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Predicting removal kinetics of biochemical oxygen demand (BOD) and nutrients in a pilot scale fed-batch algal wastewater treatment system

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ABSTRACT

Our previous reports have presented single-step treatment of primary-settled urban wastewater by a mixotrophic alga, *Galdieria sulphuraria*. Here, we present an approach to predict the operational cycle time, t^*, required to meet the discharge standards for ammoniacal-nitrogen (NH_3-N), phosphate (PO_4) and biochemical oxygen demand (BOD) in fed-batch mode, under varying influent concentrations. First order removal rates of NH_3-N, PO_4 and BOD, determined using data from 10 fed-batch cycles in a pilot scale system (700 L), were used to predict their temporal fate and t^* in 30 other cycles. Predicted concentrations of NH_3-N, PO_4, and BOD and t^* over a wide range of influent concentrations agreed well with the measured ones, with root mean square errors, respectively, of 2.06 mg/L, 0.23 mg/L, 2.88 mg/L and 0.30 days. The coefficient of determination between the measured and the predicted values were as follows: for NH_3-N: $r^2 = 0.82$, $n = 90$; for PO_4: $r^2 = 0.87$, n = 90; for BOD: $r^2 = 0.70$, $n = 30$; and for t^*: $r^2 = 0.61$, $n = 30$. The validity of the model in predicting temporal concentration profiles of NH_3-N, PO_4 and BOD reflected by Janus quotient (respectively of 0.85, 0.87 and 0.61) is comparable to or better than those reported in the literature. Sensitivity analysis procedures identified the influent concentration of PO_4 as the most sensitive parameter in predicting t^*.

1. Introduction

Publicly Owned Treatment Works (POTWs) across the US are responsible for protecting public health and the ecosystem by collecting and treating municipal wastewaters and controlling their discharge into the Nation's waterways [1]. To provide this critical public service, POTWs have continued to rely on a sequence of pollutant-specific removal technologies, each with their own dedicated reactor and hydraulic residence time [2]. These technologies were developed in the 1950s for the express purpose of meeting the respective discharge standards without due regard to secondary emissions or life-cycle impacts. Direct greenhouse gas emissions, for example, from wastewater treatment processes in 2016 are reported as 14.8 MMT of CO_2-equivalent of methane and 5.0 MMT of CO_2-equivalent of nitrous oxide; indirect greenhouse gas emissions associated with the energy consumption by these processes is estimated as 54.3 MMT of CO_2 [3]. On the other hand, valuable components of the wastewater such as phosphorous, nitrogen, organic carbon, and internal energy are wastefully dissipated or destroyed by the current processes without much recovery for reuse. It is estimated that urban wastewaters are laden with about 20% of manufactured nitrogen and phosphorous, that could potentially be recovered for reuse, whereby natural reserves and the energy used in their manufacture could be conserved [4].

With increasing concerns regarding energy conservation, resource recovery, greenhouse gas emissions, lifecycle impacts, and carbon footprint, much attention is now being paid to develop greener and sustainable systems, not only for treating wastewaters efficiently but also for recovering their energy- and nutrient-contents [5]. Algal-based systems have been investigated in recent years as a sustainable pathway for doing so [6–8]. Compared to the current electro-mechanical wastewater treatment (WWT) systems, algal-based systems can conserve energy by eliminating artificial aeration, while generating energy- and nutrient-rich biomass which can be subsequently processed to recover fertilizer, biofuel, and biogas [9,10].

1.1. Algal-based wastewater treatment

Co-cultivation of photoautotrophic algae and heterotrophic bacteria in wastewater to reduce its organic carbon- and nutrient-contents has been well demonstrated [9,11,12]. These systems, however, suffer from limitations stemming from low transfer efficiency of gaseous carbon dioxide (CO_2) [13] and diurnal and seasonal variations in sunlight [14]. Recently, it has been shown that certain algae can be cultivated mixotrophically utilizing the dissolved organic carbon (DOC) and the

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nutrients in domestic wastewaters [15]. Additionally, mixotrophic systems can also utilize CO_2 via photosynthesis to rectify any carbon-to-nitrogen-to-phosphorus (C:N:P) imbalance. This metabolic flexibility of mixotrophic algal systems enables single-step wastewater treatment (WWT) with minimal energy input [10]. Additional advantages of the mixotrophic systems over photoautotrophic and heterotrophic systems including higher growth and pollutant removal rates and potential for higher energy recovery have been reported [16].

Our previous reports have documented the development of a mixotrophic algal WWT system from lab-scale [10,17] to a pilot-scale facility deployed at a local POTW [18]. This system exploits an extremophilic alga, *Galdieria sulphuraria* (hereafter, *G. sulphuraria*), that has been shown to grow heterotrophically on DOC and autotrophically under sunlight, when available [19]. A novel component of this system is an enclosed raceway design fitted with a paddlewheel. This enclosed reactor design affords several advantages over the traditional open raceways: minimizes loss of water by evaporation and ammonia by volatilization; lower odor emissions; protection against invasion by predators and competitors; controlled headspace for better gas transfer; and capture of solar heat to maintain above-ambient temperatures. Results from long-term operation of this pilot-scale system (700 L) fed with primary effluent have confirmed that it can achieve discharge standards for DOC (measured as biochemical oxygen demand, BOD) and nutrients in a single step, within a fed-batch processing time of 3 days.

1.2. Goals of this study

The goal of the current study is to refine fed-batch cultivation of *G. sulphuraria* in our pilot-scale system. In fed-batch mode cultivation, the cycle time to meet all the discharge standards (t^*) is a critical design and operational parameter. The number of parallel reactors required to manage a given wastewater flow rate depends on t^*. Additionally, given the day-to-day variations in the influent concentrations of BOD, ammoniacal nitrogen, and phosphates, the ability to rapidly pre-determine the cycle time t^* is essential for smooth operation of the system.

This paper presents the development of a simulation model for the above purpose. Kinetic rates of the *G. sulphuraria*-based algal WWT system in removing BOD and nutrients are first determined from data collected over 10 fed-batch cycles and compared with those reported in the literature for other algal species. These rate constants are then used to predict day-by-day concentrations of BOD and nutrients in 30 other fed-batch cycles, and to estimate the fed-batch cycle time to meet the discharge standards for BOD and nutrients. The validity of this approach is demonstrated by comparing the predicted concentrations against the ones measured in 30 other fed-batch cycles. A sensitivity analysis is performed to identify the critical parameter to monitor to ensure the discharge standards are met. Results of this study are integrated to develop a field guide for aid in the operation of the fed-batch system.

2. Materials and methods

2.1. Cultivation method and equipment used

The algal strain used in this study, *G. sulphuraria*, was obtained from the Culture Collection of Microorganisms from Extreme Environments (CCMEE), University of Oregon. The algae were cultivated in primary effluent in the pilot-scale system deployed at the Las Cruces Wastewater Treatment Plant. This pilot-scale system consisted of two identical bioreactors in parallel, each with an active volume of 700 L and a culture depth of 20 cm. Both reactors were mixed by a motor-driven paddle wheel at a speed of 0.3 m s^{-1} and operated in fed-batch mode. Each fed-batch cycle was initiated with 300 L of preadapted culture to which 400 L of primary-settled wastewater were added, and the pH of the mixture was adjusted to 2.5 using 10 N sulfuric acid (optimal pH range for *G. sulphuraria* is 0–4 [17,19]). Upon reaching the stipulated discharge standards, the cycle was terminated, and the biomass was allowed to settle for 24 h. Thereafter, 400 L of the supernatant was discharged and replaced with a fresh batch of primary effluent to start a new cycle. Both reactors were operated for 20 such cycles each (40 reactor-cycles in all) between June–September in 2017. At the start of each cycle, samples from each reactor and primary effluent were analyzed for five-day BOD; ammoniacal nitrogen (NH_3-N); and phosphate (PO_4) concentrations. Initial biomass concentration in each reactor was recorded in terms of optical density at 750 nm (OD750). Thereafter, biomass growth and nutrient (NH_3-N and PO_4) concentrations in each reactor were measured daily; BOD was measured every other day. Characterization of primary effluent at the Las Cruces Wastewater Treatment Plant and the procedures followed in this study are summarized in the Supplementary information section.

2.2. Model development

The simulation model developed here is founded on the presumption that removal of NH_3-N, PO_4, and BOD in the mixotrophic system follow first-order kinetics according to

$$S_j = S_{j,0}\, exp(-k_j t) \tag{1}$$

where, S_j is the concentration of j at any time, j = NH_3-N, PO_4 or BOD; $S_{j,0}$ is the initial concentration of j; k_j is the first order rate constant for j; and t is the time. The time required to meet the discharge standard for j, t^*_j, was then found from,

$$t^*_j = -\frac{1}{k_j} ln\left(\frac{S_{j,std.}}{S_{j,0}}\right) \tag{2}$$

where, $S_{j,std.}$ is the discharge standard for j. In this study, all discharge standards refer to secondary treatment (ammoniacal nitrogen – 10 mg/L; phosphate – 1 mg/L; and BOD_5 – 30 mg/L). The fed-batch cycle time to meet the discharge standard for all three pollutants (NH_3-N, PO_4 and BOD), t^*, was then determined from

$$t^* = max(t^*_{NH3-N}, t^*_{PO4}, t^*_{BOD}) \tag{3}$$

Data from the two parallel reactors (40 reactor-cycles) were pooled in the analysis. Concentrations of NH_3-N, PO_4 or BOD measured in initial 10 fed-batch cycles (5 cycles per reactor × 2 reactors) were used to validate the presumption of first-order kinetics and to determine the respective first-order kinetic constants (k_j, 1/day), following standard procedures summarized in the Supplementary information section. These k_j values were used to predict temporal variations of BOD and nutrients in the remaining 30 fed-batch cycles (15 cycles per reactor × 2 reactors) to validate the approach. The t^* values in 30 cycles, calculated from Eq. (2), were also compared with the actual values for further validation.

2.3. Criteria to evaluate the model fit

The following measures [26,27] were adopted to evaluate the quality of the model fit. In the first one, root mean square error, RMSE, calculated using the following equation, was used for assessing the degree of prediction:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i}^{n}(y_{pred,j,i} - y_{meas,j,i})^2} \tag{4}$$

where, $y_{pred,\ j,\ i}$ is the ith predicted observation of the jth parameter; $y_{meas,\ j,\ i}$ is the ith measured observation of the jth parameter; n is the total number of observations. RMSE values close to zero would indicate a high-quality prediction. In the second one, Janus quotient (J), calculated using the following equation, was used to estimate differences between predictions when the calibration and prediction datasets (in this case, initial 10 cycles and remaining 30 cycles, respectively) are

predicted using the model [26,28]:

$$J^2 = \frac{\frac{1}{n_{val}}\sum_i^{n_{val}} (y_{pred,j,i} - y_{meas,j,i})^2}{\frac{1}{n_{cal}}\sum_i^{n_{cal}} (y_{pred,j,i} - y_{meas,j,i})^2} \quad (5)$$

where, n_{val} and n_{cal} are the total observations in validation and calibration datasets, respectively. A value of J close to 1 indicates that the predictive ability of the model is similar within and out of the calibration dataset [26]; values lower and higher than 1 indicate that the model outcomes for the validation dataset are better or worse, compared to those for the prediction dataset [28].

3. Results

3.1. Kinetic constants

Temporal profiles of NH_3-N, PO_4 or BOD recorded in the first 10 fed-batch cycles were used to establish the respective kinetic rates. The first order model fitted the field data well, yielding average k_N = 0.505 1/day (average r^2 = 0.97) and k_P = 0.663 1/day (average r^2 = 0.95). These strong correlation coefficients validated our presumption about first-order removals by *G. sulphuraria* in the fed-batch mode. Since the BOD in the field experiments dropped below the discharge standard of 30 mg/L within two days, BOD concentrations were measured only on the initial and second days of operation. As such, first order reduction of BOD could not be validated with the 2-day concentration profiles. However, results from our previous laboratory experiments [10] on *G. sulphuraria* grown in primary-settled wastewater from the same POTW had confirmed first-order behavior with an r^2 of 0.89. Thus, BOD removal in the current field study was also assumed to follow a first order reduction. Based on this assumption, using the 2-day data in the initial 10 cycles, the rate constant was found as k_{BOD} = 0.311 1/day.

Table 1 compares the first order kinetic constants, k_j (1/day) determined in this study against those compiled from literature for different algal species. Literature values for k (1/day) were either taken directly from the publications, if reported, or were calculated based on the data presented. As can be noted from Table 1, average literature values of k_N (0.469 1/day, lab conditions) are slightly lower than that in our field study; while, the average k_{BOD} values (0.360 1/day, lab conditions) are slightly higher than that in our field study. k_N and k_{BOD} for *G. sulphuraria* found from our previous lab studies [10] were somewhat higher than those found here under field conditions. The average k_{PO4} in the current study was lower than the average of those in literature studies (0.804 1/day); but similar to that found in our previous lab studies [10].

3.2. Ammoniacal nitrogen removal

Using the rate constant k_N = 0.505 1/day found from the calibration data set, daily concentration profiles of NH_3-N in 30 other fed-batch cycles in the validation set were predicted; Fig. 1 compares these predictions with the measured ones for the 30 cycles, each lasting 3 days. As can be noted, concentrations predicted by the model correlated well with the measured ones with r^2 = 0.82 (n = 90). The average volumetric removal rate of NH_3-N (4.14 ± 0.91 mg/L-d) and the removal efficiency of NH_3-N (78.0%) calculated from the predicted data for the 30 cycles were similar to the measured values (4.14 ± 0.94; mg/L-d; 78.9%). The RMSE for the 30 predicted fed-batch cycles in days 1, 2 and 3 were 2.05 mg/L, 2.27 mg/L, and 1.82 mg/L, respectively. The RMSE for the overall model after pooling all the observations was 2.06 mg/L.

The quality of prediction of NH_3-N removal in this study is similar to or better than those reported in the literature. In a laboratory study of NH_3-N removal from chemically precipitated primary effluent [35], the reported results had an RMSE of 2.67 mg/L. In another laboratory study on *Desmodemus* sp. fed with filtered domestic wastewater [25], the reported results had an RMSE of 6.67 mg/L. A study by Sin et al. [27] to evaluate a Monte Carlo-calibrated ASM2d model for activated sludge had reported low RMSE of 1.40 mg/L. The Janus quotient for NH_3-N removal model (J_N) in this study was found to be 0.85 indicating an acceptable level of prediction. The study by Sin et al. [27] had reported a J_N of 0.6. Another study by Wágner et al. [28] simulating the growth of *Chlorella sorokinian* and *Scenedesmus* sp. on synthetic wastewater under laboratory conditions had reported J_N values ranging from 1.42 to 9.62.

3.3. Phosphate removal

Using the rate constant k_P = 0.663 1/day found from the calibration data set of 10 cycles, daily concentration profiles of PO_4 in the 30 fed-batch cycles in the validation set were predicted; Fig. 2 compares the predicted PO_4 concentrations against the measured ones for these 30 cycles. Again, a strong correlation between the two could be observed, with an r^2 = 0.87 (n = 90). The RMSE for the 30 predicted fed-batch cycles in days 1, 2 and 3 were 0.28 mg/L, 0.23 mg/L and 0.17 mg/L, respectively. The RMSE for the overall model after pooling all the observations was 0.23 mg/L. The average volumetric removal rate of PO_4 (0.84 ± 0.32 mg/L-d) and the removal efficiency (86.3%) calculated from predicted data for the 30 cycles were consistent with the measured values (0.82 ± 0.34 mg/L-d; 83.0%).

The quality of prediction of PO_4 removal in this study is similar to or better than those reported in the literature. In a laboratory study of PO_4

Table 1
First order kinetic constants for ammoniacal nitrogen, phosphate, and BOD: results of this study vs. literature results.

Species	Wastewater tested	Experiment condition	First order kinetic constant (d^{-1})			Reference
			NH_3-N	PO_4	BOD	
G. sulphuraria	Primary effluent	Field	0.505	0.663	0.311	This study
G. sulphuraria	Primary effluent	Lab	0.774	0.631	0.552	[10]
Phomedium sp.	Secondary effluent	Lab	0.606	1.354	–	[9]
Chlorella reinhardtii	Secondary effluent	Lab	0.946	1.442	–	[9]
Chlorella vulgaris	Secondary effluent	Lab	0.465	1.501	–	[9]
Scenedesmus rubescens	Secondary effluent	Lab	0.490	0.600	–	[9]
Anabaena oryzae	Primary effluent	Lab	–	–	0.204	[31]
Anabaena variabilis	Primary effluent	Lab	–	–	0.388	[31]
Anabaena oryzae	Primary effluent	Lab	–	–	0.296	[31]
Chlorella vulgaris	Synthetic wastewater	Lab	0.437	0.835	–	[32]
Scenedesmus rubescens	Synthetic wastewater	Lab	0.351	0.984	–	[32]
Chlorella vulgaris	Nutrient supplemented, secondary wastewater	Lab	0.070	0.912	–	[33]
Planktothrix isothrix	Nutrient supplemented, secondary wastewater	Lab	0.108	0.272	–	[33]
Chlorella vulgaris	Nutrient supplemented, sterilized secondary effluent	Lab	0.703	0.308	–	[34]
Desmodemus sp.	Filtered domestic wastewater	Lab	0.209	0.013	–	[25]

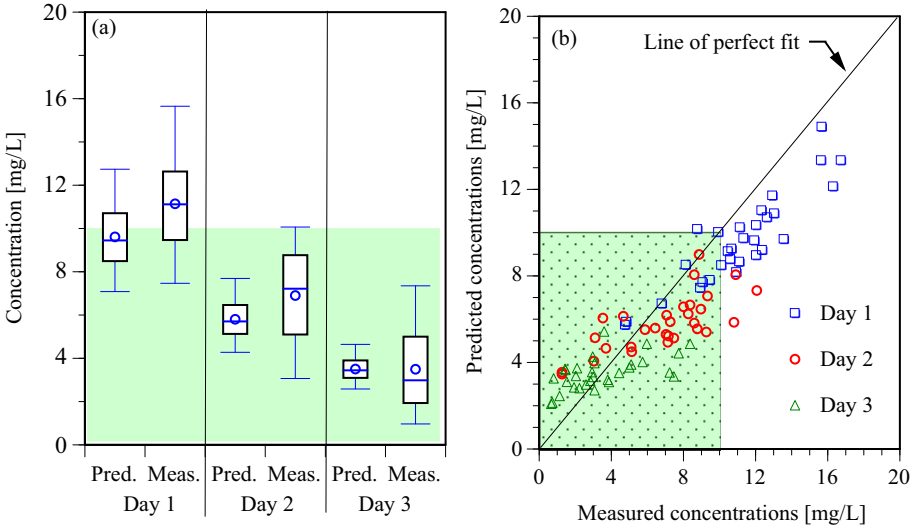

Fig. 1. a) Temporal variation of measured and predicted concentrations of ammoniacal nitrogen; b) the correlation between predicted and measured concentrations of ammoniacal nitrogen [overall $r^2 = 0.82$, $n = 90$]. Data from 30 fed-batch cycles, each 3 days long. Shaded areas indicate concentrations below discharge standards of 10 mg/L.

removal from filtered domestic wastewater [25], the reported results had an RMSE of 0.75 mg/L. In the study by Wágner et al. [28], Root Mean Square Normalized Error (RMSNE) values ranged from 0.58 to 0.76 for 4 different operation cycles. In comparison, the RMSNE values in our study were calculated as 0.17, 0.26, 0.39 and 0.29 respectively for Days 1, 2 and 3 and overall. The Janus quotient for phosphate (J_P) was found to be 0.87 in this study demonstrating a high quality of fit compared to the values found by Wágner et al. [28] under lab conditions that ranged from 0.44 to 1.21.

3.4. Biochemical oxygen demand (BOD) removal

Using the rate constant $k_{BOD} = 0.311$ 1/day found from the calibration data set of 10 cycles, daily concentration profiles of BOD in the 30 fed-batch cycles in the validation set were predicted; Fig. 3 compares the BOD concentrations predicted for these 30 cycles against the measured ones. As can be noted, concentrations predicted by this approach correlated moderately with the measured concentrations with $r^2 = 0.70$ ($n = 30$). The RMSE of the 30 predicted fed-batch cycles for BOD on day 2 was calculated as 2.88 mg/L. The predicted average volumetric removal rate of BOD (8.51 ± 1.64 mg/L-d) and the predicted average removal efficiency (46.3%) for the 30 cycles were comparable to the measured ones (8.19 ± 1.95 mg/L-d and 44.8%).

To the best of our knowledge, models for predicting BOD removal by algae do not exist; however, several models for predicting BOD removal by activated sludge have been reported. In a study presented by Lee et al. [38], RMSE values were reported as 0.61 mg/L and 0.59 mg/L. The Janus quotient in the case of BOD (J_{BOD}) in our study was 0.61, which is slightly lower than J_N and J_P.

3.5. Fed-batch cycle time

Fig. 4(A) compares the predicted and actual fed-batch cycle times to reach the individual discharge standards for NH_3-N (a); PO_4 (b); and BOD (c), and the time to reach discharge standards for all three (d). Fig. 4(B) depicts the correlation between the measured and predicted cycle times for 30 fed-batch cycles in the validation set. As can be noted in Fig. 4(B), the correlation between the measured and predicted fed-batch processing time, t*, was moderate, with an r^2 of 0.61. The RMSE for the cycle times to achieve the individual discharge standards for NH_3-N, PO_4, and BOD were 0.4 days, 0.28 days and 0.33 days; the RMSE for the overall fed-batch cycle time t* to meet the discharge standards for all three was 0.30 days. Although literature reports of similar studies for comparison could not be found, the RMSE reported

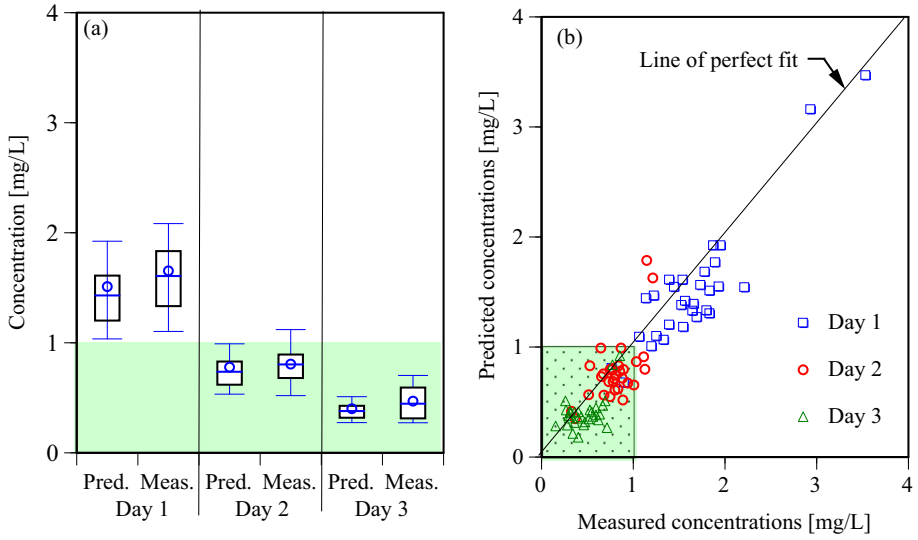

Fig. 2. a) Temporal variation of measured and predicted concentrations of phosphate; b) correlation between predicted and measured concentrations of phosphate [overall $r^2 = 0.87$, $n = 90$]. Data from 30 fed-batch cycles, each 3 days long. Shaded areas indicate concentrations below discharge standards of 1 mg/L.

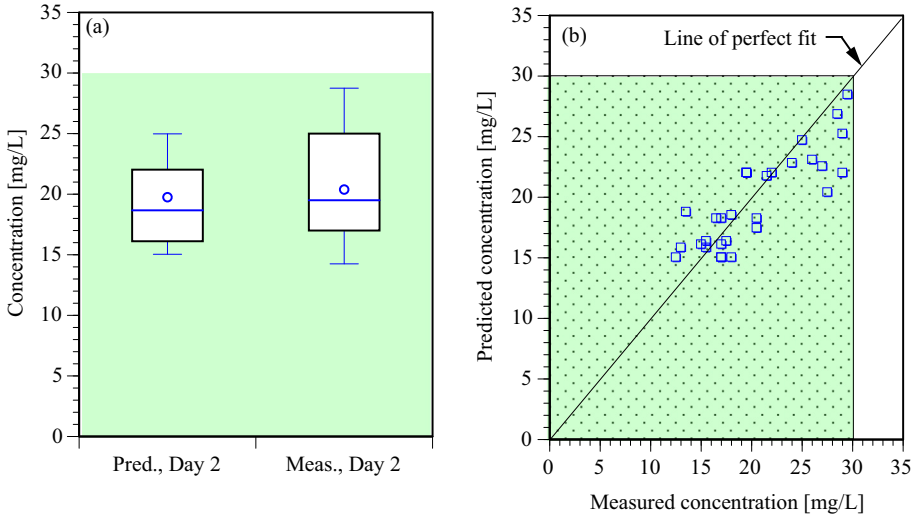

Fig. 3. a) Temporal variation of measured and predicted concentrations of BOD on day 2; b) correlation between predicted and measured concentrations of BOD on day 2 [overall $r^2 = 0.70$, $n = 30$]. Data from 30 fed-batch cycles, each 3 days long. Shaded areas indicate concentrations below discharge standards of 30 mg/L.

here are deemed acceptable for a field-scale algal wastewater treatment simulation.

In all cycles, the BOD discharge standard was attained in less than 2 days. Moreover, the predicted cycle time also depended heavily on the slow nutrient removal rate (in 97.5% of cycles), which was also consistent with the actual results (in 90% of cycles). Therefore, the effect of the moderate correlation established between actual and predicted concentrations of BOD on process time prediction could be considered minimal.

3.6. Sensitivity analysis

The sensitivity of mathematical models to process parameters is often performed by quantifying the relative deviations in the outcome of the model when a parameter of interest is changed by a certain amount [39]. In this study, the sensitivity of the overall cycle time, t*, to the three kinetic constants k_j was evaluated following two sensitivity analysis methods reported in the literature [39]. In one method, the base values of k_{BOD}, k_N and k_{PO4} established earlier were varied one-at-a-time by ± 10% and the resulting t^* values were predicted in the 30 cycles in the validation set. Thus, if the processing time of a parameter (t^*_j) is significant in determining the t*, t* will increase as k_j is reduced and will reduce as k_j is increased. From the results of this simulation, summarized in Fig. 5, it can be noted that reduction of k_{NH3}, k_{PO4}, and k_{BOD} by 10% from the base value increased t^* on average by 0.5%, 10.3%, and 1%. Similarly, increase of k_j values by 10% resulted in the reduction of the corresponding t^* values by 0%, 8%, and 0.5%. Based on these results, k_{PO4} can be concluded as the most sensitive parameter of the three in predicting the fed-batch cycle time t^*. A hypothesis test conducted to compare the process times to meet the individual discharge standards for NH_3-N, PO_4 and BOD also affirmed that $t_{PO4}{}^* > t_N{}^*$ and $t_{PO4}{}^* > t_{BOD}{}^*$, at a significance level of 0.05.

The second method used a sensitivity index (SI) defined as the percentage difference observed in an output when the minimum and maximum values of the considered parameter are used [39]. Based on the SI values calculated for k_N (= 0.06), k_{PO4} (= 0.64), and k_{BOD} (= 0.0), k_{PO4} was again seen as the most sensitive of the three in predicting the overall fed-batch cycle time, t^*.

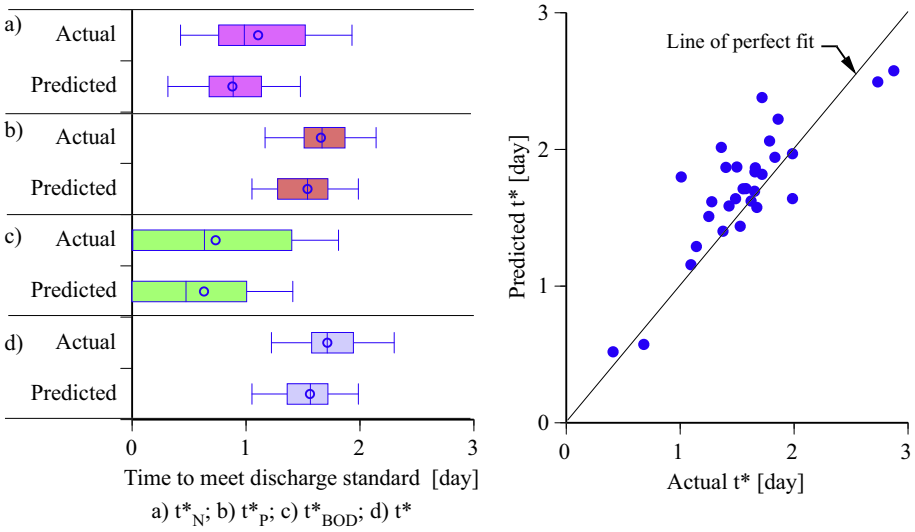

Fig. 4. A) Predicted and actual cycle times to reach individual discharge standards for ammoniacal nitrogen, phosphates, and BOD and for all three. B) Correlation between predicted and measured batch cycle time t* to achieve all three discharge standards [$r^2 = 0.61$, $n = 30$].

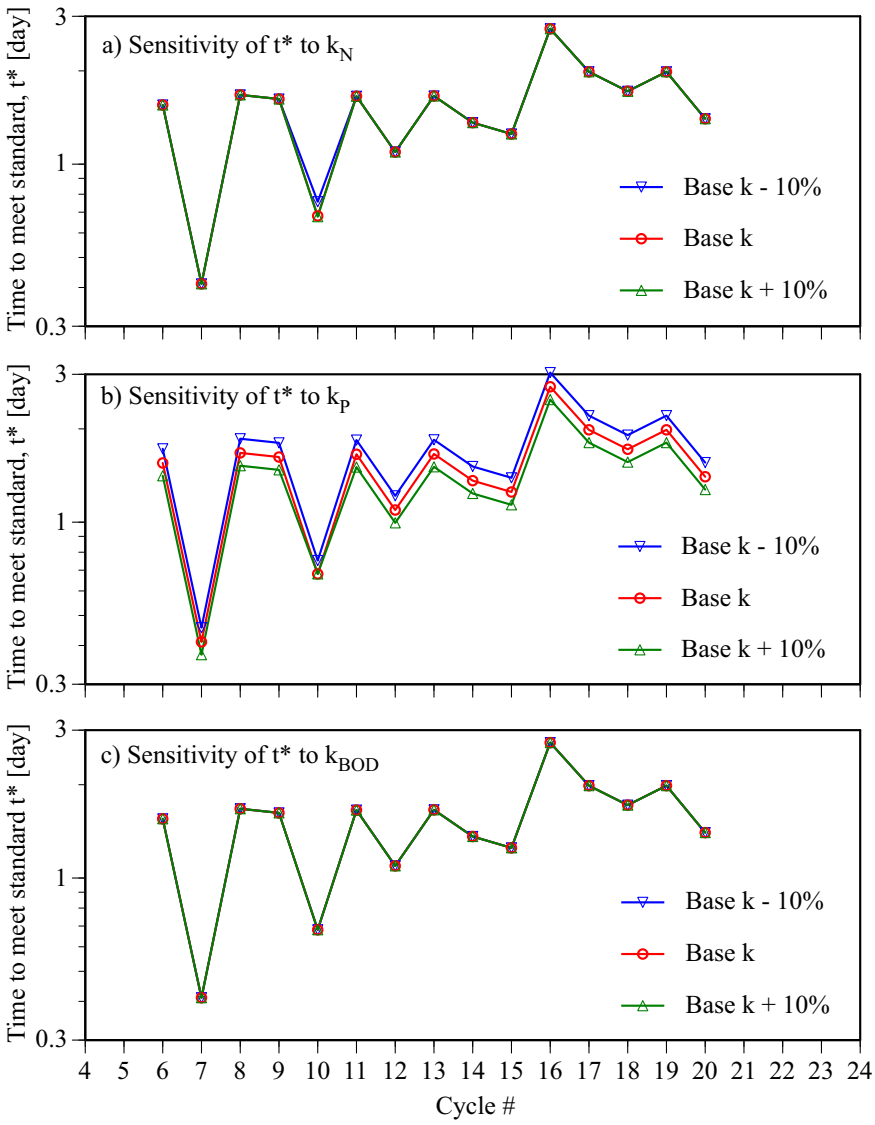

Fig. 5. Sensitivity of cycle time, t*, to meet discharge standards to ± 10% change in the rate constants for ammoniacal nitrogen (k_{NH3}), phosphate (k_{PO4}), and BOD (k_{BOD}).

3.7. Cycle time vs. initial concentrations

The cycle time t^* required to meet the discharge standards for all j is a function of the initial concentration of j in the reactor, $S_{j,0}$. Fig. 6 shows contours of t* required to achieve the discharge standards for PO_4 and NH_3-N as a function of their respective initial concentrations, $S_{PO4,0}$ and $S_{NH3\text{-}N,0}$. As mentioned in Section 3.6, Fig. 6 also indicates that the initial concentration of PO_4 is the dominant factor impacting cycle time, unless $S_{PO4,0}$ is lower than 3 mg/L. A similar diagram can be developed for t* as a function of $S_{PO4,0}$ and $S_{BOD,0}$ to confirm that the removal rate of PO_4 is still the rate limiting factor. This contour plot affords easy and rapid forecasting of the cycle time under field conditions to ensure that all the discharge standards are met for any given cycle.

4. Conclusion

This study demonstrated that removal of BOD and nutrients in the *Galdieria sulphuraria*-based mixotrophic wastewater treatment system followed first-order kinetics. Kinetic rate constants estimated from 10 fed-batch cycles were used to predict the concentration profiles of NH_3-N, PO_4, BOD, and the fed-batch cycle times to meet the discharge standards in a validation set of 30 cycles. Predicted concentrations agreed well with the measured data. Predicted fed-batch cycle time to meet all three discharge standards had an RMSE of 0.30 days. Fed-batch cycle time was found to be most sensitive to the first-order rate constant for phosphate.

Declaration of authors' contribution

All authors whose names listed this manuscript certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of authors' agreement to authorship and submission of the manuscript for peer review

All authors whose names are listed in this manuscript have contributed significantly to the work, have read the manuscript, attest to

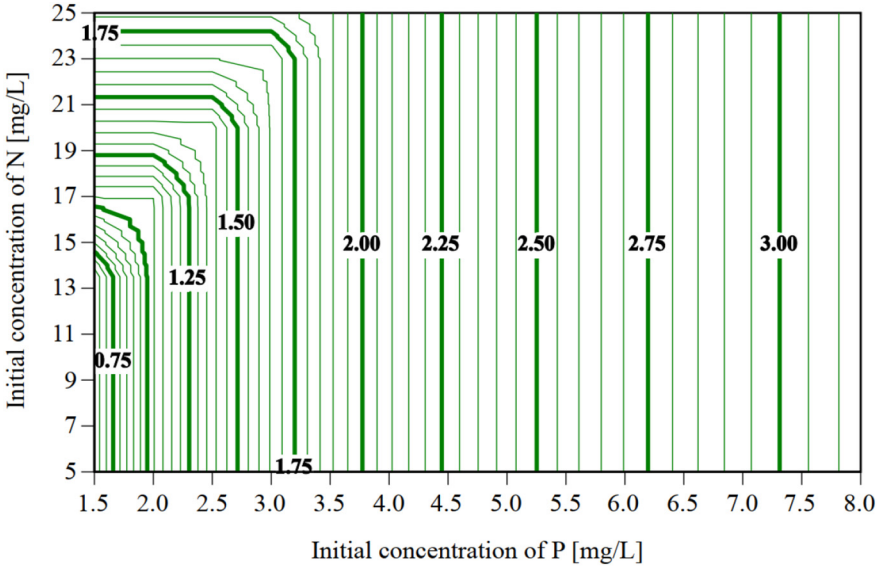

Fig. 6. Contours of overall cycle time t^* to achieve discharge standards for NH_3-N, PO_4 and BOD as a function of initial concentrations of phosphate [P] and ammoniacal nitrogen [N].

the validity and legitimacy of the data and its interpretation, and agree to its submission to Algal Research for peer review.

Declaration of competing interest

All authors whose names are listed in this manuscript certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2019.101643.

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