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Monitoring the Activated Sludge Activities Affected by Industrial Toxins via an Early-Warning System Based on the Relative Oxygen Uptake Rate (ROUR) Index

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Abstract: Shock load from industrial wastewater is known to harm the microbial activities of the activated sludge in wastewater treatment plants (WWTPs) and disturb their performance. This study developed a system monitoring the activated sludge activities based on the relative oxygen uptake rate (ROUR) and explored the influential factors with wastewater and the activated sludge samples collected from a typical WWTP in the Taihu Lake of southern Jiangsu province, China. The ROUR was affected by the concentration of toxic substances, mixed liquid suspended solids (MLSS), hydraulic retention time (HRT) and pH. Higher toxin contents significantly decreased the ROUR and the EC_{50} value of Zn²⁺, Ni²⁺, Cr(VI), Cu²⁺, and Cd²⁺ was 13.40, 15.54, 97.56, 12.01, and 14.65 mg/L, respectively. The ROUR declined with the increasing HRT and MLSS above 2000 mg/L had buffering capacities for the impacts of toxic substances to some extent. The ROUR remained stable within a broad range pH (6–10), covering most of the operational pH in WWTPs and behaving as an appropriate indicator for monitoring the shock load. A toxicity model assessing and predicting the ROUR was developed and fitted well with experimental data. Coupling the ROUR monitoring system and toxicity model, an online early-warning system was assembled and successfully used for predicting the toxicity of different potential toxic metals. This study provides a new universal toxicity model and an online early-warning system for monitoring the shock load from industrial wastewater, which is useful for improving the performance of WWTPs.

Keywords: potential toxic elements (PTEs); relative oxygen uptake rate (ROUR); toxicity; wastewater

1. Introduction

With the rapid economic development and urbanization, the quantity of wastewater discharge increases and the demands for freshwater resources are increasing worldwide [1,2]. Wastewater treatment plants (WWTPs) suffer from many difficulties to meet the rigorous discharge standards nowadays, e.g., high pollutant concentration, complicated composition, poor biodegradability, and unstable process [3,4]. It is important for WWTPs to maintain high microbial activities of the activated sludge, which are critical in removing pollutants, particularly persistent organic pollutants (POPs), emerging contaminants or potential toxic elements (PTEs). For instance, biological treatment in WWTPs was reported to effectively degrade phenol [5], polycyclic aromatic hydrocarbons (PAHs) [6,7], polychlorinated biphenyls (PCBs) [8], imidazole [9], 1-methyl-imidazole [9], *N*,*N*'-alkyl-imidazolium



chlorides [9], and SS-ethylenediamine-*N*,*N*′-disuccinic acid [10,11], and the activated sludge was also capable of adsorbing and removing PTEs [12,13]. However, the biochemical treatment units in WWTPs are sensitive to high-level of toxic emerging substances or PTEs in influents and the paralysis often occurs in case of a shock load. It is reported that the spill of cyanide and other illegal chemical dumping can cause the shutdown of WWTPs and consequently release contaminants to the surrounding environment [14]. Toxicity assessment methods require development for monitoring the change of influent toxicity and guiding the rapid response strategies to protect WWTPs from the shock load [15].

One important source of the shock load in WWTPs is industrial wastewater, which is characterized by its extreme toxicity, inhibitive ability and high concentrations of complex pollutants [16–18]. Industrial wastewater harms the biochemical treatment units of WWTPs and seriously disturbs their operation by inhibiting the activated sludge activities in WWTPs [19]. The ratio of 5-day biological oxygen demand (BOD₅) to chemical oxygen demand (COD), designated as B/C, can be significantly reduced by industrial wastewater to <0.3, indicating a poor biodegradability and causing the paralysis of the biochemical treatment units [20,21]. Due to the shock load and intermittent impacts of PTEs, cyanide and other toxic substances in industrial wastewater, WWTPs might lose efficiencies in removing pollutants to some extent and the effluent quality is affected [21,22], consequently increasing the operation cost [23]. It is therefore necessary to monitor the industrial shock load and improve the resistance of WWTPs to maintain the removal efficiency of refractory pollutants. However, due to the uncertainty and complexity of pollutants in industrial wastewater discharge, most of which are not conventional contaminants to be regularly measured in WWTPs, systems monitoring a broad spectrum of pollutants and integrally evaluating the influent toxicity are of great urgency in WWTP management.

Biological monitoring is an alternative approach to evaluate the influent quality by assessing the toxicity, instead of the accurate composition of wastewater [24]. Many studies on biological early-warning systems use various indicators to monitor the influent toxicity in WWTPs, e.g., animals and plants [25,26], special bacterial strains [27,28], microbial current [29–31], and respiratory rate [32]. Among them, luminescent bacteria are widely studied [33–35] but suffer from the difficulties in online cultivation and monitoring [36–39]. Additionally, most of these approaches are still in laboratory test, leaving a huge gap between the state-of-art concept and industrial practices. Besides, these biological measurements are indirect methods as the indicator organisms are exogenous to the activated sludge and unable to represent the real domestication and adaptability of the activated sludge during wastewater treatment process [14]. An early-warning system directly monitoring the change of the activated sludge activities can represent the real performance of WWTPs and provide a basis for emergency measures of toxic substances in industrial wastewater, for effectively evaluating the influent toxicity and alarming the shock load of industrial wastewater [3,40].

The oxygen uptake rate (OUR) is a theoretical indicator for characterizing the activated sludge activities, representing the in situ changes of microbial activities in removing pollutants [41–43]. The OUR has been used for several cases of toxicity assessment like organic compounds, PTEs, and complex wastewater [14,44]. For instance, the OUR is able to estimate the kinetic parameters in a model including substrate hydrolysis, biomass growth and endogenous metabolism [45]. In addition, the degree of the OUR also indicates the toxic and inhibitory level of pollutants [46,47]. Studies have shown that the sizes and concentrations of pollutants inhibit the OUR of the activated sludge to different extent [46]. Other operation parameters in WWTPs, like the activated sludge contents and hydraulic retention time (HRT) have distinct influence on the OUR [48]. From the OUR of the activated sludge in WWTPs, the influence of toxic substances on the biochemical treatment units is intuitional. However, as the activated sludges have unique compositions across WWTPs, it is difficult to apply one type of early-warning system in different WWTPs [49]. Therefore, a robust, reliable and universal model simulating the OUR response to different environmental variables is needed to calculate and predict the influent toxicity across WWTPs.

The aim of this work is to assemble an online early-warning system to monitor the activated sludge activities in WWTPs affected by industrial toxins via the relative OUR (ROUR) and develop a model to discuss the impacts of environmental variables on the ROUR, with a case study in a typical WWTP in the Taihu Lake of southern Jiangsu Province, China. With the typical toxicity and inhibitory sources in industrial wastewater as the target toxic substances, e.g., Zn²⁺, Ni²⁺, and phenol, we also studied some influential factors, e.g., types and concentrations of toxins, the activated sludge contents and HRT, to comprehensively explore their influence on the ROUR and predict the ROUR under different conditions. Our findings help in better understanding and applying the online early-warning system for the effective operation of different WWTPs response to the shock load from industrial wastewater.

2. Materials and Methods

2.1. Sample Collection and Instrumental Analysis

Wastewater and the activated sludge were collected from Nancao WWTP, Yixing city, Jiangsu Province (E119°58′48′′, N31°30′20′′) in March, 2015. After transport to laboratory at 4 °C, they were stored under 4 °C within 24 h before experiments.

The measurement of MLSS followed the standard method [50]. The PTEs contents in the activated sludge were detected by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8300DV, Perkin Elmer, Waltham, MA, USA, 2011). Phenol concentration was measured by a modified spectrophotometric method [5]. Briefly, 100 μ L of the activated sludge sample was diluted in 900 μ L of deionized water, added with 400 μ L of NH₄OH (2.0 M), 200 μ L of aminoantipyrine (2% *w/w*) and 400 μ L of K₃Fe(CN)₆ (2% *w/w*). The absorbance at 500 nm was measured for the mixture using a microplate reader (Synergy II multimode, BioTek Instruments, Inc., Winooski, VT, USA, 2014).

2.2. Laboratory Oxygen Uptake Rate (OUR) Monitoring System

The laboratory OUR monitoring system was consisted of a wastewater pump, an air pump, a reaction chamber, an aeration device, a BOD bottle, a magnetic stirrer and a portable dissolved oxygen (DO) detector, as illustrated in Figure 1. After mixing about 100 mL of synthetic wastewater and 20 mL of the activated sludge in a BOD bottle, the activated sludge mixture was aerated until saturated DO (around 10 mg/L in 20 min). The BOD bottle was then sealed and kept stirring by a magnetic stirrer. The change of BOD was measured by a portable DO detector every 1 min at 20 °C until DO declined to 1.0 mg/L. OUR (mg O₂/L·min) was calculated from the dynamic of DO change following the linear regression. The ROUR was calculated according to Equation (1).

$$ROUR = \frac{OUR_n}{OUR_c} \times 100\%$$
(1)

where, OUR_n (mg O_2/L ·min) refers to the OUR postexposure to the *n*th toxins, and OUR_c (mg O_2/L ·min) represent the OUR in negative control.



Figure 1. Laboratory oxygen uptake rate (OUR) monitoring system. ① wastewater pump; ② air pump; ③ reaction chamber; ④ aeration probe; ⑤ BOD bottle; ⑥ stirring bar; ⑦ DO probe; ⑧ magnetic stirrer; ⑨ portable DO detector.

2.3. Online Early-Warning System for Water Quality Based on the Relative Oxygen Uptake Rate (ROUR) Index

To apply the developed method for in situ monitoring the ROUR in WWTPs, an online early-warning system was assembled, consisted of a wastewater tank, a sludge tank, a filter, an aerator, a water pump, a sludge pump, a batch reactor for DO measurement, a DO probe, and a programmable logic controller (PLC), as illustrated in Figure 2a. The wastewater influent was continuously pumped into the wastewater tank after filtering and the activated sludge samples were directly pumped into the sludge tank. They were then mixed together in the batch reactor. The batch reactor was kept stirring (200 rpm), completed aerated (10 min), DO-monitoring (10 min) and finally discarded through the effluent tube. The whole online early-warning system was built up on a mobile platform (650 mm \times 400 mm \times 1200 mm, length \times width \times height) manufactured with galvanized steel plain sheets (Figure 2b,c). The flow rate of wastewater and the activated sludge was 350 and 50 mL/min, respectively. The aeration was achieved by a microporous aerator and the DO after aeration maintained above 6 mg/L. The PLC software was programmed for either automatically or manually controlling operational parameters of the early-warning system, including a main menu (menu selection), a trend menu (real-time illustration of OUR change), a sampling menu (sample injection and discard control), a device menu (device check), a warning menu (alarm for low ROUR and system fault), and a setting menu (parameter setting).



Figure 2. Online early-warning system. (a) Schematic program of the online early-warning system. (b) The mobile platform for the online early-warning system. (c) Inside look of the mobile platform for the online early-warning system. Up-layer, programmable logic controller (PLC); down-layer, reaction system.

2.4. Experimental Procedure

From previous studies, some industrial toxic substances, e.g., Zn²⁺, Cu²⁺, Ni²⁺, Pb²⁺, aromatic hydrocarbons, and antibiotics, were dominant in municipal WWTPs in Jiangsu Province [51–54]. Accordingly, Zn²⁺, Ni²⁺, and phenol were selected as the target toxins representing those substances in this study. In the laboratory OUR monitoring system, the stock solution was prepared by dissolving

1.041 g of ZnCl₂, 1.104 g of NiCl₂, and 0.500 g of phenol in 1.0 L of deionized water. They were then serially diluted in wastewater with the activated sludge and the final concentration ranged from 0 to 60 mg/L (details see Table S1). According to the real operation parameters in most WWTPs in Jiangsu Province (MLSS = 4000–5000 mg/L), MLSS content of the activated sludge was set from 1000 to 5000 mg/L to evaluate the impacts of MLSS on the ROUR. As MLSS had buffering capacities for the inhibitory effects of toxic substances (see Section 3.2), the optimal MLSS content was 2000 mg/L for the measurement of the ROUR and it was set for the following tests to assess the influence of pH and HRT. The pH was adjusted by H_2SO_4 (1.0M) to 2, 4, 5, and 6 in acidic treatments, whereas under alkaline conditions, NaOH (1.0M) was used to adjust the pH value to 8, 9, 10, and 12. The optimal HRT was 20 min and set to 1–60 min to evaluate the impacts of HRT on the ROUR. For all the experiments, the operation temperature was 20 °C.

In the online early-warning system, the MLSS content was 500 mg/L and the HRT was fixed as 10 min. The target toxins included Zn^{2+} , Cu^{2+} , Ni^{2+} , Cr(VI), and Cd^{2+} , prepared by dissolving 1.041 g of ZnCl₂, 1.054 g of CuCl₂, 1.104 g of NiCl₂, 1.951 g of KCrO₄, and 0.816 g of CdCl₂ in deionized water as stock solution. From metal speciation predicted by MINTEQ program [55], all the PTEs were in cation form, except for Cr (90% in form of HCrO₄⁻ and 10% in form of CrO₄²⁻). Accordingly, the final concentration was 0–10 mg/L for Zn²⁺, Cu²⁺ and Ni²⁺, 0–15 mg/L for Cd²⁺ and 0–40 mg/L for Cr(VI), respectively (details see Table S2).

2.5. Data Analysis

All the data are mean \pm standard deviation (SD) from triplicates. Statistical analysis was performed using SPSS software (version 20.0, International Business Machines Corporation, Armonk, NY, USA, 2012). All the data were validated for normality by the Brown-Forsythe test prior to a one-way analysis of variance (ANOVA), and then evaluated using ANOVA followed by Duncan's test. Significant differences (p < 0.05) between the treatments are highlighted.

The decrease in the ROUR is caused by direct inhibition effects of toxic substances in industrial wastewater, which suppress the cell activities and cell metabolisms. Various types of toxic effects are found across toxins, such as membrane integrity loss as the result of cell lysis and protein activity inhibition [56]. Accordingly, the general microbial activity inhibition can be expressed in the following Equation (2), where $k_{Toxicity}$ refers to the toxicity constant for different toxic substances, $k_{Toxicity}^{-1}$ represents the effective concentration 50 (EC₅₀), and $K_{Toxicity}$ is the dynamic toxicity coefficient.

$$K_{Toxicity} = \frac{k_{Toxicity}^{-1}}{k_{Toxicity}^{-1} + [Toxin]}$$
(2)

3. Results and Discussions

3.1. Dose-Effect of Toxic Substances on the ROUR

PTEs exhibited significant inhibition effects on the ROUR (Figure 3). Generally, the ROUR decreased dramatically with the increasing concentration of PTEs, $12.0 \pm 5.3\%$ and $22.0 \pm 15.1\%$ for Zn^{2+} and Ni²⁺ (60 mg/L), respectively. As for phenol, a similar declining behavior was found and the ROUR decreased to $36.0 \pm 5.5\%$ when phenol concentration was 60 mg/L. Additionally, our results indicated a positive correlation between the ROUR-reciprocal and the concentration of toxic substances, suggesting that Hill equation could describe the equilibrium inhibition state [57], as expressed in Equation (3).

$$ROUR = \frac{k_{Toxicity}^{-1}}{k_{Toxicity}^{-1} + [Toxin]}$$
(3)

where $k_{Taxicity}^{-1}$ was 13.40, 15.54, and 8.21 mg/L for Zn²⁺, Ni²⁺, and phenol, respectively.



Figure 3. Dose-effects of Zn^{2+} (**a**), Ni^{2+} (**b**), and phenol (**c**) on the relative oxygen uptake rate (ROUR).

The ROUR reflects the metabolic activities of the activated sludge, which are normally catalyzed by enzymes in microbial cells [41]. As the biodegradation of pollutants by the activated sludge depends on the microbial activities [58], higher concentration of metals can damage enzymatic activities, consequently resulting in the inhibited activities or even death of cells [59,60]. Accordingly, the ROUR decreased gradually with the increasing PTE concentrations. In the present study, the EC_{50} of Zn^{2+} for the activated sludge (13.40 mg/L) was similar with those obtained from individual bioreporter strains, e.g., 10.9 mg/L by Psychrobacter sp. bioreporters [24] and 16 mg/L by Pseudomonas Shk1 bioreporters [42], but much lower than other previous studies on the activated sludge (around 50 to 60 mg/L) [42,61]. Similarly, the EC₅₀ of Ni²⁺ and phenol in the present study was also lower than previous studies on the activated sludge (21–190 mg/L for Ni²⁺ and 416–608 mg/L for phenol) [48,62, 63] or by Pseudomonas Shk1 bioreporters (96 mg/L for Ni²⁺ and 482 mg/L for phenol) [64,65]. Different experimental system might explain this discrepancy. Most of the previous studies used an enclosed system to test the oxygen consumption after 10 min-aeration [42,61], which was easy in operation but hardly applied for online OUR monitoring as the wastewater samples required sequential collection. In contrast, our laboratory monitoring system used the batch reactor by injecting wastewater and the activated sludge simultaneously and cultivating the mixture with different HRT (ranging from 1 to 60 min, 10 min for the dose-effect of PTEs), consequently resulting in relative longer exposure of the activated sludge to the toxic substances and lowering the EC_{50} . Our results suggested that the EC_{50} varied across toxic compounds, and the developed laboratory OUR monitoring system could simulate WWTP-like process and provide accurate EC_{50} values to evaluate the impacts of the shock load by industrial wastewater.

3.2. Impacts of Mixed Liquid Suspended Solids (MLSS) on the ROUR

MLSS had buffering capacities for the inhibitory effects of toxic substances, as the ROUR increased with the MLSS contents for both PTEs and phenol (Figure 4). When the MLSS content was 5000 mg/L, the ROUR reached 99.0 \pm 5.5% and 79.8 \pm 21.5% for Zn²⁺ and Ni²⁺, respectively. Phenol had the same trend and the ROUR increased to 66.6 \pm 12.7% at 5000 mg/L of MLSS. Accordingly, the ROUR-reciprocal was positively correlated with MLSS-reciprocal, as expressed in Equation (4).

$$\mathrm{ROUR}^{-1} \propto [\mathrm{MLSS}]^{-1} \tag{4}$$

As the carrier of microbes in the activated sludge, MLSS content represents the amount of microbial biomass [66]. At higher MLSS or biomass, microbes tend to form aggregates and improve their resistance to toxic substances by decreasing the surface area of single microbial cells directly exposure to toxins [67]. In addition, microbes are capable of excreting extracellular polymeric substances (EPS), which are involved in the detoxification process [68,69]. Accordingly, higher MLSS can improve the resistance of the activated sludge to the shock load from toxins in industrial wastewater, designated as the buffering effect.



Figure 4. The ROUR in treatments with different mixed liquid suspended solids (MLSS) post exposure to toxic substances. (a) Zn^{2+} , (b) Ni^{2+} , and (c) phenol.

3.3. Impacts of Hydraulic Retention Time (HRT) on the ROUR

HRT also showed significant impacts on the ROUR (Figure 5), which declined with the increasing HRT, from $51.0 \pm 5.1\%$ to $41.0 \pm 2.7\%$ (Zn²⁺), $58.0 \pm 10.7\%$ to $22.0 \pm 5.0\%$ (Ni²⁺) and $32.0 \pm 5.8\%$ to $16.0 \pm 2.8\%$ (phenol), respectively. The reciprocal of distance between ROUR and 1.0 (the ROUR value in the absence of toxic substances) was positively correlated with HRT, as expressed in Equation (5). We speculate that the remaining activated sludge activities decreased according to the integral of toxin exposure and linked to the function of HRT, as defined in Equation (6), where ROUR_t represents the ROUR at time = *t*.

$$1 - \text{ROUR} \propto [\text{HRT}]^{-1} \tag{5}$$

$$ROUR = 1 - \int_0^{HRT} ROUR_t dt$$
(6)

Figure 5. The ROUR in treatments with different hydraulic retention time (HRT) post exposure to toxic substances. (a) Zn^{2+} , (b) Ni^{2+} , and (c) phenol.

HRT represents the duration of direct interaction between the activated sludge and toxic substances [48]. As the persistent toxicity causes gradually inactivation of proteins in the active cells [70], the ROUR is dependent on the exposure time or HRT. Previous studies also reported that the increasing HRT led to the decrease of MLSS, EPS concentration, sludge viscosity, and the ratio of food to microorganisms [71]. Therefore, the inhibition of microbial activities in the activated sludge increased with higher HRT.

3.4. Impacts of pH on the ROUR

The effects of pH on the ROUR behaved as a bell shape, as illustrated in Figure 6. The ROUR peaked at pH 6–10 and achieved above 80% comparing to that in the absence of toxic substances. It declined dramatically to below 10% under extremely acidic (pH < 4) or alkaline (pH > 10) conditions. The activated sludge was reported to be sensitive to pH, as most bacteria can only survive in a relatively narrow range of pH [72] and the activities of some microorganisms were inhibited under

low or high pH conditions [73]. For example, pH from 5.1 to 6.1 markedly depressed the metabolisms of the activated sludge [74]. Additionally, pH control was critical for optimizing the performance of WWTPs [75]. Our results suggested that the ROUR was quite stable within a broad range of pH (6–10), covering most of the operational pH in WWTPs, and was suitable as an indicator for online early-warning system measuring the shock load of industrial wastewater.

Figure 6. Impacts of pH on the ROUR.

3.5. Toxicity Model Predicting the ROUR under Different Conditions

As discussed above, the ROUR was significantly affected by the concentration of toxic substances, MLSS content, and HRT. It is therefore important to develop a mathematical model simulating the effects of both toxic substances and environmental variables to compare the ROUR in different WWTPs with distinct operation parameters. In the present study and considering the microbial activity inhibition by toxic substances as described in Equation (1), the integral effects of these parameters were expressed in the following Equation (7) to predict the ROUR of the activated sludge.

$$ROUR = 1 - \frac{\int_{1}^{t} OUR_{t} \cdot dt}{OUR_{0,0}} = 1 - \frac{\int_{0}^{t} \left(\frac{k_{Toxicity}^{-1}}{k_{Toxicity}^{-1} + [Toxin]/[MLSS]} \times OUR_{0,t}\right) \cdot dt}{OUR_{0,0}}$$
(7)

Here, $OUR_{0,0}$ and $OUR_{0,t}$ represent the OUR in the absence of toxic substances at time = 0 and t, respectively. If the OUR of the activated sludge is consistent along with time, the equation can be converted as Equation (8) and fit well with Equations (3) to (7) above.

$$ROUR = 1 - \frac{k_{Toxicity}^{-1}}{k_{Toxicity}^{-1} + [Toxin]/[MLSS]} \times HRT$$
(8)

Although numerous studies have reported the impacts of toxin concentration and other parameters on the OUR or other indicators representing the activated sludge activities [27,28,32], our work is the first attempt to explain the toxic effects in a theoretical model, and the results suggested that toxic substances, MLSS and HRT were all key factors affecting the ROUR.

3.6. Online Monitoring the ROUR for Industrial Wastewater Containing Potential Toxic Elements (PTEs)

The performance of the online early-warning system based on the ROUR index was illustrated in Figure 7. Different PTEs showed distinct effects on the ROUR and the predicted ROUR by the developed toxicity model matched the experimental data. All the ROUR decreased with the increasing concentrations of PTEs but to different extent. According to the model calculation, the EC₅₀ value of Zn²⁺, Ni²⁺, Cr(VI), Cu²⁺, and Cd²⁺ on the activated sludge activity was 13.40, 15.54, 97.56, 12.01, and

14.65 mg/L, respectively (Figure 7f). It is worth mentioning that the metal toxicity followed the order: $Cu^{2+} > Zn^{2+} \approx Cd^{2+} \approx Ni^{2+} > Cr(VI)$, consistent with the results from previous studies as $Cu^{2+} > Zn^{2+} \approx Cd^{2+} > Ni^{2+}$ [59] and Ni²⁺ > Cr(VI) [49].

Figure 7. Impacts of potential toxic elements (PTEs) on the ROUR by the online early-warning system. (a) Zn^{2+} , (b) Ni^{2+} , (c) Cr(VI), (d) Cu^{2+} , (e) Cd^{2+} , (f) $k_{Toxicity}$ of different PTEs.

Zn²⁺ and Ni²⁺ in industrial wastewater are mainly originated from electroplating in various industries including automobiles, construction, shipbuilding, light, etc. [48,76,77]. The predominant sources of Cr(VI) are industrial wastewater derived from the processing of chrome ore, metal surface treatment, leather tanning, printing, and dyeing [78]. High concentrations of Cu²⁺ are often found in the discharge from electrical and electronic industries, light industry, machinery manufacturing, construction industry, and national defense industry [79–81]. The majority of Cd^{2+} in industrial wastewater is from the activities of electroplating, pigments, plastic stabilizers, alloys, and battery manufacturing [82,83]. Data from eight typical WWTPs of five cities in China showed that the metal concentrations in industrial wastewater are averagely 1755 mg/L for Zn^{2+} , 68 mg/L for Ni²⁺, 317 mg/L for Cr(VI), 524 mg/L for Cu²⁺, and 17 mg/L for Cd²⁺, respectively [84]. Comparing to the EC₅₀ value obtained in this study (Figure 7f), municipal wastewater with 0.76% (volume) of the shock load from industrial wastewater containing Zn²⁺ will lose 50% of the activated sludge activities and might cause the failure of WWTPs. The volume percentage of the shock load from industrial wastewater potentially resulting in the WWTP paralysis was 22.85% (Ni²⁺), 30.78% (Cr(VI)), 2.29% (Cu²⁺), and 86.18% (Cd²⁺). Accordingly, WWTPs are more sensitive to the shock load of industrial wastewater containing Zn^{2+} , followed by Cu²⁺, Ni²⁺, Cr(VI), and Cd²⁺. Zn²⁺ and Cu²⁺ in industrial wastewater are very sensitive toxic substances affecting the performance of WWTPs and should be defined as prior pollutants for WWTP management. Ni²⁺ and Cr(VI) are considerably sensitive toxins in industrial wastewater, whereas Cd^{2+} is the least sensitive metal on the activated sludge activities in WWTPs although its EC_{50} value is similarly as other PTEs.

4. Conclusions

In the present study, we assembled an online early-warning system and successfully applied it for monitoring the activated sludge activities under the shock load from industrial wastewater based on the measurement of the ROUR. The ROUR was sensitive to the concentration of toxic substances (0–60 mg/L), MLSS content (1000–5000 mg/L), and HRT (0–60 min), remaining high stability in a broad range of pH (6–10). A toxicity model was developed to simulate the influent toxicity under different operation parameters and could be used in the online early-warning systems to predict the influence of toxic substances from industrial wastewater across WWTPs. Both experimental data and model prediction suggested that PTEs remarkably affected the activated sludge activities and the influence was dependent on metal species. Considering the order of PTE's EC₅₀ values (Cu²⁺ < Zn²⁺ < Cd²⁺ < Ni²⁺ < Cr(VI)), the performance of WWTPs showed different sensitivity and response to the shock load from industrial wastewater containing different PTEs. This study provides an effective on-line toxicity monitoring system for early-warning WWTPs facing the shock load of industrial wastewater and is helpful for WWTP management to achieve stable and satisfactory performance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/9/1/154/s1, Table S1: Concentrations of toxic compounds for the laboratory ROUR system, Table S2, Concentrations of toxic compounds for the online early-warning system.

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