod kinetic model, which only accounts for external
710 nutrient concentrations and neglects the role of internal nutrient reserves. In reality,
711 duckweed can mobilise internal stores of phosphorus and nitrogen to sustain growth
712 under nutrient scarcity [44]. Consequently, the original model assumed that plant

growth would cease once an external nutrient was depleted (i.e., N and P in the media), leading to underestimations of biomass accumulation.

To overcome this shortcoming, the kinetic model was modified to accommodate a finite growth rate under nutrient-depleted conditions by adopting a logistic growth formulation (9). This modification introduces two new parameters: a specific growth rate (μ_i), which takes on distinct values (μ_{P_0}, μ_{N_0}, or μ_{P_0,N_0}) depending on whether phosphorus, nitrogen, or both are absent in media, and a carrying capacity (L_i) that represents the maximum duckweed coverage achievable under nutrient limitation. In parallel, the expression for the relative growth rate of *Wolffia angusta* was adjusted () to integrate the effects of internal nutrient reserves, thereby refining the model's representation of plant growth in the absence of external nutrients.

$$r_X = \frac{dX}{dt} = \begin{cases} \mu * X, & [P],[N] > 0 \\ \mu_i * X * \left(\frac{L_i - X}{L_i}\right), & [P],[N] = 0 \end{cases} \quad (9)$$

$$\mu_i = \begin{cases} \mu_1 = \mu_{max} * \frac{P}{K_P + P} * \frac{N}{K_N + N} \\ \mu_2 = \mu_{P_0} * \frac{K_P}{K_P + P} * \frac{N}{K_N + N} \\ \mu_3 = \mu_{N_0} * \frac{K_N}{K_N + N} * \frac{P}{K_P + P} \\ \mu_4 = \mu_{P_0,N_0} * \frac{K_P}{K_P + P} * \frac{K_N}{K_N + N} \end{cases} \quad (10)$$

Prior to calibration, the model exhibited variable predictive ability, particularly under conditions characterised by poor nutrient concentrations in the medium. This is evident in the lower *R²* values and wider spread of data points around the 1:1 line in the corresponding correlation plots. For instance, in the pre-calibration plots (Figure 4, left panel), R^2 values were noticeably moderate to low, suggesting only a modest

proportion of the variance in observed values was explained by the model. In addition, *RMSE* values were relatively high, indicating greater discrepancies between predicted and observed values. The *RPD* values, which offer insight into the practical utility of the model (with values below 1.5 typically indicating poor predictions), were also unsatisfactory before calibration.

Following calibration (Figure 4, right panel), a substantial improvement in model performance is evident across all conditions. R^2 values increased significantly, in some cases approaching or exceeding 0.9, which reflects a much stronger agreement between observed and predicted values. The *RMSE* decreased notably across calibrated conditions, demonstrating a reduction in prediction errors. Correspondingly, *RPD* values improved, often exceeding the threshold of 2.0, suggesting the model's predictions became reliable and suitable for analytical purposes. Particularly under conditions with low nutrient concentrations, the calibration procedure had a marked impact. The improvement in prediction is highlighted by the narrowing of the 95% confidence intervals and the tighter clustering of data points along the ideal 1:1 line, reducing bias and enhancing model robustness.

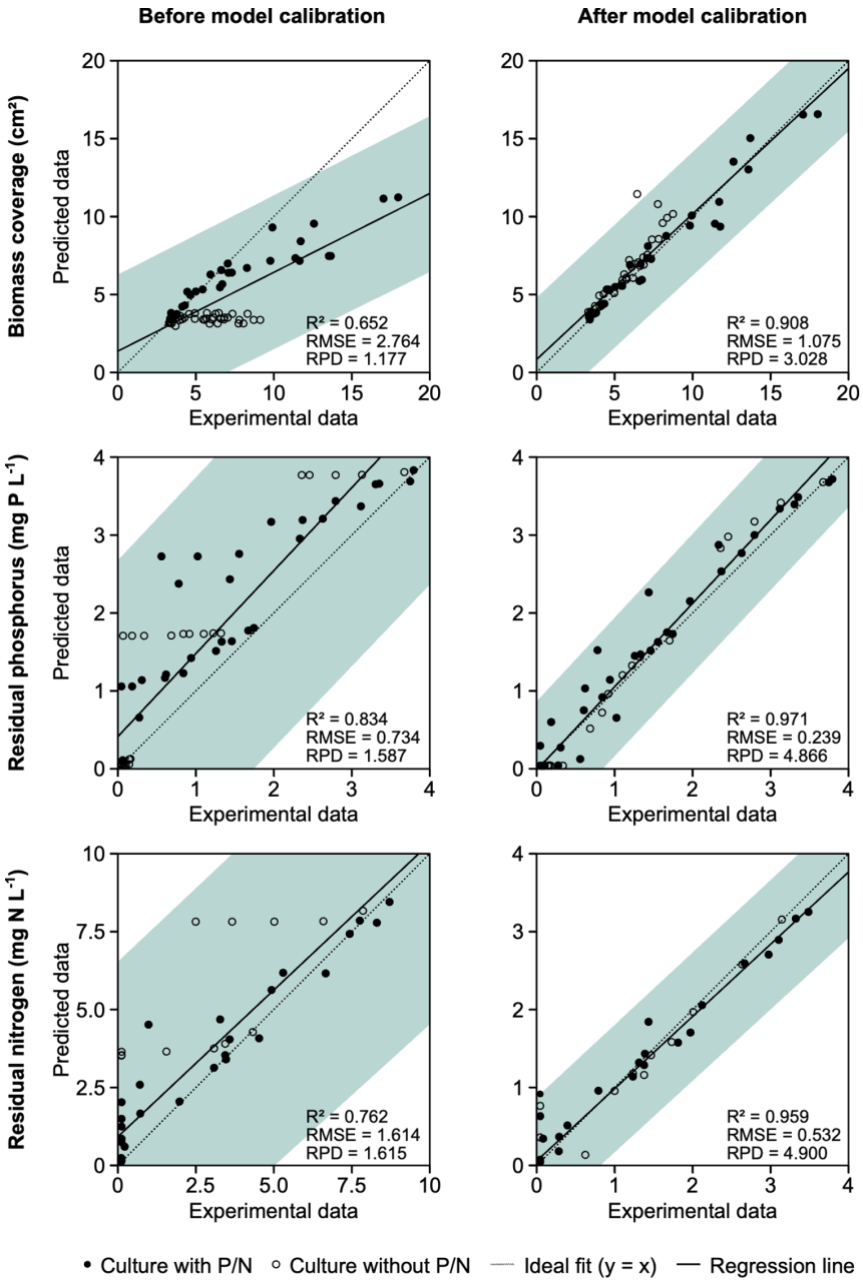


Figure 4. Correlation plots between experimental and predicted data from uncalibrated and calibrated kinetic batch models

The figure shows six scatterplots comparing observed and predicted under different modelling conditions, illustrating model performance before (left panel) and after calibration (right panel). Shaded areas represent the 95% confidence intervals around the predicted values, indicating the uncertainty of model estimates. Three statistical metrics, the coefficient of determination (R^2), the root mean square error (RMSE), and the ratio of performance to deviation (RPD), are reported within each plot to quantitatively assess model accuracy. Data points represent experimental results from cultures with high (solid dots) and low (hollow dots) nutrient availability.

3.5. Model application: Design of duckweed-based polishing systems

To illustrate a potential application of the calibrated kinetic model beyond the prediction of biomass production and nutrient uptake under tested experimental

765 conditions, we performed a scenario-based simulation of a *Wolffia*-based polishing
766 system treating effluent from a hybrid anaerobic baffled reactor (HyABR) system,
767 under continuous flow and tropical conditions in Indonesia. The purpose of this
768 simulation was not to validate a full-scale treatment design, but to explore how the
769 model could be used to compare theoretical polishing-system configurations under
770 clearly defined assumptions.
771
772 The simulated scenario was based on decentralised urban wastewater management
773 conditions relevant to Indonesia, where HyABR systems have been reported as a
774 popular treatment technology with limited nutrient control capacity [66, 67]. The
775 simulations assumed a freshwater consumption of 120 L person^{-1} day^{-1}, with 80%
776 returned as wastewater [68]. HyABR effluent was modelled with nutrient
777 concentrations of 20 mg N L^{-1} and 5 mg P L^{-1} [67] and assuming that aeration
778 chambers in HyABRs achieve full nitrification. The number of duckweed polishing
779 ponds arranged in series was varied from 1 to 12 under simulated tropical conditions
780 (25°C, 12-hour photoperiod, and non-limiting light intensity). Initial duckweed surface
781 coverage in each pond was set at 50% and allowed to increase until full (100%)
782 coverage was reached, after which sufficient biomass was harvested to restore
783 coverage to 50%.
784
785 The simulations were set to identify the hydraulic retention (HRT) required to meet a
786 set regulatory phosphorus discharge limit of 1 mg P L^{-1} to prevent eutrophication in
787 receiving waters. The resulting model outputs are presented in Figure 5. Under the
788 assumptions used, the results indicate that increasing the number of duckweed ponds
789 in series reduces both the total HRT and the treatment area per person needed to

meet the effluent phosphorus target (Figures 5A and 5B). However, this also results in lower duckweed biomass productivity (Figure 5C). Specifically, the single-pond configuration requires a HRT of 52.5 days and a treatment area of 10.1 m^2 per person, producing 1.448 tonnes of fresh duckweed biomass per day. Increasing the number of ponds from 1 to 12 reduces total HRT, treatment area, and biomass productivity by 28.8%, while the corresponding per-pond values decreased by 94.1%.

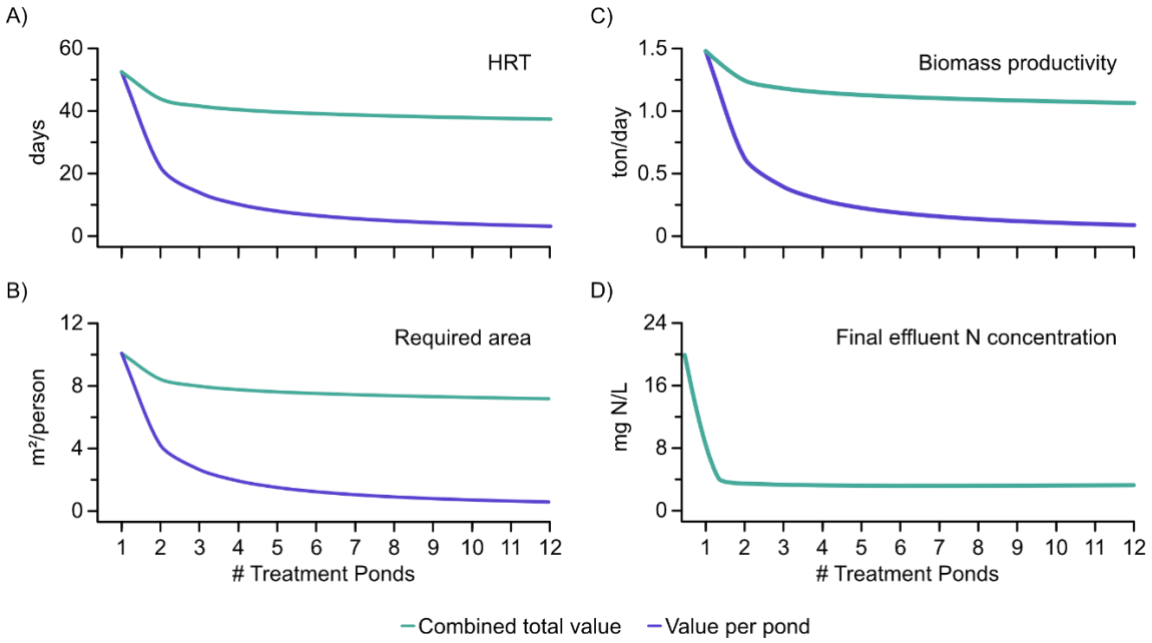


Figure 5. Scenario-based simulated performance of a duckweed-based polishing system treating hybrid anaerobic baffled reactor (HyABR) effluent under tropical conditions. The figure illustrates a theoretical model application rather than an experimentally validated system design. System performance was evaluated based on (A) hydraulic retention time (HRT), (B) treatment area required per person, (C) fresh duckweed biomass productivity, and (D) concentration of nitrogen in the final effluent, across systems with varying numbers of ponds. Results are shown as both per-pond values and total values for the entire treatment system. Simulations assumed typical tropical conditions (25°C, 12-hour photoperiod, and sufficient light intensity), for a population of 2,000 people using 120 L/person/day of freshwater. Wastewater return was set at 80%. Post-ABR effluent concentrations were assumed to be 20 mg N L^{-1} and 5 mg P L^{-1}. Duckweed coverage in each pond was initially set at 50% and was reset to the same value whenever the plants covered the entire pond surface. In all cases, the simulation aimed to achieve a final effluent phosphorus concentration of 1 mg P L^{-1}.

In all simulated cases, nitrogen removal exceeded 80%, with final concentrations below 4 mg N L^{-1} (Figure 5D). These simulated values are in compliance with current

regulatory thresholds in Indonesia (< 10mg N L^{-1}, Ministry of Environment and Forestry of the Republic of Indonesia, Regulation No. P.68/2016), and thresholds applied in sensitive areas in the UK, where total nitrogen limits of < 10mg N L^{-1} and minimum nitrogen removal efficiencies of 70-80% may apply [69]. However, because the complete polishing-system configuration was not experimentally validated, these results should be interpreted as model-based estimates rather than evidence of regulatory compliance under field conditions.

Under the assumptions used in this scenario, a polishing system consisting of two to three ponds in series appears to provide favourable balance between treatment performance, surface area requirements, and biomass productivity. This configuration achieved the set phosphorus target in the final effluent while also maintaining predicted nitrogen concentration below selected nitrogen thresholds. The addition of further ponds does not provide significant benefits, as it neither reduces the required surface area nor increases biomass production. Therefore, the two- to three-pond configuration may represent a useful starting point for future experimental or pilot-scale evaluation, rather than a validated optimal design.

Beyond treatment performance, the model application also suggests that duckweed-based polishing ponds could provide opportunities for resource recovery through harvested biomass. At this scale and based on the simulated biomass production and the reported composition of duckweed biomass, including 28% protein on a dry-weight basis and 12.1 g P kg^{-1} dry weight [40], the harvested *W. angusta* biomass in the simulated system could theoretically enable the recovery of approximately 0.44 tonnes of phosphorus and 1.65 tonnes of nitrogen per year. These estimates are intended to

illustrate the potential relevance of duckweed polishing systems for resource recovery and circular-economy strategies in urban wastewater treatment, but they require validation under representative operating conditions.

The potential use of harvested *W. angusta* biomass for animal feed, fertiliser, or other valorisation pathways would depend on its safety and regulatory acceptability. Biomass grown on wastewater may accumulate contaminants such as pathogens, heavy metals, organic micropollutants, and residual pharmaceuticals, depending on the wastewater source and treatment conditions. Therefore, although duckweed biomass has nutritional and nutrient-recovery potential, its direct use as feed or fertiliser cannot be assumed without appropriate characterisation and risk assessment. Future work should evaluate the microbiological quality, contaminant accumulation, nutrient composition, and post-harvest treatment requirements of biomass produced on real wastewater before recommending specific reuse pathways.

4. Conclusion

This study presents a robust kinetic model that accurately predicts duckweed biomass productivity and nutrient depletion under varying tropical conditions, including nutrient-limited scenarios. The model offers a practical tool for optimising decentralised, nature-based wastewater treatment systems using *Wolffia angusta*, particularly in tropical regions impacted by nutrient-rich discharges.

Consistent with previous studies on duckweed systems, our findings reveal temperature as a key driver of biomass growth and nutrient removal by *W. angusta*, with optimal performance between 25 and 30°C. Within the analysed pre-saturation growth phase, higher temperatures promoted biomass accumulation without a

proportional increase in nutrient uptake rates, indicating that growth governs nutrient removal. Nutrient availability strongly influenced both biomass production and nutrient uptake patterns, with low nutrient concentrations limiting growth, and different N:P ratios leading to distinct responses in growth and nutrient removal efficiency. Batch systems performed best at high nutrient concentrations, maximising both growth and nutrient recovery. Beyond controlled experiments, the model was applied to simulate a duckweed-based unit to polish the effluent from a hybrid anaerobic baffled reactor (HyABR), a common decentralised treatment approach in Indonesia. These simulations illustrate the potential use of the model for preliminary design exploration, but they should not be interpreted as validated design recommendations. The model was developed and evaluated under controlled experimental conditions and was not validated using real wastewater, which is a limitation of the present study. Future work should validate the model using real wastewater and pilot- or field-scale duckweed polishing systems and should include a comprehensive mass balance of nutrients and duckweed biomass characterisation (protein, starch fibre, bioactive compounds, etc.) to further explore internal nutrient reserves, microbial interactions, system resilience under dynamic environmental conditions and downstream valorisation of harvested biomass.

Declaration of Competing Interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Johan Pasos-Panqueva: Conceptualisation, Experimental design, Experimental work, Data curation and processing, Model development and validation, Writing – original draft, review and editing. Anie Yulistyorini: Writing – review and editing. Alison Baker: Conceptualisation, Experimental design, Data curation, Supervision, Resources, Writing – review and editing. Miller Alonso Camargo-Valero: Conceptualisation, Experimental design, Data curation, Supervision, Resources, Funding acquisition, Writing – review and editing.

Data access statement

All data underlying the results are available as part of the article and no additional source data are required.

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Present Address Notes

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