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**Enhanced carbon and nitrogen removal performance of
simultaneously anammox and denitrification (SAD) with mannitol
addition treating saline wastewater**

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Abstract

BACKGROUND: Simultaneously anammox and denitrification (SAD) can remove carbon and nitrogen. However, its performance would be suppressed under saline surroundings. In this work, mannitol was used to enhance SAD process treating saline wastewater.

RESULTS: The optimal carbon and nitrogen removal was achieved at 0.2 mM mannitol, during which ammonium removal efficiency (ARE), nitrite removal efficiency (NRE) and chemical oxygen demand (COD) removal efficiency were 96.95 %, 93.70 % and 90.05 %, respectively. The maximal ammonium removal rate (ARR), nitrite removal rate (NRR) and the specific anammox activity (SAA) were increased by 25.49 %, 55.84 % and 33.83 % with optimal addition (0.2 mM mannitol) respectively. The diameter of sludge was enlarged with the addition of mannitol (≤ 0.2 mM). Tseng-Wayman model was more suitable to simulate the whole process of SAD. The modified Logistic model, the modified Boltzman model and

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the modified Gompertz model were all appropriate to describe nitrogen removal in a typical cycle with addition of mannitol.

CONCLUSION: Mannitol was effective to enhance SAD process treating saline wastewater, and the maximum nitrogen removal was achieved at mannitol = 0.2 mM. Tseng-Wayman model could well predict the whole process of SAD treating saline wastewater with mannitol addition.

Keywords: simultaneously anammox and denitrification (SAD); mannitol; saline wastewater; kinetic analysis; sludge properties.

INTRODUCTION

As a new type of biological technology, simultaneous anammox and denitrification (SAD) has attracted wide attention worldwide due to advantages such as requiring no addition of an external carbon source and less sludge production.¹ Anammox has been regarded as a cost-effective and energy-saving way for nitrogen removal.² Compared with traditional nitrification-denitrification, anammox is the autotrophic oxidation of ammonium to N₂ using nitrite as an electron acceptor without the presence of organic carbon and oxygen.³ Therefore, anammox process can save 60 % aeration, 100 % organic carbon.^{4,5} Except the advantages described above, AnAOB had some drawbacks too. It was sensitive to the outside environment, such as heavy metal,⁶ certain chemicals⁷ and so on. Organic carbon which is ordinary in wastewater was also harmful to anammox process.⁸ SAD process can remove nitrate produced in anammox process. Besides, it can also remove organic matter because denitrifiers can

reduce nitrate to N_2 using organic carbon as an electron donor.⁹

Wastewater originated from agriculture, aquaculture, and many industrial sectors usually contains high salinity with a variety of organic compounds.¹⁰ It is significant challenge to treat this kind of wastewater due to the adverse effect of salinity, which has negative influence on microbial metabolic activity, pollutant removal and sludge settleability.^{11,12,13} Salinity exerts toxic effects on microorganisms during biodegradation processes. One common mitigating strategy is to acclimatize the microbial culture by progressively increasing salt concentrations.¹⁴ However, acclimatization time may be quite long and microbial salt resistance can be easily lost if the salt concentration selection pressure is removed.¹⁵ Although microorganisms are able to survive in high salinity environments through bio-augmentation with halophilic species to achieve more stable and durable process performance, it is not easy to maintain dominance of such species in mixed culture systems.¹⁶

An alternative approach to deal with saline wastewater was the addition of compatible solutes to enhance sludge activity. The previous study has shown the enhancement effect of compatible solutes under salinity stress and found that compatible solutes addition was one of the feasible solution counteract saline condition.¹⁴ Many microorganisms accumulate organic solutes (called “compatible solutes”) to balance osmotic pressure between the cytoplasm and the outer surroundings. The reason why these organic compounds are called compatible solutes is that they can function inside the cell without the need for special adaptation of the intracellular enzymes, and it also serve as protein stabilizers in the presence of high

ionic strength.¹⁷

Cyplik et al.¹⁸ investigated the effect of ectoine and trehalose on denitrification at 15 g NaCl/L by employing a denitrifying consortium. It was found that ectoine resulted in faster denitrification and trehalose had no effect on nitrate removals.¹⁸ Liu et al.¹⁶ found that 1 mM glycine betaine addition was most effective in alleviating salt toxicity in anammox biomass obtained from a pilot-plant UASB. To the best of our knowledge, no studies have focused on the effect resulting from mannitol addition on the SAD process to treat saline wastewater. Mannitol as a polyol, is also a kind of compatible solutes. The objective of present work was to evaluate carbon and nitrogen removal performance of SAD process treating saline wastewater with mannitol addition. Besides, kinetics was performed to analyze the enhanced effect resulting from mannitol addition.

MATERIALS AND METHOEDS

Reactor configuration and operation

A sequencing batch reactor (SBR) made of polymethyl methacrylate was used in this work (Fig. 1). The reactor was double-jacketed with an effective volume of 7.0 L. Its temperature was controlled at 35 ± 0.5 °C by a water bath with water recirculation through the outer chamber. The reactor was covered with aluminum caps to prevent the potential growth of phototrophic micro-organisms. The operating mode of the reactor consisted of 0.5 h influent feeding, 3 h anoxic stirring reaction, 0.5 h sludge settling and 0.5 h effluent discharging. Influent pH was controlled around 7.4 ± 0.2 by

adding 1 M NaOH or HCl. The addition concentration of mannitol was gradually increased from 0 to 0.6 mM. N₂ (99.99 %) was purged into the influent for 15 min to maintain anaerobic conditions.

----- **Figure. 1** -----

Biomass and wastewater characteristics

Before this study, the reactor had been operated more than 2 years in SAD mode and steady state was achieved. The average concentration of mixed liquor suspended solids (MLSS) and average sludge settling ratio (SV, %) was 14.47 g/L and 8%, respectively. The diameter of granular biomass was 0.53-1.53 mm, with a mean value of 0.84 mm. The digital and microscopic images of the sludge were presented in Fig. S1. Synthetic feed was prepared with seawater which was taken from Jiaozhou Bay of Qingdao (Shandong Province, China). The wastewater salinity was 32 ± 2 g/L. The concentration of NH₄⁺-N, NO₂⁻-N and COD (glucose) in influent were 80 ± 5 , 160 ± 5 , and 214 ± 5 mg/L, respectively. The composition of mineral medium was (g/L): KH₂PO₄ 0.029; CaCl₂·2H₂O 0.136; MgSO₄·7H₂O 0.06; KHCO₃ 1.4. Trace element solutions I and II (1.4 mL/L) were also added. The trace element solution I contained (g/L): FeSO₄·7H₂O 5; EDTA 5. The trace element solution II contained (g/L): EDTA 15; H₃BO₃ 0.011; MnCl₂·4H₂O 0.99; CuSO₄·5H₂O 0.25; ZnSO₄·7H₂O 0.43; NiCl₂·6H₂O 0.19; Na₂MoO₄·2H₂O 0.22; CoCl₂·6H₂O 0.24; NaSeO₄·10H₂O 0.21.

Analytical methods

Samples were withdrawn and centrifuged at 6000 rpm for 30 min. The supernatants were analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ and COD concentrations according to the standard methods.¹⁹ The sludge volume index (SVI, ml/g) and SV was also measured based the standard methods.¹⁹ 30 ml granular sludge were sampled and the size of granular sludge was analyzed according to method described by Tang.²⁰ Each sample was analyzed in triplicate and the mean value was reported. Values of pH were monitored by using probes with an FE20 pH meter (Mettler Toledo). Morphology characteristics and superficial structure of the biomass specimens were observed using scanning electron microscopy (SEM) model Hitachi S-4800 (Japan). Biomass concentration was determined after filtering the washed samples through 0.45 μm membrane filter and drying at 105 °C to constant weight. The measurement of specific anammox activity (SAA) was according to the previous research of our group.²⁰ Ammonium removal rate (ARR) was calculated by Eq. (1) below, nitrite removal rate (NRR) was calculated by Eq. (2).

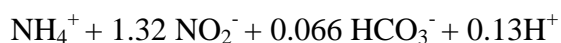
$$ARR = \frac{(C_{\text{NH}_4^+\text{-N}_{\text{Inf.}}} - C_{\text{NH}_4^+\text{-N}_{\text{Eff.}}}) \times 24}{1000 \times HRT} \quad (1)$$

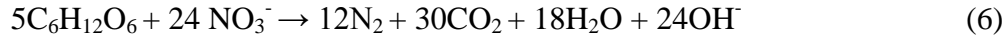
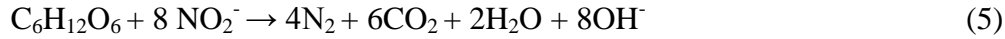
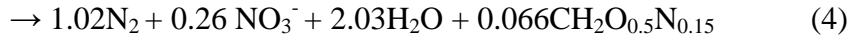
$$NRR = \frac{(C_{\text{NO}_2^-\text{-N}_{\text{Inf.}}} - C_{\text{NO}_2^-\text{-N}_{\text{Eff.}}}) \times 24}{1000 \times HRT} \quad (2)$$

$$SAA = \frac{V_{\text{max}}}{MLSS} \quad (3)$$

where: V_{max} was the maximum removal rate of $\text{NH}_4^+\text{-N}$ ($\text{mgNH}_4^+\text{-N}/(\text{L}\cdot\text{h})$).

The SAD process mainly included anammox, denitrification and denitrification reactions as follows:





Based on nitrogen mass balance and Eqs. (4) - (6), the percentages of nitrogen removal (p) by anammox and denitrification can be calculated as follows:

$$P_{ana}(\%) = \frac{1.32\text{NH}_4^+{}_{rem}}{\text{NO}_2^-{}_{inf} + \text{NO}_3^-{}_{inf} + 0.26\text{NH}_4^+{}_{rem} - \text{NO}_2^-{}_{eff} - \text{NO}_3^-{}_{eff}} \times 100 \quad (7)$$

$$P_{den}(\%) = 100 - P_{ana}(\%) \quad (8)$$

where the subscripts represent anammox (ana), denitrification (den), removal (rem), influent (inf), and effluent (eff); and the concentrations of NH_4^+ , NO_2^- and NO_3^- are all expressed in mg/L as N.²¹

Kinetic analysis

Haldane,^{22,23} (Eq. 9) Aiba,²⁴ (Eq. 10) and Edwards²⁵ (Eq. 11) models were first applied in SAD process in present work. They are suitable to describe the relationship between the effluent substrate (NH_4^+ -N and NO_2^- -N) concentrations and substrate removal rate.

$$r = \frac{r_{max}S}{K_S + S + S^2 / K_{IH}} \quad (9)$$

$$r = \frac{r_{max}S}{K_S + S} \exp\left(-\frac{S}{K_{IA}}\right) \quad (10)$$

$$r = r_{max} \left(\exp\left(-\frac{S}{K_{IE}}\right) - \exp\left(-\frac{S}{K_S}\right) \right) \quad (11)$$

The luong model²⁶ was also used for determining the substrate removal kinetics in

the anammox process.

$$r = \frac{r_{\max} S}{K_S + S} \left(1 - \frac{S}{S_m}\right)^n \quad (12)$$

Han and Levenspiel model²⁷ was applied in the growth of a mixed culture with n-pentane. Niu et al.²⁸ reported it fitted the the experimental data well though it was seldom used in anammox process.

$$r = r_{\max} S \frac{1 - (S/S_m)^n}{S + K_S (1 - S/S_m)^m} \quad (13)$$

The Tseng-Wayman model²⁹ was used to describe inhibitory effect resulting from gluconic acid and glucose on *Gluconobacter* quantitatively. Zhu et al.³⁰ used it to demonstrate nitrite inhibition on anammox performance quantitatively.

$$r = r_{\max} \left(\frac{S}{S + K_S} \right) - K_{IT} (S - S_{\text{crit}}) \quad (14)$$

where: r is substrate removal rate, $\text{kgN}/(\text{m}^3 \cdot \text{d})$; r_{\max} is maximum substrate removal rate, $\text{kgN}/(\text{m}^3 \cdot \text{d})$; S is effluent substrate concentration, mg/L ; S_m is effluent substrate concentration above which net growth ceases; S_{crit} is threshold substrate concentration; K_S is half-saturation coefficient, mg/L ; K_{IH} is Haldane coefficient, mg/L ; K_{IA} is Aiba coefficient, mg/L ; K_{IE} is Edwards coefficient, mg/L ; K_{IT} is Tseng-Wayman coefficient, mg/L ; n and m are coefficients.

The modified Logistic model (Eq. 15) was the first to describe hydrogen production process in batch test.³¹ The modified Boltzmann model (Eq. 16) could be used to indicate the potential of anammox process³² and describe the recovery performance.³³ The modified Gompertz model (Eq. 17) was suitable to describe the process of substrates degradation in anammox process. The modified Logistic model, modified

Boltzmann model and modified Gompertz model were applied in present work to describe nitrogen removal process of SAD in an operating cycle.

$$H = \frac{H_{\max}}{1 + \exp[4R_{\max}(\lambda - t)/H_{\max} + 2]} \quad (15)$$

$$H = H_{\max} + \frac{H_{\min} - H_{\max}}{1 + \exp[(t - t_c)/t_d]} \quad (16)$$

$$H = H_{\max} \exp\left[-\exp\left(\frac{R_{\max} e}{H_{\max}}(\lambda - t) + 1\right)\right] \quad (17)$$

where: H is total nitrogen removal efficiency; NRE_{\max} is the maximum NRE, %; NRE_{\min} is the minimum NRE, %; R_m is maximum NRR, $\text{kgN}/(\text{m}^3 \cdot \text{d})$; λ is lag time, h; t is operating time, h; t_c is the time of half NRE_{\max} , h; t_d is time constant.

Jin et al.³⁴ reported that the modified Logistic model, modified Boltzmann model and modified Gompertz model can be used to simulate the recovery process of anammox bacteria (AnAOB) under the inhibition of phenol and sulfide.

$$r = \frac{r_{\max}}{1 + \exp[4R_{\max}(\lambda - t)/r_{\max} + 2]} \quad (18)$$

$$r = r_{\max} + \frac{r_{\min} - r_{\max}}{1 + e^{(t - t_c)/t_d}} \quad (19)$$

$$r = r_{\max} \exp\left[-\exp\left(\frac{R_{\max} e}{r_{\max}}(\lambda - t) + 1\right)\right] \quad (20)$$

where: r represents substrate removal rate, $\text{kgN}/(\text{m}^3 \cdot \text{d})$; R_m represents maximum nitrogen recovery rate, $\text{kgN}/(\text{m}^3 \cdot \text{d})$; λ represents recovery lag time, h; t_c represents centre recovery time, h; t_d represents recovery time constant.

Results and discussion

Long-term effect resulting from mannitol addition on SAD process

The concentrations of nitrogen species and COD at different mannitol concentrations are presented in Fig. 2a. The variations of ARE, NRE and organic removal efficiency (ORE) are presented in Fig. 2b. When mannitol was no more than 0.2 mM, effluent $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and COD decreased with increasing mannitol. Optimal ARE (96.95 %), high NRE (93.70 %) and high ORE (90.05 %) were achieved when the optimal dose of mannitol was 0.2 mM. However, when the mannitol ≥ 0.25 mM, the effluent $\text{NH}_4^+\text{-N}$ continuously ascended. The effluent $\text{NO}_2^-\text{-N}$ and COD decreased with increasing mannitol at first, then both $\text{NO}_2^-\text{-N}$ and COD could be removed completely at mannitol ≥ 0.3 mM, these results indicated that moderate amount of mannitol (≤ 0.2 mM) could strengthen SAD process. With optimal mannitol addition (0.2 mM), the maximal ARE was increased by 18.44 %.

The nitrogen and COD removal rates at different mannitol concentrations are presented in Fig. 2c. The ARR gradually increased with increasing of mannitol, and peaking at 0.2 mM which accounting for $0.622 \pm 0.016 \text{ kg}/(\text{m}^3 \cdot \text{d})$ at $\text{OLR} = 2.059 \pm 0.037 \text{ kg}/(\text{m}^3 \cdot \text{d})$. Then it gradually decreased with further increasing of mannitol. It noted that mannitol ≤ 0.2 mM could enhance anammox performance. The NRR increased and reached a maximum value ($1.194 \pm 0.331 \text{ kg}/(\text{m}^3 \cdot \text{d})$), when mannitol was less than 0.2 mM. Then it maintained stable. The ORR increased with increasing mannitol continuously. The reason was that heterotrophic denitrifying bacteria (HDB) adapt to a high OLR. Wang et al.³⁵ reported that the simultaneous partial nitrification, anammox and denitrification process was affected more by nitrogen loading rate

(NLR) than by OLR. Li et al.²¹ reported that SAD process was greatly affected by OLR, which in agreement with present work. These results indicated that low mannitol (≤ 0.2 mM) feeding could result in enhancement of SAD performance treating saline wastewater. Cyplik et al.¹⁸ pointed out that the addition of 1 mM ectoine accelerated the denitrification process at 15 g/L NaCl. The same study also investigated that relative enzyme activities of lipase and nitrate reductase increased by 32 % and 35 %, respectively, in the presence of 1 mM ectoine.¹⁸ Liu et al.¹⁶ found that 1mM glycine betaine was the most effective in alleviating salt toxicity in anammox biomass. It is likely that different compatible solutes have different mechanisms and different optimum addition on AnAOB and HDB. Therefore, an optimal concentration of mannitol should be added to adjust the osmotic pressure and maintain the internal and external osmotic pressure cellular equilibrium.

The percentages of nitrogen removal by anammox and denitrification are presented in a Fig. 2d. Anammox accounted for 73.91 ± 2.73 % of nitrogen removal when there was in absence of mannitol. Nitrogen removal through anammox dropped with increasing mannitol content. In contrast, nitrogen removal through denitrification increased with increasing mannitol content. Nitrogen removal through anammox only accounted for 22.99 ± 2.16 % when mannitol was 0.6 mM. This indicates that a higher mannitol concentration does not necessarily contribute to anammox performance. It is likely that when mannitol concentrations exceed the optimum. Some of mannitol is no longer absorbed by AnAOB and HDB as an osmotic regulator, but as an organic carbon source for HDB. The presence of organic carbon was not suitable for

anammox. Another reason for this could be the biodegradation of mannitol. It is possible that at higher mannitol concentrations, specific genes are triggered and overexpressed, thus the osmoprotectant mechanisms of mannitol are suppressed.¹⁷ However, more research needs to be conducted to understand how this effect works in mix culture biological systems.

The stoichiometric ratios of nitrite and nitrate to ammonium (i.e., $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ and $\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$) can be applied as evidence indicating the performance of SAD. The theoretical stoichiometric ratio of $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ and $\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ for anammox is 1.32 and 0.26, respectively.³ As shown in Fig. 2e, a higher $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ and lower $\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ were found in this study. In absence of mannitol, the $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ was around 1.55 while the $\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ was around 0.020. The $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ gradually increased with increasing mannitol then it deviated obviously when mannitol was more than 0.3 mM. In contrast, the $\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ was more stable. When mannitol was taken as an organic carbon source by HDB, the growth of AnAOB would be limited resulting from substrate competition for nitrite between AnAOB and HDB. This may be a factor for more nitrite consumption and less nitrate formation. Therefore, high mannitol (≥ 0.35 mM) concentration could induce a deviation on the $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$. This was accordance with previous report¹⁷ that high COD concentration could result in a significant disturbance on the $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$. The recovery period for high mannitol inhibition was shown in Fig. S2.

----- **Figure. 2** -----

SAD performance in a typical cycle

To investigate the synergistic mechanism of anammox and denitrification with mannitol addition, nitrogen and COD removal within different operating cycles treating saline wastewater were analyzed (as shown in Fig. 3). At mannitol = 0.1 mM, ammonium, nitrite and COD concentrations decreased with reaction time. At the end of the cycle, the effluent nitrate concentration was 6.29 mg/L. The pH increased from 7.41 to 7.98. At mannitol = 0.15 mM, all the effluent ammonium, nitrite and COD were lower than that of mannitol = 0.1 mM. In the first 0.5 h, ammonium, nitrite and COD decreased by 23.46, 59.45, 50 mg/L, respectively. This result was higher than that when mannitol was 0.1 mM. The effluent nitrate concentration was 0.57 mg/L. This highlights the facts that proper mannitol concentration could enhance substrate conversion rate within shorter reaction time and could improve the removal of nitrate by denitrification. The pH increased from 7.40 to 8.04. At mannitol = 0.2 mM, the optimal removal of ammonium, nitrite and higher removal of COD were achieved at the reaction time of 3 h. The pH increased from 7.40 to 8.07. At mannitol = 0.3 mM, nitrite and COD was removed almost completely at the reaction time of 3 h while the removal of ammonium was slightly inhibited. The effluent nitrate was 0.43 mg/L indicating that denitrification could effectively remove nitrate resulting from anammox. It noted that 0.3 mM mannitol could slightly inhibit anammox but strengthen denitrification. The pH increased from 7.40 to 8.06. When mannitol was 0.6 mM, both nitrite and COD were completely removed at the reaction time of 2.5 h. The effluent ammonium was 49.06 mg/L, indicating that anammox was severely

inhibited. Meanwhile, the inhibiting effect on anammox resulting from high mannitol concentration was also showed within the first 1 h. Therefore, the maximum inhibition was acquired at 0.6 mM mannitol in present study. The pH increased from 7.40 to 8.10 at the reaction time of 2.5 h and then decreased to 7.93 at the end of the reaction.

As was shown in Fig. 3e, the pH inflection point appears at the reaction time of 2.5 h when nitrite and COD were completely removed. After 2.5 h operation, no residual nitrite and COD were found, which led to the cease of anammox and denitrification. The pH decreased in the last 0.5 h. This could be due to the chemical balances between ammonium and free ammonia. The OH^- was consumed which resulted in the descent of pH value. The variation tendency of pH may be used as an indicator for the inhibition of anammox in the SAD process.

Regarding to V_{\max} , in general, it first increased with increasing mannitol content and then decreased until the maximum inhibition. SAA was quite similar with V_{\max} . The small difference is that, the value of SAA at 0.6 mM was higher than it at 0.5 mM. Optimal SAA and V_{\max} increased by 24.00 % and 57.05 % over no mannitol addition, respectively. At 0.6 mM, V_{\max} decreased by 63.45%, and at 0.5 mM, SAA decreased by 24.98%.

AnAOB activity was inhibited at mannitol > 0.2 mM, while HDB activity would be accelerated. The anammox and denitrification could exhibit a good synergy when mannitol was less than 0.2 mM. Compatible solutes such as mannitol could equalize the high external osmotic pressure within the cytoplasm without the need for special

adaptation of the intracellular enzymes, and compatible solutes also serve as protein stabilizers at high ionic strength inside the cell.^{36,37} Oren³⁷ reported that the concentration of compatible solutes in the cell can range from millimolar to 1 - 2 M in response to the extracellular osmolarity. It depends on wastewater salinity and microorganism. The optimal ammonium removal was achieved at 0.2 mM in this study during which almost all ammonium, 93.70 % nitrite and 87.80 % COD could be removed. However, the inhibition resulting from high mannitol (0.6 mM) could severely affect AnAOB activity.

----- **Figure. 3** -----

Sludge properties

The particle size distributions of anammox and denitrification with addition of mannitol were presented in Fig. 4. Mannitol addition (≤ 0.2 mM) could promote the size of granular sludge. When there was in absence of mannitol, the diameter of anammox and denitrification sludge ranged from 0.53 to 1.53 mm with the mean diameter of 0.84 mm. The diameter of anammox and denitrification sludge gradually boost along with the improvement of mannitol addition. The diameter of anammox and denitrification sludge was 0.61 - 2.26 mm and 0.68 - 3.08 mm with the mean diameter of 1.1 mm and 1.23 mm at 0.1 mM and 0.2 mM mannitol, respectively. Apparently, it was larger than that with no mannitol addition. The frequency of sludge with diameter larger than 0.9 mm was 75 % and 88 % at 0.1mM and 0.2 mM respectively, while it was only 16 % at 0 mM mannitol.

Especially, a phenomenon was simultaneously observed with the increasing addition of mannitol. The diameter of anammox sludge gradually boosted when mannitol was no more than 0.3 mM in the present study. It is likely that the density of AnAOB considerably increased and these AnAOB cells spontaneously converged with the addition of mannitol. The aggregated distribution of the AAOB cells might have been linked to the re-activation of the AnAOB resulting from the positive effect of mannitol treating saline wastewater. It was similar with the results reported by Vyrides et al.³⁸ that methane produced was three times higher with glycine betaine addition during batch anaerobic digestion in salinity of 35 gNaCl/L. However, the mean diameter of granular sludge was 0.73 mm at 0.6 mM, which was smaller than that at 0 mM mannitol. This indicated that 0.6 mM mannitol seriously affected the activity of AnAOB. The inhibiting concentration of mannitol could lead to a certain amount of broken anammox sludge. The decay and cell lysis of AnAOB resulted in soluble organic carbon, which was used as electron donors by HDB. Therefore, it is a significant factor for the higher activity of HDB even though the concentration of mannitol remains high. It still needs further research in our research group.

----- **Figure. 4** -----

The sludge concentration, SV and SVI were presented in Fig. 5. The sludge concentration first increased from 1.41 g/L to 1.69 g/L when mannitol was no more than 0.2 mM. Then continuously decreased to 0.68 g/L (0.6 mM mannitol), due to the mechanism mentioned above. The small particle formed with high concentration (0.6 mM) of mannitol had fragment of AnAOB lead to descent of MLSS in SBR. In

general, the SV and SVI were quite similar at 0.1 and 0.2 mM mannitol. At low addition concentration (≤ 0.2 mM), mannitol elevated the sedimentation of sludge, which increased from 8 % to 12 % and induced augment the biomass retention. In present study, SVI of anammox and denitrification values ranges from 56.66 mL/g to 71.21 mL/g in the activity improvement period and from 71.21 mL/g to 50.24 mL/g in the inhibited period. Dapena-Mora et al.³⁹ and Dosta et al.⁴⁰ reported that SVI of anammox granule ranged from 40 - 110 mL/g. Sludge floatation was also observed from 0.3 mM which could lead to the increase of SVI. Furthermore, the sludge was not easy to precipitate. The image of sludge was presented in the supplementary materials (Fig. S2).

----- **Figure. 5** -----

Kinetic analysis

The experimental data, simulated results and the parameters predicted by different models were presented in Fig. 6. Although Haldane model, Aiba model, Edwards model, Luong model, Han-Levenpiel model and Tseng-Wayman model were widely used in substrate inhibition,^{41,34,42,43} they are seldom applied in SAD process. The high value of the correlation coefficient ($R^2 > 0.97$) was obtained from these models which were applied for describing the relationship between effluent substrate ($\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$) and substrate removal rate with the addition of mannitol in SBR. By comparison, R^2 (0.9987) obtained from Han-Levenpiel model was the highest, while R^2 (0.9774) obtained from Luong model was the lowest. S_m and n obtained from

Luong model and Han-Levenpiel model do not have biological significances. The fitted r_{\max} deserved from Haldane model, Aiba model, Edwards model, Luong model, Han-Levenpiel model and Tseng-Wayman model were 2.16, 1.92, 1.92, 1.83, 2.09 and 1.76 kgN/(m³·d), respectively. The relative error of the fitted r_{\max} deserved from these models and NRR_{\max} obtained from experiment were 22.72 %, 9.09 %, 9.09 %, 3.98 %, 18.75 % and 0 %, respectively. That is to say, r_{\max} fitted from Tseng-Wayman model was closest to the experimental value compared to other models. However, r_{\max} from Haldane model gravely deviates from the experimental value. Therefore, Tseng-Wayman model could provide an effective tool for process control. Furthermore, the parameters fitted from Tseng-Wayman model could more directly reflect the reaction process. As a result, Tseng-Wayman model was the most suitable to simulate the whole process of SAD.

----- **Figure. 6** -----

Nitrogen removal process with mannitol addition were described by modified Logistic model, modified Boltzamn model and modified Gompertz model. As depicted in Fig. 7 and Table1, kinetic parameters were deserved from the three models. High R^2 obtained indicating that they are all appropriate for describing the nitrogen removal kinetics and predicting the performance of SAD with the addition of mannitol. The TNRE_{\max} , TNRE_{\min} , R_m , and λ could be predicted well. The λ of nitrogen removal at different mannitol concentration were 0, indicating that the SAD process had no lag phase, which were in good accordance with experimental data. TNRE_{\max} value obtained from modified Logistic model, modified Boltzamn model and

modified Gompertz model were 91.17 %, 91.15 %, 93.01 % respectively, which were quite close to experimental data (92.78 %).

The minimum t_c were acquired at 0.86 h at mannitol = 0.1 mM. TNRE was 47.76 % at 1 h with 0.1 mM mannitol addition, which was almost half of $TNRE_{max}$. The validation of three models was also presented by analyzing the linearity between the observed and the predicted values in Fig. 7. At mannitol ≥ 0.2 mM, the predicted value deserved from modified Logistic model were high than the experimental data. Besides, the predicted value obtained from the modified Boltzman model and the modified Gompertz model were both higher than the experimental data. The t-test was performed to determine the bias of the models. The high value of R^2 (0.9635, 0.9857, 0.9815) from the three models dictated a good linear relationship between the experimental data and the predicted value. The t-value (0.06, 2.54E-06, 2.56E-05) were much low than the value of the t_{crit} (0.703) indicating that the models were unbiased.^{43,44} Theses findings were demonstrated that all these three models could be suitably applied for determining the TNRE with addition of mannitol in SAD process. The kinetic analysis for high mannitol inhibition recovery period was shown in Fig. S4 and S2.

----- **Figure. 7** -----

----- **Table. 1** -----

CONCLUSIONS

The SAD process treating saline wastewater with mannitol addition was

investigated in present work. The optimal carbon and nitrogen removal were achieved at 0.2 mM mannitol, during which ARE, NRE and ORE were 96.95 %, 93.70 % and 90.05 %, respectively. The maximal ARR, NRR, SAA, V_{\max} , the diameter of sludge and MLSS were improved. Tseng-Wayman model was more suitable to simulate the whole process of SAD. The modified Logistic model, the modified Boltzman model and the modified Gompertz model were all appropriate to describe nitrogen removal in a typical cycle.

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Table

Table 1. Simulated parameters acquired through different kinetic models

Table 1. Simulated parameters acquired through different kinetic models								
Models	Models parameters							Equations
	Mannitol (mM)	TNRE _{max} [kgN/m ³ ·d]	TNRE _{min} [kgN/m ³ ·d]	R _m (mg/L)	t _c (h)	t _d (h)	λ (h)	
Modified Logistic model	0.1	70.70		40.90				y=70.70/1+exp[4×40.90(0-x)/70.70+2]
	0.15	89.52		43.91				y=89.52/1+exp[4×43.91(0-x)/89.52+2]
	0.2	81.78		43.36				y=81.78/1+exp[4×43.36(0-x)/81.78+2]
	0.25	89.81		44.31				y=89.81/1+exp[4×44.31(0-x)/89.81+2]
	0.3	87.26		40.90				y=87.26/1+exp[4×40.90(0-x)/87.26+2]
	0.35	82.16		42.79				y=82.16/1+exp[4×42.79(0-x)/82.16+2]
	0.4	91.17		49.53				y=91.17/1+exp[4×49.53(0-x)/91.17+2]
	0.5	79.63		42.54				y=79.63/1+exp[4×42.54(0-x)/79.63+2]
	0.6	76.00		43.16				y=76/1+exp[4×43.16(0-x)/76+2]
Modified Boltzman model	0.1	70.99	0		0.86	0.45		y=0-70.99/1+exp[(x-0.86)/0.45]
	0.15	85.44	0		1.06	0.58		y=0-85.44/1+exp[(x-1.06)/0.58]
	0.2	91.15	0		1.02	0.58		y=0-91.15/1+exp[(x-1.02)/0.58]
	0.25	90.04	0		1.01	0.52		y=0-90.04/1+exp[(x-1.01)/0.52]
	0.3	88.39	0		1.07	0.58		y=0-88.39/1+exp[(x-1.07)/0.58]
	0.35	82.15	0		0.98	0.48		y=0-82.15/1+exp[(x-0.98)/0.48]
	0.4	92.28	0		0.92	0.51		y=0-92.28/1+exp[(x-0.92)/0.51]
	0.5	80.51	0		0.93	0.52		y=0-80.51/1+exp[(x-0.93)/0.52]
	0.6	76.25	0		0.88	0.45		y=0-76.25/1+exp[(x-0.88)/0.45]
Modified Gompertz model	0.1	72.58	42.80				0	y=72.58exp[-exp(42.80e/72.58(0-x)+1)]
	0.15	85.26	44.20				0	y=85.26exp[-exp(44.20e/85.26(0-x)+1)]
	0.2	91.78	46.76				0	y=91.78exp[-exp(46.76e/91.78(0-x)+1)]
	0.25	93.01	46.55				0	y=93.01exp[-exp(46.55e/93.01(0-x)+1)]
	0.3	90.66	42.51				0	y=90.66exp[-exp(42.51e/90.66(0-x)+1)]
	0.35	85.07	44.22				0	y=85.07exp[-exp(44.22e/85.07(0-x)+1)]
	0.4	93.15	52.87				0	y=93.15exp[-exp(52.87e/93.15(0-x)+1)]
	0.5	81.25	45.53				0	y=81.25exp[-exp(45.53e/81.25(0-x)+1)]
	0.6	77.96	45.66				0	y=77.96exp[-exp(45.66e/77.96(0-x)+1)]

Figure captions:

Figure 1. Schematic diagram of the SBR configuration: The peristaltic pump a was used for influent. The peristaltic pump b was used for water recirculation to balance temperature of water bath.

Figure 1

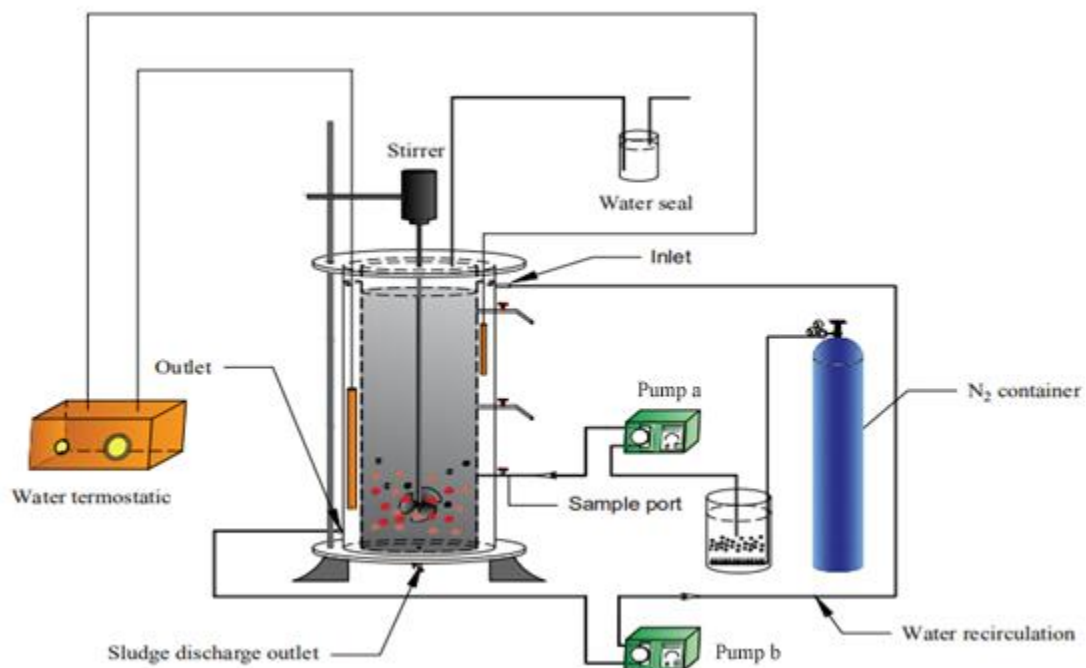


Figure 2. Performance of SAD at various mannitol concentrations: (a) concentrations of three nitrogen species and COD in influent and effluent; (b) removal efficiency of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and COD; (c) loading rate and removal rate of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and COD; (d) percentage of nitrogen removal by anammox and denitrification; (e) stoichiometric ratios of $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ removal.

Figure 2

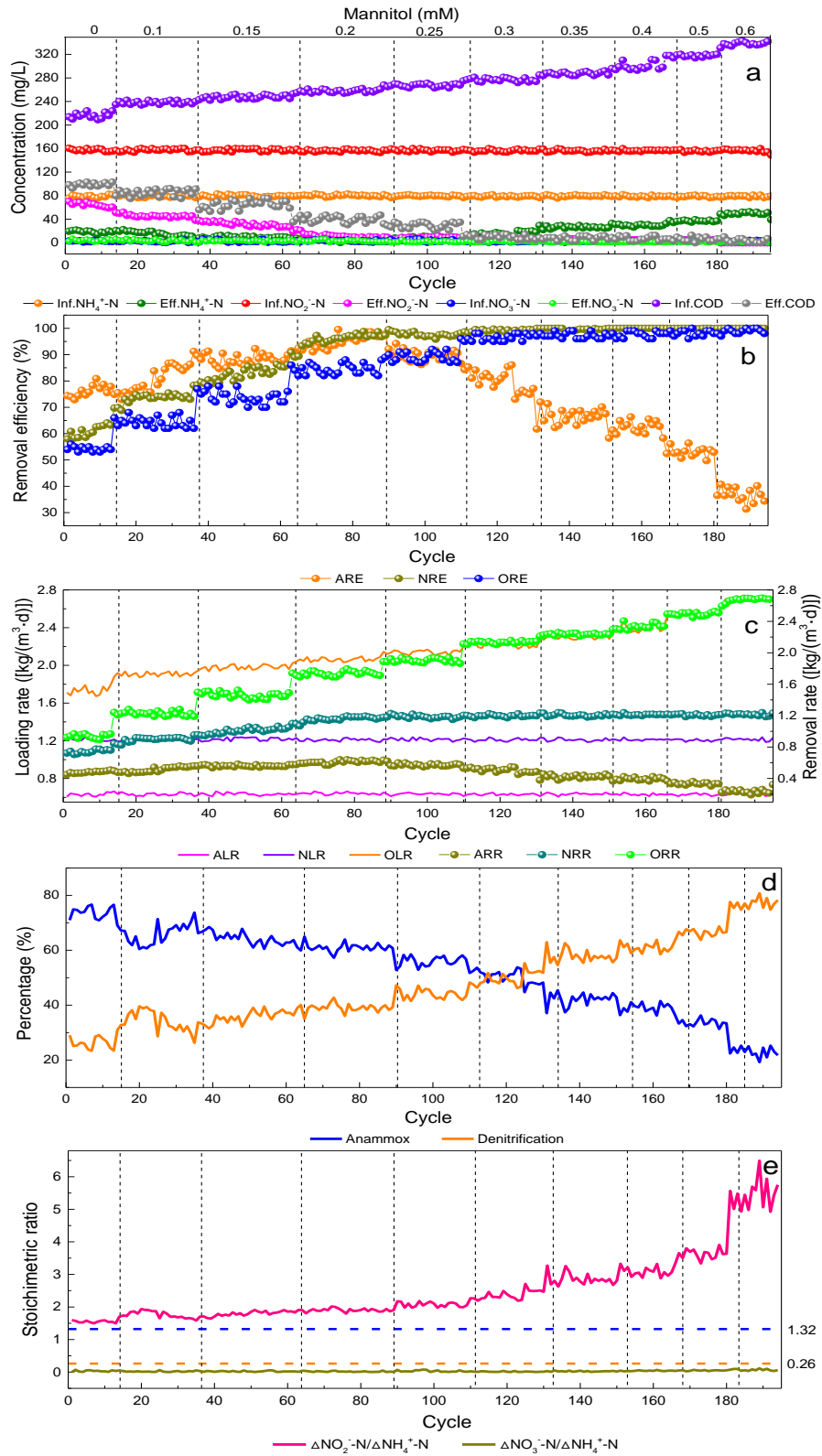


Figure 3. Nitrogen removal in a typical operation cycle of SBR at various mannitol concentrations: (a) 0.1 mM; (b) 0.15 mM; (c) 0.2 mM; (d) 0.3 mM; (e) 0.6 mM; (f) SAA mgNH₄⁺-N/(g·h) and V_{max} mgNH₄⁺-N/(L·h).

Figure 3

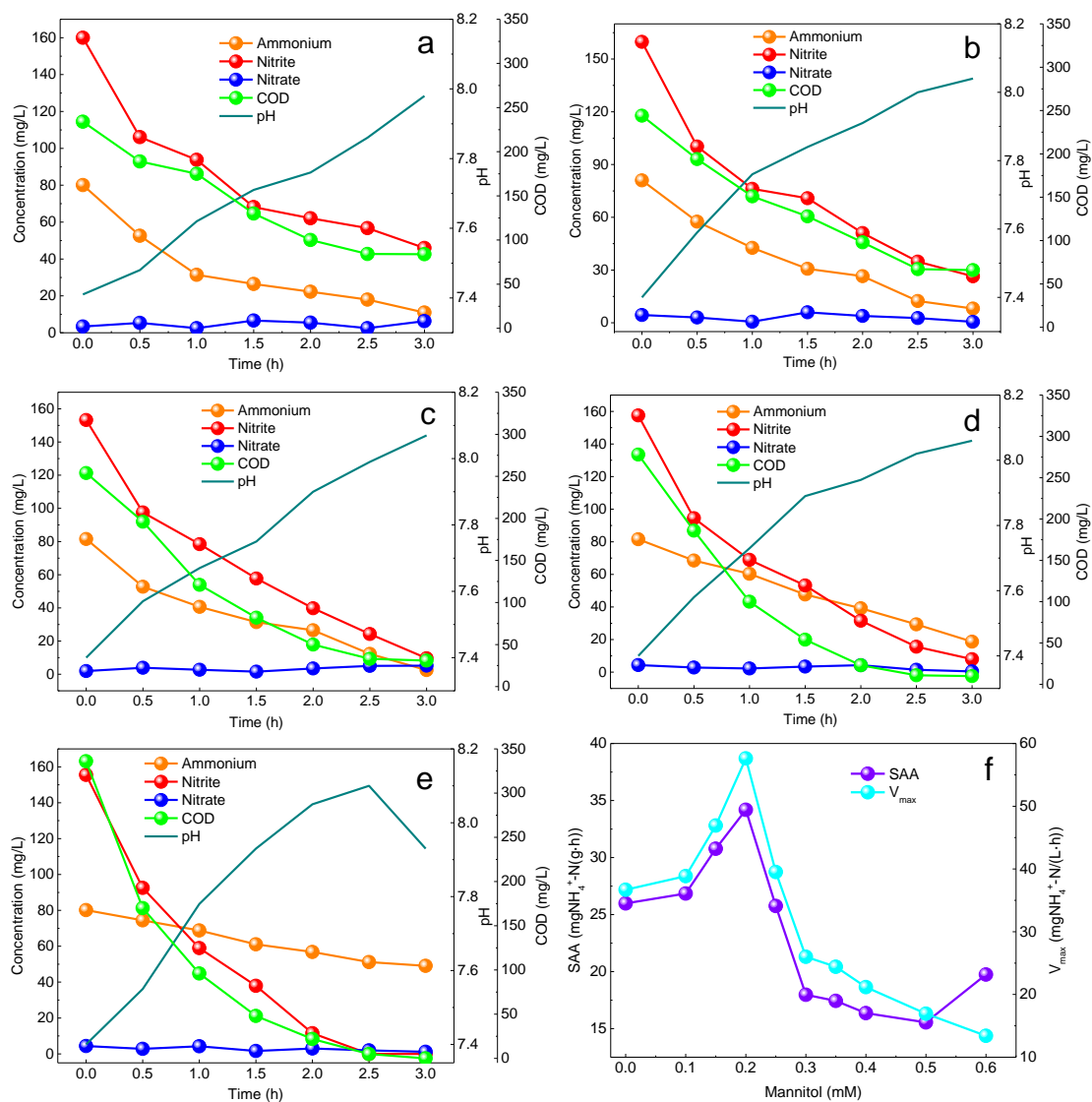


Figure 4. Particle size distribution of sludge at various concentration of mannitol: (a) 0 mM mannitol (b) 0.1 mM mannitol (c) 0.2 mM mannitol (d) 0.3 mM mannitol (e) 0.5 mM mannitol (f) 0.6 mM mannitol

Figure 4

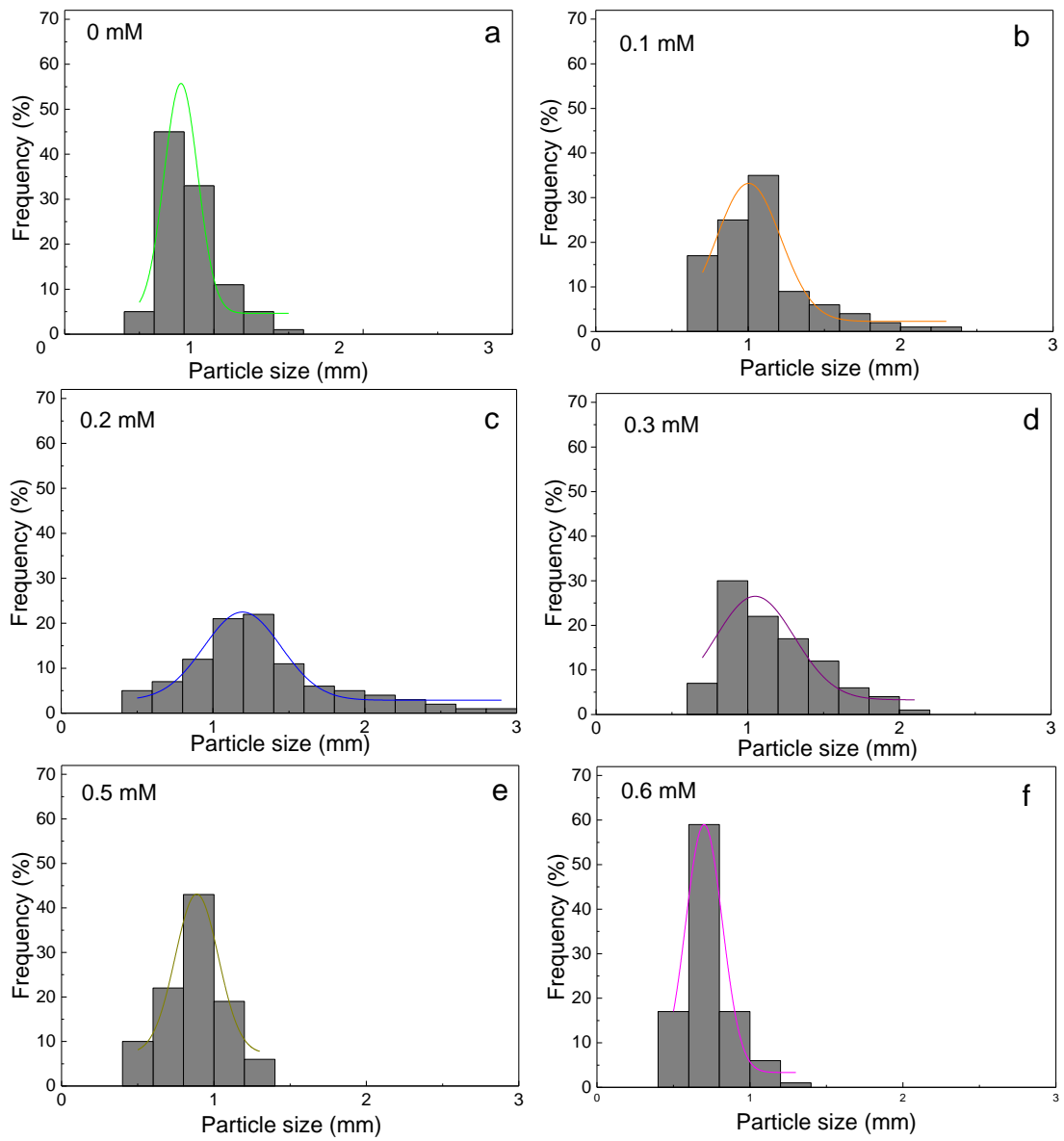


Figure 5. Sludge concentration, sludge settling ratio and sludge volume index at different concentration of mannitol.

Figure 5

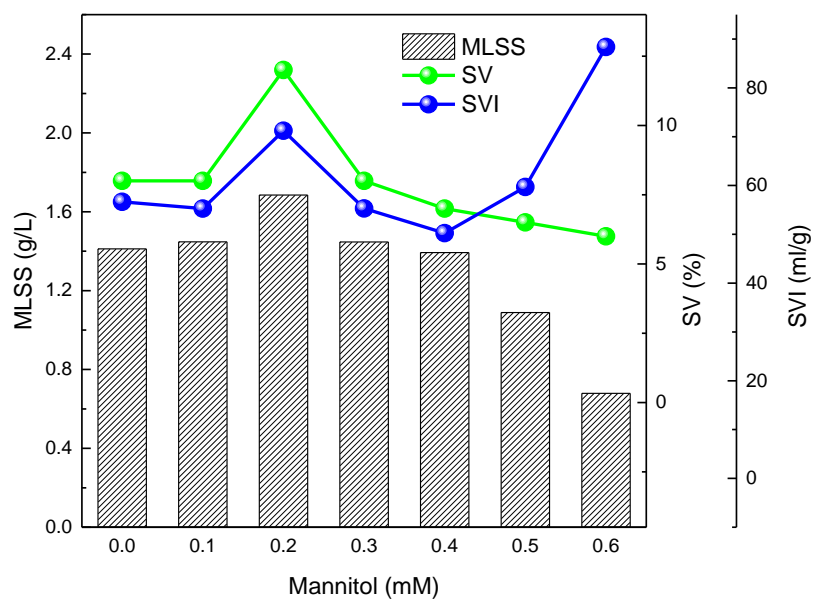


Figure 6. Model plots for substrate removal kinetics: (a) Haldane model; (b) Aiba model; (c) Edwards model; (d) Luong model; (e) Han-Levenspiel model; (f) Tseng-Wayman model.

Figure 6

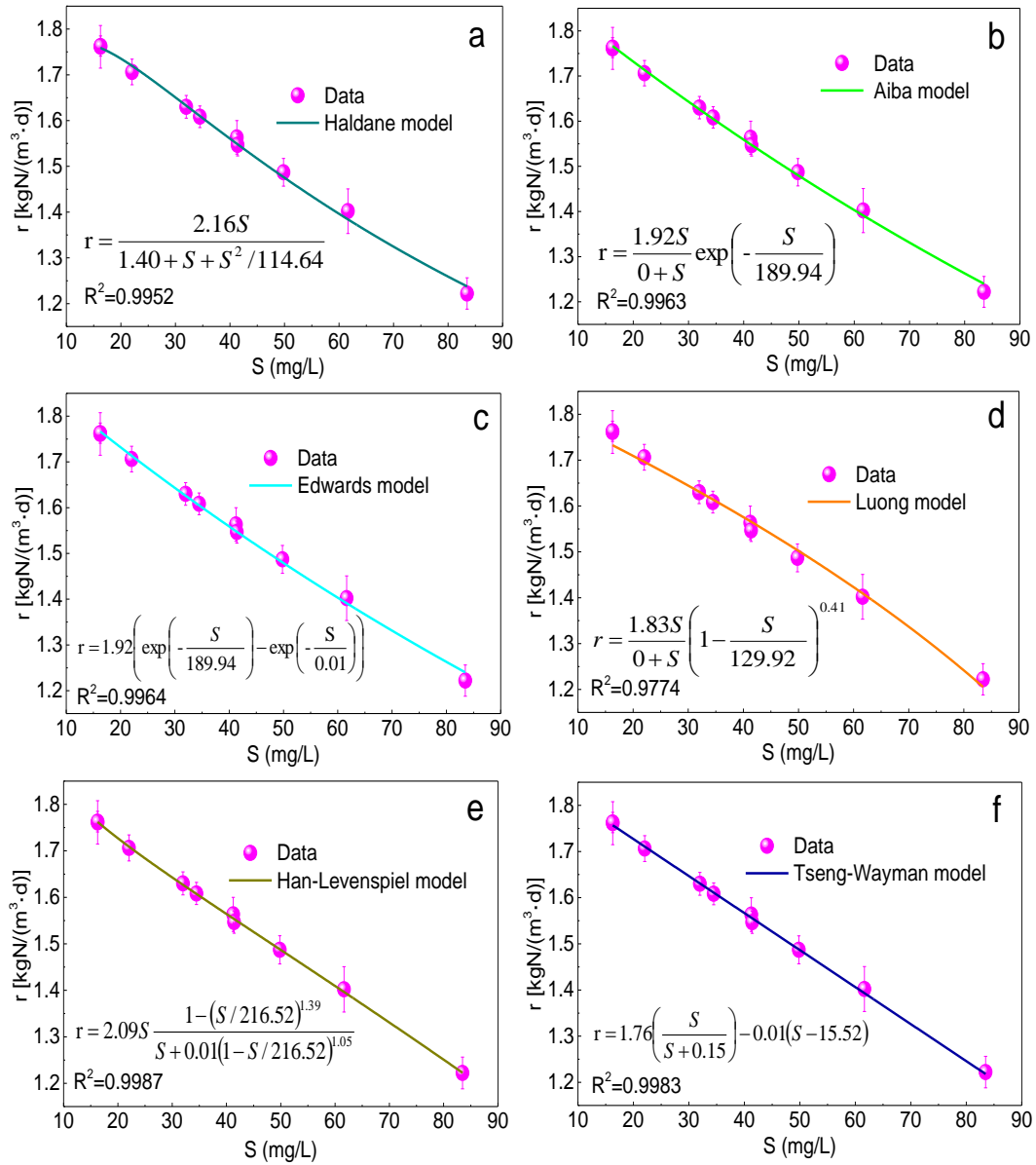


Figure 7. Kinetic analysis of nitrogen removal at various mannitol concentrations and comparison of predicted value and experimental data: (a) modified Logistic model; (b) modified Boltzman model; (c) modified Gompertz model; (d) modified Logistic model; (e) modified Boltzman model;

(f) modified Gompertz model.

Figure 7

