Adsorption mechanisms and impact factors of oxytetracycline on activated sludge

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Abstract: The adsorption mechanisms and the effect of Oxytetracycline (OTC) onto activated sludge were studied. The results show that the adsorption of Oxytetracycline (OTC) onto activated sludge was coincident with the Pseudo-second-order kinetic model which suggested that chemical adsorption mechanism was dominant. The influences including pH and metal ions on the OTC were examined. It was demonstrated that the adsorption process was highly pH-dependant, which indicate that cationic exchange mechanisms may play an important role in the adsorption process. Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cd²⁺ ions more or less inhibited the adsorption of OTC on activated sludge while Cu²⁺ enhanced the adsorption ability. The phenomenon may reflect the result that a surface complexation mechanism could involved in the adsorption.

1. Introduction
Tetracycline (TC), has been used extensively world wide as a feed additive to treat diseases and thus improve the growth rate of livestock. As in the case with other antibiotics, tetracycline is poorly adsorbed in the digestive tract. As a result, 50 – 80% of the initial TCs dose was discharged in livestock waste [1]. TCs residues have been found everywhere in the environment, including soils [2], sediments [3], surface water [4], and wastewater [5,6]. The presence of low levels of antibiotics and their byproducts in the environment is favorable for the transfer and spread of antibiotic resistant determinants among microorganisms, and cause public health issues [7]. Therefore, it is of great importance to fully understand the occurrence, fate, transport and transformation of antibiotics in the environment [1].

The activated sludge process is the most common form of secondary treatment employed in China. Tetracycline originating from livestock is discharged into the sewer (with or without in situ pretreatment) and ends up in municipal wastewater treatment plants (WWTPs) [8]. The problem of Tetracycline pollution has drawn great attention of researchers all over the world in recent years. However, knowledge gaps still exist on the interaction between TCs and activated sludge. Some previous studies on pure clays, soils, and sediments had proven that the adsorption capacity of sorbent to TCs was influenced by pH due to different mechanisms [9-11], but little research has been focused on the effect of the adsorption of OTC on activated sludge [12]. So, it is of significant importance to understand the factors that affect tetracycline adsorption by activated sludge and the adsorption mechanism.

In this study, Oxytetracycline, was selected as a representative compound for the investigation of the adsorption behavior of TCs on activated sludge. The study investigates the effect of pH and metals on adsorption - in aqueous solution using batch experiments. The overall objectives of this study were...
(1) To evaluate the kinetics of OTC adsorption onto activated sludge, (2) To investigate effects of pH and heavy metals on the adsorption of OTC on activated sludge, and (3) to discuss the interaction mechanisms between OTC and activated sludge based on OTC molecular structure and the results of FTIR analyses.

2. Materials and methods

2.1 Sorbents
The activated sludge used in this study was obtained from the stage 1 aeration basin in the wastewater treatment plant for the Technical and Economic Development Area (TEDA), Tianjin, China. After collection, the aerobic activated sludge samples were aerated in a well mixed 12L sequencing batch reactor (SBR). Conditions in the reactor were maintained at an agitation rate of 150 rpm, temperature of 25°C, pH of 6.5-6.8, hydraulic retention time (HRT) of 24 h, sludge retention time (SRT) of 15 days and dissolved oxygen (DO) from 2 to 4 mg/L. Synthetic wastewater[13] was fed into the reactor to keep the organic loading rate of the system at about 500 mg COD/L·d. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentration were 1.2 ± 0.1 and 0.9 ± 0.1 g/L, respectively. After 30 days, the activated sludge in SBR reactor achieved stable properties and adsorption efficiency.

In sorption tests, mature activated sludge was used. The sludge was washed three times using deionized water to remove the surface soluble ions, and then sealed into flasks for storage at 4 °C. The treated sludge was diluted to the required experimental concentrations in subsequent adsorption experiments[14].

2.2 Chemicals
Hydrochloride salt of OTC (98% purity), acetonitrile (HPLC grade), and oxalic acid (99% purity) were purchased from sango biotech. (Shanghai, China). All other chemicals, including Cadmium, sodium, potassium, copper, calcium, and magnesium salts, were A.R. grade bought from Sinopharm Chemical Reagent Co. (Shanghai, China).

2.3 Analysis of OTC
The concentration of OTC in the supernatant filtrate was analyzed directly using a high performance liquid chromatography system HPLC (Waters 2695, USA) equipped with a Supercol C18 reverse-phase column (5μm, 4.6 × 200 mm) with column temperature at 30°C. The mobile phase consisted of acetone trile (23%) and 10 MM oxalic acid (77%) at a flow rate of 1.0 mL/min. OTC was analyzed by a UV detector at 355nm. The detection limit of HPLC was 0.1 mg/L. The calibration curve was statistically significant and linear in the concentration range of 0.5-20 mg/L (p<0.05, R2=0.9996).

3. Results and discussion

3.1 Adsorption kinetics of OTC
The adsorption kinetic parameters of OTC onto activated sludge are illustrated in table 1. The high correlation coefficient (R2>0.999) shows that pseudo-second-order kinetic model was more suitable for description of sorption kinetics for the adsorption of OTC than pseudo-first-order kinetic model, while the theoretical values of qe calculated from pseudo-second-order kinetic corresponed well with the experimental value of maximum sorption amount. The pseudo-second-order kinetics model is used to describe chemisorptions and has been widely applied to the adsorption of pollutants from aqueous solutions in recent years[6]. The pseudo-second-order rate equation is as follows:

\[ \frac{dq_t}{dt} = k_2(q_e - q_t)^2 \]

Where \( k_2 \) (g mg\(^{-1}\) h\(^{-1}\)) is the rate constant of adsorption, \( q_e \) (mg g\(^{-1}\)) is the amount of OTC
adsorbed at equilibrium, and \( q_e \) (mg g\(^{-1}\)) is the amount of OTC adsorbed on the surface of the adsorbed at any time.

Rearranging Eq. (1) into a linear form, we obtained

\[
\frac{t}{q_i} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \tag{2}
\]

Where \( k_2 q_e^2 \) (h) is the initial rate (mg g\(^{-1}\) h\(^{-1}\)). The pseudo-second-order rate constants \( k_2 \) and \( q_e \), were calculated from the slope and intercept of the plots of \( t/q_i \) versus \( t \). As shown in the Fig. 1, the plots showed good linearity under different conditions of MLSS and R’s were greater than 0.999. Kinetics of OTC adsorption on activated sludge followed the pseudo-second-order model, suggesting that the biosorption of OTC onto activated sludge is a chemical-sorption process. The process involves exchange or sharing of electrons mainly between cation and functional groups (hydroxyl and carboxyl groups) of the biomass cells\(^8\). The equilibrium adsorption capacity \( q_e \) (mg g\(^{-1}\)) of activated sludge is varies depending on the initial concentration of adsorbent. Table.1 and Fig. 1 show that, adsorption capacities decreases with an increase in the initial concentration of adsorbent. A certain amount of activated sludge can adsorb a certain ratio of OTC. If the initial concentration of adsorbent increase while OTC amount kept constant, the specific removal efficiency of OTC by activated sludge is decreased.

**Table 1.** Pseudo-first-order and pseudo-second-order adsorption rate constants of OTC on activated sludge in different MLSS.

| MLSS (mg L\(^{-1}\)) | First-order constants | Second-order constants |
|----------------------|-----------------------|-----------------------|
|                      | \( c_0 /\text{mg L}^{-1} \) | \( q_d /\text{mg g}^{-1} \) | \( K_1 /\text{h}^{-1} \) | \( R^2 \) | \( K_2 /\text{g (mg h)}^{-1} \) | \( q_e /\text{mg g}^{-1} \) | \( R^2 \) | \( h /\text{mg h}^{-1} \) |
| 400                  | 10                    | 13.9                 | 0.216                 | 0.962 | 0.107 | 25.00 | 0.999 | 66.7       |
| 800                  | 10                    | 4.98                 | 0.169                 | 0.885 | 0.390 | 12.66 | 0.999 | 62.5       |
| 1600                 | 10                    | 1.51                 | 0.197                 | 0.850 | 1.976 | 6.49  | 1     | 83.3       |
| 2000                 | 10                    | 0.91                 | 0.154                 | 0.692 | 3.725 | 5.18  | 1     | 100.0      |

The initial adsorption rates were 62.5, 66.7, 83.3 and 100 mg g\(^{-1}\) h\(^{-1}\), when the initial MLSS concentration was 400 mg/L, 800 mg/L, 1600 mg/L, and 2000 mg/L, respectively. This is may be because the mass transfer driving increases with increasing MLSS concentration resulting in OTC molecule adsorbing to the sludge surface in a short period of time\(^7\). These initial rates are slow when compared to TC adsorption on palygorskite\(^8\) and smectites\(^9\) due to a lower TC adsorption capacity of 25 mg g\(^{-1}\) on activated sludge compared to 30 mg g\(^{-1}\) on paly-gorskite\(^7\) and 441 mg g\(^{-1}\) on smectite\(^9\). However, the fitted rate constant was 3.725 g (mg-h\(^{-1}\)), when the initial MLSS concentration was 2000 mg/L, which was much larger than 0.13 and 0.43 g (mg-h\(^{-1}\)) for TC adsorption on palygorskite\(^8\) and smectite\(^7\). Such a large rate constant indicates that activated sludge is a good adsorbent for OTC removal.
Fig. 1. Curve of the Sorption kinetic of oxytetracycline (OTC) fitted into the pseudo-second-order kinetics model.

3.2 The effect of metal ions on Adsorption of OTC on activated sludge
In Figure 2, compared with the control group, tests containing Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and Cd\(^{2+}\) suppressed the adsorption of OTC on activated sludge to varying degrees. Cations initially present on the activated sludge surface affect OTC adsorption by competing for surface sites. These may also interfere with cation exchange mechanism between OTC and the activated sludge surface in low pHs. Generally, the replacing power of a cation increases with its charge, which may explain why Ca\(^{2+}\), Mg\(^{2+}\) and Cd\(^{2+}\) suppressed the adsorption of OTC on activated sludge more than Na\(^+\) and K\(^+\). This phenomenon was consistent with previous study examining OTC adsorbing to clay \([15-16]\).

![Fig. 2. Amount of oxytetracycline (OTC) adsorbed on activated sludge at three pH values as affected by metal ions.](image)

As seen in Fig 2, Cu\(^{2+}\) enhanced the adsorption of OTC to activated sludge at all pH values. Previous studies showed that Cu\(^{2+}\) could form strong inner-sphere complexes (binding with amide I group) with activated sludge \([17]\) and that OTC can easily complex with metal cations \([18]\). It is known that Cu\(^{2+}\) chelates with OTC at a ratio of 1:1 and the stability constant of the chelate complex is 1012.4 \([15]\). This value is much higher than those of the Ca\(^{2+}\) and Mg\(^{2+}\) ions with OTC (106.4, and 105.8, respectively) \([19]\). Therefore, Cu\(^{2+}\) might act as a bridge between activated sludge surfaces and OTC by forming surface species. Ca\(^{2+}\), Mg\(^{2+}\), and Cd\(^{2+}\) cannot act as a bridge ion to increase the adsorption of OTC \([20]\). So, different effects of metal ions on the adsorption of OTC derive from the interaction mechanisms of metal ions with OTC and activated sludge surface functional groups.

As seen in Fig 2, it is noteworthy that pH influences the adsorption process in different ways depending on which metal ion is being tested. Maximum adsorption was obtained at pH 5.5 which is consistent with the above experiment.

3.3 FTIR analysis
A qualitative and preliminary analysis of the vibrational frequency changes of the main functional groups on the activated sludge that might be involved in adsorption process can be assessed by FT-IR. The peaks at 3345, 2924, 2361, 1742, 1644, 1604, 1542, 1440, 1193, and 883 cm\(^{-1}\) showed that native activated sludge had characteristic bands of chitin, acidic polysaccharides, lipids, amino acids proteins and cellulose \([21]\), which may be able to react with OTC. Some similar functional groups were found in biosorption of nickel (II) onto activated sludge.
Fig. 3. FTIR analysis of native activated sludge, after OTC biosorption, after OTC + Cd²⁺ biosorption, and after OTC+Cu²⁺ biosorption, indicating the involvement of various functional groups OTC and metal ions biosorption.

A significant change after the contact of OTC with activated sludge was a shift in the peak of C-O and phenolic -OH from 1193 cm⁻¹ to 1190 cm⁻¹. This shift indicated that direct complexation between OTC and functional groups of activated sludge might have occurred. What’s more, a slight increase of wave numbers from 1542 cm⁻¹ to 1550 cm⁻¹ after reaction with OTC was probably caused by electronic induced effects, indicating a possible adsorption mechanism involving cation-π interactions between the OTC functional groups and activated sludge.

The presence of Cu²⁺, during the OTC treatments, lead to a shift in the peak of COOH from 1644 cm⁻¹ to 1629 cm⁻¹, a shift in the peak of C-O and phenolic-OH from 1190 cm⁻¹ to 1150 cm⁻¹, and enhancement of the peak that was assigned to aromatic C=O and carboxylate groups to 1383 cm⁻¹. What’s more, a combination band from the -N-H bending mode and the C-N stretching mode of the amide (II) moiety at 1604 cm⁻¹ shifted to lower energy. These changes suggested that the carboxylic, amide (II) moiety of activated sludge and phenolic groups reacted with Cu²⁺ to form inner-sphere surface complexes, thus forming activated sludge-O≡Cu-OTC ternary complexes [15]. Therefore, Cu²⁺ could have played a bridging role, indicating a possible OTC adsorption mechanism of metal bridging and proving the above hypothesis. The presence of Cd²⁺ caused the peak of -N-H and C-N to shift from 1604 cm⁻¹ to 1598 cm⁻¹. The shift indicates Cd²⁺ interacts to some extent with amide (II). There was no evidence that Cd²⁺ interacts with aromatic compounds in FT-IR spectrum. Therefore, Cd²⁺ could not play a bridging role like Cu²⁺ but filled more adsorption points thereby suppressing the adsorption of OTC on activated sludge.

4. Conclusions

Activated sludge was an effective sorbents to OTC. Pseudo-second-order kinetic model was suitable for describing adsorption kinetics of OTC. The initial rate attain to 100 mg g⁻¹ h⁻¹ at MLSS concentration was 2000 mg/L. The presence of heavy metals had varying effects on OTC adsorption to activated sludge. Cu²⁺ enhanced the adsorption apparently while Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cd²⁺ suppressed adsorption. Multivalentions suppress adsorption onto activated sludge more than monovalent ions. According to FTIR analysis, the main adsorption mechanisms of OTC to activated sludge include cation exchange, surface complexation, metal bridging and cation-π interactions.

Acknowledgments

This work was financially supported by the Tianjin Science and Technology Research Projects (14TXSYJC00453) and (16YDLJSF00030).
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