

Early warning activated sludge bulking by margin of safety for processing indicators in municipal sewage treatment plant

Yuying Dong*, Songmei Zhu, Yuting Chen, Jian Jiao, Zhixin Sha, Xuejun Zou

College of Environment and Resource, Dalian Minzu University, Dalian 116600, China, emails: dong_yuying@163.com (Y.Y. Dong), zhushm0905@163.com (S.M. Zhu), 2891163858@qq.com (Y.T. Chen), 439939684@qq.com (J. Jiao), 649499934@qq.com (Z.X. Sha), zouxuejun@dlmu.edu.cn (X.J. Zou)

Received 17 November 2020; Accepted 20 March 2021

ABSTRACT

Activated sludge (AS) bulking is a thorny problem which leads to serious issues in municipal sewage treatment plant (MSTP). An approach based on the margin of safety (MOS) was proposed to alarm AS bulking. Chemical oxygen demand (COD) serving as a representative chemical index for effluent was introduced to obtain the MOS. It was shown that the area of observation was divided into safety, alarm, and risk zones by the MOS with a range of COD between 25.63 and 50.00 mg L⁻¹. The MOS of invertase under the influence of bisphenol A and triclosan was 534.40–902.59 mg L⁻¹ and 904.91–1,870.81 mg L⁻¹, respectively. The goodness of fit to the practical situations for the MOS of COD in several MSTPs was further certified. The MOS-based method using different types of indicators to predict AS bulking demonstrated many advantages of precision, ease-of-use, and feasibility in complex situations. The findings in this work are valuable to evaluate the environmental risks of emerging organic contaminants and the disturbance risks of sewage treatment processes.

Keywords: Activated sludge bulking; Margin of safety; Biochemistry indicators; Early warning

1. Introduction

Activated sludge (AS) bulking is a challenging problem in municipal sewage treatment plants (MSTP) due to its poor sedimentary properties or sometimes even system collapse. Especially in Northern China, in more than 50% of AS municipal sewage treatment plants, this phenomenon was observed every year because of the complex influent compositions, unstable pH, climate, and many other factors [1,2]. AS bulking will directly affect the secondary sedimentation with the characteristics of occurring frequently, hard to control and long recovery time, that is, it is difficult to separate the AS from the effluent water [3,4].

It is important to take urgent measurements to recover the normal state of AS to avoid any potential outbreak of

AS bulking. The MSTP has to artificially improve the settlement ability of AS in order to maintain a high bacterial concentration in a reactor for effective removal of biological pollutants [5]. However, the process will increase not only the workload but also the cost of the MSTP which may lead to a huge economic loss. In addition to pH and temperature, other factors affecting AS bulking include sludge load, chemical and biological indicators, types of sewage, and unbalance nutrients, etc. [6]. The MSTP process indexes such as sludge volume index (SVI), chemical oxygen demand (COD), total organic carbon (TOC), dissolved oxygen (DO), and functional enzymes can be used as the indicators to alarm sludge bulking including filamentous and non-filamentous bulking [2,7]. The DO is used to characterize the self-purification ability of water bodies, which

* Corresponding author.

is one of the important indicators to measure the water quality and to reflect the status of organic compounds [8]. The low DO concentration has long been regarded as one of the main factors that cause filamentous bulking. The COD has a significant negative correlation with the DO; the greater the COD concentration, the more serious the water is contaminated by organic substances [9]. TOC, an important indicator for assessing the degree of contamination, indicates the organic carbon content in water. Since there is a significant positive correlation between the COD and the TOC, using the margin of safety (MOS) of the COD will be the main research focus in this paper [10,11].

There are also many functional enzymes in the AS treatment of sewage, in which urease (URE) is a hydrolase that promotes the hydrolysis of nitrogen-containing organic compounds. The urea is hydrolyzed specifically to ammonia and carbon dioxide by urease, which is of great significance for the decomposition of nitrogen-containing organic matters in wastewater [12]. And invertase (INVERT), a hydrolase, which can increase the soluble nutrients in AS, plays an important role in the carbon cycle in AS [13]. These two enzymes are all responsible for purifying sewage. Research has shown that the responses of microorganisms to sludge settlement performance may be more direct. For example, filamentous bulking occurs in most cases caused by some types of filamentous bacteria overgrowth [14]. Some of our studies found that enzymes were more sensitive than conventional chemical indicators, therefore, susceptible functional enzymes serving as the crucial indicators would have practical significance in sludge bulking multivariate alarming in the future.

The margin of safety (MOS) is mainly caused by the uncertainties between the pollution load and the capacity of the water body. The mathematical modeling and calculations were often included in the MOS to reduce energy costs during combined design operations [15]. Based on the spectrum norm and the singular value analysis, the mathematical basis of the safety margin is expounded [16]. In the field of food safety, Esposito et al. [17] assessed the dietary intake of acrylamide across six groups of consumers to evaluate the margin of exposure related to neurotoxic and carcinogenic critical endpoints. Nugraha et al. [18] gathered all available occurrence data of Aflatoxin B1 in maize and peanut originating from Indonesia to evaluate the risk of exposure to Aflatoxin B1 using the margin of exposure and the established quantitative liver cancer risk approaches.

In the environmental field, the MOS was applied to evaluate the risk-based phosphorus of total maximum daily load (TMDL, which is the maximum amount of pollutant that a water body can assimilate without violating water quality standards and the allocation of that amount to contributing sources of the pollutant plus a margin of safety) in lakes and the non-point source contamination of TMDL, and to assess the effectiveness of uncertainty in watershed phosphorus load and reservoir phosphorus concentration [19–21]. Patil and Deng [22] used the Bayesian approach to estimate the MOS for TMDL development as well. The MOS also was used to calculate the total amount of pollutants by TMDL for monitoring the water quality in rivers and lakes, point source, and non-point source pollution control [23,24]. Jordan et al. [25] studied the risk

quotients, the values of margin of safety of 10% (MOS10) calculated by using runoff water concentrations were used to estimate risk indicating that the MOS10 values were more sensitive than risk quotients in estimating risk. Our research work was focused on comparing with the sensitivity of chemical and biological indicators in order to make functional enzymes become potential sensitive indicators to alarm AS bulking.

According to the investigations, the MSTP process indicators-based sludge bulking monitoring was not explored sufficiently. Nevertheless, there were still a few studies and applications of the relationship between the MOS and the MSTP process indicators. Developing a comprehensive approach for predicting the state of activated sludge will be a promising idea in this direction. The objective of this study was to develop a MSTP process indicators-based, using the margin of safety aiming to fill up the gap of the existing technology and to predict the evolution of chemical and biological indicators as well as further control sludge bulking in an earlier stage.

The MOS was first proposed by analyzing the relationship between the MOS and the MSTP process indicators in predicting sludge bulking. Secondly, due to the uncertainty and instability of a single variable prediction result, the multidimensional and multivariate integrating strategies were explored to avoid abnormal situations. The SVI is an empirical measurement would be combined with other indices to characterize the sludge bulking [26]. Lastly, in order to find more sensitive alternative indicators than the COD, the biological indicator-enzyme was considered to propose MOS_{enzyme} based on the existing research. Due to the limited utilized data in situ, further studies are needed to test the validation of the MOS stemming from functional enzymes. The results of this study can not only contribute to the new usage of the MOS but also serve as the scientific basis to provide a process-index-based strategy for AS bulking control. The introduction of the MOS has a remarkable and great prospect in reducing and alarming the occurrence of uncertain events in the sewage treatment process.

2. Materials and methods

2.1. Methods

2.1.1. Method of urease determination

The urease activity was determined by the sodium phenol colorimetric method [27]. 5 mL of sludge suspension was mixed with 1 mL of toluene in a 50 mL volumetric flask, shaken at 200 rpm for 20 min, added with 1 mL of 10% urea solution and 4 mL of citric acid buffer solution (pH 6.7), and cultured in a 37°C incubator for 24 h. Remove the solution from the incubator, waved fully, and filter the mixture with filter paper into a clean tube. 3 mL of the filtrate was transferred to a 50 mL volumetric flask with 4 mL of 0.28 mol L⁻¹ sodium phenolate and 3 mL of 1% sodium hypochlorite. The solution was well mixed and allowed to stand for 20 min to appeared indigo-blue. Finally, it was diluted to 50 mL of the scoreline and its absorbance was measured at 578 nm. Urease activity is represented as the mass of NH₃-N produced by the sludge per 1 g dry weight within 24 h.

$$\text{URE} = \frac{aVn}{m} \quad (1)$$

where the letter a is the mass of $\text{NH}_3\text{-N}$ calculated from the absorbance value of the sample; V is the volume of chromogenic solution (mL); n is the ratio of the sample filtrate volume/the aspirated filtrate volume; m is the mass of dried sludge (g).

2.1.2. Method of invertase determination

Invertase activity was determined by 3,5-dinitrosalicylic acid method [28]. 5 mL of phosphate citric acid buffer solution (pH 5.5), 15 mL of 10% sucrose matrix solution and 0.2 mL of toluene were added to 4 mL of the sludge suspension, and the mixture was placed in a 37°C incubator for 24 h. After filtering the mixture, a little filtrate was taken into a 50 mL volumetric flask, 2 mL of 3,5-dinitrosalicylic acid solution was added, which was heated in a boiling water bath for 5 min, diluting the solution to 50 mL after cooling and measure the absorbance at 540 nm. The invertase activity is represented as the mass of glucose formation per 1 g of dried sludge after 24 h.

$$\text{INVERT} = \frac{bn}{m} \quad (2)$$

where the letter b is the mass of glucose calculated from the absorbance value of the sample; n is the ratio of the sample filtrate volume/the aspirated filtrate volume; m is the mass of dried sludge (g).

2.1.3. Method of TOC determination

The TOC was determined using a multi-N/C 3100 TOC assay analyzer. The calculation of the analytical results is based on a calculated calibration function of total carbon (TC) and inorganic carbon (IC).

$$C_{\text{TOC}} = C_{\text{TC}} - C_{\text{IC}} \quad (3)$$

where C_{TC} is the calculated calibration function of TC; C_{IC} is the calculation calibration function of IC.

2.2. Data sources

The determined data in Tables 3–5 and Fig. 3 were obtained from a municipal sewage treatment plant in Dalian, the reported data were obtained from Wuhu and Tianjin which have been cited and marked [29,30]. All data were obtained by the anaerobic/anoxic/oxic (A²/O) process.

2.3. MOS determination

Mathematically, MOS can be defined as the difference between the load associated with a maximum limiting concentration with the predefined MSTP standard (LC_{max}) and the load associated with a critical concentration ($\text{LC}_{\text{critical}}$) [31]. A failure event is defined as an event in which the MSTP process specification exceeds the system design

limit. The indicators of MSTP include chemical indicators: COD, TOC, and biochemical oxygen demand, DO, etc. and biological indicators, that is, functional enzymes.

$$\text{MOS} = \text{LC}_{\text{max}} - \text{LC}_{\text{critical}} \quad (4)$$

The variable W is defined as the ratio of the concentration of the MSTP process indicator N to the established limiting standard LC_{max} , the success event (the system meets the standard) and the failure event (the system does not meet the standard) can be expressed as $W < 1$ and $W > 1$, respectively.

$$W = \frac{N}{\text{LC}_{\text{max}}} \quad (5)$$

Sludge bulking is a failure event, that is, $W > 1$, defined as in Eq. (6),

$$P(W > 1) = \int_{W=1}^{+\infty} P(W) dW \quad (6)$$

where $P(W)$ is the probability density function of W , and W is a normal distribution [19]. The critical value $\text{LC}_{\text{critical}}$ can be considered as the difference between the maximum limit value LC_{max} and MOS. When the MSTPs process indicator is normally distributed, the calculation can be performed by Eq. (7),

$$\text{MOS} = \text{LC}_{\text{max}} - (\text{LC}_{\text{max}} - Z_{\text{risk}} S^*) = Z_{\text{risk}} S^* \quad (7)$$

where S^* is the standard deviation related to the process indicator of the MSTPs, $S^* = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$ ($1 \leq i \leq n$), where x_i is the value of the i -th process indicator, \bar{x} is the average value of i measurements, i is the number of measurements and n is the maximum number of measurements; Z_{risk} is the standard normal quantile for any given acceptable level of risk.

2.4. Approach procedure

Proposed approach frame of alarming AS bulking through MOS by the following steps shown in Fig. 1. The chemical indicators and biological indicators of MSTP were screened, taking COD, urease and invertase as the research objectives. This paper first determined that the MOS of COD was used to warn the sludge bulking, and then further research and comparison found that the biological index was more sensitive than the chemical index. It was also proposed to use MOS of a sensitive functional enzyme to predict sludge bulking may be more valid.

3. Results and discussion

3.1. Development of a predicted method for the MOS of COD

The COD of the system designed was 50.00 mg L⁻¹, that is, 50.00 mg L⁻¹ was LC_{max} of the normal effluent COD in

MSTP. The removal rate of the effluent COD given in most literature was over 60.0% for MSTP. The MSTP system has no obvious effect on the removal of organic compounds when the COD removal rate is less than 60.0%. The COD removal rate of this plant was between 62.3% and 97.0%, so the effect of sewage treatment was stable. The measured mean value of effluent COD within 10 samples was 33.6 mg L⁻¹, S* was 12.955. The failure event is expressed as $W > 1$ and $W^* = 9.70$, so the interval of W is set to (1, 10) (Table 1, Eq. (5)). The value of $P(W > 1)$ calculated by Eq. (6) was 0.97, Z_{risk} obtained by the standard normal quantile table was 1.88079. Finally, it could be deduced that MOS_{COD} was 24.37 mg L⁻¹ according to Eq. (7).

3.2. Verification the observed MOS of COD

Based on the calculations in Section 3.1, the $\text{LC}_{\text{critical}}$ of COD calculated by Eq. (4) was 25.63 mg L⁻¹. Therefore, the alarm zone suggested that close attention should be paid to analyze the condition of the process to prevent unsystematic risk when the MOS_{COD} was between 25.63 and 50.00 mg L⁻¹. The safety zone has shown that the MSTP system was stable and the treatment effect meets the requirement of sludge condition when COD was less than 25.63 mg L⁻¹. The area enclosed by the dotted line, abscissa and normal distribution curve was the risk zone (Fig. 2) when the COD was higher than 50.00 mg L⁻¹ implied that the possibility of sludge bulking.

The sedimentation performance of AS is related to many factors, including SVI, pH, nitrogen and phosphorus concentration, DO, temperature, etc., which may contribute to filamentous or non-filamentous bulking due to the uncertainty of these indicators [32]. The goodness of fit to the practical situations for the MOS of COD in several MSTPs was further certified. Although the COD during the non-bulking period was within the risk and alarm zone, the values between bulking and non-bulking periods changed very sharply (Table 2) that suggested giving more attention.

This approach was reasonable and accurate to predict the sludge bulking by MOS, but it cannot distinguish the bulking types. Prediction of filamentous and non-filamentous bulking still needs to be improved. Liu et al. [33] proposed integration of image analysis with enhanced Multi-output Gaussian Processes Regression model. Though the present research could handle the above problem to some degree, the application of MOS will be more extensive. Future work should be aimed to test and validate this method in MSTPs.

3.3. Comparison of the sensitivity of chemical and biological indicators

At present, the environmental monitor still uses chemical indicators. The lack of fusion methods using chemical and biological indicators was the bottleneck for monitoring the ecological impacts effectively when the complex component wastewater treatment systems were evaluated. Predicting the impact of emerging organic contaminants (EOCs) on MSTP, screening sensitive indicators affecting the AS ecological functions and evaluating potential risks of MSTP scientifically were helpful to warn the change of sludge activity in MSTP timely.

The dynamic variation rate emphasizes the change in the interval between adjacent detecting points.

$$\Delta R_E = \frac{A_n^E - A_{n-1}^E}{A_0^E} \times 100\% \quad (n \geq 2) \quad (8)$$

where ΔR_E is the dynamic rate of enzyme activity; A_n^E is enzyme activity of the measuring point; A_{n-1}^E is enzyme activity at the last measuring point; A_0^E is the enzyme activity value of the blank control, that is, the exposure concentration is 0. The activity of urease and invertase were calculated by Eqs. (1) and (2), respectively.

$$\Delta R_{\text{TOC}} = \frac{C_{\text{TOC}}^n - C_{\text{TOC}}^{n-1}}{C_0^{\text{TOC}}} \times 100\% \quad (n \geq 2) \quad (9)$$

Table 1
COD and corresponding W measured from 10 samplings of a MSTP

COD _i (mg L ⁻¹)	COD* (mg L ⁻¹)	W*	COD _e (mg L ⁻¹)	W	Removal rate, %
336	201.6	4.03	48	0.96	85.7
168	100.8	2.02	48	0.96	71.4
208	124.8	2.50	32	0.64	84.6
172	103.2	2.06	8	0.16	95.4
106	63.6	1.27	40	0.80	62.3
568	340.8	6.82	48	0.96	91.6
432	259.2	5.18	32	0.64	92.6
200	120.0	2.40	24	0.48	88.0
260	156.0	3.12	32	0.64	87.7
808	484.8	9.70	24	0.48	97.0

Notice: (1) The COD data of inlet and outlet water comes from the study of Qian et al. [29] on the effects of inlet wastewater quality on the diversity of bacterial communities in aero-tank of the wastewater treatment plant. (2) COD_i was influent COD, COD_e was effluent COD. According to Eq. (5), W and W^* were obtained in Table 1. COD* was COD with a removal rate of 60.0%; W^* was W corresponding to COD* removal rate of 60.0%.

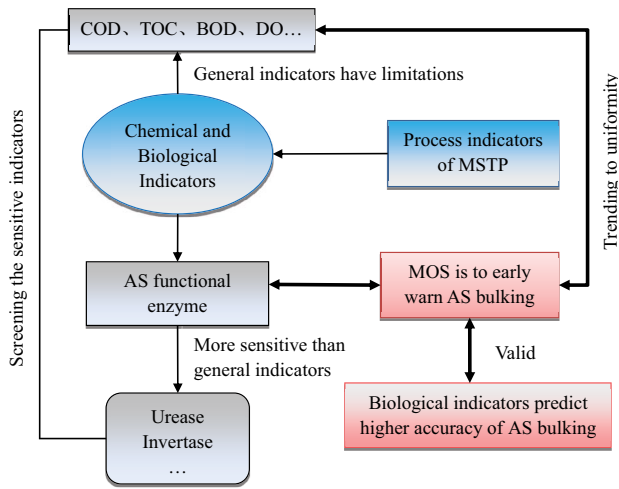


Fig. 1. Proposed approach frame of early warning AS bulking through MOS.

Table 2

COD comparison between bulking and non-bulking periods in different treatment processes

Treatment process	COD (mg L ⁻¹)	
	Non-bulking period	Bulking period
Pre-anoxic tank + A ² /O + filler	25	27
A ² /O + filler	32	34
A ² /O	44	46

Notice: The data comes from the study of Gao et al. [30] on the effect of denitrification and phosphorus removal microorganisms in activated sludge bulking caused by filamentous bacteria.

where ΔR_{TOC} is the degradation rate of TOC; C_n^{TOC} is the concentration of TOC at measuring point; C_{n-1}^{TOC} is the concentration of TOC at the last measuring point; C_0^{TOC} is the TOC of the blank control, that is, the exposure concentration is zero. The values of TOC were calculated by Eq. (3).

The concentration of bisphenol A (BPA) was 4.0 $\mu\text{g L}^{-1}$, although the difference among indicators was insignificant, the relative change rate of enzyme activity was higher than TOC. The concentration of BPA was 5.0 $\mu\text{g L}^{-1}$, the dynamic rate of enzyme activity was significantly higher than TOC (Table 3, Eqs. (8) and (9)). The decrease of urease and invertase activity will affect nitrogen removal and sucrose decomposition, which may cause poor effluent quality. Therefore, it is important to alarm the state of sludge in the AS process by monitoring the enzyme activity.

Biological indicators were generally superior to chemical indicators [34]. Some differences in the sensitivity among various biological indicators due to the different types of pollutants and action mechanism. Urease can facilitate the hydrolysis of the nitrogenous organic matter; the activity of hydrolase such as urease might be decreased when there were toxic substances in AS. The increase of invertase activity provided sufficient carbon source for

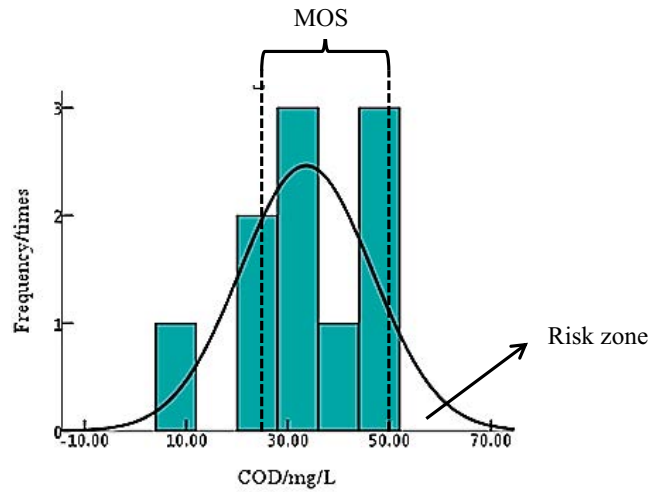


Fig. 2. Normal distribution curve of COD has been measured by 10 samples.

denitrifying bacteria to supply nitrate reductase for biological nitrogen removal, therefore, there were some differences in functional enzyme responses.

3.4. Development of a predicted method for the MOS of enzyme

Since the precision and accuracy of TOC were better than COD, we determined the value of TOC [35]. Based on the linear correlation between TOC and COD, COD can be calculated and listed in Tables 4 and 5, which was used to explore the relationship between biological and chemical indicators [36].

Eight unary linear regression equations were obtained according to Fig. 3, among which four were COD under the influence of triclosan, and the pollutant concentration corresponding to the MOS_{COD} was obtained according to these equations (Figs. 3a and c). Finally, the enzyme activity corresponding to pollutant concentration can be deduced, that is, the MOS of enzymes were obtained based on the other four linear equations: the MOS_{INVERT} under the influence of BPA and triclosan was 534.40–902.59 mg L^{-1} and 904.91–1,870.81 mg L^{-1} (Fig. 3b), the MOS_{URE} under the influence of BPA and triclosan was 27.06–32.33 mg L^{-1} and 15.94–19.64 mg L^{-1} (Fig. 3d). Although the MOS of these two enzymes observed in this study were stable, the invertase was much more sensitive than urease. Further research is needed to verify these findings to ensure veracity. What should be noticed is the composition of wastewater will fluctuate with the influent or other factors, so the equation was not unique in Fig. 3 which needs to be corrected by the actual situation.

Some studies have proved that the magnitude of enzyme activity sometimes shows opposite effects, inhibitory or facilitating in the ecosystems. Taking urease existing in various organisms as an example, on the one hand, it catalyzes the hydrolysis of urea and helps MSTP denitrification; On the other hand, it leads to an increase in pH value, which has a negative impact on human health and agriculture, just like the urea hydrolysis reaction shown in the chemical Eq. (10) [37].

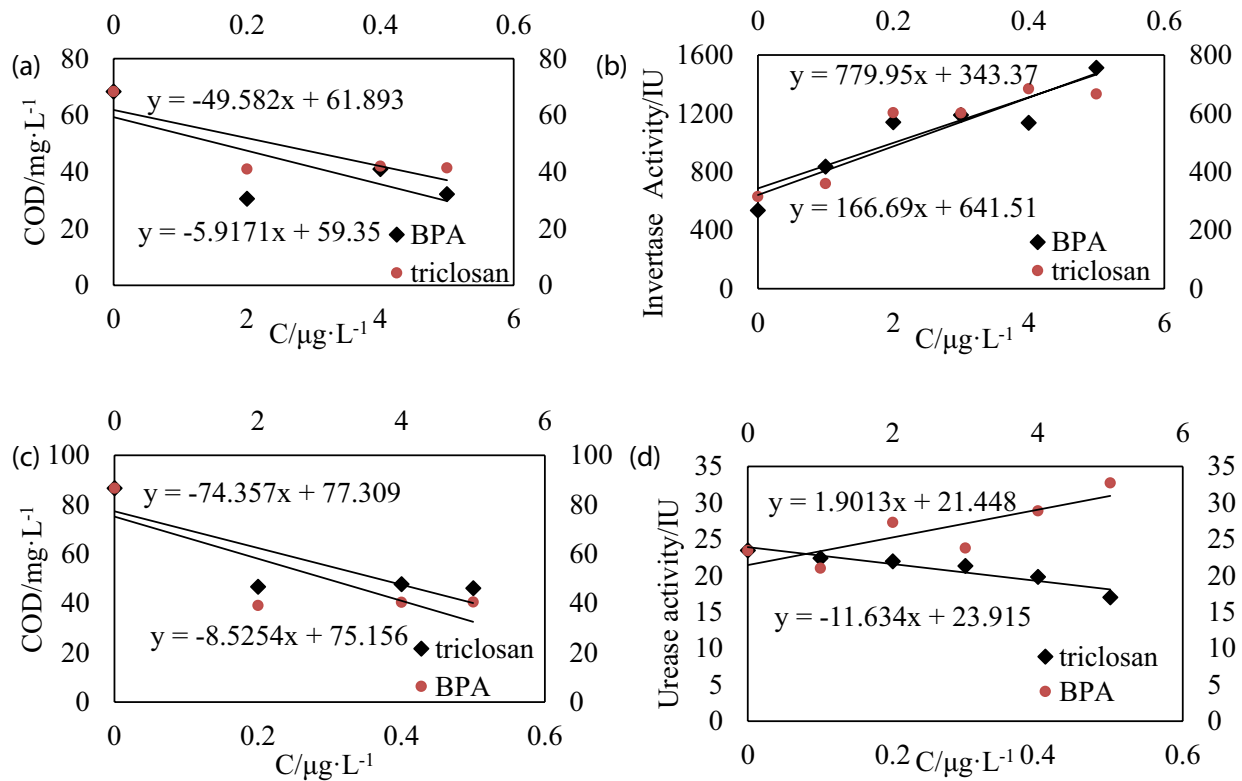
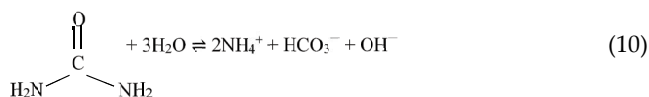


Fig. 3. The effect of BPA and triclosan on process indicators of MSTP. Effect of BPA and triclosan on (a) COD (exposure time is 0.5 h), (b) invertase (exposure time is 0.5 h), (c) COD (exposure time is 5 h) and (d) urease (exposure time is 5 h).

Table 3
Effects of different concentrations of bisphenol A (BPA) and triclosan on biological and chemical indicators

Concentration ($\mu\text{g L}^{-1}$)	ΔR_{TOC} (%)	ΔR_{URE} (%)	ΔR_{INVERT} (%)	
BPA	4.0	-9.90	10.95	22.48
	5.0	2.61	54.08	34.31
Triclosan	0.4	-0.99	33.33	0
	0.5	1.62	-27.77	34.09



We considered the enzyme is a kind of paradoxical enzyme, which is combined with MOS to monitor the

Table 4
Calculation of COD under the influence of BPA and triclosan by TOC (exposure time 0.5 h)

C_{BPA} ($\mu\text{g L}^{-1}$)	TOC (mg L^{-1})	COD (mg L^{-1})	$C_{\text{triclosan}}$ ($\mu\text{g L}^{-1}$)	TOC (mg L^{-1})	COD (mg L^{-1})
0	34.82	68.43	0	34.82	68.43
2.0	18.99	30.60	0.2	23.38	41.09
4.0	23.36	41.04	0.4	23.78	42.04
5.0	19.68	32.25	0.5	23.54	41.47

operation state of wastewater treatment system by controlling enzyme activity. The interaction between EOCs and AS functional enzymes was studied in order to obtain the risk data of their impact on system function, which laid the foundation for characterizing the performance of AS, evaluating the stability of AS and preventing the risk of MSTP.

4. Conclusions

In this paper, the MOS was firstly introduced in predicting sludge bulking. Verified by actual situation analysis, the MOS based approach using different types of indicators to predict sludge bulking demonstrated the advantages of precision, ease-of-use, and feasibility. The proposed method can track and predict the variations of the COD or enzymes combined with other indicators, which can overcome the limitations of sludge bulking in MSTP to a certain extent. It has been confirmed that the biological indicators are more

Table 5

Calculation of COD under the influence of BPA and triclosan by TOC (exposure time 5 h)

C_{BPA} ($\mu\text{g L}^{-1}$)	TOC (mg L^{-1})	COD (mg L^{-1})	$C_{\text{triclosan}}$ ($\mu\text{g L}^{-1}$)	TOC (mg L^{-1})	COD (mg L^{-1})
0	36.95	86.64	0	36.95	86.64
2	17.08	39.15	0.2	20.28	46.80
4	17.64	40.49	0.4	20.71	47.83
5	17.67	40.56	0.5	20.02	46.18

sensitive than chemical indicators and further studies are needed in biological indicators in order to provide basic data for risk assessment of wastewater biochemical treatment processes.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (21477001), the LIAONING S&T Project (20180550107) and the Basic Scientific Research Funds of DLNU (2020fwgj016).

References

- [1] P. Wang, Z.S. Yu, R. Qi, H.X. Zhang, Detailed comparison of bacterial communities during seasonal sludge bulking in a municipal wastewater treatment plant, *Water Res.*, 105 (2016) 107–166.
- [2] J.H. Guo, Y.Z. Peng, S.Y. Wang, X. Yang, Z.G. Yuan, Filamentous and non-filamentous bulking of activated sludge encountered under nutrients limitation or deficiency conditions, *Chem. Eng. J.*, 255 (2014) 453–461.
- [3] P.L.P. Goldwyn, M.K. Saseetharan, J. Jeyanthi, Influence of addition of chlorine in the design of secondary settling tank by controlling the bulking in dairy activated sludge, *J. Inst. Eng. (India): Chem. Eng. Div.*, 91 (2010) 9–13.
- [4] B. Xie, X.-C. Dai, Y.-T. Xu, Cause and pre-alarm control of bulking and foaming by *Microthrix parvicella*—a case study in triple oxidation ditch at a wastewater treatment plant, *J. Hazard. Mater.*, 143 (2007) 184–191.
- [5] C.C. Ye, X.P. Yang, F.-J. Zhao, L.F. Ren, The shift of the microbial community in activated sludge with calcium treatment and its implication to sludge settleability, *Bioresour. Technol.*, 207 (2016) 11–18.
- [6] L.J. Gui, Y.Z. Peng, G.X. Gan, Z.X. Peng, H.X. Hou, G. Wang, H.R. Shi, Activated sludge bulking initiated by cooperation of low DO and sludge loads, *CIESC J.*, 62 (2011) 2042–2048.
- [7] D.M. Lü, Y.Z. Peng, W.H. Zhao, S.Y. Wang, Occurrence and control of filamentous bulking in A²O-BAF denitrifying phosphorus removal process, *China Environ. Sci.*, 35 (2015) 3026–3031.
- [8] R.J. Diaz, Overview of hypoxia around the world, *J. Environ. Qual.*, 30 (2001) 275–281.
- [9] F.-X. Yang, H.-M. Jian, L. Tian, Q.-Z. Yao, Distribution characteristics of COD and DO and its influencing factors in the Daliaohe estuary, *Environ. Sci.*, 35 (2014) 3748–3754.
- [10] X.L. Yu, X.D. Zhang, L.N. Pan, M. Chang, L. Wu, The research on relationship between COD and TOC, BOD and its application on the water environmental monitoring, *Appl. Laser*, 34 (2014) 455–459.
- [11] F. Chen, J.F. Xu, A. Wang, Y.Y. Cao, Association analysis of TOC and COD in sewage treatment plants, *Environ. Sci. Surv.*, 37 (2018) 75–78.
- [12] S.Y. Zhu, M.X. Huo, K. Zhou, J. Wang, X. Yang, Effect of photodegraded quinoline aqueous solutions on enzyme activities in activated sludge, *Adv. Mater. Res.*, 374 (2012) 1025–1030.
- [13] Y. Li, R.J. Chróst, Microbial enzymatic activities in aerobic activated sludge model reactors, *Enzyme Microb. Technol.*, 39 (2006) 568–572.
- [14] C. Meunier, O. Henriët, B. Schoonbroodt, J.-M. Boeur, J. Mahillon, P. Henry, Influence of feeding pattern and hydraulic selection pressure to control filamentous bulking in biological treatment of dairy wastewaters, *Bioresour. Technol.*, 221 (2016) 300–309.
- [15] M.S. Yakhnenko, N.V. Snetkova, A.V. Mironov, Computational and mathematical modelling during calculation of margin of safety as the way of decrease in expenses of energy during the operation of combined designs, *Adv. Mater. Res.*, 853 (2013) 526–530.
- [16] Z.P. Qiu, D.L. Liu, Safety margin analysis of buckling for structures with unknown but bounded uncertainties, *Appl. Math. Comput.*, 367 (2020) 124759 (1–10), doi: 10.1016/j.amc.2019.124759.
- [17] F. Esposito, A. Nardone, E. Fasano, M. Triassi, T. Cirillo, Determination of acrylamide levels in potato crisps and other snacks and exposure risk assessment through a margin of exposure approach, *Food Chem. Toxicol.*, 108 (2017) 249–256.
- [18] A. Nugraha, K. Khotimah, I.M.C.M. Rietjens, Risk assessment of Aflatoxin B1 exposure from maize and peanut consumption in Indonesia using the margin of exposure and liver cancer risk estimation approaches, *Food Chem. Toxicol.*, 113 (2018) 134–144.
- [19] D.E. Langseth, N. Brown, Risk-based margins of safety for phosphorus TMDLs in lakes, *J. Water Res.*, 137 (2011) 276–283.
- [20] R.A. Camacho, J.L. Martin, T. Wool, V.P. Singh, A framework for uncertainty and risk analysis in total maximum daily load applications, *Environ. Modell. Software*, 101 (2018) 218–235.
- [21] M. Karamouz, M. Taheriyoun, M. Seyedabadi, S. Nazif, Uncertainty based analysis of the impact of watershed phosphorus load on reservoir phosphorus concentration, *J. Hydrol.*, 521 (2015) 533–542.
- [22] A. Patil, Z.-Q. Deng, Bayesian approach to estimating margin of safety for total maximum daily load development, *J. Environ. Manage.*, 92 (2011) 910–918.
- [23] B. Liang, X.Y. Wang, L.P. Cao, Application of total maximum daily load (TMDL) program to control of non-point source pollution, *Water Resour. Protect.*, 4 (2004) 37–41.
- [24] G. Sehlke, D.F. Hayes, D.K. Stevens, An Approach to Establishing Risk Based Margins of Safety for Total Maximum Daily Loads for Phosphorus in Lakes, World Water and Environmental Resources Congress, Salt Lake City, Utah, United States, June 27–July 1, 2004, pp. 1172–1181.
- [25] L.T. Jordan, D.H. Amanda, M.M. Tracey, E.H.H. Kara, Y.F. Courtney, J.L. Michael, Fate and risk of atrazine and sulfentrazone to nontarget species at an agriculture site, *Environ. Toxicol. Chem.*, 36 (2017) 1301–1310.
- [26] G. Sürücü, S. Soyupak, Effects of operational parameters on the settling properties of activated sludge, *Environ. Technol. Lett.*, 10 (1989) 471–478.
- [27] Z.H. Lv, Y.L. Yao, Z.M. Lv, H. Min, Effect of tetrahydrofuran on enzyme activities in activated sludge, *Ecotoxicol. Environ. Saf.*, 70 (2008) 259–265.
- [28] Y.Y. Dong, H.Q. Wang, L.W. Wang, X.J. Zou, Effects of salt stress on different enzyme activities of aerobic sludge in municipal sewage treatment plant, *J. Dalian Minzu Univ.*, 19 (2017) 11–13+20.

- [29] F. Qian, J. Zhao, C.P. Nie, Effects of inlet wastewater quality on diversity of bacterial communities in aero-tank of wastewater treatment plant, *Environ. Pollut. Control*, 34 (2012) 10–14.
- [30] C.C. Gao, J. You, Y. Chen, X.C. Chen, W. Shang, W.A. Zhang, Effect of denitrification and phosphorus removal microorganisms in activated sludge bulking caused by filamentous bacteria, *Environ. Sci.*, 39 (2018) 2794–2801.
- [31] S. Franceschini, C.W. Tsai, Incorporating reliability into the definition of the margin of safety in total maximum daily load calculations, *J. Water Res.*, 134 (2008) 34–44.
- [32] S.S. Ai, X.H. Zhang, Y.B. Xiao, X. Tian, D.J. Bian, Study on characteristics of activated sludge at low temperature, *Appl. Mech. Mater.*, 675–677 (2014) 574–577.
- [33] Y.Q. Liu, Y.P. Pan, D.P. Huang, Q.L. Wang, Fault prognosis of filamentous sludge bulking using an enhanced multi-output gaussian processes regression, *Control Eng. Pract.*, 62 (2017) 46–54.
- [34] Y.Y. Dong, J.J. Zhao, L.W. Wang, H.Q. Wang, X.J. Zou, J.G. Zhang, Effect of bisphenol A and pentachlorophenol on different enzymes of activated sludge, *Sci. Total Environ.*, 671 (2019) 1170–1178.
- [35] M. Tang, P.J. Fang, Relationship between TOC and COD_{Mn} of surface water, *Meteorol. Hydrol. Mar. Instrum.*, 27 (2010) 93–96.
- [36] X. Xie, Correlation analysis of COD and TOC in urban sewage, *China Resour. Compr. Util.*, 33 (2015) 53–55.
- [37] L. Mazzei, M. Cianci, S. Benini, S. Ciurli, The structure of the elusive urease–urea complex unveils the mechanism of a paradigmatic nickel-dependent enzyme, *Angew. Chem. Int. Ed.*, 58 (2019) 7415–7419.