Identifying critical components causing seasonal variation of activated sludge settleability and developing early warning tool
Xiaodong Wang, Xuejun Bi, Changqing Liu and Harsha Ratnaweera

ABSTRACT
Settleability of activated sludge is one of the most common problems that restricts the efficiency of activated sludge system. Obvious seasonal variation of settleability was found in the activated sludge system of a full scale wastewater treatment plant (WWTP) during 2 years of observation. Principal component analysis (PCA) was applied to study the correlation between diluted sludge volume index (DSVI), operational and environmental factors. As a result, temperature and mixed liquid suspended solids (MLSS) were found as the most significant variables relating with DSVI variation. Multivariate regression, partial least squares regression and support vector machine regression were applied to develop early warning models for DSVI prediction. The multivariate regression model was proved as a simple and easy-to-interpret early warning tool to be applied in practice. Based on the ratio of volatile substances in biomass, the original cause of seasonal variation of settleability was further discussed by referring the storage-biodegradation mechanism. Moreover, the results of this work also suggested that modern statistical techniques were important to investigate complicated engineering problems. This study provided insights of seasonal variation of activated sludge settleability by systematic investigation of long-term data of a full scale WWTP.

Key words | activated sludge, early warning, principal component analysis, settleability, substance storage, wastewater treatment

INTRODUCTION
The flocs of activated sludge are formed by microorganisms, which grow in biological wastewater treatment systems, while the size, shape and microbial composition of flocs will vary due to changes of operational and environmental conditions. Poorly formed activated sludge flocs settle slower in sedimentation tanks and may lead to poor solid–water separation or even process failure. Activated sludge settling remains one of the most common problems that restricted the efficiency of biological wastewater treatment.

The causes of poor settleability have been studied from several aspects, such as filamentous bulking (Arelli et al. 2009), biomass density (Schuler et al. 2001; Jones & Schuler 2010) and the role of extracellular polymeric substances (Ding et al. 2015). On the one hand, these works well explained the biomass structure and physiochemical characteristics of settling flocs. On the other hand, they reflected the difficulties in figuring out the original causes of variable settleability of activated sludge.

Diluted sludge volume index (DSVI) is an empirical indicator of activated sludge settleability. Seasonal variation of DSVIs has been reported in full-scale wastewater treatment plant (WWTP) (Jones & Schuler 2010). Jassby et al. (2014) combined biomass density and filament content in biomass to explain the variation of DSVI, and a settleability model was developed as a function of biomass density and filament content. However, the original causes of variable settleability of activated sludge were influent characteristics, operational and environmental factors of the biological system, rather than the physiochemical property or biostructure of biomass. For early warning purpose in practice, it is important to develop a DSVI prediction model based on the original causes of the variation of activated sludge settleability.
Multivariate statistical methods are getting additional attention in the optimization of wastewater treatment system. Principal component analysis (PCA) was increasingly used in complexity analysis of full-scale WWTPs (Avella et al. 2011) and pattern detection (Amaral et al. 2013). Partial least squares regression (PLSR) is a useful tool to perform prediction (Amaral et al. 2013). Support vector machine regression (SVMR) and multivariate regression had been proved as capable tool for wastewater treatment process surveillance (Liu et al. 2016a; 2016b; Wang et al. 2017). These multivariate statistical methods can also be used to develop early warning tool for poor settleability of activated sludge. Moreover, the correlation of DSVI and other environmental or operational variables may be figured out by conducting a comprehensive study by applying multivariate statistics.

The aim of this work is to figure out the impact factors of settleability from the complexity of full-scale activated sludge system, and to suggest an early warning tool for poor sludge settleability. This work was performed to explain how operational and environmental factors affect biomass settling and provide better understanding of seasonal variation of activated sludge settleability for troubleshooting.

METHODS AND MATERIALS

Activated sludge system and data collection

The WWTP locates in Qingdao, China, where the temperature in biological reactor varies from 10.5 °C to 28.2 °C. The WWTP receives municipal wastewater from the city Qingdao, and the effluent is discharged to marine recipient after treatment. The WWTP is configured with primary treatment, secondary treatment and tertiary treatment, where secondary treatment is performed by an activated sludge system. Following by secondary treatment, flocculation and filtration system consist tertiary treatment. The activated sludge system was an enhanced biological phosphorus removal (EBPR) system equipped with anaerobic, anoxic and aerobic chambers, which enabled the biological treatment stage to remove nitrogen and phosphorus. The settled activated sludge was recycled back to anaerobic chamber from the secondary clarifiers. The activated sludge sedimentation of the biological system was observed for over a 2-year period.

Influent samples were collected from the outlet of primary settler by an automatic sampler, which fetched a sample every hour and mixed 24 samples together as daily average sample for laboratory analysis. Effluent samples were collected from the outlet of secondary clarifier. The measurement of all the contaminants was carried out according to the standard methods (APHA et al. 2012). DSVI were measured immediately after the mixed liquid obtained from the end of aeration tank according to Lee et al. (1985). Other operational data were either obtained from surveillance system or calculated from pre-known data. The range of these data and some variables used in this study were listed in Table 1.

Statistical analysis

PCA is a multivariate statistical method for studying covariance of variables and detecting collinearity of dataset. The mathematical procedure had been well explained by Tao et al. (2013). We applied PCA to study the correlation between DSVI and other environmental/operational variables. To develop early warning tools of poor activated sludge settleability, three methods were adopted and compared: multivariate regression, PLSR and SVMR. Multivariate regression is already a commonly used statistical method. PLSR is also a regression method, which has the advantage to avoid overfitting when there are too many variables. SVMR is a machine learning algorithm, which can be used to perform both linear and non-liner modelling. The mathematical procedures of PLSR and SVMR had been well explained in literature (Mevik & Wehrens 2007; Seshan et al. 2014). All these multivariate analyses were performed in Unscramber X version 10.4 (CAMO Software 2016).

RESULTS AND DISCUSSION

Settleability variation and the consequences

Significant variation of activated sludge settleability was observed during the 2-year period. As is shown in Figure 1(a), higher DSVIs appeared in cold season when the temperature of the biological reactor was lower than 15 °C, while lower DSVI values were observed in warm season when the temperature was higher than 23 °C. Generally, when the DSVIs were higher than 350 mL/g, the poorly settled activated sludge would be washed out of the sedimentation tank. As a consequence, extremely high effluent SS was quite often observed during cold season when the influent temperature was lower than 15 °C (Figure 1(b)).
The result agreed well with Jones & Schuler (2010), who investigated four full-scale WWTPs in New Mexico, USA, and similar variation of activated sludge settleability was found in their study.

The results in Figure 1 provide evidence of the seasonal variation of activated sludge settleability, indicating the impact of temperature on activated sludge property. The bacterial community of activated sludge varied in a seasonal pattern (Flowers et al. 2013), which may be a possible explanation of the seasonal variation of settleability. However, the variable characteristics of activated sludge were the combined effect of operational conditions, influent characteristics and environmental conditions (Ju et al. 2014; Jo et al. 2016). To figure out the original causes of seasonal variation, a systematic investigation based on multivariate statistical analysis was carried out.

The correlation of 10 variables that represent influent characteristics, contaminant loadings, environmental and operational conditions were assessed together with DSVI by applying PCA (Figure 2). Generally, PCA generated 11 new independent components, which were linear combination of original variables. Figure 2(a) shows the variance explained by the first seven principal components (PCs). The rest of the PCs represented less than 0.1% of the total variance of the dataset, which can be ignored. PC-1 and PC-2 together represented almost 100% of the variation of the entire dataset, which suggested the high collinearity of the original variables.

Further, Figure 2(b) shows the loading of original variables on the plane formed by the first principal component (PC-1) and second principal component (PC-2). DSVI, MLSS and temperature are the most significant variables due to the higher PC-1 or PC-2 loading. These three variables mainly determined the variation of PC-1 and PC-2 as well as the entire original data. Both temperature and MLSS were negatively correlated with DSVI, and therefore they could be used to predict the settleability of activated sludge. Besides, the other variables located in the inner circle of Figure 2(b) were not significant variables because they had either too low PC-1 or PC-2 loading. It indicated that MLSS and temperature correlated well with DSVI, while the other variables were not significant to the

<table>
<thead>
<tr>
<th>Disturbance (variables)</th>
<th>Notation</th>
<th>Unit</th>
<th>Min</th>
<th>Mean</th>
<th>Max</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent flow rate</td>
<td>Flow.in</td>
<td>m³/h</td>
<td>13.722</td>
<td>44.305</td>
<td>64.935</td>
<td>6.540</td>
</tr>
<tr>
<td>Temperature of bio-reactor</td>
<td>Temperature</td>
<td>°C</td>
<td>10.50</td>
<td>18.93</td>
<td>28.20</td>
<td>4.93</td>
</tr>
<tr>
<td>Influent pH</td>
<td>pH</td>
<td></td>
<td>7.08</td>
<td>7.87</td>
<td>8.94</td>
<td>0.25</td>
</tr>
<tr>
<td>Influent chemical oxygen demand</td>
<td>COD.in</td>
<td>mg/L</td>
<td>126.0</td>
<td>424.1</td>
<td>2,249.1</td>
<td>149.1</td>
</tr>
<tr>
<td>Influent biological oxygen demand</td>
<td>BOD.in</td>
<td>mg/L</td>
<td>65.0</td>
<td>186.4</td>
<td>748.0</td>
<td>65.53</td>
</tr>
<tr>
<td>Influent total nitrogen</td>
<td>TotN.in</td>
<td>mg/L</td>
<td>26.15</td>
<td>62.74</td>
<td>113.53</td>
<td>10.70</td>
</tr>
<tr>
<td>Influent total phosphorus</td>
<td>Tot.in</td>
<td>mg/L</td>
<td>1.00</td>
<td>6.18</td>
<td>31.80</td>
<td>2.10</td>
</tr>
<tr>
<td>Total suspended solids in influent</td>
<td>SS.in</td>
<td>mg/L</td>
<td>25.0</td>
<td>181.3</td>
<td>756</td>
<td>130.5</td>
</tr>
<tr>
<td>Influent ammonium</td>
<td>Ammonia.in</td>
<td>mg/L</td>
<td>18.80</td>
<td>53.71</td>
<td>90.75</td>
<td>10.55</td>
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<tr>
<td>Influent chloride</td>
<td>Chloride.in</td>
<td>mg/L</td>
<td>99.26</td>
<td>334.43</td>
<td>1,825.0</td>
<td>192.3</td>
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<tr>
<td>Influent alkalinity</td>
<td>Alkalinity.in</td>
<td>mg/L</td>
<td>75.08</td>
<td>335.25</td>
<td>675.68</td>
<td>143.06</td>
</tr>
<tr>
<td>BOD/COD ratio</td>
<td>BOD/COD</td>
<td></td>
<td>0.22</td>
<td>0.45</td>
<td>0.56</td>
<td>0.10</td>
</tr>
<tr>
<td>Suspended solids of secondary clarifiers outlet</td>
<td>SS.out</td>
<td>mg/L</td>
<td>1.00</td>
<td>35.16</td>
<td>482.0</td>
<td>74.3</td>
</tr>
<tr>
<td>Sludge concentration (Mixed liquid suspended solids)</td>
<td>MLSS</td>
<td>mg/L</td>
<td>938</td>
<td>2,023</td>
<td>5,372</td>
<td>472</td>
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<tr>
<td>Diluted sludge volume index</td>
<td>DSVI</td>
<td>ml/g</td>
<td>62</td>
<td>251.2</td>
<td>634</td>
<td>114</td>
</tr>
<tr>
<td>Volatile matter ratio in sludge</td>
<td>MLVSS/MLSS</td>
<td></td>
<td>0.52</td>
<td>0.74</td>
<td>0.94</td>
<td>0.03</td>
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<tr>
<td>COD/Nitrogen ratio</td>
<td>COD/TotN</td>
<td></td>
<td>2.26</td>
<td>6.79</td>
<td>19.81</td>
<td>1.91</td>
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<tr>
<td>COD/Phosphorus ratio</td>
<td>COD/TotP</td>
<td></td>
<td>20.11</td>
<td>71.06</td>
<td>469.46</td>
<td>25.35</td>
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<tr>
<td>COD loading</td>
<td>COD loading</td>
<td>kg COD/ (kgMLSS.day)</td>
<td>0.05</td>
<td>0.48</td>
<td>2.00</td>
<td>0.27</td>
</tr>
<tr>
<td>BOD loading</td>
<td>BOD loading</td>
<td>kg BOD/ (kgMLSS.day)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.67</td>
<td>0.13</td>
</tr>
<tr>
<td>Sludge retention time</td>
<td>SRT</td>
<td>days</td>
<td>0.98</td>
<td>18.73</td>
<td>184.3</td>
<td>25.00</td>
</tr>
</tbody>
</table>

The potential disturbances of activated sludge settleability used for this study are listed in Table 1.
Figure 1  |  (a) Variation of sludge volume index (SVI) along with temperature changing in 678 days of monitoring; (b) scatter plot of secondary clarifier SS outlet versus temperature.

Figure 2  |  Principal component analysis (PCA) of DSVI and the other 10 variables: BOD/COD ratio, pH, MLVSS/MLSS ratio, SRT, MLSS, BOD loading, COD loading, COD/total phosphorus ratio, COD/total nitrogen ratio, temperature and MLSS. (a) The proportion of total variance being explained by different numbers of principal components; (b) correlating loading plot projected to the plane of PC-1 and PC-2.
Variation of DSVI in this activated sludge system. Therefore, for early warning purpose, temperature and MLSS can be used to develop a prediction model for DSVI.

**Activated sludge settleability prediction for early warning**

During the 2 years’ operation of the WWTP, we concluded the experience that if activated sludge settleability went out of control in the cold season, it will never be self-healing until the warm season. As a consequence, activated sludge would be washed out of the system together with the supernatant, and it will lead to low removal efficiency of organic matter and ammonia. Therefore, it is necessary to develop early warning tools to predict DSVI values. Three methods: multivariate regression, PLSR and SVMR were compared to find proper DSVI prediction modelling tools.

As was suggested by PCA in Figure 2(b), temperature and MLSS were selected as predictors to perform linear regression to predict DSVI. Equation (1) is the formula of multivariate regression for DSVI prediction, and the prediction results were shown in Figure 1. The prediction validated by leverage correction indicates that the predicted DSVI can explain 64% variation of the measured DSVI (Figure 3(a)). The prediction accuracy was not high enough compared with those models based on filamentous bulking (Smets et al. 2006; Liu et al. 2016b). However, we were aimed to study the original causes of the seasonal variation of activated sludge settleability rather than predicting the status of activated sludge, e.g. filamentous bulking or non-filamentous bulking. Considering the uncontrollable disturbances and the complexity of the full-scale system, the R-square of more than 0.64 was fine to indicate the tendency of settleability changing.

The multivariate regression model was built upon the assumption that the prediction errors had a normal distribution with constant variance. In another word, no pattern should be detected on the residual plot (Figure 3(b)). The residuals were the differences between predicted DSVI and measured DSVI. However, an obvious pattern was found in Figure 3(b), where the residuals were getting greater with the increasing of DSVI. Therefore, the capability of this model was limited, because prediction error would be unnecessary larger for greater DSVI values.

\[
DSVI = 724.6325 - 14.6889 \cdot \text{Temperature} - 0.0953 \cdot \text{MLSS}
\]  

To build a proper early warning tool based on influent characteristics and environmental/operational conditions, PLSR and SVMR were also applied to compare if there was a better solution for DSVI prediction. Figure 4 shows the goodness of fitting of PLSR and SVMR. All the variables listed in Table 1 were employed as explanatory variables to build PLSR and SVMR models, because both PLSR and SVMR can naturally avoid overfitting.

The \( R^2 \) of cross validated PLSR model was 0.64 (Figure 4(a)), while it was 0.67 for SVMR (Figure 4(b)). Support vector machine captured some nonlinear characteristics of DSVI variation and resulted in better fitting in terms of \( R^2 \). Although PLSR and SVMR applied more explanatory variables for model construction, the goodness of fit was not significantly improved compared with multivariate regression. The multivariate regression

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Figure 3 | Multivariate regression for DSVI prediction with two explanatory variables: temperature and MLSS. (a) Predicted DSVI versus the measured DSVI; (b) residuals plot versus predicted DSVI.
model, Equation (1), used less computational power and it was easier to interpret. A semi-empirical model of settleability was developed as a function of biomass density and filament content by Jassby et al. (2014). Although that model performed well in DSVI prediction ($R^2 > 0.95$) (Jassby et al. 2014), the complexity of density measurement and filament quantification would limit the usage of the model in practice. Compared with the model developed by Jassby et al., the multivariate regression model in this study lost some accuracy of DSVI prediction. In spite of this, the multivariate regression model indicated the correlation of settleability and its original causes. Secondly, the multivariate regression is more convenient to be adopted as an early warning tool in practice, because the explanatory variables can be easily measured in full-scale WWTPs.

**Volatile substances storage and settleability**

An interesting finding during 2 years’ observation was that the volatile substances of biomass show a seasonal variation pattern against temperature changing. As is shown in Figure 5, the ratio of MLVSS/MLSS was varied within 0.65–0.82. Generally, MLVSS/MLSS was relatively lower in warm season and it was relatively higher in cold season.

![Figure 4](https://iwaponline.com/wst/article-pdf/77/6/1689/242844/wst077061689.pdf)

Figure 4 | (a) Scatterplot of predicted DSVI by partial least squares regression versus measured DSVI. (b) Scatterplot of predicted DSVI by partial least squares regression versus measured PLSR.
The dramatic drop of MLVSS/MLSS in the first 70 days was caused by the addition of external coal ash, which was not the natural decrease of volatile proportion of biomass. The inorganic coal ash was supposed to improve the settling of activated sludge during that period.

Higher settling velocity was usually accompanied with higher inorganic substances in the biomass (Schuler & Jang 2007; Jassby et al. 2014) because inorganic substances contributed positively to biomass buoyant density (Jones & Schuler 2010). The seasonal changing of volatile substances in this study agreed with these literature reports. Since temperature was the original cause of seasonal variation of the volatile substances as well as activated sludge settleability, how temperature impact activated sludge settleability via altering volatile substances of biomass was further discussed.

Activated sludge model No. 3 (ASM3) introduced storage of organic substance to split COD flux (Gujer et al. 1999). A study of the distribution of COD flows to direct growth or storage showed that storage contributed 65–92% of the total COD utilization (Makinia et al. 2006). The temperature-dependent expression of simplified storage and aerobic heterotrophic growth process based on ASM3 is shown as Equations (2) and (3), and the list of symbols were shown in Table 2.

\[
\frac{dX_{STO}}{dt} = k_{STO,20^\circ C} \cdot e^{\theta T (T-20^\circ C)} \cdot \frac{S_s}{K_s + S_s} \cdot X_H \tag{2}
\]

\[
\frac{dX_H}{dt} = \mu_{H,20^\circ C} \cdot e^{\theta_T (T-20^\circ C)} \cdot \frac{X_{STO}/X_H}{K_{STO} + (X_{STO}/X_H)} \cdot X_H \tag{3}
\]

The storage process transforms the soluble COD \( (S_s) \) into internal particulate COD \( (X_{STO}) \) and stores these in the biomass, which may increase volatile substances of the biomass. Both storage and heterotroph growth process are temperature-dependent, while the growth process is more sensitive to temperature change than that of storage process. On the one hand, the consumption of stored substance would be significantly slower in the cold season, but on the other hand, the formation of volatile substances (storage process) is slightly affected by temperature. Therefore, more volatile substances were accumulated in the biomass and led to higher MLVSS/MLSS ratio. It explains the seasonal variation of MLVSS/MLSS as is indicated in Figure 5. If the inorganic content reaches 50%, the setting velocity of activated sludge could be 50% higher than the activated sludge with only 5% inorganic content (Trelles et al. 2017).

Overall, the seasonal variation of MLVSS/MLSS and the storage-consumption mechanism of volatile substance provided insight to understand the seasonal variation of activated sludge settleability.

### CONCLUSION

- An obvious seasonal variation pattern of activated sludge settleability was found during 2 years’ observation in a full-scale WWTP. The DSVI decreased to poor-settling level in cold season, and it would gradually rise to fast-settling level with the increase of temperature in warm season.
- Principal component analysis shows that temperature and MLSS were the most significant variables correlated with the variation of DSVI in this system. Even though SRT and influent loading rate are important factors

<table>
<thead>
<tr>
<th>Notation</th>
<th>Definition</th>
<th>Units</th>
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<tbody>
<tr>
<td>( X_H )</td>
<td>Heterotrophic biomass</td>
<td>mg COD/L</td>
</tr>
<tr>
<td>( X_{STO} )</td>
<td>Organics stored by heterotrophs</td>
<td>mg COD/L</td>
</tr>
<tr>
<td>( k_{STO,20^\circ C} )</td>
<td>Storage rate constant at 20°C</td>
<td>g ( S_s/(g \ X_H \cdot \text{day}) )</td>
</tr>
<tr>
<td>( \theta_T )</td>
<td>Temperature coefficient for organic storage</td>
<td>°C⁻¹</td>
</tr>
<tr>
<td>( T )</td>
<td>Temperature</td>
<td>°C</td>
</tr>
<tr>
<td>( S_s )</td>
<td>Readily biodegradable COD</td>
<td>mg COD/L</td>
</tr>
<tr>
<td>( \mu_{H,20^\circ C} )</td>
<td>Max. growth rate of heterotrophs at 20°C</td>
<td>d⁻¹</td>
</tr>
<tr>
<td>( \theta_H )</td>
<td>Temperature coefficient for heterotrophic growth</td>
<td>°C⁻¹</td>
</tr>
<tr>
<td>( K_s )</td>
<td>Saturation constant for ( S_s )</td>
<td>mg COD/L</td>
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<tr>
<td>( K_{STO} )</td>
<td>Saturation constant for ( K_{STO} )</td>
<td>mg COD/L</td>
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![Figure 5](https://iwaponline.com/wst/article-pdf/77/6/1689/242844/wst077061689.pdf) | Variation of volatile substances in biomass in terms of terms of MLVSS/MLSS, along with seasonal temperature shifting.
determining biological process performance, they are weakly correlated with the variation of activated sludge settleability.
- A multivariate regression model was built to predict DSVI for the purpose of early warning ($R^2 = 0.64$). The model was simple and easy to be adopted in full-scale WWTPs, but we should be aware of that the prediction capability decreased for higher DSVI prediction.
- Due to the differences in temperature sensitivity of substance storage and biodegradation, volatile content of biomass would increase when temperature decreased in the cold season. The negative correlation of volatile content with biomass density led to higher MLVSS/MLSS ratio as well as higher DSVI in the cold season. This biomass storage-biodegradation mechanism based on ASM3 provides insight of the seasonal variation of activated sludge settleability.

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