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Investigating the performance of three modified activated sludge processes treating municipal wastewater in organic pollutants removal and toxicity reduction

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ABSTRACT

This study investigated the treatment performance of three types of modified activated sludge processes, i.e., anoxic/oxic (A/O), anaerobic/anoxic/oxic (A2/O) and oxidation ditch process, in treating municipal wastewater by measuring physicochemical and spectroscopic parameters, and the toxicity of the influents and effluents collected from 8 full-scale municipal wastewater treatment plants (MWTPs). The relationships between spectroscopic and physicochemical parameters of the wastewater samples and the applicability of the nematode *Caenorhabditis elegans* (*C. elegans*) bioassays for the assessment of the toxic properties of municipal wastewater were also evaluated. The results indicated that the investigated MWTPs employing any of A/O, A2/O and oxidation ditch processes could effectively control the discharge of major wastewater pollutants including biochemical oxygen demand (BOD), chemical oxygen demand, nitrogen and phosphorus. The oxidation ditch process appeared to have the advantage of removing tyrosine-like substances and presented slightly better removal efficiency of tryptophan-like fluorescent (peak T) substances than the A/O and A2/O processes. Both ultraviolet absorbance at 254 nm and peak T may be used to characterize the organic load of municipal wastewater, and peak T can be adopted as a gauge of the BOD removal efficacy of municipal wastewater treatment. Using *C. elegans*-based oxygen consumption rate assay for monitoring municipal wastewater toxicity deserves further investigations.

1. Introduction

Municipal wastewater treatment plants (MWTPs) receive domestic and industrial sewage and remove solids, organic matters and nutrients by physical, chemical and biological treatment methods to achieve a significant reduction in pollutants and ecotoxicity in the receiving surface or ground water (Morris et al., 2017). The most commonly utilized biological treatment method is an activated sludge process, which is designed to substantially remove biodegradable dissolved and colloidal organic matter (Hashimoto et al., 2014). To further treat specific wastewater constituents (nitrogen, phosphorus, etc) that can't be effectively removed by the conventional activated sludge process, certain modified activated sludge processes are used more frequently by MWTPs (Wang et al., 2017).

The anoxic/oxic (A/O), anaerobic/anoxic/oxic (A2/O) and oxidation ditch processes are the three typically used types of modified activated sludge processes. In China, these processes have been adopted in 60% of the wastewater treatment plants and treat 51% of the total volume of wastewater generated (Zhang et al., 2016). The A/O process includes anoxic and aerobic reactors. Organic carbon is removed by aerobic microorganisms; meanwhile, ammonia is converted aerobically to nitrate, which is then reduced to nitrogen gas in an anoxic reactor (Cui and Jahng, 2004). Compared with the A/O process, the A2/O process adds an anaerobic reactor prior to the anoxic and aerobic reactors and thereby provides an environment that encourages the growth of phosphorus accumulating organisms that uptake excessive

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phosphate in aerobic reactors, and phosphorus is removed with the waste sludge (Kim et al., 2013). Thus, A2/O process can attain the effective removal of both nitrogen and phosphorus. The oxidation ditch process utilizes large round or oval ditches (channel reactors) with one or more horizontal aerators, which allows for the simultaneous removal of carbon, nitrogen, and phosphorus from sewage with long solid retention times and repetitive aerobic/anaerobic treatment phases (Terashima et al., 2016; Xu et al., 2017). Oxidation ditch technology is a good choice for small-scale and medium-scale MWTPs because of its simple infrastructure and convenient management, but for large-scale MWTPs, the A2/O and A/O technologies are more appropriate due to the lower capital investment per unit of wastewater treatment (Jin et al., 2014). Although the selection of a wastewater treatment process is a comprehensive consideration of technological, economical and environmental factors, the effective removal performance is the key point to ensure qualified effluent.

Physicochemical parameters, particularly the chemical oxygen demand (COD) and biochemical oxygen demand (BOD), are traditionally used to evaluate the pollutant removal efficiency of the MWTPs. However, the frequent monitoring of COD and BOD is relatively expensive and time-consuming. Spectral measurements, including ultraviolet (UV)-visible absorbance and fluorescence signals, are cheap and rapid analyses that offer particularly promising solutions for the surrogate monitoring of COD and BOD (Henderson et al., 2009; Thomas et al., 1996). Moreover, a series of UV indices such as the absorbance at 254 nm (UV_{254}) and the ratio of the absorbance at 250 nm to that at 365 nm (UV_{250}/UV_{365}) may provide insight into the nature of the organics present in municipal wastewater. UV_{254} is generally linked to the high molecular weight and hydrophobic (aromatic) content of natural organic matter in water (Liu et al., 2016). The UV_{250}/UV_{365} ratio has been introduced to characterize the aromaticity and molecular size of organic matter. This ratio increases as the aromaticity and molecular size decrease (Uyguner and Bekbolet, 2005). A three-dimensional fluorescence excitation-emission matrix (3D-EEM) can be generated by measuring the fluorescence intensity of an aqueous sample at consecutive excitation and emission wavelengths. Thus, complex dissolved organic matter can be characterized according to its fluorescent components. Compared to the UV absorbance, the EEM provides more information regarding the organic matter fractions and their chemical characteristics, and offers at least an order of magnitude more sensitivity for the detection of organic matter (Leenheer and Croué, 2003). Characterizing the changes in organic matter during wastewater treatment can provide valuable information for the selection of the optimum operation conditions and the best biological treatment process. The use of UV–visible absorbance technology and fluorescence EEM technology for performance evaluations of MWTPs is growing as a strong complement to conventional technology that often focus only on a set of quantitative variables that typically includes COD, BOD, and nutrients. To date, however, comprehensive studies investigating the fluorescence of wastewater subjected to modified activated sludge processes, such as the A/O, A2/O and oxidation ditch processes, in full-scale MWTPs are scarce.

Municipal wastewater is a complex mixture of largely unknown substances that may be hazardous to humans and aquatic organisms. Nevertheless, the current physicochemical analysis used for compliance assessment of wastewater and the above-mentioned optical approaches can detect only a limited number of chemicals, and both approaches are unable to evaluate toxic and interactive (additive, antagonistic or synergistic) toxic effects of the chemicals coexisting in wastewater. Therefore, biotoxicity assays that measure the effect of all bioavailable contaminants in wastewater are essential to complement the physicochemical and optical measures of wastewater quality and provide deeper understanding of the process performance of MWTPs. In addition, toxicity testing is vital for assessing the potential harmful effects of wastewater that is discharged into ecosystems and monitoring the toxicants present in wastewater that reduce the efficiency of biological treatment due to the intoxication phenomena (Liwarska-Bizukojc et al., 2016; Xiao et al., 2015).

Caenorhabditis elegans (*C. elegans)* is a highly attractive model for assessing aquatic toxicity. *C. elegans* has a short life cycle and a small body size, is easy to maintain under laboratory condition and allows for the use of high throughput techniques (Chu and Chow, 2002; Tejeda-Benitez and Olivero-Verbel, 2016). *C. elegans* has a high tolerance to pH, salinity, and water hardness and offers a wide variety of ecologically and toxicologically relevant endpoints, such as mortality, growth, and reproduction (Chu and Chow, 2002; Tejeda-Benitez and Olivero-Verbel, 2016). A *C. elegans* bioassay with the mortality as endpoint has been used to evaluate the toxicity of municipal and industrial wastewater (Hitchcock et al., 1997). Oxygen is a key metabolite of aerobic organisms, and the rate of oxygen uptake reflects metabolic activity, health and responses to various stimuli (Schouest et al., 2009). Respirometric toxicity assay based on measuring the oxygen consumption rate (OCR) of organisms offers a sub-lethal parameter and has been used to assess the toxicity of different types of toxicants (e,g., metals, pesticides, polycyclic aromatic compounds, drinking water disinfection by-products) and industrial wastewater samples on various species including microorganisms, mammalian cells and whole animals (Kungolos, 2005; Schouest et al., 2009; Zitova et al., 2009; Zuo et al., 2017). Although *C. elegans* is a valuable toxicity model, to the best of our knowledge, no study has been conducted utilizing *C. elegans* respirometric toxicity assays for the evaluation of the municipal wastewater toxicity properties.

Understanding the treatment performance of modified activated sludge processes is important for the selection and design of biological wastewater treatment technologies. This study focuses on revealing the differences in organic pollutants removal and toxicity reduction in MWTPs using A/O process, A2/O process, and oxidation ditch process, respectively. Treatment efficiency of 8 MWTPs was investigated by traditional physicochemical analysis in combination with UV–visible and EEM spectroscopy and *C. elegans* biotoxicity assays. The links between physicochemical and spectroscopic parameters of the wastewater samples were also assessed to determine whether the spectra analysis could be used as a monitoring tool of treatment efficiency in MWTPs. In addition, the applicability of the *C. elegans*-based respirometric bioassays in the assessment of the toxic properties of municipal wastewater was initially evaluated.

2. Methods and materials

2.1. Wastewater sampling

Sixteen untreated influent and final-treated effluent samples were collected from eight MWTPs, namely, the Longwangzui (LWZ), Erlangmiao (ELM), Shahu (SH), Sanjintan (SJT), Nantaizihu (NTZH), Huangjiahu (HJH), Tangxunhu (TXH), and Luobuzui (LBZ) MWTPs, in Wuhan, which is the capital city of Hubei Province in Central China. Wuhan is located at the confluence of the Han and Yangtze Rivers and has a population of more than 10 million. The wastewater emission of Wuhan in 2015 reached 924 million tons, including 155 million tons of industrial wastewater and 769 million tons of domestic wastewater, and approximately 85.61% of the wastewater is treated in 26 MWTPs. The average volume of wastewater treated daily in the eight MWTPs investigated accounted for 70% of the total average volume of wastewater treated daily in all MWTPs in Wuhan city. At the eight MWTPs, the influent wastewater undergoes the following four major processes: preliminary treatment by coarse and fine screeners for the removal of coarse solids and other large materials, primary treatment by sedimentation for the removal of settleable organic and inorganic solids, secondary treatment using biological digestion by bacteria for the removal of biodegradable dissolved and colloidal organic matter and the nutrients nitrogen and phosphorus, and tertiary treatment using chlorine to kill pathogens, such as bacteria and viruses. The major