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Mixed cultured algal and bacterial remediation of dissolved organic nitrogen under low solid retention time condition

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ABSTRACT

The impact of low solid retention times (SRT) on the biodegradability of wastewater derived dissolved organic nitrogen (DON) was studied using a continuous stirred tank reactor (CSTR). Laboratory scale experiments were conducted at different low SRTs (0.2, 0.4, 0.5, 1 and 2 d) using mixed culture bacteria and a pure strain algae *Chlamydomonas reinhardtii*. Results indicated that more than 80% effluent DON removal was achieved at SRT 2 d. However, no significant removal was observed for the rest of the SRTs conducted. Nearly complete nitrification (98%) was observed at SRT 2 d and about 53% to 73% of dissolved ammonia was nitrified to dissolved nitrite or nitrate in SRTs 0.2 to 1 d , respectively. Nitrite accumulation was observed, however, it did not affect the degradability of DON. Model simulation using MATLAB R2016b was conducted to validate the model. The R-square values were calculated to validate the goodness of fit of the model.

Keywords: Algae; Dissolved organic nitrogen; Chemostat; Wastewater; Solid retention time; Biological treatment

1. Introduction

Nitrogen is an essential nutrient source for plant and animal nutrition that controls the productivity of aquatic ecosystem. Optimal amount of nitrogen is important in water environment; however, in high concentrations it can be a contaminant. Dissolved organic nitrogen (DON) is commonly found in various aquatic environments including lakes, streams, rivers, estuaries, and oceans. Natural and/or anthropogenic inputs of DON increase deterioration of the water quality. Sewage and industrial wastewater discharge, agricultural and urban runoff, riverine delivery, groundwater discharge, atmospheric deposition, and biotic water column processes are potential sources of DON [1–8].

The chemical composition of DON varies depending on its origination from numerous natural and anthropogenic sources, and autochthonous production [9–11]. DON consists of complex macromolecules and has been partially characterized; some of the known DON compounds in the environment include urea, dissolved free amino acids (DFAA), dissolved combined amino acids (DCAA), peptides, amino sugars, purines, pyrimidines and other complex macromolecules such as humic and fulvic acid and a variety of uncharacterized components [12,13]. Urea in raw wastewater can be readily converted to ammonium carbonate and it can be found as ammonium instead of urea in receiving waters [3,14,15].

Total dissolved nitrogen (TDN) consists of dissolved inorganic nitrogen (DIN) and DON. DON is a potential nitrogen source for bacterial, algal, and phytoplankton communities in aquatic environment and its fraction can vary from 8 to 83% of the TDN. It is a dynamic contributor of the nitrogen cycle and spontaneously produced, consumed, and transformed in the various oligotrophic water systems due to microbial and photolytic reactions [1].

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Water scarcity in some arid areas might force the communities to reclaim, recycle, and reuse of wastewater effluent or surface waters that receive discharges from wastewater treatment plants (WWTPs), as an alternative water supply to meet the water demands of their growing population. However, DON content in these water sources is a major concern since DON has adverse effect on reuse potential of the water sources [16].

The major portion of nitrogen flux from estuaries and some rivers and lakes is DON, yet it is not considered to effect surface water quality because of its assumed refractory (biologically unavailable) nature. However, recent studies have proved that DON in natural surface waters is metabolically important source since it supplies nutrients to bacterial and algal communities [3,6,17]. Due to its complex structure, most of the compounds in wastewater derived DON cannot be identified with current technologies. While some portion of DON are readily biodegradable and/or bioavailable to bacterial communities in biological treatment systems, some portion of it are recalcitrant [9,18–20].

Previous investigations proved that at least 50–85% of the refractory portion of DON became biodegradable and/or bioavailable to living organisms in water ecosystems when the optimum environmental conditions, such as the concentration of initial DON, residence time, type and amount of bacterial and algal communities, DO level, and temperature were met [13,14,19,21]. DON in urban runoff, animal feedlot runoff, and from indigenous production is readily available to bacteria and algae, while DON from forest, wetlands, agricultural runoff, lagoons, and wastewater treatment plant effluent has limited availability [3,4,9,22].

Municipal wastewater effluent DON is one of the most important autochthonous nitrogen sources to receiving waters and its reduction is crucial for especially nutrient sensitive surface waters. Main design and operational parameters, such as solids retention time (SRT), hydraulic retention time (HRT), temperature, and bacterial community are influencing parameters to DON removal. Understanding the fate and characteristics of DON in the wastewater influent and across the biological treatment process are of great interest in order to reduce effluent TDN concentration. Bacterial activity and food/microorganism ratio (depending on the characteristics of substrate and microorganisms) are crucial factors for DON removal. The scope of this study is to evaluate and understand how low SRTs can impact the removal of DON in chemostat reactor when seeded with bacteria + *Chlamydomonas reinhardtii*.

2. Materials and methods

2.1. Sample collection and preparation

Grab samples were collected from the City of Fargo WWTP (Fargo, ND). The plant has two-stage trickling filter processes, which are biochemical oxygen demand (BOD) and nitrification trickling filter (TF) units. The samples were collected after BOD TF process, which mainly contain ammonia, nitrate, and DON. The plant treats an average 15 million gallons per day (MGD) with a peak pumping capacity of 29 MGD. The samples collected were filtered and immediately taken for parametric analysis. The samples were preserved in the refrigerator at 4°C for later experimentation and analysis. The reactor influent sample was refreshed every 3–4 d by collecting new sample from the plant.

2.2. Algal and bacterial inoculum preparation

The algae *C. reinhardtii*was obtained from University of Texas, Austin. The strain was grown in Bristol Medium with continuous aeration using fine bubble diffusers with 12 h light/dark cycle at 20°C. *C.reinhardtii* is a single-cell green algae which is widely available in soil and fresh water. This particular strain of green algae is known for its ability to provide stable nitrogen removal systems and photoproduce ammonia from wastewater contaminants [8,23–26].

At the beginning of each chemostat reactor operation, about 3 L of wastewater sample placed into the reactor. Approximately 500 ml of a mixed bacterial culture (mixed liquor suspended solids, MLSS) and about 500 ml of a pure algal strain *C.Reinhardtii* were used to inoculate the reactor. The MLSS was obtained from the aeration tank of City of Moorhead WWTP, Moorhead, MN. Before beginning the experiments, the system was aerated continuously at an SRT of 4 d or more to facilitate the growth of the microbes without washout. Since there was not any media available in the reactor for the organisms to attach and grow (not an attached growth system), hydraulic retention time (HRT) was considered as equal to SRT in the biological reactor. Therefore, only SRT was used as a terminology in entire study.

2.3. Experimental design

During the experiments, the influent sample was continuously pumped using a peristaltic pump (Cole-Parmer Masterflex Peristaltic Pump, Vernon Hills, IL, USA) to the reactor with a desired SRT from the refrigerated (4ºC) sample. The reactor was operated at low SRTs of 0.2, 0.4, 0.5, 1 and 2 d. At the beginning of each SRT operation, about 3 L of wastewater samples placed into the reactor. Approximately 500 ml of a mixed bacterial culture (mixed liquor suspended solids, MLSS) and about 500 ml of a pure algal strain *C.reinhardtii* were used to inoculate the reactor.

The dimensions of the reactor were 45 cm × 25 cm × 25 cm with a working volume of 8 L. The reactor was made of polyvinyl chloride (acrylic). The reactor was maintained at a constant room temperature of 25°C and continuously aerated using fine bubble diffusers. The pump was calibrated at the beginning of each SRT and tested regularly to monitor for desired performance and accuracy. Dissolved oxygen (DO) level of 4 to 6 mg/L was maintained in the reactor.

2.4. Sample analyses

Reactor effluent samples were collected after the steady state conditions were achieved. Reactor influent and effluent samples were analyzed to determine the nitrogen (N) species and soluble chemical oxygen demand (SCOD) concentration. About 50 ml of filtered samples were used to measure dissolved ammonia, nitrite, nitrate, SCOD and total nitrogen. The samples were filtered initially using 1.2 µm pore size glass microfiber filter paper (Whatman Inc., Kent, UK) and further re-filtered using 0.45 µm membrane filter of the same brand. The inorganic nitrogen and SCOD concentration were measured using TNT test kits from Hach. The details of the TNT kits are presented in Table 1.

All the TNT test kits were measured using Hach DU 6000 spectrophotometer at varied wavelengths. The dissolved nitrate was analyzed using a second derivative UV spectrophotometric method whereas TDN was measured by following the standard per sulfate digestion method using an Agilent Cary 60 UV-Vis spectrophotometer. DON was calculated using the traditional subtractive method of calculating from the difference between TDN and DIN species using the mass balance equation (Eq. (1)).

$$\text{DON (mg N/L)} = \text{TDN} - \text{DNH}_3\text{-N} - \text{DNO}_2\text{-N} - \text{DNO}_3\text{-N} \quad (1)$$

2.5. Model development

A kinetic model was developed with the concept of continuous stirred tank reactor (CSTR) to simulate the biodegradation of wastewater derived DON from the Fargo WWTP. The conversion of DON was carried out in three steps: mineralization of DON by mixed culture bacteria, generation of ammonia, and finally nitrification of ammonia with uptake of nitrate by algae. The mass balance equations were developed considering the first order kinetic Monod model. A number of parameters would influence the biodegradation of DON; however, to simplify the complexity of the model the mineralization rate, ammonia oxidation growth rate, and the rate of nitrification has only been considered as rate limiting parameters for the model. The model was calibrated using an observed dataset obtained during the experimental study. A goodness of fit was performed to understand the model fit to the observational data. It should be noted that only steady state performance is of interest.

MATLAB (R2016b; The MathWorks, Natick, MA) was used to develop the code to solve the mathematical model and validated against the observed experimental data. The experimental data were incorporated into an excel spreadsheet and imported into MATLAB for the calculation purpose. The ordinary differential equations were solved using ODE 45 solver which is designed based on 4th and 5th Range-Kutta methods. Model performance was evaluated by statistical analysis between simulated and observed data using mean absolute error (MAE), root mean square error (RMSE) and coefficient of determination (R^2).

Table 1
Parameters and measurement methods

Parameters	Test kits	Detection limit
DNH_3-N	TNT 830 LR	0–2 mg/L
	TNT 832 HR	2–42 mg/L
DNO_2-N	TNT 839 LR	0–0.6 mg/L
	TNT 840 HR	0.6–6 mg/L
SCOD	TNT 821 LW	3–150 mg/L
	TNT 822 HR	20–1500 mg/L

LW: Low range, HR: High range, SCOD: Soluble chemical oxygen demand.

2.6. Mathematical modelling

As a first task, the kinetic model of the DON degradation must be performed. The considered chemical reactions are as follows:

$$DON \xrightarrow[ammonification]{} NH_3\text{-}N \xrightarrow[nitritation]{} NO_2\text{-}N \xrightarrow[nitratation]{} NO_3\text{-}N \quad (2)$$

2.7. Mass balance equation

The CSTR model has been conceptualized in Fig. 1 to understand the mass balance variables. The state variables considered are concentrations of DON (x_1), NH_3-N (x_2) and NO_3-N (x_3) and are function of time (t).

The assumptions are:

1. Working volume (V) of the tank remains constant throughout the experiment.
2. Influent flow rate(Q) remains invariant with time (t).
3. Outflow rate is equal to the influent flow rate.
4. There is perfect mixing in the tank, indicating that the solute concentration in the tank is uniform and invariant with space.
5. Effluent solute concentration (x (t)) is same as in the tank and is a function of time.
6. The initial concentration (u (t)) for a particular SRT remains constant throughout the experiment.

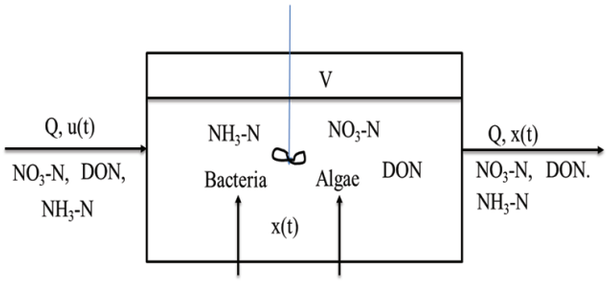

Fig. 1. Continuous stirred tank reactor based schematic diagram.

The mass balance equations were derived from ordinary differential equations (ODE) of first order described below:

- In mathematical term:

$$d[Vx(t)]/dt = Qu(t) - Qx(t) - V[k_c x(t)] + V[k_g x(t)] \quad (3)$$

where;
k_c = constant first order rate of consumption (day^{-1})
k_g = constant first order rate of generation (day^{-1})

- System mass balance equation

$$\text{DON: } d[V_{x1}(t)]/dt = Q_{u1}(t) - Q_{x1}(t) - V[k_c x_1(t)] + V[k_g x_1(t)] \quad (4)$$

$$NH_3\text{-N: } d: [V_{x2}(t)]/dt = Q_{u2}(t) - Q_{x2}(t) - V[k_c x_2(t)] + V[k_g x_2(t)] \quad (5)$$

$$NO_3\text{-N: } d[V_{x3}(t)]/dt = Q_{u3}(t) - Q_{x3}(t) - V[k_c x_3(t)] + V[k_g x_3(t)] \quad (6)$$

The net constant first order rate of change $k = k_g - k_c$. The model parameters are presented in Table 2. The influent data has been the same for all the SRTs performed in the experimental study.

3. Results and discussion

3.1. Dissolved inorganic nitrogen, TDN, and DON

Dissolved ammonia decreased as the retention time increased gradually from 0.2 to 2 d. Complete nitrification of DNH_3-N was observed at SRT 2 d (98% and above) (Fig. 2a). A previous study showed similar results that full nitrification occurred at SRTs 2, 3, and 4 d in a chemostat reactor inoculated with bacteria only inoculum [27]. Also, a study reported higher rate of nitrification by a microalgae-bacteria consortia in a flat panel photo-bioreactor from artificial wastewater at SRT 2 d [28]. Incomplete nitrification at SRTs 0.2, 0.4, 0.5, and 1.0 d in this study showed that low residence time did not provide appropriate time for bacteria and algae to uptake and degrade the ammonia. However, at low residence times nitrification was achieved between 53 and 73%. The rate of conversion of dissolved ammonia to other forms of nitrogen were statistically insignificant between 0.2 and 0.4 d. SRT 1-day could nitrify only 9% of the influent ammonia achieving the lowest fraction of nitrified ammonia. Reactor influent NO_2-N values were <0.91 mg/L while effluent nitrite values ranges between 1.92 to 5.65 mg/L in all the SRTs showing that even SRT 2-day was short to reduce nitrite (Fig. 2b). Since the reactor was continuously aerated, DO level of the reactor was approximately 6 mg/L. However, this nitrite accumulation did not affect DON biodegradability. Since the ammonia degradation is higher than nitrite accumulation in the reactor, it can be explained that some portion of ammonia nitrified to nitrate. Reactor influent nitrate values in SRTs 0.2, 0.4, 0.5 and 1 d reduced (Fig. 2c) due to denitrification facilitated by nitrite oxidizing bacteria and nutrient uptake by algae *C. Reinhardtii* (Fig. 2c). A study [29] on the nitrification of artificial wastewater using photo-bioreactors seeded with algae and/or bacteria consortium reported that about 81–85% of ammonium was nitrified by bacteria in an SRT of 15 d rather than algae-only consortium. It was also observed that the concentration of nitrate increased and achieved the complete nitrification [29].

Influent dissolved nitrate nitrogen varied between 7.02 and 7.65 mg/L. The SRTs of 0.2 to 1-day show reduction in the concentration of effluent nitrate favoring uptake of nitrate by algae. However, unlike the rest of the SRTs, the nitrate levels at SRT 2 d significantly increased to 16.83 mg/L. This increase advocates the degradation of DON to lower molecular compounds at SRT 2 d.

The wastewater sample from after BOD trickling filter location has low SCOD concentration of an average of 48 mg/L. Since the wastewater sample is already treated under biological process, no further reduction was observed in the SCOD profile in the reactor effluent at low SRTs. The

Table 2
Model input parameters

SRTs (days)	Rate of ammonification, k_1, (day^{-1})	Rate of bacteria growth, k_2(day^{-1})	Rate of nitrification, k_3(day^{-1})
0.2	0.2071	0.0999	0.0309
0.4	0.0755	0.1012	0.0350
0.5	0.1120	0.2372	0.0196
1	0.0222	0.0961	0.1135
2	0.0923	0.1459	0.0066

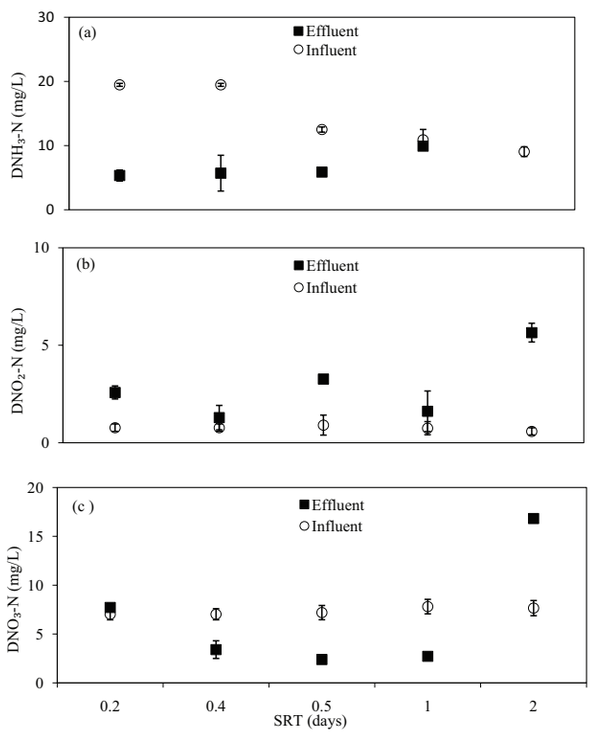

Fig. 2. (a) DNH_3-N in influent and effluent, (b) DNO_2-N in influent and effluent, (c) DNO_3-N in influent and effluent.

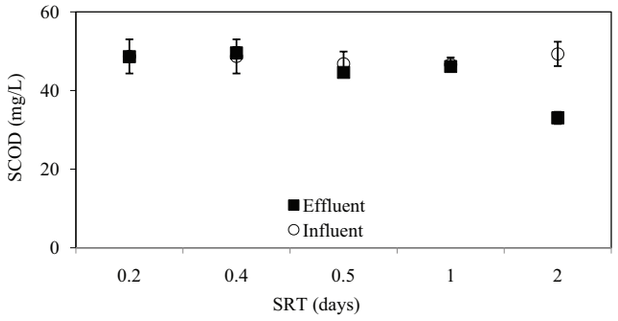

Fig. 3. Influent and effluent SCOD at different SRTs.

SCOD was mostly between 48 mg/L and 46 mg/L (Fig. 3). However, 67% removal of SCOD was observed at SRT 2.0 d being the maximum removal efficiency compared to the remaining SRTs.

Influent TDN concentration decreased in the plant from about 34 to 24 mg/L when the experiments were carried out between SRT 0.2 through 2 d since the samples were collected in different months. Effluent TDN reduced in all SRTs; however, the highest reduction (54.66%) was observed at SRT 0.2 d (Fig. 4a). TDN reduction was lowest at SRT 2 d. Only 5% reduction was observed from which it could be inferred that DON was mineralized by bacteria and utilized by algae in the system. This also justifies the reduction of DON (83%) in the system at SRT 2 d recording highest removal when compared to the rest of the SRTs conducted.

Significant removal of effluent DON was observed in SRT 2 days compared to influent concentration. The effluent DON values ranged between 4.44 mg N/L (SRT 0.2) and 1.24 mg N/L (SRT 2.0 d). However, at low SRTs the removal capacity of DON by the reactor reduced. Cell washout was also observed in the tank during lower SRTs. At SRT 2 d, the reduction of DON was more than 80% compared to approximately 30% in SRT 0.2 d. A gradual increase in the reduction of DON could be observed in Fig. 4b. However, SRT 0.4 and 0.5 d showed higher removal than that of 1 d.

About 70–80% of DON was observed to be bioavailable to algal bacterial mixed culture in the treatment of wastewater samples obtained from a trickling filter wastewater treatment plant. It was also suggested that *C. reinhardtii* could be chosen as a standard test species in the removal of nitrogen in wastewaters [30]. A symbiotic system of algae (*C. vulgaris*) and bacteria (*B. licheniformis*) in the ratio of 1:3 achieved TDN removal of 88–95% from synthetic wastewater [31].

The DON/TDN ratio decreased with the increase in SRT. Although there is no statistical significance between the DON/TDN ratio at low SRTs of 0.2, 0.4 and 0.5 d, Fig. 4c shows that there is a decrease in the concentration of DON to the concentration of total nitrogen at SRT 2 d. Higher residence time gives sufficient time for the microbes to degrade high molecular organic compounds into simpler inorganic forms of nitrogen.

3.2. Model simulation

Fig. 5 shows individual simulated vs observed data curve to exhibit a clearer picture of the model validation. The dotted line represents the observed data and the smooth curve represent the simulated model data. The straight line represents the steady state value for the observed data. The straight line in the above figures represents the proximity of the simulated vs observed data. The x-axis represents the time taken by each SRT to reach steady state. The observed time taken by each SRT agrees with simulated data. The model simulation data showed that the optimum reduction of DON was achieved at SRT 2 d which aligns with the observational data. Significant removal of effluent DON was observed at SRT 2 d compared to influent. The effluent DON concentration at SRT 2 d was 1.24 mg/L. At low SRTs the removal capacity of DON by the reactor reduced. At SRT 2 d, the reduction of DON was more than 80% compared to approximately 50% in SRT 0.2 d. The concentration of DON in SRTs 0.2, 0.4, 0.5 and 1 d ranged between 2–4 mg/L. The degradation of DON at SRT 0.2, 0.4 and 0.5 d were statistically insignificant. SRT 1 day showed lower reduction in the degradation of DON, which was recorded as 3.5 mg/L. This could be due to cell wash out that was observed inside the tank during operation. The high inflow rate could have caused the washout of the algae and bacteria from the reactor.

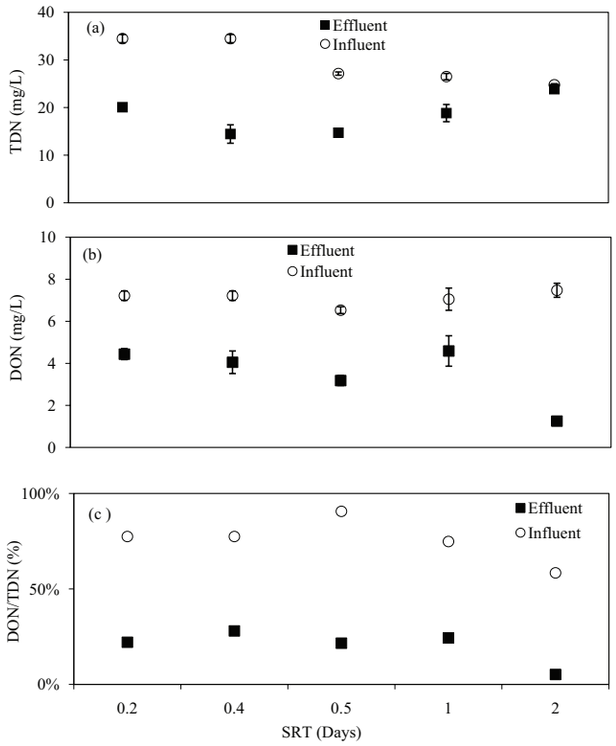

Fig. 4. (a) TDN in influent and effluent (b) DON in influent and effluent (c) DON/TDN ratio for influent and effluent.

The experimental data has been presented in Fig. 3b which provides a comparison in the reduction capability of DON when operated at different SRTs. The influent data has been included to provide a clearer picture of the degradation in the concentration of DON.

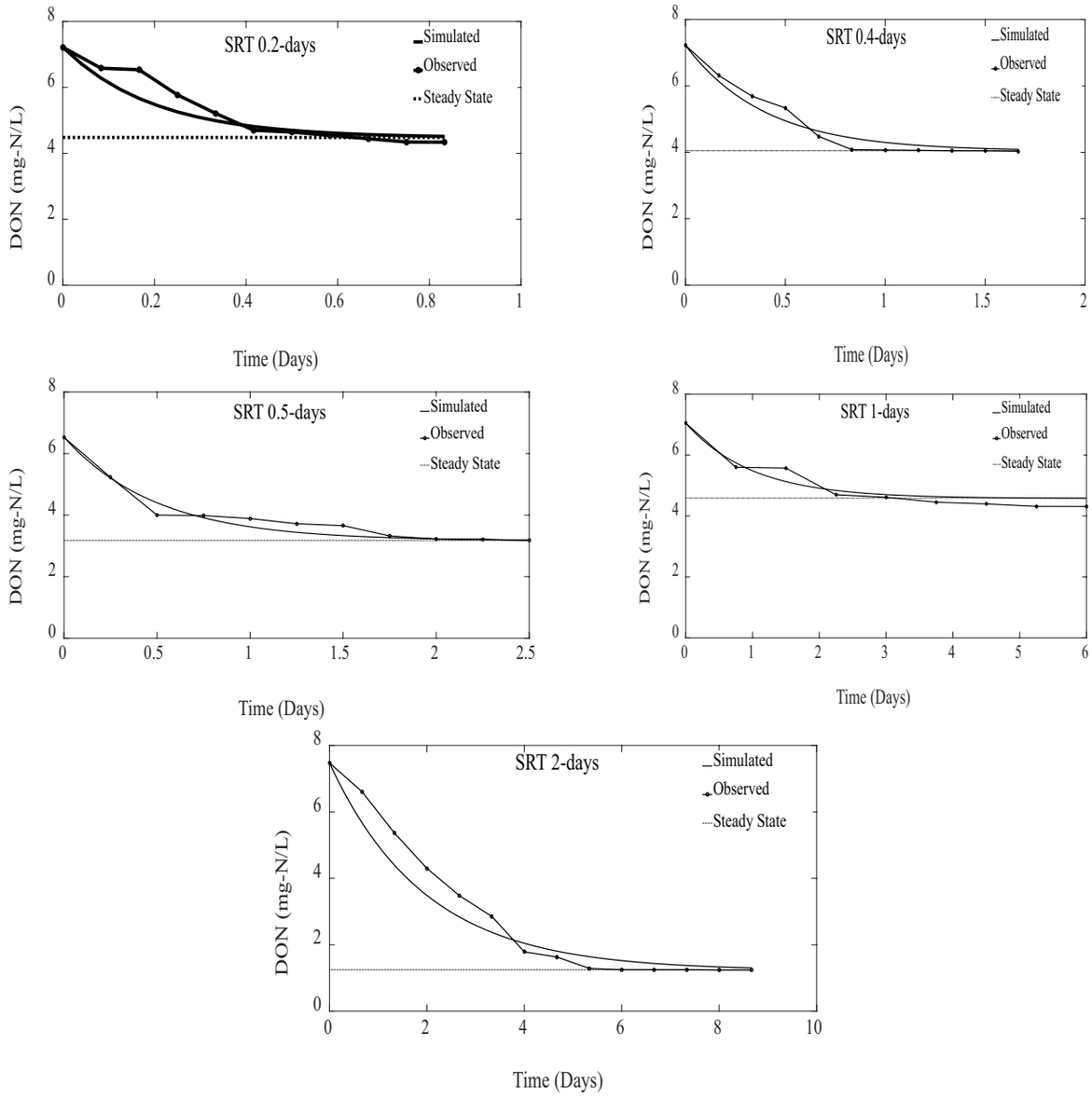

Fig. 5. Dynamic and steady state curve for observed vs steady state for each SRT.

3.3. Statistical analysis

The model performance was validated conducting statistical analysis incorporated in MATLAB. Statistical analysis between simulated and observed data using mean absolute error (MAE), root mean square error (RMSE), coefficient of determination (R^2) and percent bias (PBIAS) were performed. Since each SRT operated at different time scale, thus, error calculation was performed on individual SRTs only. The statistical analysis has been tabulated in Table 3. The MAE ranged between 13% and 19% at SRTs 0.4, 0.5 and 1d. However, SRT 0.2 and 2 had MAE of 24% and 38% respectively. The MAE was further validated with RMSE calculation. The RMSE predicted the difference in the values predicted by the model and the observational data. Both RMSE and MAE values suggests that the model overestimated the prediction values of SRT 0.2, and 2 d. The R^2 was performed to check the model fit with the experimental design. R^2 value for each SRT was more than 90% indicating that the model was a good fit to the experimental design.

4. Conclusion

The following conclusions were drawn from this study:

- SRT 2 d shows optimum degradation of DON to nitrate. The steady state concentration of wastewater derived DON at an inflow rate of SRT 2 d was 1.24 mg/L which is more than 80% removal efficiency. The reduction at

Table 3
Statistical parameters for input (inorganic nitrogen and total dissolved nitrogen) and output (dissolved organic nitrogen)

SRT (Days)	R-squared (R^2)	MAE	RMSE
0.2	0.9120	0.2411	0.3402
0.4	0.9688	0.1776	0.2129
0.5	0.9660	0.1314	0.1962
1	0.9479	0.1963	0.2301
2	0.9649	0.3842	0.4978

MAE: Mean absolute error, RMSE: Root mean square error

SRTs 0.2, 0.4, 0.5 and 1 d was statistically insignificant and could partially remove DON from the wastewater sample.

- The model developed showed a close proximity between the predicted values and the observed values. The simulated data indicated the time taken by each SRT to reach the steady state for DON, NH_3-N and NO_3-N concentration. However, since the study mainly aims at achieving steady state conditions, thus, the dynamic change in the operation has been neglected. The data comparison has been purely done based on steady state conditions.
- Statistical analysis indicates the model to be a good fit to the experimental design with R^2 value ranging from 84% to 95% for each SRT. The MAE and RMSE analysis signifies the difference between the predicted and the observed values and provides the basis of estimation for the model predicted values.

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