Water Science & Technology

 Water Science & Technology Vol 86 No 6, 1325 doi: 10.2166/wst.2022.260

# A techno-economic assessment of nutrient recovery from wastewater using microalgae: scenario in India collected from published literature

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## ABSTRACT

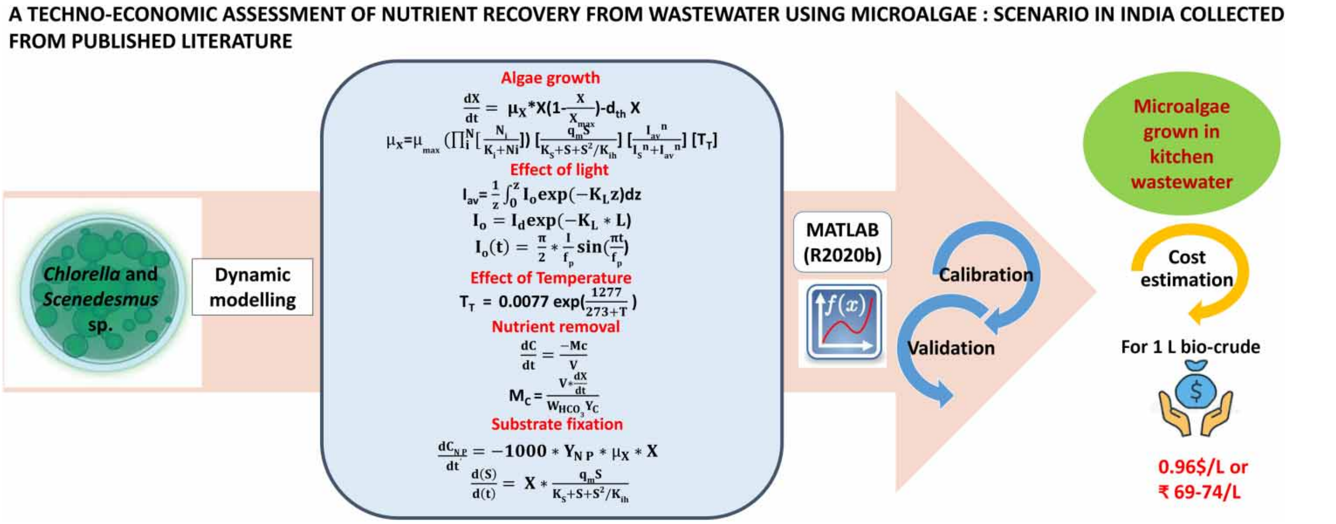
The true potential of the microalgae-based wastewater treatment (MWT) process is determined based on whether the process will provide a positive energy output and whether it is economically viable. The objectives of this study are dynamic modelling of microalgae growth based on initial wastewater concentration, temperature, solar radiation and a techno-economic assessment for an MWT scheme for application in a hot, dry climate. Through reference to relevant literature data on MWT in the Indian subcontinent, a selection of appropriate microalgal species *Chlorella* and *Scenedesmus* was made. The dynamic model developed was successfully calibrated and validated using independent experimental data collected from the published literature. Cost of production of bio-crude from microalgae grown in a hybrid photobioreactor and pond system in kitchen wastewater of Indian Institute of Technology, Hyderabad was calculated. A break-even selling price (BESP) of US$0.549/kg was obtained for the microalgae biomass. The cost of production of 1 L bio-crude was US$0.96 (Rs 69–74), which is comparable with crude oil cost. The model developed can be used by practising engineers to predict biomass growth and nutrient removal, thereby achieving a break-even point for cost efficiency.

**Key words:** break-even selling price, dynamic microalgae modelling, Indian conditions, nutrient removal, techno-economic analysis, wastewater treatment

## HIGHLIGHTS

- Dynamic modelling of microalgae growth and nutrient removal in a warm arid climate.
- *Chlorella* and *Scenedesmus* were the most suitable microalgae species for nutrient recovery.
- Microalgae grown in a hybrid photobioreactor and pond system in kitchen wastewater.
- Break-even selling price of US$0.549/kg for algal biomass to cover operating costs.
- Cost for 1 L bio-crude production US$0.96 (Rs 69–74) under optimum conditions.

## GRAPHICAL ABSTRACT




## INTRODUCTION

Nutrients in wastewater, if released untreated, can induce eutrophication in surface water bodies and disrupt the ecosystem's equilibrium. Therefore, wastewater treatment systems are built to reduce nutrient levels to permissible levels before discharge and reuse. Microalgae have been shown to be effective in removing nutrients from a variety of wastewaters, including municipal and industrial wastewater. (Li *et al.* 2019).

In the process of remediating wastewater, microalgae can also generate by-products such as biofuel. Microalgae produced in wastewater have the benefit of not competing with crops for cultivable land and freshwater because they may be cultivated in wastewater and on non-cultivable land (Cai *et al.* 2013). Second, with a dry weight oil content of 20–50%, they may develop swiftly. They can also fix carbon dioxide, lowering greenhouse gas emissions and improving air quality (Al Ketife *et al.* 2019). They can utilise nutrients from a wide range of wastewaters, assisting in effluent treatment (Li *et al.* 2019), and algal biomass residue, a by-product of microalgae production after lipid extraction, may be used as a nitrogen source for crops or protein-rich animal feed (Cai *et al.* 2013). So wastewater treatment process based on microalgae has a wide variety of applications, including carbon dioxide reduction, biofuel production and wastewater treatment. Considering the nutrient requirements of microalgal growth, 1 $Mm^3$ of sewage can produce around 1 ton of microalgal biomass (Acién *et al.* 2016). Using industrial fertilisers, microalgae production costs can be around US$100/kg of biomass (Delrue *et al.* 2016).

The composition of the wastewater has a considerable impact on the microalgal treatment performance. Microalgae require three essential nutrients to grow and function: carbon (C), nitrogen (N), and phosphorus (P). The difference in carbon, nitrogen and phosphorus concentrations between wastewater streams has been shown to alter microalgal removal efficiency (Mohsenpour *et al.* 2021). A study by Cabanelas *et al.* (2013) found that the microalgae *Chlorella vulgaris* strain had different treatment efficiency in different types of wastewater streams. Experiments for each wastewater stream were done on samples from two different wastewater treatment plants. Higher total nitrogen (TN), total phosphorus (TP), and COD removal rates were found when cultivated in raw wastewater compared to secondary treated. The raw samples had higher *C. vulgaris* growth rates, ranging from 111 to 125 mg/L/d, compared with 63 to 68 mg/L/d in the secondary treated samples.

When nutrients (such as N and P) become scarce, the indigenous microbial population in wastewater may compete for resources with exogenous microalgae (which are added to the wastewater). As a result, it is critical to create an environment that favours the growth of microalgae over bacteria and fungi. Temperature and light have a considerable impact in this context (Mohsenpour *et al.* 2021). Microalgae productivity and nutrient removal efficiency have been shown to be influenced by light intensity, light frequency, photoperiods and environmental temperature in many studies.

The potential of freshwater microalgae *Chlorella protothecoides* in tertiary treatment of municipal wastewater was investigated by Binnal & Babu (2017) using a laboratory-scale externally lighted photobioreactor. The impact of variables such as light intensity, photoperiod, pH, carbon dioxide content in the air, temperature, and aeration rate on wastewater nutrient removal efficiency was investigated. The highest removal of COD (78.03% on the 10th day), 100% removal of TN (on the 7th day), and 100% removal of TP (on the 6th day) was observed under optimum conditions (light intensity – 6 klux, photoperiod – 16 h:8 h, pH 6.8, the concentration of carbon dioxide in air was 6%, temperature 25 °C, and aeration rate 3 lpm). Under ideal conditions, the greatest biomass concentration was 1.96 g/L. According to Binnal & Babu (2017), low irradiance and photoperiods (2 klux and 8 h:12 h) resulted in low treatment efficiency (17.97% COD, 29.44% total nitrogen, and 23.91% total phosphorous). Operating the PBR at a non-optimal lower temperature (20 °C) or higher temperature (30 °C) did not improve nutrient removal efficiency.

While screening microalgae strains for wastewater treatment, the factors considered are high nutrient removal efficiency, fast growth rate, strong adaptability to local climate and a variety of wastewater, and high biomass productivity. Usually, all these criteria might not be satisfied simultaneously. When numerous criteria cannot be met, the first choice of 'rapid growth rate' should be prioritised as it demonstrates the adaptability of a selected strain, which may be associated with a high nutrient removal rate and strain efficacy. A wide variety of microalgae species has been utilised to remediate wastewater. Chlorophytes are one of the biggest phyla of microalgae, with a diverse range of species and geographical distribution. *Chlorella* and *Scenedesmus* are the most often employed chlorophytes for nutrient recovery from wastewater. Supplementary Table 1 gives the nitrogen and phosphorus removal from diverse waste streams in India using various microalgae.

According to the Stumm empirical molecular formula for microalgae ($C_{106}H_{263}O_{110}N_{16}P$), 1 g algal biomass requires 0.063 g nitrogen and 0.009 g phosphorus (Shukla *et al.* 2020). In most wastewater, N:P ratios may be as low as 4–5 (Eladel *et al.* 2019). The ideal N:P ratio for *Chlorella* has been reported to be 7, which is consistent with the N:P ratio of 7.2 determined using the Stumm empirical formula for microalgae. *Scenedesmus*, on the other hand, requires an N:P ratio of around 30 to develop without being hampered by any of the nutrients. (Park *et al.* 2010).

Nitrogen and phosphorous (N&P) removal efficiencies, in batch cultures, from different wastewater using *Chlorella* are in the range 75–100 and 55–100% (Supplementary Table 1), while that for *Scenedesmus* reported removal efficiencies are in the range 80–100% and 55–100%, respectively. Typically, *Chlorella* has been reported to contain around 15–25%, 50–60% and 35–65% of carbohydrate, proteins and lipids, respectively, while *Scenedesmus* has around 20–50%, 40–50% and 40–50% of carbohydrate, proteins and lipids, respectively (Li *et al.* 2019). While growing on wastewater, both the species have been reported to accumulate up to 30% of lipid (Supplementary Table 1).

The proportions of different compositions of carbohydrates, proteins and lipids can vary significantly among species and are based on the variation in culture conditions, and it can vary even in the same strain. However, the largest percentage is usually found to be proteins, followed by lipids and carbohydrates. *Scenedesmus* microalgae strain has been observed to be more tolerant of high $CO_2$ concentrations (10–80%) than the *Chlorella* strain, but both species can thrive at lower (10–30%) $CO_2$ concentrations as well (Molazadeh *et al.* 2019).

One of the most challenging issues in microalgae-based technology is properly understanding the kinetics of microalgal growth, which is required for the optimal design and operation of microalgae reactors for wastewater treatment. Also, the true potential of the algae-based approach is determined based on whether the process will provide a positive energy output and whether it is economically viable. There comes the role of techno-economic assessment in wastewater treatment and nutrient recovery using microalgae. In order to predict commercial feasibility, any forthcoming technology must undergo a thorough review of its economic potential. Microalgae-based technologies, for example, are still in the early stages of commercialisation in several developing countries, such as India, and their future is uncertain; therefore, industrial feasibility studies and sensitivity analyses based on peer-reviewed data and industrial expertise are required. If cost-effective and sustainable microalgal technologies can be created, the potential benefits that appear to be compelling can be proven, which will help policymakers develop research and development investment policies in the near future (Kumar *et al.* 2020).

Many techno-economic analyses on microalgae wastewater treatment and biomass production have been published worldwide. Some of the analyses are based on impractical productivities impossible to attain with solar radiation, which are extrapolated from theoretical photosynthetic efficiencies (Tredici *et al.* 2016). Most analyses do not target specific products except for biofuels. Some of the analyses have not considered location, which is an important parameter since environmental conditions like temperature and light strongly affect productivity (Tredici *et al.* 2016). So, there is a lot of room for experimentation when it comes to the economics of employing microalgae for wastewater treatment and subsequent by-product development in the subtropical environment of the Indian subcontinent, where there is an abundance of natural light. This exercise might make microalgal technologies doable in this region.

This paper aims to the dynamic modelling of expected microalgae growth based on initial wastewater concentration, temperature and solar radiation since a well-built microalgae kinetic model is critical for predicting biomass growth, removing nutrients, and optimising operating conditions. A techno-economic assessment of sustainable microalga-based nutrient recovery and wastewater treatment schemes for use in a hot, dry climate is also performed, allowing for the strategic planning of microalgae-based treatment systems in India. The model developed can be used by practising engineers for predicting biomass growth and nutrient removal and thereby achieving a break-even point for cost efficiency.

## MATERIALS AND METHODS

The study starts with the collection of valid literature data of microalgae-based wastewater treatment related to the Indian subcontinent and other countries having similar climatic conditions. The influence of algal strain, environmental conditions and wastewater concentrations on nutrient removal efficiency and algal growth was studied. The selection of suitable algal species by a literature review of available information was made (Supplementary Table 1). This was followed by model development, in which the simulation of algae growth was done based on initial wastewater concentration, temperature and light. The model was successfully calibrated using four separate sets of experimental data obtained from the available literature. After validating the model with three experimental data from the literature, cost estimation was done for the bio-crude production from kitchen wastewater at Indian Institute of Technology (IIT), Hyderabad.

### Model development

Monod and Droop models were the most favoured kinetic models used to study microalgae growth (Eze *et al.* 2018). In both the models specific growth rate of microalgae is represented as a function of major substrates like nitrogen, phosphorus and inorganic carbon. Here maximum growth rate of microalgae is determined by nutrient limitation. The maximum growth rate in Monod's equation is the specific growth rate at infinite external nutrient concentration, while in Droop's equation, it is at infinite internal nutrient cell count, which is named the cell quota. Monod's equation and Droops equation are given in Equations (1) and (2) (Eze *et al.* 2018):

$$\mu_m = \mu_{max} * \frac{[N]}{K_N + [N]} \tag{1}$$

$\mu_m$: Monod specific growth rate (time$^{-1}$), $\mu_{max}$: Monod maximum specific growth rate (time$^{-1}$), [N]: concentration of the nutrient N (mg/L), $K_N$: half-saturation constant for nutrient N (mg/L)

$$\mu_D = \mu_{Dmax} * \left(1 - \frac{Q_0}{q}\right) \tag{2}$$

$\mu_D$: Droop specific growth rate (time$^{-1}$), $\mu_{Dmax}$: Droop maximum specific growth rate (time$^{-1}$)

$Q_0$: minimum cell quota; q: cell quota

The available literature shows that the dynamics of microalgae is accurately reproduced by Droop's model. However, the model is not widely utilised since it is challenging to experimentally measure microalgae cell quotas. The Monod model is widely used by researchers as it is easy to measure the external nutrient media concentration (Eze *et al.* 2018). Monod model can be extended to include carbon limitation as well as dual nutrient-deficient microalgae growth, which is given in Equation (3) (Zinn *et al.* 2004):

$$\mu_m = \mu_{max} * \frac{[C]}{KC + [C]} * \frac{[N]}{KN + [N]} * \frac{[P]}{KP + [P]} \tag{3}$$

But the kinetics of microalgae growth depends not only on the presence of nitrogen, phosphorus and inorganic carbon source but also on the light intensity and media temperature. So, the integrated Monod model for microalgae-specific growth rate, according to (Bernard *et al.* 2001), is:

$$\mu_x = \mu_{max}\left(\prod_i^N\left[\frac{N_i}{K_i + N_i}\right]\right)\left[\frac{q_m S}{K_S + S + S^2/K_{ih}}\right]\left[\frac{I_{av}^n}{I_S^n + I_{av}^n}\right][T_T] \tag{4}$$

$N_i$: Nitrogen, phosphorus and total carbon concentration of the culture, respectively, $K_i$: Related half-saturation constants for nutrients/substrate N, $q_m$: Maximum specific transformation rate, S: Organic matter concentration (mg/L), $K_S$: Half-saturation coefficient for organic matter, $K_{ih}$: Self-inhibition constant (mg/L), $I_S$: Saturation light intensity, $I_{av}$: Average radiant energy defined by Equation (6), $T_T$: Temperature in °C according to Equation (9), n: Shape factor for the reactor.

The model equations adapted from (Al Ketife *et al.* 2019) are as follows:

## Growth equation

The general logistic model to predict algal biomass growth rate $\frac{dX}{dt}$ is given by,

$$\frac{dX}{dt} = \mu_X * X\left(1 - \frac{X}{X_{max}}\right) - d_{th}X \tag{5}$$

where $\mu_X$: specific growth rate in (time$^{-1}$) (From Equation (4)). Here $d^{-1}$. $d_{th}$: Biomass death rate. $X_{max}$: Maximum biomass concentration in the cultivation time

The average light intensity for the total culture volume of a thoroughly mixed liquid culture is given by Sciandra (1986),

$$I_{av} = \frac{1}{z}\int_0^z I_o \exp(-K_L z)dz \tag{6}$$

z: reactor depth, $K_L$ overall light extinction coefficient and $I_o$ is the light intensity from Equation (7).

Light intensity drops exponentially with distance from the reactor wall due to an increase in algal concentration. So $I_o$ can be written as,

$$I_o = I_d \exp(-K_L * L) \tag{7}$$

$I_d$: Radiant energy at the incident reactor surface after integrating the Equation (8) for culturing time, $K_L$: Light extinction coefficient, L: Distance from the reactor wall

Variation of light intensity diurnally, $I_d$ by integrating (Smith 1980):

$$I_o(t) = \frac{\pi}{2} * \frac{I}{fp}\sin\left(\frac{\pi t}{fp}\right) \tag{8}$$

I: Total daily light intensity at the reactor surface, t: Time of culturing, $f_P$: Photoperiod fraction in a day

The effect of temperature is given by (Hossain *et al.* 2020):

$$T_T = 0.0077\exp\left(\frac{1277}{273 + T}\right) \tag{9}$$

T: Temperature in (°C).

### Nutrient removal/substrate fixation

The dissolved inorganic carbon (C) molar balance in a fully mixed liquid phase of volume (V) (Cabello *et al.* 2014):

$$\frac{dC}{dt} = \frac{-M_C}{V} \tag{10}$$

$$M_C = \frac{V * \frac{dX}{dt}}{W_{HCO3} Y_C} \tag{11}$$

$M_C$: Rate at which biomass absorbs carbon dioxide, dX: Change in algae biomass concentration, $Y_C$: biomass yield per carbon, $W_{HCO3}$: Molecular weight of bicarbonate

Mass balance equation for the total dissolved nutrients, nitrogen (N) and phosphorus (P), which are not engaged in the gas-liquid mass transfer process,

$$\frac{dC_{N,\,P}}{dt} = -1000 * Y_{N\ P} * \mu_X * X \tag{12}$$

$[N\ P] = [N_{init}, P_{init}]$ at t=0 and $Y_{N\ P}$ : Yield coefficient for nitrogen and phosphorus

The dissolved organic matter biodegradation rate (Anderson *et al.* 2002):

$$\frac{d(S)}{d(t)} = X * \frac{q_m S}{K_S + S + S^2/K_{ih}} \tag{13}$$

S: Organic matter concentration (mg /L), $q_m$: Maximum specific transformation rate, X: Biomass concentration, $K_S$: Half-saturation coefficient, $K_{ih}$: Self-inhibition constant (mg/L).

### Cost equations

The amount of biomass required to produce 1 $m^3$ of biofuel is,

$$X = \frac{\rho_{oil}}{F_{Xoil}} \tag{14}$$

$F_{Xoil}$ is the possible producible oil content (% w/w, on a dry weight basis) of density $\rho_{oil}$ (kg $m^{-3}$).

The operational expenditure cost (OPEX) is estimated by taking into account the expenses of algae cultivation and oil extraction, as well as the cost of wastewater delivery mitigated by the value of resources recovered, such as biofuel and nutrients. The bio-crude production cost:

$$B_{Co}\ (\$/L) = \frac{\rho_{oil}}{F_{Xoil}} \times 10^{-3} \times (\text{Total cost} - \text{Credits from nutrient removal}) \tag{15}$$

Total cost includes the cost of inoculation, cost of cultivation, cost of wastewater treatment, cost of solar drying and disinfection, cost of delivered wastewater, cost of labour and cost of bio-crude production. Credits include credit for the recovery of carbon, nitrogen, phosphorus and BOD removal from the wastewater.

Assume that 1 L of crude oil is transformed into different usable energy products at a cost that is almost equal to turning X kg of biomass into bioenergy. A parameter break-even selling price (BESP) can be used, which is the highest acceptable price that might be paid for algal biomass with specific oil content. (Amanor-Boadu *et al.* 2014):

$$\text{BESP}\ (\$/L) = \frac{\text{1 barrel petroleum price}}{\text{No. of equivalent bio-crude in litres}} \tag{16}$$

OPEX includes land, energy, insurance, materials, maintenance, taxes, labour and loan payments.

### Sensitivity analysis

A sensitivity analysis is done to identify which input parameters affect the results (biomass growth) of the mathematical model more. It was done by changing the input parameters and seeing how much change was happening for the value of output X (Hussain 2008). Sensitivity is given by $\sigma_x$

$$\sigma_X = \frac{\Delta X}{\Delta I} * \frac{I}{X} \tag{17}$$

I: Nominal input value, X: Model response using nominal input value, $\Delta$I: $\pm$20% variation in input applied to obtain $\Delta$X.

### F-test

An F-test was conducted in MATLAB using 'vartest2' to determine the variance between the predicted and measured values. The two sample F-test returns a test decision for the null hypothesis that the data in vectors x and y come from normal distributions with the same variance. The alternative hypothesis is that they come from normal distributions with different variances. The result h is 1 if the test rejects the null hypothesis at 5% significance level, and 0 otherwise. p is the probability of observing a test statistic as extreme as, or more extreme than, the observed value under the null hypothesis. Small values of p cast doubt on the validity of the null hypothesis.

## RESULTS AND DISCUSSION

A robust and simple prediction model was developed using a mix of literature data and MATLAB numerical simulations of growth kinetic equations for microalgae to include the temporal microalgae biomass concentration X (g/L) and nutrient uptake expressions with temperature and intensity of light. The combination of ordinary differential equations (ODEs) in Equations (4)–(13) were numerically modelled in MATLAB (R2020b) using ODE45 solver (Runge-Kutta method), and the parameters of the model were calibrated by minimising the root mean squared errors (RMSE $< 0.5$) between the predicted and experimental data using the fmincon function. The kinetic model developed for the growth of microalgae and nutrient removal was validated with the use of the second set of experimental data. Table 1 gives the wastewater concentrations used for calibration, validation and cost estimation along with source data.

The data used for calibration supplying applicable experimental results on microalgae concentration X and nutrient removal as residual concentration i.e. TC, TN and TP are taken from four different studies as mentioned in Table 1. For validation, the data were taken from three different studies treating wastewater. The data used for calibration and validation cover a diverse set of input parameters for carbon (C), nitrogen (N), phosphorus (P), light (I) and temperature (T). For cost estimation, feed water concentrations of kitchen wastewater in the IIT Hyderabad (IITH) are taken from Babu *et al.* (2017).

### Calibration

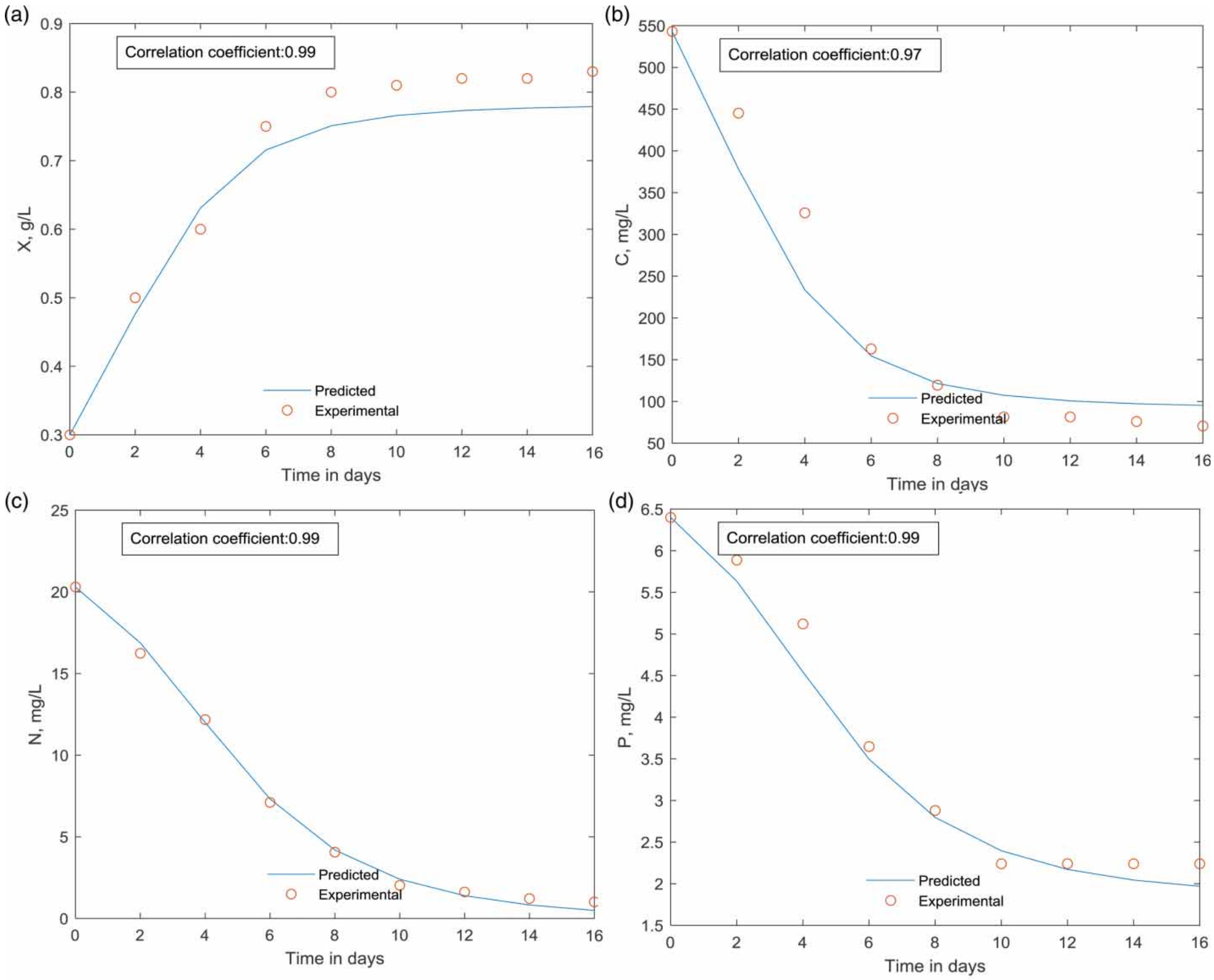
The time-dependent algae concentration and nutrient removal curves are obtained using the model (Equations (4)–(13)). The computed dynamic response and experimental values for biomass concentration (X), carbon removal (C), phosphorus removal (P) and nitrogen removal (N) for the data set 1 are given in Figure 1, and for the datasets 2, 3 and 4 are given in Supplementary Figure 1, 2 and 3.

It is observed that the predicted algae concentration profile had a RMSE of 0.0525 for dataset 1 and 0.1326, 0.1092, 0.33 for the other datasets, respectively, which means that the estimated parameters can be used for further study. The correlation coefficients of predicted and experimental values of X, C, N and P of dataset 1 are 0.99, 0.97, 0.99 and 0.99, respectively. The most appropriate numerical values of main algal growth parameters (the parameter values in which RMSE $< 0.5$ between predicted and experimental data) from calibration and the parameters used for calibration are shown in Table 2.

The maximum specific growth rate of algae obtained was 1.088/day, and half-saturation constants for C, N, and P were 129.97 mg/L, 129.97 mg/L and 30.52 mg/L. A maximum specific growth rate of 0.9991/day was reported by Bello *et al.* (2017). Eze *et al.* (2018) reported the half-saturation constants for C, N and P as 124.9 mg/L, 31.5 mg/L and 10.5 mg/L, respectively.

**Table 1** | Wastewater concentrations used for the study

| | Type of wastewater | Microalgae species | Avg TN(mg/L) | Avg TP (mg/L) | Avg TC (mg/L) | Avg S (mg/L) | T (°C) | I (μE/m$^2$ s) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Calibration (Dataset 1) | Brewery wastewater effluent | *Chlorella vulgaris* | 20.3 ± 3.1 | 6.4 ± 4.2 | 543 ± 32.5 | 1,250 ± 104.2 | 30 | 200 | Choi (2016b) |
| Calibration (Dataset 2) | Municipal wastewater | Algal consortium having *Chlorella* and *Scenedesmus* species | 64.6 ± 4.2 | 28 ± 4.3 | 219 ± 14 | 676 ± 17.2 | 30 | 100 | Mahapatra *et al.* (2014) |
| Calibration (Dataset 3) | Domestic wastewater | *Chlorella* species | 45.1 | 12.8 | 93 | 201 | 25 ± 2 | 100 | Gupta *et al.* (2016) |
| Calibration (Dataset 4) | Municipal wastewater | *Chlorella protothecoides* | 14.56 ± 0.15 | 2.25 ± 0.18 | 48.25 ± 1.2 | 200 | 25 | 100 | Binnal & Babu (2017) |
| Validation (Dataset 1) | Palm oil mill effluent | Algal consortium having *Chlorella* and *Scenedesmus* species | 210–360 | 105–150 | 2,500–3,000 | 7,500–7,800 | 30 | 100 | Babu *et al.* (2016) |
| Validation (Dataset 2) | Parboiled rice mill wastewater | Algal consortium having *Chlorella* species | 141.66 ± 2.96 | 47.32 ± 2.56 | 326.4 ± 4.53 | 1,666 ± 56.57 | 28 | 250 | Mukherjee *et al.* (2016) |
| Validation (Dataset 3) | Diary wastewater treatment | *Chlorella vulgaris* | 28.9 ± 9.4 | 9.4 ± 4.6 | 264 ± 86.4 | 356, 356 ± 124 | 30 | 200 | Choi (2016a) |
| Cost estimation | Kitchen wastewater | Algal consortium of *Chlorella* and *Scenedesmus* species | 20–30 | 16–18 | 260–300 | 1,000–1,200 | 30 | 100 | Babu *et al.* (2017) |




(X: Biomass, C: Carbon, N: Nitrogen, P: Phosphorus)

**Figure 1** | Predicted and experimental concentration during calibration for dataset 1 (a) Biomass concentration (X). (b) Carbon removal (C). (c) Nitrogen removal (N). (d) Phosphorus removal (P).

Half-saturation constants describe the maximum quantity of nutrients or substrates that microalgae may absorb for growth and are defined as the concentration of substrate at which the rate of response is half that of the maximum. As a result of the high half-saturation constant, a large quantity of substrates is required to achieve the maximal reaction rate. Hence, the lower half-saturation constant also indicates a higher affinity for the substrate and vice versa (Ermis & Altinbas 2019). Here the lowest half-saturation constant for phosphorus indicates a higher affinity for phosphate.

The computed dynamic response for total nutrients is given in Figure 1(b), 1(c) and 1(d). Figure 1(b) shows carbon removal curves from the calibration. The yield coefficient for TC obtained is 0.095. The yield coefficient is the ratio of biomass generated to substrate consumed (g biomass produced/g substrate consumed). It denotes the quantity of biomass created during substrate removal (Mardani *et al.* 2011).

Figure 1(c) shows N removal curves after calibration. The yield coefficient for N obtained in this study is 28.787. Chaudhary *et al.* (2020) reported different yield coefficients for N as 26.77. Figure 1(d) shows P removal curves from calibration. The yield coefficient of P obtained in this study is 9.995. Ermis & Altinbas (2019) in a study of nutrient removal from anaerobic liquid digestate by *Chlorella* and *Scenedesmus* mixed microalgae culture reported a yield coefficient of P as $PO_4^{2-}$ as 5.03. In the present study, the yield coefficient for N is 2.8 times more than that for phosphorus and microalgae uptake rate is 2.8 times more for nitrogen than phosphorus. The experimental results from Ermis & Altinbas (2019) showed that nitrogen is 10 times more preferred than phosphorus for microalgae growth.

**Table 2** | Numerical values of main algal growth parameters

| Parameters | Units | Estimated values | Values used for calibration | References |
|---|---|---|---|---|
| $\mu_{max}$ | $d^{-1}$ | 1.088 | 0.9991 | Bello *et al.* (2017) |
| $Y_N$ | – | 28.787 | 26.77 | Chaudhary *et al.* (2020) |
| $Y_P$ | – | 9.995 | 5.03 | Ermis & Altinbas (2019) |
| $Y_C$ | – | 0.095 | 0.99 | Al Ketife *et al.* (2019) |
| $K_S$ | mg/L | – | 225.96 | |
| $K_{ih}$ | mg/L | – | 5,380.31 | |
| $K_N$ | mg/L | 129.972 | 31.5 | Eze *et al.* (2018) |
| $K_P$ | mg/L | 30.529 | 10.5 | |
| $K_C$ | mg/L | 129.972 | 124.9 | |
| $I_S$ | $\mu E/m^2$ s | 28.34 | 16 | Al Ketife *et al.* (2019) |
| $K_L$ | $mg/m^2$ | 32.48 | 15 | |
| $f_p$ | – | | 0.583 | |
| $d_{th}$ | $d^{-1}$ | $7.77*10^{-10}$ | 0.09 | |
| n | – | 0.304 | 0.12 | |

### Validation

After successful calibration, the microalgae kinetic model was validated using experimental data from three different studies, which are given in Table 1. The result demonstrates that the kinetic model can predict the microalgae growth efficiently since predicted values are in line with experimental data. The correlation coefficients of predicted and experimental X, C, N and P are 0.95, 0.93, 0.99 and 0.99 respectively. According to Taylor (1990), a correlation coefficient greater than 0.9 shows a high or strong correlation between the data sets. Figure 2 gives the predicted and experimental values of X, C, N and P after validation. For validation data set 1, test statistic h for X, C, N and P was found to be 0 using the F-test, that means it fails to reject the null hypothesis with p-values of 0.967, 0.7929, 0.9706, 0.7711, respectively. Thus the null hypothesis that the data in predicted and experimental values come from normal distributions with the same variance is accepted here. This indicates that the model is giving accurate predictions for biomass growth, N removal and possible predictions for C and P removal.

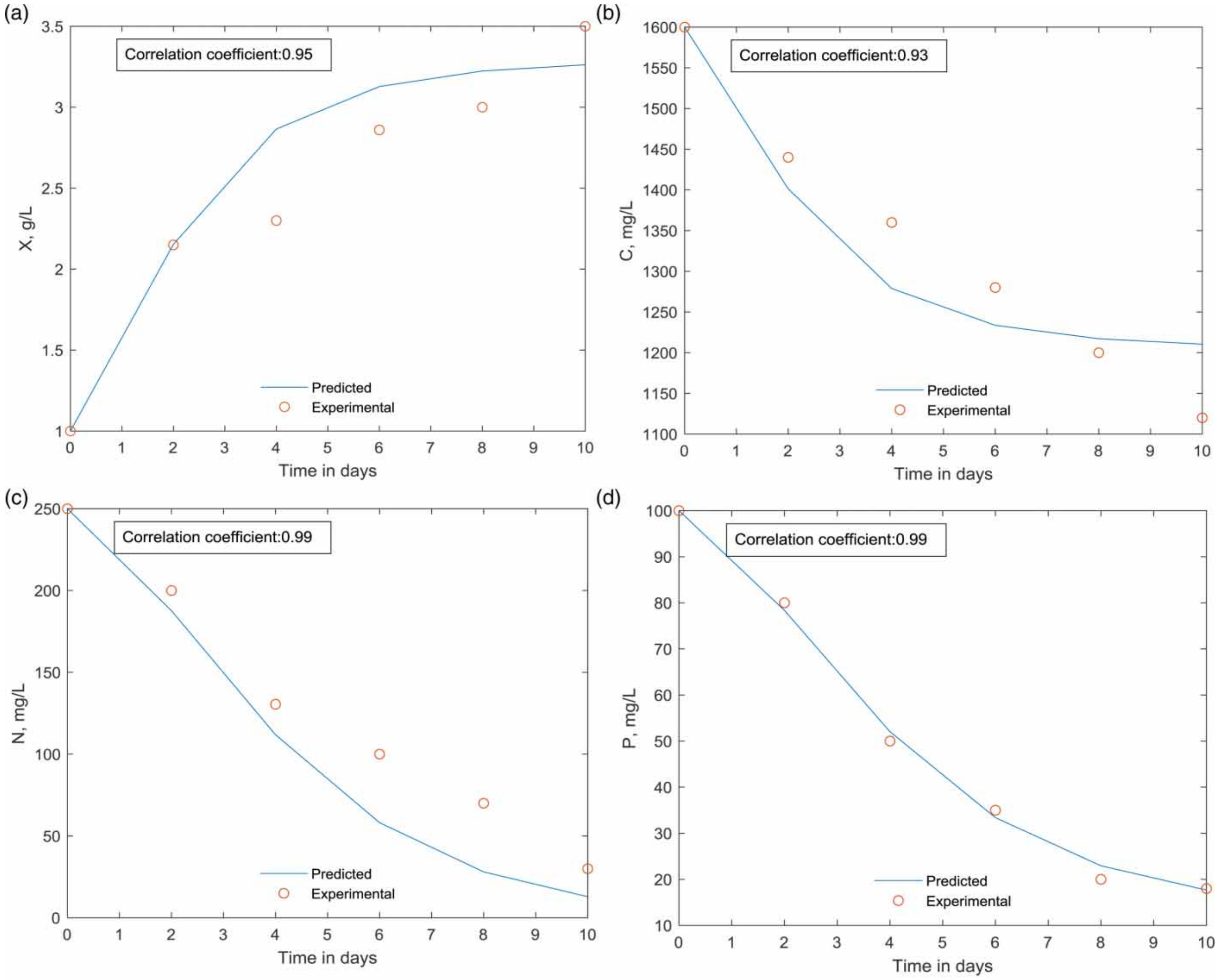
### Sensitivity analysis

The sensitivity $\sigma_x$ of biomass growth to the values of input parameters such as carbon (C), nitrogen (N), phosphorus (P), temperature (T) and light intensity (I) is given in Figure 3. Biomass growth is found to be sensitive to all input parameters in both directions. Growth of biomass is most sensitive up to 3.347 and 3.341 to carbon content and nitrogen content, respectively in the positive direction and to 3.344 to phosphorus content in the negative direction. So the biomass growth rate is sensitive to carbon, nitrogen and phosphorus content.

### Effect of light and temperature on biomass growth and nutrient removal

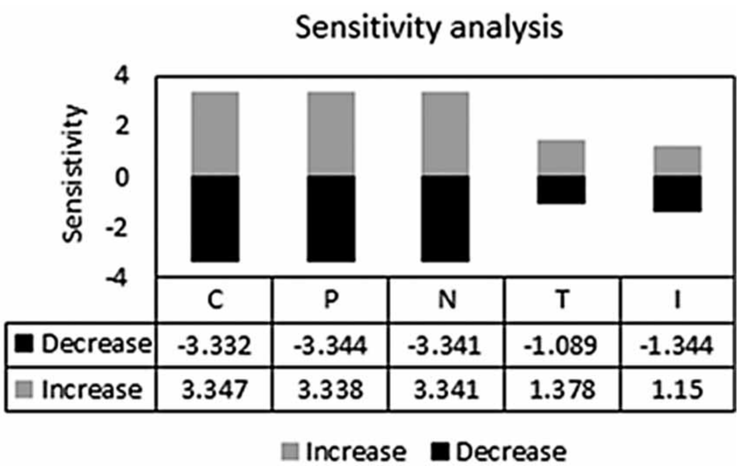
In terms of light, its availability is critical for proper microalgal function. The process of $O_2$ evolution is fueled by light energy, which provides ATP and the reducing agents needed to fix $CO_2$ into organic carbon (Williams & Laurens 2010). The rate of photosynthetic activity is related to the irradiance intensity below the light saturation point, with intensities over this point generating photo inhibition as receptor systems are destroyed. Depending on the microalgal species and temperature, the illumination intensity at which saturation occurs may vary (Singh & Singh 2015).

Most studies showed that freshwater microalgae's light saturation point is between 100 and 400 $\mu E/m^2$ s (Mohsenpour *et al.* 2021). Maintaining an algal culture at or below the saturation point has a practical component because extra light is not used by the algae, resulting in a waste of energy expenditure in the form of excess electricity usage. Here in this study, most literature data had light intensities ranging from 100 to 200 $\mu E/m^2$ s. That means the light intensity of the Indian subcontinent is feasible for sustainable microalgae cultivation and wastewater treatment.




(X: Biomass, C: Carbon, N: Nitrogen, P: Phosphorus)

**Figure 2** | Validation (a) Predicted and Experimental X, (b) Predicted and Experimental C (c) Predicted and Experimental N (d) Predicted and Experimental P.




| | C | P | N | T | I |
|---|---|---|---|---|---|
| Decrease | -3.332 | -3.344 | -3.341 | -1.089 | -1.344 |
| Increase | 3.347 | 3.338 | 3.341 | 1.378 | 1.15 |

**Figure 3** | Sensitivity analysis of biomass growth with respect to input data.

Most microalgae can survive at temperatures ranging from 10 to 30 °C, with the ideal temperature commonly falling between 15 and 25 °C (Singh & Singh 2015). Although higher temperatures are associated with higher growth rates and nutrient uptake rates because of increased metabolic activity, these circumstances are not necessarily compatible with wastewater treatment conditions. Artificially maintaining an ideal temperature in a microalgal wastewater treatment process is impractical due to the massive volumes and energy input required. In mid-latitude settings, wastewater temperatures have been found to range from 3 to 27 °C (Eddy 2014). Because photosynthetic carbon absorption (i.e. the Calvin cycle) is enzymatically mediated, the reaction rate is temperature dependent, with a lower rate observed at lower temperatures. Microalgae respond by lowering their chlorophyll concentration at lower temperatures compared to cells at temperate settings under the same lighting intensity to adjust for the imbalance of more light being absorbed than can be utilised for carbon fixation (Falkowski & Raven 2013). The temperature found in most of the literature used in this study is between 27 and 30 °C which is ideal for the algae growth. This proves that microalgae cultivation and wastewater treatment is viable in the climatic conditions of Indian subcontinent.

## Limitations of the model

From the validation studies, it is clear that the model will give a possible prediction of microalgae growth and nutrient removal but not accurate predictions. To predict N removal, $NH_3$ volatilisation was not included in the model and only phosphorus precipitation was considered but not phosphorus accumulation. Also, the model was found to be under predicting carbon removal. The reason can be that in the model development, the rate of $CO_2$ transferred from gas to liquid phase was not considered. The model considered most of the biokinetic parameters listed in the published literature ($\mu_x$: Specific growth rate, Kc, $K_N$, $K_P$: half-saturation constants for nutrients carbon, nitrogen, phosphorus, $q_m$: Maximum specific transformation rate, $K_S$: Half-saturation coefficient for organic matter, $K_{ih}$: Self-inhibition constant, $d_{th}$: Biomass death rate. $Y_{N\ P}$ : Yield coefficient for nitrogen and phosphorus, Yc: Yield coefficient of carbon) and the wastewater treatment in ponds and photo bioreactors. Other parameters like endogenous respiration constant, inhibition constant of photorespiration, coefficient of excess dissolved oxygen, saturation concentration of oxygen in the air, etc., are not considered in the model due to unavailability of enough data. This may have an impact on the accuracy of predicting biomass growth rate and nutrient removal. Since the model takes into account the majority of the biokinetic parameters of microalgae growth, it can be used to describe and predict the performance of microalgae wastewater treatment processes, as well as to design, operate, and control them. Biokinetic models are used to develop microorganism and substrate balance, predict effluent substrate concentration, develop process design parameters, and ensure a more scientific and reliable process performance and stability. In this context, the current model performs commendably. The model's capacity to forecast biomass concentrations under diverse cultivation settings will be improved if a more detailed study of the microalgae nutrient removal mechanism and other biokinetic, chemical and physical parameters are incorporated in the modelling equation.

## Cost estimation

The dynamic model for time-dependent microalgae biomass concentration X (g/L) and expression of microalgae nutrient absorption with the intensity of light and temperature was used for the economic analysis of the treatment of kitchen wastewater from the IIT Hyderabad with reference to nutrient removal, as well as biomass or biofuel production. A population of 5,000 and 75 lpcd kitchen wastewater production (Manna 2018) from the canteen is assumed. The technology design and costs are adapted from Al Ketife *et al.* (2019). To maintain efficient light penetration, the initial microalgae biomass concentration is considered to be 0.5 k $gm^{-3}$. The schematic of the treatment, cultivation and biofuel production process is given in Figure 4. The treatment and cultivation system is a mix of photo bioreactors and ponds. The entire system consists of wastewater treatment system (WWT), cultivation system and a bio-crude production system. The WWT system is designed to remove suspended matter prior to UV radiation. To avoid contamination of the algal culture, disinfection is required (Agulló-Barceló *et al.* 2013). Clarification is required to ensure adequate UV transmittance and thus disinfection capacity. Compound parabolic collectors (CPCs) that track the sun are used to provide solar energy for both the UV unit and the biomass solar drying process (McGuigan *et al.* 2012). Following the WWT system, there is a set of green wall panels (GWPs) and algal ponds (APs), with the GWPs producing the inoculum and the APs mass producing it. Following that are algal harvesting, hydrothermal liquefaction (HTL), extraction of bio-crude, and biofuel production. The dewatering and harvesting process includes natural settling and filter press. The extraction is assumed to be done by HTL to produce bio-crude. During HTL, the product phases spontaneously separate, yielding bio-crude, an aqueous phase, char and a gas phase rich in $CO_2$.

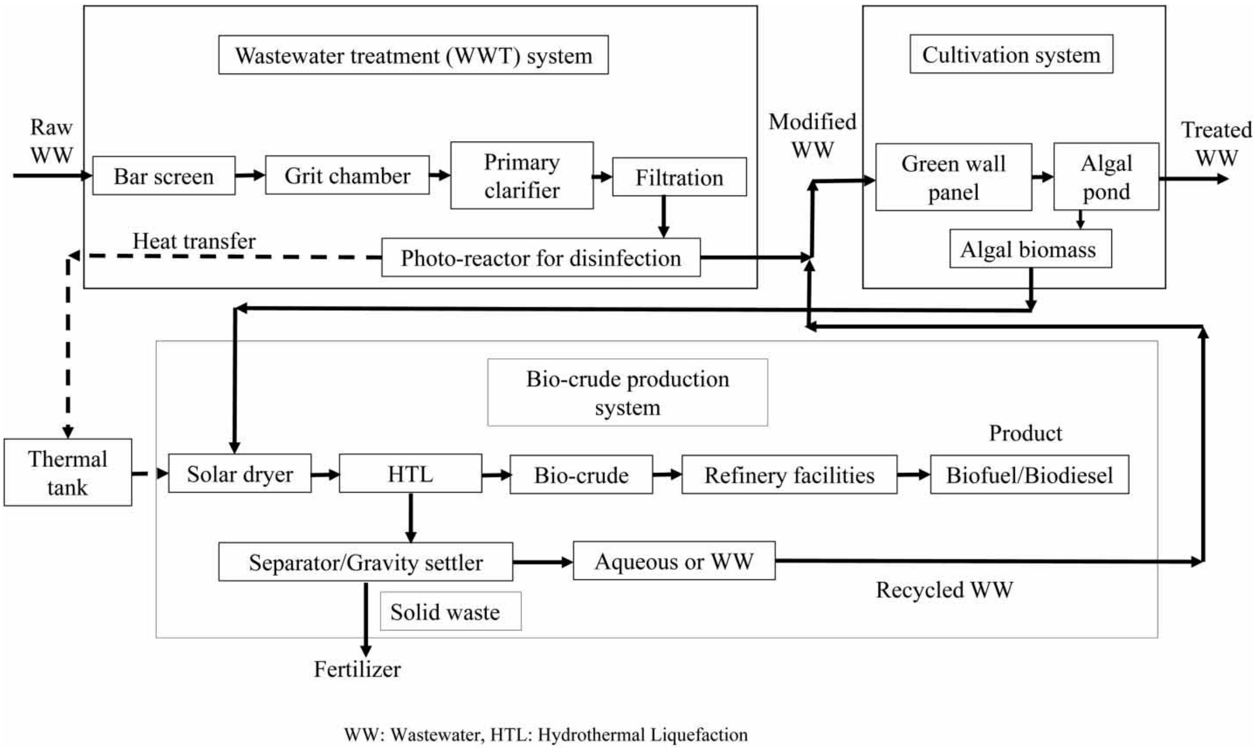



**Figure 4** | Schematic of the treatment system.

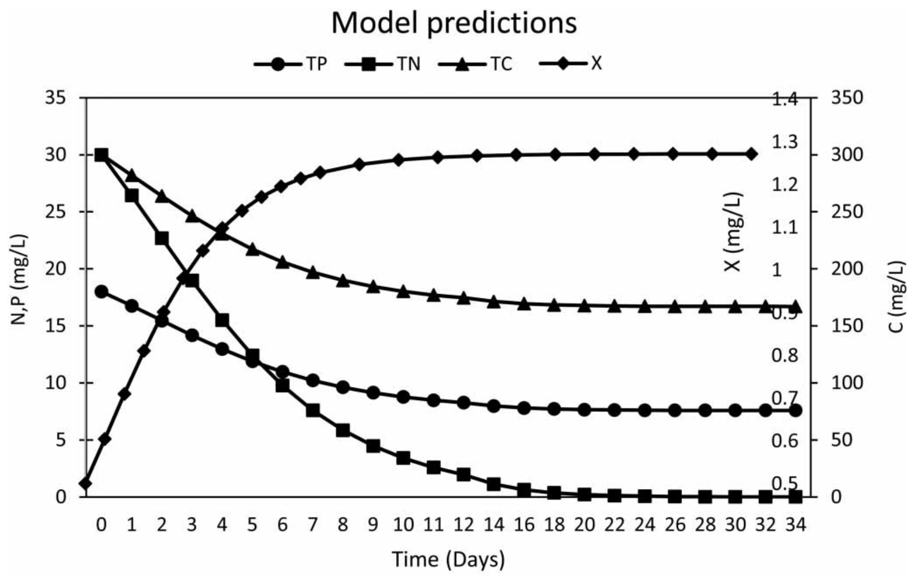
Cost parameter values in US$/kg dry biomass according to Al Ketife *et al.* (2019) are: inoculation – 0.36, cultivation – 0.09, wastewater treatment – 0.012, delivered wastewater – 0.04, solar drying and disinfection – 0.012, bio-crude production – 0.00401, labour – 0.083 which gives a total cost of reactor as 0.601 US$/kg dry biomass. The credits from carbon, nitrogen, phosphorus and BOD removal are 0.04, 1.48, 1.48, 1.48*0.02 US$/Kg dry biomass, respectively.

The time-dependent algal biomass concentration and nutrient removal predictions were obtained for microalgae grown in kitchen wastewater of IITH for 12 days and 34 days. The algae concentration curve and nutrient removal curves for 34 days are given in Figure 5. The final biomass concentration at 12th day is found to be 1.228 g/L and 1.272 g/L for 34 days. The algae concentration is found to be in the stationary phase after 34 days. A 41.81% removal of carbon is seen in 12 days and 44.32% removal in 34 days from 300 mg/L. A 54% removal of nitrogen is seen in 12 days from an initial concentration of 30 mg/L, and a 57% removal is seen in 34 days. For phosphorus, a 93% removal and 99.98% removal are seen in 12 days and 34 days from an initial concentration of 18 mg/L.

The operating parameters of the treatment system are given in Table 3. The cost analysis was conducted for a sustainable microalgae-based wastewater treatment system with harvest of biomass every 12 days. The estimated BESP (Equation (15)) was calculated based on credit from nutrient removal with expenditure incurred by inoculation, wastewater delivery, cultivation, wastewater treatment, disinfection, solar drying and HTL, as shown in Supplementary Table 2.

According to the current investigation, the nutritional content of the wastewater is adequate to support the requisite microalgae biomass concentration of 0.5 kg $m^{-3}$. Treatment of kitchen wastewater gives a credit of 0.004 (US$0.04/kg $\times$ 300 mg/L $\times$ 1.36 $\times$ $10^8$ L $\times$ 0.41/$10^6$ $\times$ 167,008 kg), 0.0337, 0.0117 and 0.0036 US$/kg dry biomass for carbon, nitrogen, phosphorus and organic matter, respectively.

The total cost of the reactor was assumed as 0.601 US$/kg (Al Ketife *et al.* 2019), and total credits were calculated as US$0.053/Kg. The estimated BESP of the microalgae biomass is US$0.549/kg, equating to US$0.96/L or Rs. 69–74/L for the extracted bio-crude, based on the operating expenditure cost (OPEX). Crude oil price in India ranges between Rs. 50 and 55/L. A biofuel BESP that is lower than the benchmark of US$1/L is considered in the current study. A minimum production cost of dry microalgae biomass of around US$0.55/kg was reported by (Al Ketife *et al.* 2019) and (Acién *et al.* 2016). Here the production cost per litre of bio-crude is US$0.549/kg. Credits from pollution abatement are US$0.053/kg in this study.




TP: Total Phosphorus, TN: Total Nitrogen, TC: Total Carbon, X: Biomass concentration

**Figure 5** | Model predictions.

**Table 3** | Operating parameters of the treatment system

| Variable | Units | Value |
|---|---|---|
| Kitchen wastewater production | lpcd | 75 (Manna 2018) |
| Population | Number | 5,000 |
| Annual wastewater production | L/yr | $1.36*10^8$ |
| Initial microalgae concentration | g/L | 0.5[a] |
| Final microalgae concentration | g/L | 1.228 (Model prediction for 12 days) |
| Annual biomass production | kg | 167,008 |
| Microalgae bio-crude fraction | wt% | 0.5[a] |
| Oil density | $kgm^{-3}$ | 880[a] |

Lpcd, Litre per capita per day.
[a]From (Al Ketife *et al.* 2019).

Al Ketife *et al.* (2019) reported a credit of US$0.086/kg under Arabian gulf conditions. Thus, a BESP of US$0.549/kg is obtained for the microalgae biomass necessary to offset all running expenses under ideal operating circumstances.

The cost analysis based on maximum biomass growth and removal efficiency for the microalgae grown for 34 days gives a BESP of US$0.552/kg and bio-crude production cost of US$0.97/L. So the bio-crude production cost increases as the HRT increases. So an optimum HRT is also required for the sustainable working of the system. Also, the initial nutrient concentration of carbon, nitrogen and phosphorus can be adjusted to get maximum biomass growth. The cost of the reactor is not taken based on Indian conditions. So, if the cost values are calculated based on the Indian context, more accurate results can be obtained.

## CONCLUSION

Using data in Indian conditions, a dynamic model of algal growth and techno-economic assessment was developed, calibrated and validated. Under ideal operating circumstances, a BESP of US$0.549/kg is obtained for the microalgae biomass necessary to cover all operating expenses. The cost of production of 1 L bio-crude from microalgae grown in kitchen wastewater in IITH was calculated to be US$0.96 (Rs 69–74), which is comparable with crude oil cost. The model developed can be used by practising engineers for predicting biomass growth and nutrient removal to a break-even point for cost efficiency.

## FUTURE SCOPE

### Nutrient removal mechanism of microalgae

Microalgae that are photoautotrophic can use inorganic carbon, primarily $CO_2$, as their principal carbon source. Gaseous $CO_2$ dissociates into bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$) ions in aqueous solutions depending on the pH, with the precise equilibrium dependent on the environment's temperature cation concentration and salinity (Hill *et al.* 2014). $CO_2$ may easily diffuse across the plasma membrane of microalgal cells due to its non-polar nature, whereas $HCO_3^-$ requires active transport processes. To promote the fixation of inorganic carbon, $HCO_3^-$ is rapidly catalysed to $CO_2$ in the chloroplast of microalgal cells by the enzymatic action of carbonic anhydrase. Because of the low $CO_2$ concentration in aquatic conditions, most microalgae have developed carbon concentration mechanisms to minimise the loss of photosynthetic activity and improve $CO_2$ accumulation rate within the chloroplast (Arias *et al.* 2017)

Microalgae can obtain nitrogen from both inorganic ($NH_4^+$, $NO_3^{2-}$, and $NO_2^{2-}$) and organic (amino acids, urea, purines, and nucleosides) sources (Cai *et al.* 2013). When it comes to inorganic nitrogen, microalgae prefer $NH_4^+$ if it is available because its digestion and integration are more energy efficient. Ammonium is assimilated via a family of membrane transporter proteins known as the ammonium transporter family, which is found in bacteria, yeast, algae, and higher plants. $NH_4^+$ can be immediately converted into amino acids required for growth and other metabolic processes once it has crossed the membrane. $NO_3^{2-}$ and $NO_2^{2-}$ must, on the other hand, be reduced to $NH_4^+$ by the enzymes nitrate reductase and nitrite reductase, which require the reductants NADH and ferredoxin, respectively. Furthermore, $NO_3^{2-}$ transport into the cell is an energy-intensive process that consumes ATP directly (Perez-Garcia *et al.* 2011).

P is a significant element in microalgae as it is a structural component of phospholipids and nucleotides and is essential to the biological energy currency, ATP (Borowitzka 2016). Inorganic P in wastewater exists in multiple ionic forms, and the specific species depends on pH ($H_3PO_4$, 2.15; $H_2PO_4$, 2.15 to 7.20; $HPO_4^{2-}$, 7.20 to 12.33; and $PO_4^{3-}$, >12.33) (Shen *et al.* 2015). Microalgae have been observed to preferentially digest $HPO_4^{2-}$ and $H_2PO_4$, making inorganic P the most accessible form of P. $PO_4^{3-}$ enters the cell by active transport through a symporter channel, with $H^+$ or $Na^+$ ions acting as the driving force, which is established by a plasma membrane $H^+$-ATPase pump. Soluble organic P molecules are becoming a significant source of bioavailable P. The production of external membrane-bound as well as free phosphatases, which non-specifically hydrolyse bound $PO_4^{3-}$ groups, makes these available to microalgae (Mohsenpour *et al.* 2021).

So the integration of removal mechanisms for carbon, nitrogen, and phosphorus into the modelling process can improve the overall efficiency.

## ACKNOWLEDGEMENT

Authors are grateful to their institute for providing software and literature facilities. The authors thank Mr Rohan TM, PhD student at the University of Agder, Norway and Mr Nived MR, PhD student at IIT Hyderabad, for their constant support throughout the modelling study.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 6 April 2022; accepted in revised form 17 August 2022. Available online 22 August 2022