*Original Research*

# Integrated Effect of MLSS and SRT on Performance of a Full-Scale Modified A²/O Process

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## Abstract

MLSS and SRT are key parameters in process control. A full-scale modified A2/O process varying with MLSS and SRT was evaluated, and MLSS and SRT effect was distinguished by statistical methods so as to seek early diagnosis and efficient control. Results showed that complete SRT rather than MLSS resulted in considerable effluent SS fluctuation with inert SS accumulation. COD removal was more sensitive to MLSS than SRT. Increasing MLSS until 2,000 mg/L could improve COD, SS, and $NH_3$ removal efficiency, and further increases in MLSS simply enhance stability. MLSS of 2,000 mg/L was also efficient in TP removal. SRT shorter than 30 d was the guarantee for PAOs, while SRT longer than 90 d was equal to complete SRT as TP was removed by assimilation. Prolonged SRT from 30 to 90 days reduced the $Y_{obs}$ from 0.17 to 0.15 g MLSS/g COD. MLSS and SRT had no effect on TN removal. Correlation between MLSS and influent was effective for diagnosing longer SRT. MLSS/RSS could be an indicator for TP removal. The suggested control strategy was SRT-assisted MLSS control.

**Keywords**: MLSS, SRT, statistical analysis, stability, control strategy

## Introduction

Wastewater leads to organic contamination and eutrophication in receiving water. Due to increasingly stringent effluent quality requirements in China, existing wastewater treatment plants (WWTPS) need to modify configurations (such as modified A²/O, JHB, VIP process) or optimize operational parameters in order to guarantee the effluent. The JHB process introduces a pre-anoxic selector prior to the anaerobic zone to eliminate the negative effect of $NO_3^-$-N existing in the return

activated sludge (RSS) on anaerobic phosphorus release. Every zone of the VIP process consists of several tanks in order to form a concentration gradient. The modified A²/O process in this study combined both advantages of the JHB and VIP process.

Besides wastewater characteristics, mixed liquid suspended solids (MLSS) and sludge retention time (SRT) are important factors affecting effluent. MLSS is an easy and direct control parameter for biomass, which plays a significant role on the shearing thinning behavior [1] and SVI [2]. SRT is positively related with soluble microbial products (SMP) formation [3]. Actually, MLSS can be adjusted by sludge return ratio and discharge of waste sludge, so it is partially related with SRT. MLSS-based

SRT control is able to reduce COD removal variation by 90% [4]. However, in some conditions such as in winter or start-up running with low strength influent, biomass fails to grow, thus wasting sludge is not discharged (complete SRT or infinite SRT) so as to sustain sufficient MLSS, resulting in total independence of SRT and MLSS. In recent years, the high organic load-activated sludge process with complete SRT is reported to be sludge minimization [5] with sedimentation problems [6] and decreased nitrifying bacterial [7].

In view of the complexity of WWTP with many variables, statistical methods can be a useful tool to seek physical explanation of observed facts. For example, Person or Spearman correlation coefficient can reveal correlations between parameters, explaining the phenomenon of phosphorus fluctuation [8] and analyzing the effect of MLSS on bacterial composition [9]. Also, the statistical method is useful for detecting a change in WWTP operation [10] and is effective for early identification of potential problems [11].

In this study, MLSS and SRT were varied in the Anqing WWTP. Through analysis of removal stability, biomass characteristics, and factors analysis by statistical methods, the goal of the study was to evaluate the integrated effect of MLSS and SRT on performance as well as to identify parameters for irregular behavior diagnosis and control.

## Material and Methods

### Description of Wastewater Treatment Plant

The Anqing WWTP was designed to treat a flow of $12 \times 10^4$ m³/d, including 80% of domestic wastewater and 20% of industry wastewater. The plant consisted of pre-selector, anaerobic, anoxic, three-staged oxic tanks and settler. 90% of influent came into an anaerobic tank while 10% came into a pre-anoxic selector where denitrification of return-activated sludge was accomplished. Oxic tanks were composed of three rectangular tanks and aeration was provided through a porous membrane aerator. Details of process constitutes could be seen in our previous study [12]. Influent wastewater was typically of low-strength. Average concentration of influent COD, BOD₅, TN, TP, NH₃-N, NO₃-N, NO₂-N, and SS was 185, 48, 14, 1.26, 7.90, 3.58, 0.011, and 76 mg/L, respectively. The internal recycle ratio was lower than 50% in consideration of low-influent TN and operational cost. The external recycle ratio was 100%.

### MLSS and SRT Experiment

The experiment was divided into three periods, with each period operating for several months. In order to eliminate differences caused by temperature, data of November, March, and April with similar water temperature (12-16ºC) were selected for analysis. In period 1, ranging from September to December, MLSS

did not grow, and growth of biomass was equal to decay, therefore no waste activated sludge (WAS) was removed from the system. Then to stimulate growth and activity of biomass, WAS of 420 m³/d (period 2) and 140 m³/d (period 3) were discharged. As a result, the experiment was divided into period 1 (MLSS of 1,062±294 mg/L, complete SRT), period 2 (MLSS of 1,270±176 mg/L, SRT of 30 d), and period 3 (MLSS of 3,694±578 mg/L, SRT of 90 d). It was worth mentioning that initial design MLSS concentration for this WWTP was 3,500 mg/L.

### Analytical and Statistical Methods

According to standard methods [13], COD, MLSS, NH₃-N, NO₃-N, NO₂-N, TN, and TP were measured. DO and temperature were measured using a Cellox 325-3 oxygen probe. To evaluate biomass growth, observed yield $Y_{obs}$ was calculated by measuring the amount of WAS and COD removal per day, as shown in Eq. (1):

$$Y_{obs} = \frac{\Delta MLSS}{\Delta COD} \tag{1}$$

The measured results were offered as means ±standard deviation. The statistical significance of difference between operational periods was assessed using the Student's t-test at the 95% confidence level ($P<0.05$). Person's correlation coefficient (r) was calculated to indicate pairwise correlation. Principal component analysis (PCA) was conducted to extract the main factors affecting effluent quality among influent variables and operating parameters. Also, multiple linear regressions were used to quantitatively describe variables. All the statistical analysis was accomplished by SPSS 19.0 software.

## Results and Discussion

### Operation Results of three Periods

COD removal was shown in Fig. 1a). For periods 1, 2, and 3, COD removal was 70±3% , 77±2%, and 80±1%, respectively. Average BOD₅ removal was 64%, 69%, and 73%, respectively. BOD₅ removal lower than COD removal reflected the poor biodegradability of the wastewater, and some organics were only absorbed by activated sludge but not biodegraded. COD removal of period 1 with complete SRT was the lowest. COD removal of periods 2 and 3 was statistically different (p<0.01). Effluent COD in periods 1 and 3 was not associated with effluent SS, as shown in Fig. 1f), hence the main constitute of effluent COD was dissolved COD such as soluble microbial product (SMP).

SS removal from periods 1 to 3 (Fig. 1b) was 62±23%, 79±6%, and 83±4%, respectively. In period 1, SS removal fluctuated extremely from 21% to 85%, and effluent SS always exceeded 20 mg/L, which did not meet the

discharge limit. The phenomenon of deteriorated effluent SS with complete SRT was also observed by Amanatidou [6], who attributed this phenomenon to high MLSS and flotation of sludge flocs in the clarifier. However, in our study SS fluctuation was observed when MLSS was relatively low, so the explanation for SS fluctuation existed in flocculating and settling property of sludge. Measured $SV_{30}$ of 5~8% proved the aging of sludge, which made the sludge less cohesive [14]. On the other hand, the main constituent of effluent SS was inorganic or inert substances since no correlation existed among effluent SS and effluent COD, TN, and TP (Fig. 1f) in periods 1 and 3, while in period 2 effluent SS was correlated with effluent TN (r = 0.51). From the above, the reason for fluctuation in SS removal was proved to be sludge aging and inert SS accumulation. Whether the disable removal of inert SS

was caused by complete SRT or relatively low MLSS will be resolved later in the statistical analysis section.

$NH_3$-N removal from period 1 to 3 (Fig. 1c) was 91±6%, 97±3%, and 98±2%, respectively. As it is known that long SRT was beneficial for nitrifers, $NH_3$-N removal of period 3 was improved as compared to period 2 (p<0.05), but $NH_3$-N removal of period 1 was not favored despite complete SRT. With complete SRT, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) decreased [7]. TN removal (Fig. 1d) was 19±10%, 17±11% and 13±7%, respectively. TN removal of periods 1 and 3 was different (p<0.05), which was mainly correlated with influent $NH_3$ (r = 0.70).

Average TP removal (Fig.1e) was 28%, 53%, 31%, respectively. Phosphorus excessively absorbed by polyphosphate-accumulating organisms (PAOs) was
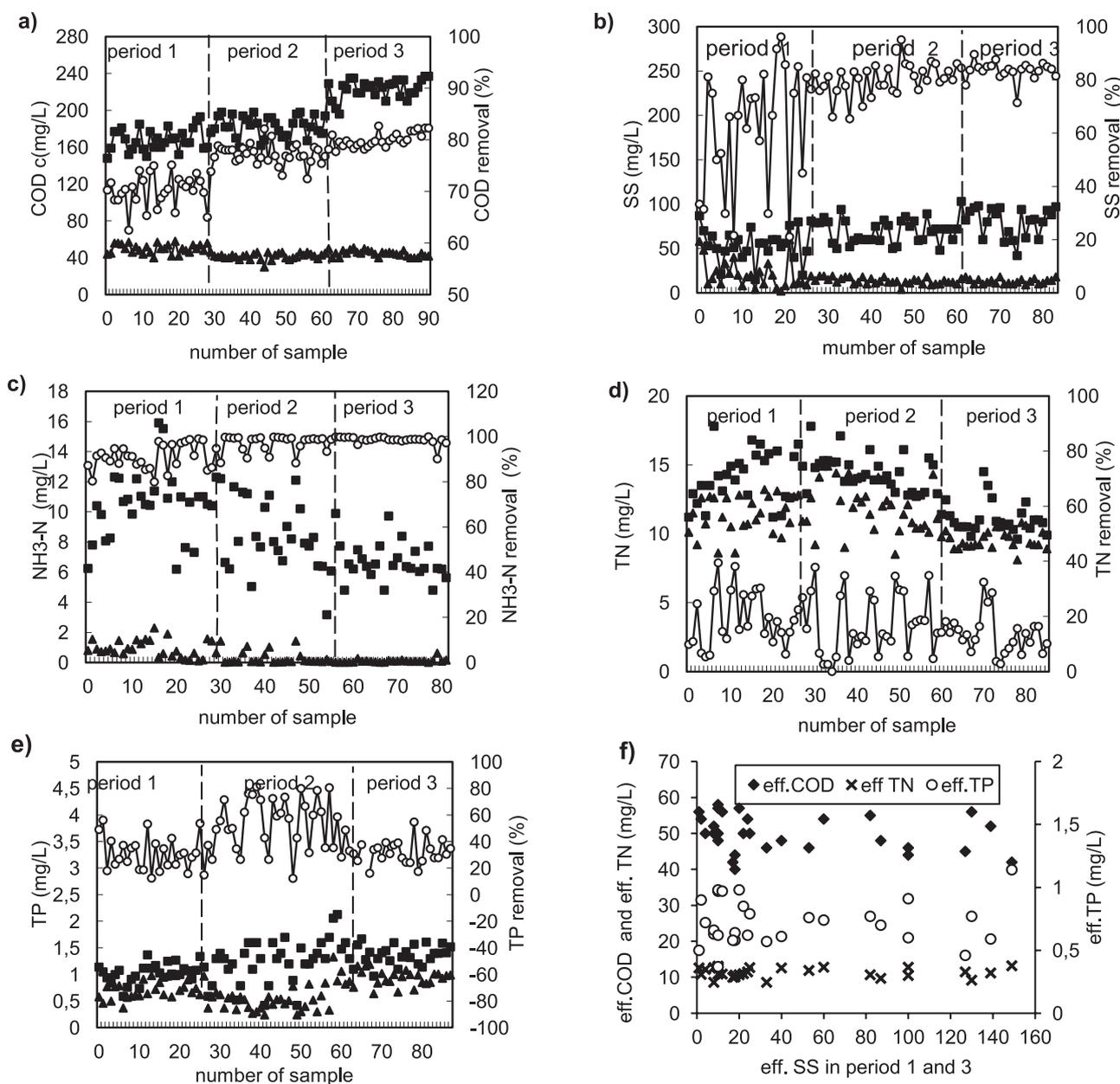


Fig. 1. Profiles of COD, SS, NH$_3$-N, TN, and TP, for influent ■, effluent ▲, and removal efficiency ○.

removed from the system along with WAS. Without discharge of waste sludge, phosphorus could only be removed for bacterial assimilation. Based on empirical bacterial formula $C_{60}H_{87}O_{23}N_{12}P$ [15], P in wasted sludge could be calculated with $2.3\% \Delta X$, and calculated TP removal through assimilation was 22% in period 3. The calculation result indicated that assimilation was the major reason for TP removal in period 3. In period 1, P release disappeared and P uptake became very weak (data not shown). F/M ratio was 0.06 kg $BOD_5$/kg MLSS.d, which negatively affected PAOs due to carbon starvation [16]. Also, prolonged SRT was not beneficial for PAO recovery of PHA and glycogen [17]. In period 2, TP removal was improved obviously, along with wide fluctuation (SD = 19%).

## MLSS and Distribution

Fig. 2 showed the MLSS, MLVSS, ash, and MLVSS/MLSS ratios and observed sludge yield $Y_{obs}$. From period 1 to 3, average MLSS of 1,062, 1,270, and 3,694 mg/L and MLVSS of 475, 760, and 2,720 mg/L were obtained,



Fig. 2. a) MLSS and $Y_{obs}$ b) average MLSS of three-staged oxic tanks.

with MLVSS/MLSS ratio of 0.48, 0.60, and 0.78. In period 1 the MLVSS/MLSS ratio dropped straight from 0.60 to 0.30. From day 15 to day 30, the increasing MLSS was absolutely due to the accumulation of ash in activated sludge (r = 0.85). Compared with period 1, both MLVSS and ash were more stable in period 2. Although influent COD increased from 180 mg/L (in periods 1 and 2) to 220 mg/L (period 3), influent $BOD_5$ increased from 50 to 60 mg/L, and it seemed inadequate for supporting the rapid increase of the MLVSS/MLSS ratio from 0.60 to 0.80. The reason for the increased MLVSS/MLSS ratio was unclear and could be further researched through stored polymers and EPS. $Y_{obs}$ of periods 2 and 3 was 0.17 and 0.15 g MLSS/g COD. The decline in $Y_{obs}$ was supported by the finding that prolonged SRT from 10 to 110 days reduced $Y_{obs}$ from 0.31 to 0.13 [18].

It was very occasional to find that MLSS distribution in three-stage aerobic tanks was uneven. In period 1, the difference of MLSS in three oxic tanks was not obvious (Fig. 2b), while in periods 2 and 3 MLSS in three aerobic tanks increased. Correlation analysis between influent SS and MLSS of each tank found that the Person coefficient gradually increased with three oxic tanks ($r_1$ = 0.41, $r_2$ = 0.49, $r_3$ = 0.64), the opposite of usually expected and thus increasing MLSS did not result from influent SS but was caused by sedimentation of MLSS in the bottom of the tank. With increased DO in three tanks ($DO_1$ = 3.15, $DO_2$ = 5.55, and $DO_3$ = 5.73 mg/L) and mixing conditions [12], biomass became well mixed and sedimentation of MLSS in the bottom was reduced. Unbalanced distribution of MLSS was also reported by Pai [19], who simulated biomass using ASM. 2 d biomass concentration was increased slightly in two aerobic tanks, and a similar result was reported by You [20] in which MLSS concentration in anaerobic/anoxic/aerobic tanks was gradually increased.

## Effect of MLSS and SRT on Pollutant Removal

The scatter plot was helpful for obtaining optimum MLSS and SRT. In Fig. 3a), when MLSS increased from 1,000 to 2,000 mg/L, COD and $NH_3$ removal linearly increased while SS and TP removal gradually increased with great fluctuation. It was obvious that high TP removal of 60~80% with maximum removal of COD, $NH_3$, and SS of 80%, 99%, and 82% were observed with MLSS of 2,000 mg/L. When MLSS increased from 2,000 mg/L to 4,000 mg/L, though COD, $NH_3$, and SS removal did not increase further, it was more stable than those with lower MLSS. TP removal dropped. TN removal was hardly affected by MLSS. Although high MLSS led to absolutely low BOD loading of 0.015 kg $BOD_5$/kg MLSS.d; endogenous denitrification favored by starvation [21] was not observed in this study. The results of pollutant removal indicated optimum MLSS was 2,000 mg/L.

In Fig. 3b), SRT of period 1 was considered the same as the operating days. COD and $NH_3$-N removal
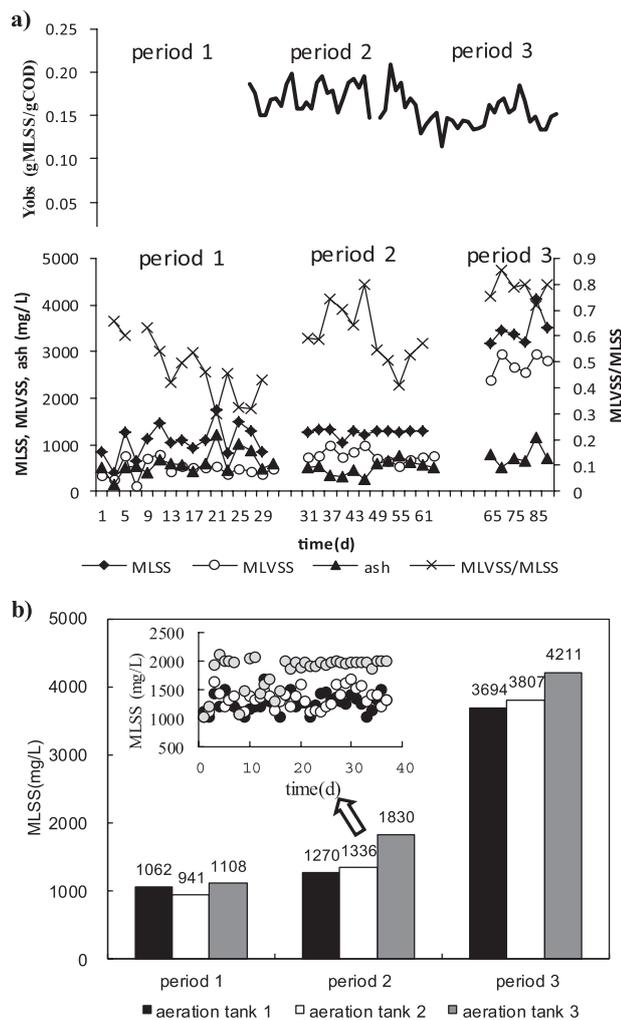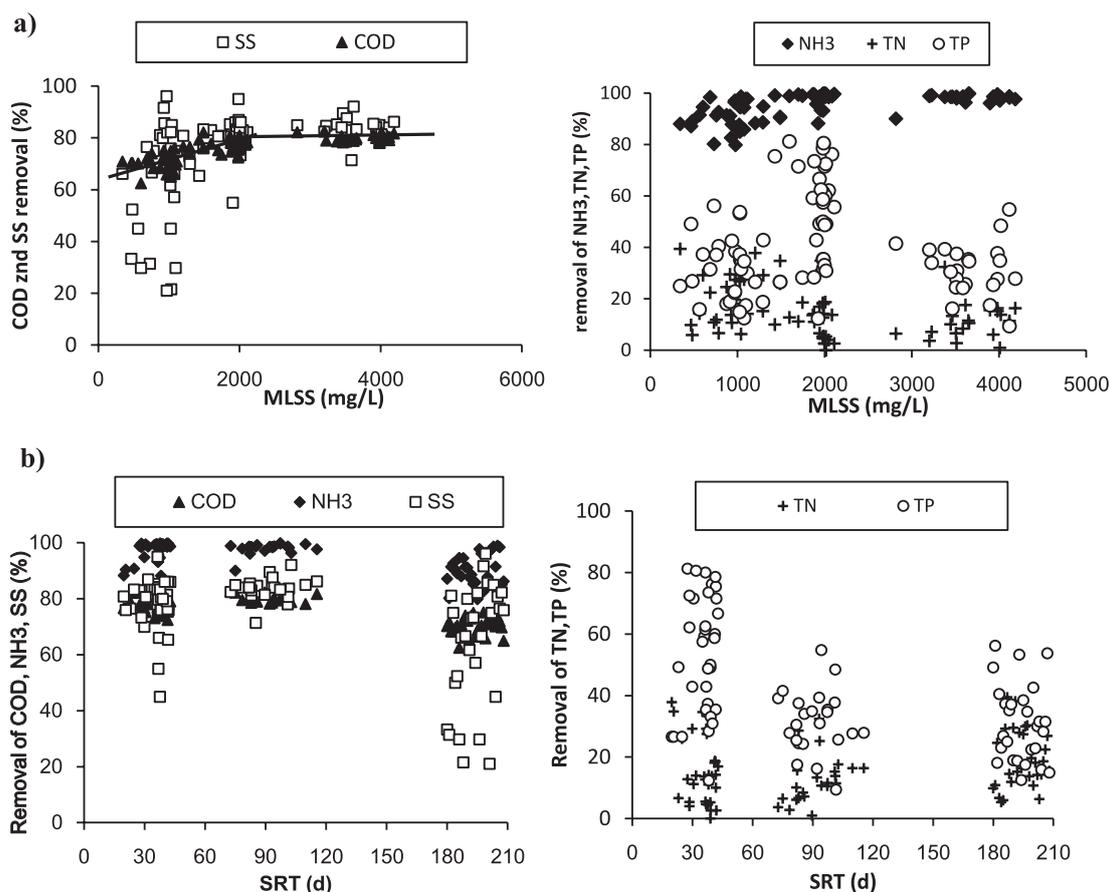
Fig. 3. a) effect of MLSS on removal of pollutants and b) effect of SRT on removal of COD, SS, $NH_3$, TN, and TP.

was stable at SRT less than 120 d, while dropping 10% and 7% at complete SRT. In terms of SS removal, SRT of 90 d was optimal since the lower SRT of 30 d and complete SRT both contributed to fluctuation of SS removal. SS removal at SRT of 30 d varied from 60% to 80%, and the fluctuation was aggravated at complete SRT. TP removal at SRT of 90 d was equal to those at complete SRT. This result confirmed that TP in periods 1 and 3 was removed by assimilation as previously calculated. Since TN removal did not respond to SRT, optimum SRT should be kept lower than 30 d.

### Correlation between MLSS, SRT, Influent, and Effluent

Potential correlation between operating parameters and water quality proved to be important for early identification of system failures. A strong relationship was identified when the Pearson coefficient was closer to the absolute value of 1. Pearson correlation analysis (Table 1) was conducted among influent, effluent, and operating parameters (MLSS, SRT) to identify bivariate relationships.

MLSS of period 2 was correlated with each influent parameter and the removal rates of $NH_3$, SS, and TN, but in periods 1 and 3 the correlation disappeared, thus a change of this correlation could be used to detect the

change of longer SRT. Moreover, in period 2, MLSS was positively correlated with influent TP, SS, and COD, while it was inversely correlated with influent $NH_3$-N. This correlation indicated that bacterial growth was affected by a shortage of influent TP (0.5-1.5 mg/L), and higher MLSS was favorable for adapting shock loading of influent SS and COD, and nitrifying bacteria growth favored by influent $NH_3$ hampered an increase of MLSS. On the other hand, closer correlation between MLSS and SRT (r = 0.95) was observed in period 2. Decreasing r with increasing SRT (r = 0.58 in period 3, r = 0.48 in period 1) indicated a separation of MLSS and SRT (in periods 1 and 3). Based on good correlation between MLSS and SRT, MLSS could be a proxy of SRT, and thus SRT-assisted MLSS control was suggested.

The correlation between SS removal and SRT revealed that the fluctuation of SS removal in period 1 was correlated with complete SRT rather than MLSS. With whole analysis of the data of periods 1-3, it can be seen that MLSS had positive effect on COD, $NH_3$, and SS removal, while SRT exerted a negative effect on COD, $NH_3$, SS, and TP removal.

### Main Factors Affecting Performance

Influent variables and operating parameters were factors of process performance. Due to the complexity and

Table 1. Person correlation coefficients between MLSS, SRT, influent, and effluent.

| Correlation | | $COD_i$ | $SS_i$ | $NH_{3i}$ | $TN_i$ | $TP_i$ | $COD_e$ | $SS_e$ | $NH_{3e}$ | $TN_e$ | $TP_e$ | COD% | $NH_3$% | SS% | TN% | TP% | MLSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | MLSS | 0.26 | -0.02 | 0.29 | 0.18 | 0.16 | -0.02 | -0.24 | -0.15 | 0.17 | 0.23 | 0.22 | 0.26 | 0.30 | 0.04 | -0.14 | -- |
| | SRT | 0.28 | 0.04 | 0.17 | 0.28 | 0.39* | -0.05 | -0.36 | -0.27 | 0.22 | 0.40* | 0.26 | 0.36 | -0.39* | -0.13 | -0.13 | 0.48* |
| P2 | MLSS | 0.41* | 0.64** | -0.50** | -0.41* | 0.65** | 0.21 | 0.00 | -0.35* | 0.25 | 0.33* | 0.15 | 0.54** | 0.56** | -0.56** | 0.30 | -- |
| | SRT | 0.20 | 0.17 | -0.17 | -0.15 | 0.27 | 0.25 | 0.13 | -0.59** | 0.16 | 0.14 | 0.17 | 0.53** | 0.60** | -0.52** | 0.28 | 0.95** |
| P3 | MLSS | 0.14 | -0.23 | -0.43* | -0.08 | 0.12 | 0.10 | 0.12 | -0.50* | -0.07 | 0.12 | -0.02 | 0.41* | -0.23 | 0.01 | -0.08 | -- |
| | SRT | 0.02 | 0.27 | -0.31 | 0.10 | 0.29 | 0.35 | 0.13 | -0.34 | -0.31 | 0.29 | -0.35 | 0.30 | 0.19 | -0.06 | -0.06 | 0.58** |
| P1-3 | MLSS | 0.88** | 0.45** | -0.49** | -0.67** | 0.43** | -0.15 | -0.23* | -0.46** | -0.44** | 0.49** | 0.67** | 0.47** | 0.45** | -0.27* | -0.16 | -- |
| | SRT | -0.36** | -0.20 | 0.43** | 0.03 | -0.31** | 0.67** | 0.29* | 0.41** | -0.11 | 0.26* | -0.71** | -0.54** | -0.48** | 0.16 | -0.48** | -- |

**Significant at $P<0.01$, *Significant at $P<0.05$

relevance of influent and operating parameters, the PCA method was employed to reduce the dimensionality and seek main factors affecting performance. Two principal components accounting for 74.6% of the total variance were extracted (Fig. 4a). The first principal component (PC 1) explained 54.3% of the total variance, and this component was dominated by influent COD, MLSS, and RSS, while influent TN and $NH_3$ was located on the opposite. In addition, the result indicated collinearity of MLSS and RSS, or that RSS was linearly correlated with MLSS. PC 1 showed that the formation of MLSS could be promoted by influent COD, SS, and TP while being inversely affected by influent TN and $NH_3$. This was consistent with the fact that the relatively low ratio of $NH_3$ to TN stabilized at 0.64. PC 2 explaining 20.3% of the total variance was dominated by MLSS/RSS, TP removal, and influent TP. PC 2 described parameters related to TP removal. MLSS/RSS could be an alternative of SRT and more convenient for controlling TP removal (Fig. 4b).

## Multivariate Regressions

WWTP operators often seek multivariate regressions to estimate parameters without measuring them, or predict slower and more expensive variables through easy and fast-responding variables [8], as well as evaluating the contribution exerted by each element [22].

Based on correlation analysis and PCA, some variables were chosen to predict removal rates of COD, $NH_3$, TN,
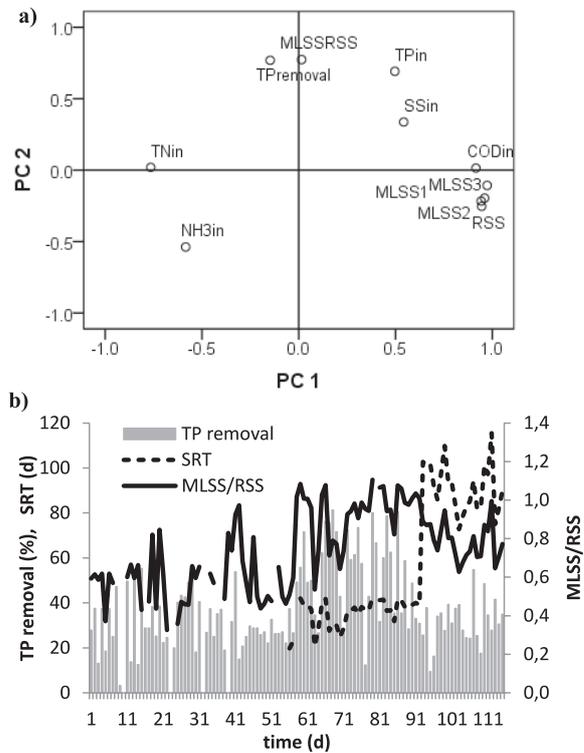


Fig. 4. a) principle component analysis and b) response of MLSS/RSS to TP removal.

TP, and SS. The multivariate linear regression model in this study attempted to forecast removal rate and evaluate the important degree of MLSS and SRT on performance. Given the complexity of full-scale WWTP, prediction accuracy of multiple regressions with $R^2$ greater than 0.7 was acceptable [23], and then removal rates of COD, $NH_3$, TP, and RSS were identified to be well predicted as Eqs. 2-6 (each parameter included in the model was significant, $p<0.05$). The regressions suggested that COD removal could be improved by increasing MLSS and decreasing SRT, with MLSS being more efficient than SRT because the standardized regression coefficient of MLSS and SRT was 0.55 and -0.23. TP removal could be enhanced with the increasing MLSS/RSS. Also, the regression-verified MLSS/RSS could be an indicator for TP removal as PCA showed. The inclusion of MLSS and SRT did not result in greater $R^2$ for $NH_3$ removal, so it was deleted from the regression, demonstrating that MLSS and SRT had little impact on $NH_3$ removal.

$$COD\% = 92 - 0.41COD_{out} + 1.42MLSS - 0.015SRT \ (R^2 = 0.88) \tag{2}$$

$$TP\% = 4.4 - 63.8TP_{out} + 44.2\frac{MLSS}{RSS} + 0.23COD_{in} \ (R^2 = 0.70) \tag{3}$$

$$NH_3\% = 99 - 9.168NH_{3out} \ (R^2 = 0.93) \tag{4}$$

$$RSS = 1.28MLSS - 0.125 \ (R^2 = 0.93) \tag{5}$$

$$MLSS = 0.055COD_{in} - 0.144TN_{in} - 0.272NO_{3in} - 5.58 \ (R^2 = 0.82) \tag{6}$$

## Conclusions

Complete SRT resulted in obvious fluctuation of COD and SS removal and declining $NH_3$-N removal. The reason for fluctuation of SS removal was disabled inert SS removal associated with complete SRT rather than MLSS. Increasing MLSS until 2,000 mg/L could improve COD, SS, and $NH_3$ removal, while further increasing MLSS could not improve removal efficiency but enhance removal stability. Maximum TP removal of 80% with fluctuations also can be obtained at MLSS of 2,000 mg/L. COD removal was more sensitive to MLSS than SRT, and SRT of lower than 30 d was required for PAOs, and SRT longer than 90 d was equal to complete SRT regarding TP removal. As for COD, SS, and $NH_3$ removal, SRT lower or higher than 90 d both led to fluctuations. The formation of MLSS was positively correlated with influent of COD, SS, and TP, and negatively affected by influent of TN and $NH_3$. Correlation between MLSS

and influent could be used to diagnose the change of longer SRT. The separation of MLSS and SRT could be recognized through Person correlation coefficient. MLSS/RSS could be an alternative of SRT to control TP removal. SRT-assisted MLSS control was suggested and could be realized by parameters of MLSS/RSS and MLSS.

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