Effects of surfactants on the combined toxicity of TiO₂ nanoparticles and cadmium to *Escherichia coli*

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ABSTRACT

The combined ecological toxicity of TiO₂ nanoparticles (nano-TiO₂) and heavy metals has been paid more attention. As the common pollutants in water environment, surfactants could affect the properties of nanoparticles and heavy metals, and thus further influence the combined toxicity of nano-TiO₂ and heavy metals. In this study, the effects of sodium dodecyl benzene sulfonate (SDBS) and Tween 80 on the single and combined toxicities of Cd^{2+} and nano-TiO₂ to *Escherichia coli* (*E. coli*) were examined, and the underlying influence mechanism was further discussed. The results showed both SDBS and Tween 80 enhanced the toxicity of Cd^{2+} to *E. coli* in varying degrees. The reaction of SDBS and Cd^{2+} could increase the outer membrane permeability and the bioavailability of Cd, while Tween 80 itself could enhance the outer membrane permeability. The combined toxicity of nano-TiO₂ and Cd^{2+} to *E. coli* in absence of surfactant was antagonistic because of the adsorption of Cd^{2+} to nano-TiO₂ particles. However, in the presence of SDBS, both SDBS and nano-TiO₂ influenced the toxicity of Cd^{2+}, and also SDBS could adsorb to nano-TiO₂ by binding to Cd^{2+}. The combined toxicity was reduced at Cd^{2+} lower than 4 mg/L and enhanced at Cd^{2+} higher than 4 mg/L under multiple interactions. Tween 80 enhanced the combined toxicity of nano-TiO₂ and Cd^{2+} by increasing the outer membrane permeability. Our study firstly elucidated the effects of surfactants on the combined toxicity of nano-TiO₂ and Cd^{2+} to bacteria, and the underlying influencing mechanism was proposed.
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Introduction

In the past decades, the ecological risks of engineered nanomaterials have attracted worldwide attention. However, in real environments, the coexistence of nanoparticles with other chemicals is inevitable, and the interaction between nanoparticles and other chemicals could change their properties alone, thereby affecting their toxicities. The combined toxicity of nanoparticles and the coexisting chemicals has been paid more attention (Canesi et al., 2015).

TiO₂ nanoparticles (nano-TiO₂) are one of the most widely used nanomaterials, which would enter environment during their production, utilization, and disposition. It was reported that the toxicity of nano-TiO₂ to aquatic organism and bacteria varied from negligible to intermediate (Adams et al., 2006; Heinlaan et al., 2008; Jiang et al., 2009; Amiano et al., 2012; Clemente et al., 2012; Li et al., 2014; Lin et al., 2014; Fekete-Kertesz et al., 2017), which mainly depended on the properties of nanoparticles, organism species, exposure time and illumination conditions. However, when the toxic chemicals such as heavy metals coexist with nano-TiO₂, the toxicity of coexist chemicals could be enhanced or alleviated. Although a lot of work has been done, the effect of nano-TiO₂ on the toxicity of heavy metals was still in dispute. For green alga, it was

reported that the toxicity of heavy metals could be decreased by nano-TiO$_2$ (Yang et al., 2012; Chen et al., 2015). In contrast, for those multi-cellular organisms, which could actively ingest nanoparticle-metal complexes and assimilate surface-adsorbed metal ions through the dietary pathway, nano-TiO$_2$ can act as a heavy metal carrier and enhance heavy metal bioavailability, such as for daphnia (Fan et al., 2011, 2012; Tan et al., 2012; Tan and Wang, 2014), ciliate (Yang et al., 2014). However, the reduced toxicities of heavy metal by nano-TiO$_2$ for *Mytilus galloprovincialis* and *Gammarus fossarum* were observed by Della Torre et al. (2015) and Rosenfeldt et al. (2015), respectively. The unchanged toxicities of Cd^{2+} in the presence of nano-TiO$_2$ were also observed for *M. galloprovincialis*, *Lumbriculus variegates*, and *Daphnia magna* by Hartmann et al. (2012) and Balbi et al. (2014). The different results could be due to different experimental conditions.

Although a lot of work has been done for the combined toxicity of nanoparticles and toxic chemicals, the effect of organic matter including natural organic matter and surfactants common in water could not be ignored, which would influence the combined toxicity of nanoparticles and heavy metals by interacting with them. Fan et al. (2016) showed that nano-TiO$_2$ significantly decreased Cu accumulation in *D. magna*, but the reducing effect of nano-TiO$_2$ was eliminated in the presence of humic acid. Besides natural organic matter, surfactants are the chemicals that are most likely to coexist with nanoparticles (NPs). They have been widely used in diverse products, such as motoroils, pharmaceuticals, detergents, and flotation agents, and modification of nanoparticles. Consequently, the coexistence of NPs and surfactants may be common in the environment. Once surfactants are coexisting with nanoparticles and heavy metals, more complicated interactions would happen to them. Surfactants would change the surface properties of nanoparticles and the bioavailability of heavy metals, and further the interaction between nanoparticles and heavy metals. There are few studies on the effect of surfactant on the combined toxicity of nanoparticles and heavy metal. The effect of surfactant on the toxicity of nanoparticles has been studied by some researchers. Wang et al. (2014) indicated that surfactants with different ion types can alter the properties of NPs (i.e., particle size and surface charge) in different ways and present complex joint effects on NP toxicities. Oleszczuk et al. (2015) reported that surfactants decreased the toxicity of ZnO, TiO$_2$ and Ni nanoparticles to *Daphnia magna* which most probably was related with the formation of engineered nanoparticles (ENPs) aggregates that inhibited the availability of ENPs for *D. magna*. The combined toxicity of surfactants and heavy metals to rainbow trout reported by Calamari and Marchetti (1973) showed that a "more-than-additive" effect existed for anionic detergents and metals, while "less-than-additive" for the mixture of nonionic detergent and metal.

To date, little is known about the effect of surfactants on the combined toxicity of nanoparticles and heavy metals, especially to bacteria. Bacteria are unicellular organisms, which play important role in the degradation of pollutants. The bacterial cells could interact with pollutants directly, and it is reported that nanoparticles could hardly enter the cells actively. The interactions among surfactants, nanoparticles, heavy metals, and bacteria are much more complicated, which are extremely difficult to clarify clearly. In the present

study, we firstly try to uncover the effects of surfactants on the combined toxicity of nano-TiO$_2$ and Cd^{2+} to bacteria, aiming at providing valuable information for the evaluation of combined toxicity of nanoparticles and heavy metals in the presence of surfactants, and exploring the underlying influencing mechanism.

1. Materials and methods

1.1. Materials

TiO$_2$ nanopartilces (anatase, 5–10 nm, purity >99%) purchased from Zhejiang Hongsheng materials and technology company, were used in this study. The stock suspension of nano-TiO$_2$ was prepared with ultrapure water by sonication for 20 min. Cd(NO$_3$)$_2$ (analytic grade) was chosen as the source of Cd^{2+} ions, which was known as a primary pollutant in water systems. Sodium dodecyl benzene sulfonate (SDBS) and Tween 80 were chosen as the representatives of anionic surfactant and nonionic surfactant, respectively. All the chemicals used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd.

Escherichia coli (Genbank No: MG388227) isolated from sewage water was used as the test organism. The bacterial stock suspension was prepared as previous study (Li et al., 2011, 2013), and the final OD$_{600}$ was adjusted with ultrapure water to 1.0.

1.2. Toxicity tests

In order to clarify the effect of nano-TiO$_2$ and surfactants on the toxicity of Cd^{2+} to bacteria, the toxicity test protocol was designed as **Appendix A Table S1** in supporting information. Besides the effects of fixed concentration of nano-TiO$_2$ and surfactant on the dose-effect of Cd^{2+}, effects of nano-TiO$_2$ and surfactant concentrations on the toxicity of Cd^{2+} with a fixed concentration were also examined. Each experiment was repeated for three times and two parallel samples were used for each time.

The toxicity test method was improved according to the previous study (Li et al., 2011). In order to exclude the effect of water chemistry and nutrition, the samples were prepared with ultrapure water. One milliliter bacteria suspension (OD$_{600}$ = 1.0) was added to a 9-mL prepared test sample in a 40-mL vial, and then the samples were placed on the constant temperature shaker (CHA-2, Aohua, Changzhou) for 3 hr at 25°C with the speed of 150 r/min. At the end of experiments, the survival bacterial number was evaluated by determining the bacterial growth in Luria-Bertani (LB) medium. One milliliter of sample from mixed suspension was transferred to a 9-mL sterilized LB liquid medium and incubated at 37°C for observation of bacterial growth. The OD$_{600}$ of the mixed culture media was recorded by UV–Visible spectrophotometer (UV759CRT, Yoke, Shanghai) with 1-hr interval until the bacterial growth reached the stable phase. The bacterial survival of samples comparing with the control group at initial stage of logarithmic phase was in coincidence with that calculated with the plate count method, which was much more tedious in procedure and prone to bring deviation

during the operation. The bacterial growth reduction values (GR, %) may be expressed as Eq. (1).

$$GR = \left(1 - \frac{OD_{it} - OD_{i0}}{OD_t - OD_0}\right) \times 100\% \qquad (1)$$

where the OD_{it} and OD_{i0} were the OD_{600} of sample i at time t and the initial time, respectively, and OD_t and OD_0 were the OD_{600} of the control group at time t and the initial time, respectively. In this study, the second hour was selected as t in Eq. (1).

One-way analysis of variance (ANOVA) was used to analyze the significance among the toxicities caused by varying surfactant concentrations. The nature (antagonistic, synergistic, or additive) of toxicity of mixture of surfactant, Cd^{2+} in absence or in the presence of nano-TiO_2 was determined using the method used by Ince et al. (1999) and Srivastava and Kumar (2017).

1.3. Determination of dissolved cadmium and characterization of nano-TiO_2

After the 3-hr toxicity exposure test, the sample was centrifuged at 4000 r/min for 15 min and then filtered through a 0.22-μm hydrophilic filter membrane, finally the filtrate was used to analyze the dissolved cadmium concentration with inductive coupled plasma emission spectrometer (Prodigy7, Teledyne Leeman Labs).

The hydrodynamic size and zeta potential of nano-TiO_2 in various exposure media were characterized with a zetasizer (Nano-ZS90, Malvern Instruments, UK). The concentrations of nano-TiO_2, surfactants, and Cd^{2+} were 100, 50, and 10 mg/L, respectively.

1.4. Settling experiments

Effect of surfactants or Cd^{2+} on the stability of nanoparticles could be reflected by the sedimentation of nanoparticles. In order to simulate the effect of hydrodynamic process, the tests were conducted in shaken condition with a speed of 150 r/min.

Effect of surfactants and Cd^{2+} on the stability of nano-TiO_2 (100 mg/L) was observed by determining the OD_{600} of the mixed suspension prepared with ultrapure water every 20 min for 3 hr.

In addition, the interaction between nanoparticles and bacteria could also be reflected by the settling experiments. The bacterial concentration was adjusted to be 5 times as much as that used in toxicity exposure tests in order to enhance the possible interaction chance. The nano-TiO_2 concentration was still 100 mg/L.

1.5. Determination of membrane permeability

The inner membrane permeability and outer membrane permeability were determined as the method described by Zhang et al. (2013). The inner membrane permeability method was as follows: 5-mL cell suspension ($OD_{600} = 1.0$) was mixed with 5-mL surfactant solution with or without Cd^{2+}, and then 500-μL ONPG (30 mmol/L) was added. After 2-hr incubation at 37°C, suspension was centrifuged at 4000×g for 5 min and the

supernatant was determined by UV–Visible spectrophotometer at 415 nm. For the outer membrane permeability, the mixed cell suspension was incubated for 1-hr at 37°C, and then a 3-mL sample and a 20-μL NPN (1 mmol/L) was vortex mixed for 10 sec. And then the fluorescence intensity was determined under the condition of that: $\lambda_{ex} = 350$ nm, $\lambda_{em} = 420$ nm, slits equal 3 and 5 nm, respectively.

2. Results and discussion

2.1. Effects of surfactants on the toxicity of Cd^{2+}

As can be seen from Fig. 1, both SDBS and Tween 80 with a concentration of 50 mg/L could not result in the lethal toxicity to *E. coli* in absence of Cd^{2+}. However, both of SDBS and Tween 80 enhanced the toxicity of Cd^{2+} to *E. coli* bacteria dramatically. Moreover, the bacterial growth reduction by SDBS was
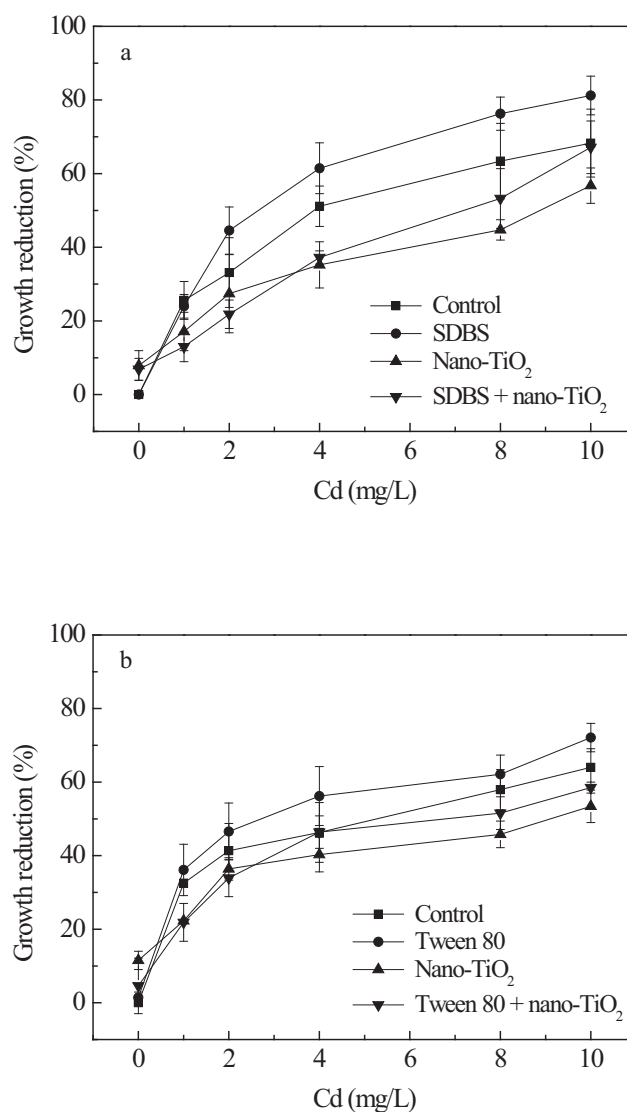
Fig. 1 – Effects of surfactants (50 mg/L, (a) SDBS and (b) Tween 80) on the toxicity of Cd^{2+} and nano-TiO_2 (100 mg/L) to *E. coli* bacteria. Data are mean ± standard deviation (n = 3).

higher than that by Tween 80. The 3-hr LC_{50} of Cd^{2+} in ultrapure water was 4 mg/L, while it was 2 mg/L with the presence of 50 mg/L of SDBS and Tween 80. Figure 2 shows the effects of surfactant concentration (0–100 mg/L) on the toxicity of Cd^{2+} (1 mg/L and 10 mg/L). The effect of SDBS and Tween 80 on the toxicity of Cd^{2+} varied at different Cd^{2+} levels. For 1 mg/L of Cd^{2+}, the toxicity of Cd^{2+} didn't change significantly with increasing SDBS concentration. For 10 mg/L of Cd^{2+}, the toxicity of Cd^{2+} increased with increasing SDBS concentration. Different from SDBS, Tween 80 with concentration higher than 10 mg/L enhanced the toxicity of 1 mg/L of Cd^{2+}, and the toxicity of 10 mg/L of Cd^{2+} didn't change dramatically with varying concentration. In order to identify the nature of mixed toxicity, the statistical testing was done, and the result is shown in **Appendix A Table S2**. The synergistic toxicity was observed for the group of Cd^{2+} (10 mg/L) and SDBS (50 and 100 mg/L), and the group of Cd^{2+}

(1 mg/L) and Tween 80 (50 and 100 mg/L) in the absence of nano-TiO_2.

It was a widely-held opinion that surfactants could cause an increase in cellular permeability and, therefore, a greater penetration of both the surfactant itself and also other substances present in the environment. In our study, the outer membrane permeability and inner membrane permeability were determined. As seen from Fig. 3, in the presence of surfactant alone, Tween 80 caused the increase of the outer membrane permeability dramatically, while SDBS hardly affected the permeability at the concentrations lower than 200 mg/L. Interestingly, in the presence of Cd^{2+}, the outer membrane permeability increased with SDBS concentration dramatically, which was much higher than that caused by Tween 80 and Cd^{2+} when the surfactant concentration was higher than 50 mg/L. Compared with the outer membrane permeability, the inner membrane permeability didn't change significantly (data not shown). The change of outer membrane permeability caused by surfactant was presumed to be in accordance with the adsorption of surfactant on the bacteria surface. SDBS was anionic surfactant, which could hardly attach to the bacteria surface with negative charges. Tween 80 as the nonionic surfactant could attach to the bacteria surface, and thus increased the outer membrane permeability significantly. However, in the presence of Cd^{2+}, SDBS could react with Cd^{2+} to form more stable SDBS-Cd (Reaction (2)).

$$C_{12}H_{25}-C_6H_4-SO_3^- + Cd^{2+} \Leftrightarrow C_{12}H_{25}-C_6H_4-SO_3-Cd^+$$
$$C_{12}H_{25}-C_6H_4-SO_3-Cd^+ + C_{12}H_{25}-C_6H_4-SO_3^- \Leftrightarrow (C_{12}H_{25}-C_6H_4-SO_3)_2Cd$$
$$(2)$$

As seen from Reaction (2), in the mixed solution of SDBS and Cd^{2+}, SDBS-Cd^+ and $(SDBS)_2Cd$ could coexist, and the amount of $(SDBS)_2Cd$ increased with the increasing SDBS concentration. Both SDBS-Cd^+ and $(SDBS)_2Cd$ could attach to the bacteria surface more easily, which better explained the increase of outer membrane permeability in the mixture of SDBS and Cd^{2+}. On the other hand, although SDBS could reduce the concentration of free Cd^{2+}, it could also enhance the entrance of Cd^{2+} to bacteria cell because of the increasing outer membrane permeability. Therefore, at low concentrations of Cd^{2+}, the
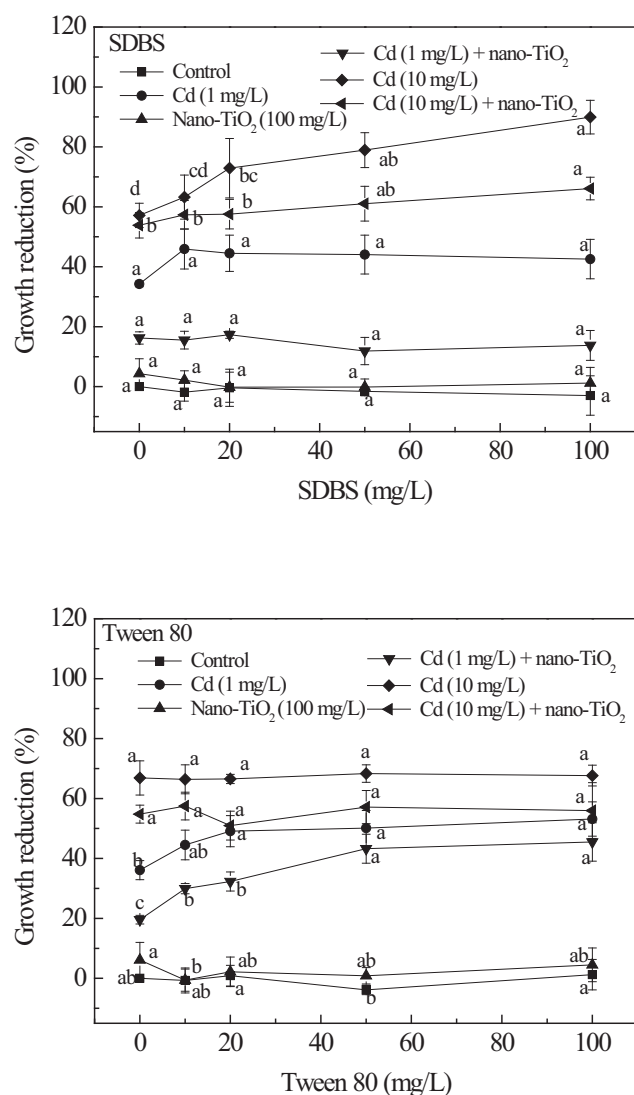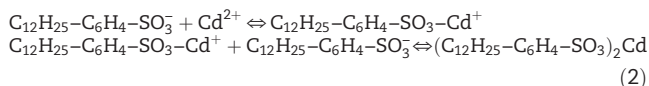

Fig. 2 – Effect of varying surfactants concentrations on the toxicity of nano-TiO_2 (100 mg/L) and Cd (1 and 10 mg/L). Significance levels were analyzed with one-way ANOVA, and the differences were labeled with a, b, c, d, if there is no same letter for two data, the significant difference exists.
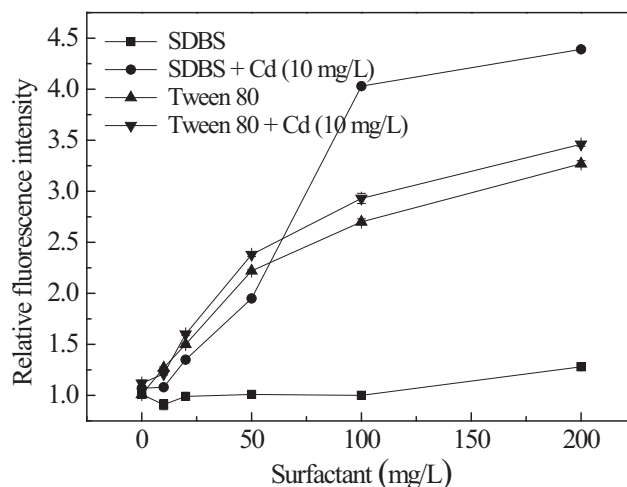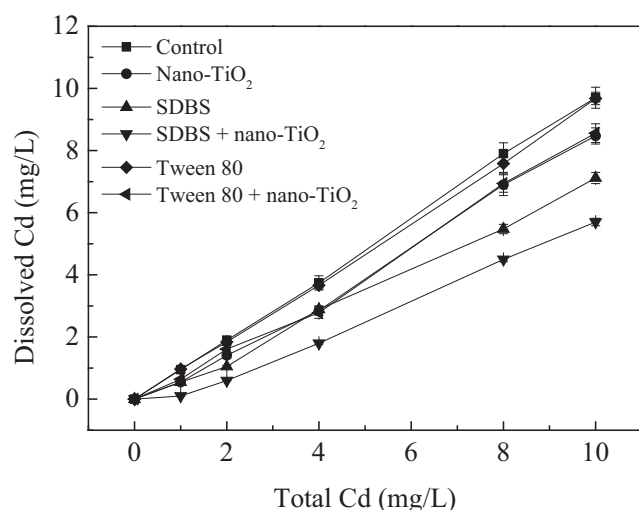

Fig. 3 – Effects of surfactant and Cd^{2+} on the outer membrane permeability.

combined toxicity of SDBS and Cd^{2+} were additive, while it changed to be synergistic with the increasing Cd^{2+} concentration. An earlier study reported by Calamari and Marchetti (1973) also indicated that a "more-than-additive" effect existed for the toxicity of mixtures of anionic detergents and metals to rainbow trout. They considered that the formation of a "surfactant-metal-surfactant" compound enhanced the toxicity of surfactant. Marchetti (1965) and Tovell et al. (1974) proposed that the increased toxicity of sodium alkylbenzenesulfonate (ABS) and sodium lauryl sulfate (SLS) in the presence of Ca^{2+} (or other bivalent cations) could be due to the replacement of bivalent cations with Na^+ in anionic surfactants. We also tried to verify this assumption, but the Ca^{2+} didn't enhance the toxicity of SDBS in our experiment. Therefore, the high toxicity caused by SDBS was mainly due to the increasing outer membrane permeability from the reaction between SDBS and Cd, which could further result in the accumulation of $SDBS-Cd^+$ in the cell membrane.

For nonionic surfactant, Tween 80 could not react with Cd^{2+}, and thus the dissolved cadmium in the presence of Tween 80 was almost the same to that in ultrapure water (Fig. 4). Although Tween 80 could increase the outer membrane permeability, the growth reduction of bacteria didn't change dramatically in the presence of Tween 80 alone. For 1 mg/L of Cd^{2+}, Tween 80 enhanced the toxicity of Cd, which could be probably that bacterial cells with higher outer membrane permeability were more sensitive to the toxic chemicals. However, for 10 mg/L of Cd^{2+}, the high toxicity caused by Cd^{2+} could mask the effect of Tween 80. In addition, the outer membrane permeability caused by SDBS and Cd^{2+} was much higher than that caused by Tween 80, which was also the reason of higher toxicity than that caused by Tween 80 and Cd^{2+}.

2.2. Effects of surfactants on the toxicity of nano-TiO₂

Figure 5 shows nano-TiO₂ ranged from 0 to 200 mg/L didn't result in the reduction of bacteria growth amount significantly. For 100 mg/L of nano-TiO₂, neither of SDBS and Tween 80 ranging from 0 to 100 mg/L enhanced the toxicity of nano-TiO₂ (Fig. 2). In contrast, the bacterial growth reduction
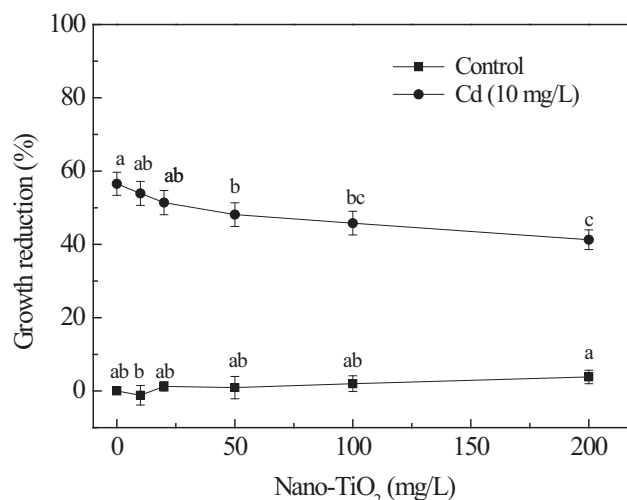
Fig. 5 – Effect of nano-TiO₂ concentration on the toxicity of Cd^{2+} (10 mg/L).

reduced slightly with increasing surfactant concentrations for both SDBS and Tween 80.

The direct contact between nanoparticles and bacteria has been considered as the main reason for the toxicity of nanoparticles to bacteria (Neal, 2008). The reactive oxygen species generated by nano-TiO₂ was not taken into account because all the experiments were conducted in absence of illumination. The surface charge and stability of nanoparticles would influence the interaction. Electrostatic repulsion dominated in the interaction between nano-TiO₂ and bacteria because of the negative charges on both of their surfaces (Table 1). Therefore, nano-TiO₂ exhibited little toxicity to bacteria. SDBS with anionic group was difficult to adsorb to the surface of nano-TiO₂ or E. coli with negative charges because of the role of electrostatic repulsion, and thus SDBS didn't enhance the toxicity of nano-TiO₂ to E. coli. In the presence of Tween 80, the zeta potential of nano-TiO₂ increased, which was probably the adsorption of Tween 80 to nano-TiO₂ (**Appendix A Fig. S1**). The greater hydrodynamic size of nano-TiO₂ in the presence of Tween 80 indicated that the agglomeration of nanoparticles could be caused by the reducing negative charges of nano-TiO₂ (Table 1). The stability of nanoparticles varying with time affected by surfactants was shown in Fig. 6A. Both SDBS and Tween80 promoted the settlement of nano-TiO₂, and 30% of nano-TiO₂ had settled down at the time of 100 min. However, the settling speed of nano-TiO₂ in the presence of Tween 80 slowed down after 100 min. In addition, no significant settlement was observed when nano-TiO₂ and bacteria coexisted, even though in the presence of SDBS and Tween 80 (Fig. 6b). All these data indicated that there were no strong interaction between nano-TiO₂ and bacteria and thus the weak effect of SDBS and Tween 80 on the toxicity of nano-TiO₂.

2.3. Effects of surfactants on the toxicity of Cd^{2+} in the presence of nano-TiO₂

As shown in Fig. 1b, nano-TiO₂ reduced the toxicity of Cd^{2+} to E. coli. The LC_{50} of Cd^{2+} increased to 10 mg/L in the presence of

Fig. 4 – Effect of surfactant (50 mg/L) and nano-TiO₂ (100 mg/L) on the dissolved Cd concentration.

Table 1 – The hydrodynamic size and zeta potential of nano-TiO$_2$ and *E. coli* bacteria suspensions. The concentrations of nano-TiO$_2$, surfactants, and Cd^{2+} were 100 mg/L, 50 mg/L, and 10 mg/L, respectively.

|	nano-TiO$_2$	+SDBS	+Tween80	+Cd^{2+}	+SDBS + Cd^{2+}	+ Tween80 + Cd^{2+}	*E. coli* (OD$_{600}$ = 1.0)
Hydrodynamic size (nm)	721 ± 3	779 ± 30	1522 ± 45	–	–	–	779 ± 127
Zeta potential (mV)	−20.4 ± 0.3	−22.6 ± 0.6	−6.8 ± 0.2	−0.3 ± 0.3	−14.0 ± 0.3	−6.1 ± 1.1	−39.3 ± 0.3

nano-TiO$_2$. The combined toxicity of Cd^{2+} (10 mg/L) and nano-TiO$_2$ reduced with the increasing nano-TiO$_2$ concentration. The combined toxicity was possibly due to the free Cd^{2+} and the interaction between nano-TiO$_2$ and bacteria. The free Cd^{2+} concentration was reduced by the adsorption of Cd^{2+} to nano-TiO$_2$, and thus decreased the toxicity of Cd^{2+}. Meanwhile, the zeta potential of nano-TiO$_2$ was neutralized by Cd^{2+} (Table 1), which could result in the self-agglomeration of nano-TiO$_2$ and the heterogeneous agglomeration between nano-TiO$_2$ and bacteria. The former weakened the interaction between nanoparticles and bacteria, while the latter enhanced. Overall,

the reduced free Cd^{2+} concentration was mainly responsible for the reduced combined toxicity.

When surfactant was added into the mixed suspensions of nano-TiO$_2$ and Cd^{2+}, the toxicity was changed. For SDBS of 50 mg/L, when the concentration of Cd^{2+} was below 4 mg/L, the joint toxicity was lower than that of mixtures of nano-TiO$_2$ and Cd^{2+}, while the toxicity was higher when Cd^{2+} was above 4 mg/L (Fig. 1). Compared to SDBS, 50 mg/L of Tween 80 enhanced the combined toxicity of nano-TiO$_2$ and Cd^{2+} slightly with Cd^{2+} higher than 2 mg/L seen from Fig. 1b. Overall, all the toxicities of mixtures of nano-TiO$_2$ and Cd^{2+} with surfactant were lower than the single toxicity of Cd^{2+}. The effect of surfactant concentration on the combined toxicity of Cd^{2+} and nano-TiO$_2$ was in accordance with that of Cd^{2+} (Fig. 2). One hundred milligram per liter of SDBS enhanced the combined toxicity of 10 mg/L of Cd^{2+} and nano-TiO$_2$ significantly, while Tween 80 enhanced the combined toxicity of 1 mg/L of Cd^{2+} and nano-TiO$_2$.

Effects of surfactant on the combined toxicity of nano-TiO$_2$ and Cd^{2+} could be illustrated from two aspects, which were discussed as follows: (1) Effect on the properties of nano-TiO$_2$. In the presence of SDBS, SDBS could react with Cd^{2+} adsorbed on the surface of nano-TiO$_2$ and, thus, increased the amount of negative charges on the nano-TiO$_2$ surface (Table 1). So the stability of nano-TiO$_2$ in coexistence of SDBS and Cd^{2+} was better than that of nano-TiO$_2$ with Cd^{2+} alone (Fig. 6). Compared to SDBS, the adsorption of Tween 80 to nano-TiO$_2$ reduced the adsorption of Cd^{2+} to nano-TiO$_2$ slightly (Fig. 4). The hypothesis could be confirmed by both the dissolved Cd^{2+} concentrations shown in Fig. 4 and the zeta potential of nano-TiO$_2$ shown in Table 1.

Seen from Fig. 6, the settling speed of nano-TiO$_2$ in absence of Cd^{2+} was slowed down. Cd^{2+} accelerated the settling speed of the mixed suspensions, which reached a stable period in 30 min. Surfactant didn't promote the agglomeration of nano-TiO$_2$ and bacteria, while Cd^{2+} enhanced the interaction between nano-TiO$_2$ and bacteria. This was probably because the adsorption of Cd^{2+} to nano-TiO$_2$ neutralized the negative charge (Table 1), which could promote the direct interaction of nano-TiO$_2$ and bacteria. According to the zeta potentials of nano-TiO$_2$, the interaction strength between nano-TiO$_2$ and bacteria in the presence of surfactant and Cd^{2+} could be: Cd^{2+} > Tween 80 + Cd^{2+} > SDBS + Cd^{2+}. However, the agglomeration of nano-TiO$_2$ in the presence of Cd^{2+} could inhibit the interaction between nano-TiO$_2$ and bacteria, which contributed less in the combined toxicity. (2) Effect on cadmium species. For the mixed suspension of SDBS, nanno-TiO$_2$, and Cd^{2+}, the reduced dissolved cadmium concentration was caused by both reaction of Cd^{2+} with SDBS and the adsorption of nano-TiO$_2$. SDBS could enhance the toxicity of Cd^{2+}, while nano-TiO$_2$ could reduce the toxicity of Cd^{2+}. On the other hand, the SDBS-Cd^{+} would also be adsorbed to the surface of
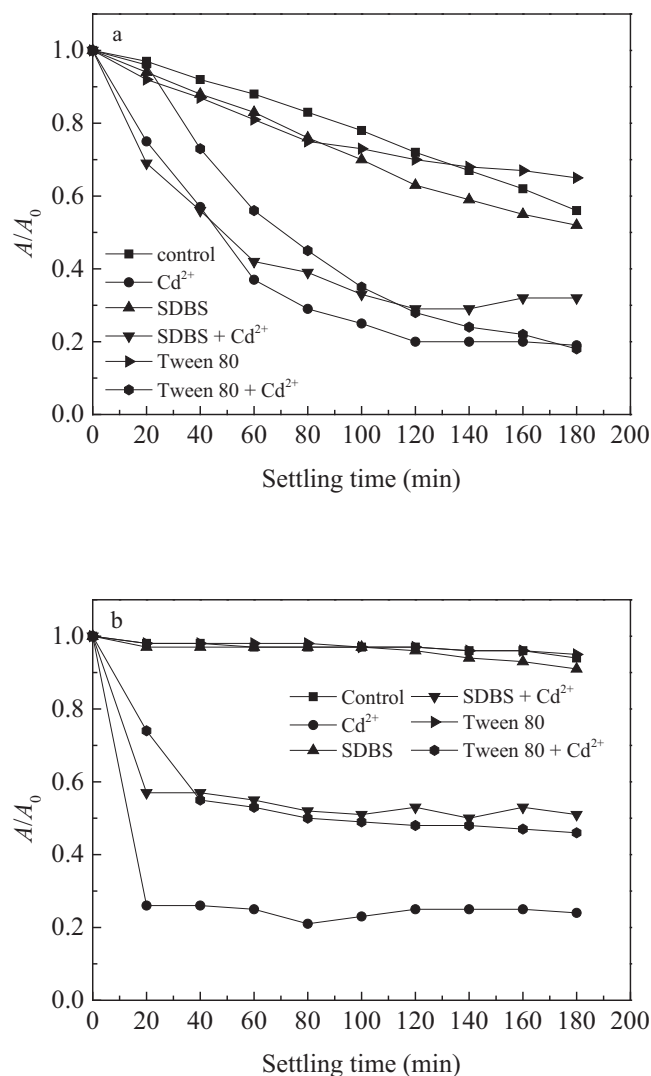
Fig. 6 – Effect of surfactants (50 mg/L) and Cd^{2+} (10 mg/L) on the stability of (a) nano-TiO$_2$ (100 mg/L), and (b) the mixtures of nano-TiO$_2$ and bacteria.

nano-TiO_2 by the electrostatic attraction, which alleviated the enhancement of SDBS on the toxicity of Cd^{2+}. Maybe this alleviation of nano-TiO_2 on the toxicity of SDBS-Cd^+ dominated for Cd^{2+} at low concentrations. When Cd^{2+} concentrations increased, the combined toxicity of nano-TiO_2 and Cd^{2+} in the presence of SDBS was higher than that without SDBS.

3. Conclusion

In the present study, the effects of surfactant on the single and combined toxicity of nano-TiO_2 and Cd^{2+} to *E. coli* were investigated, and the underlying mechanism was discussed. Some valuable information could be concluded as follows: (1) Effects of surfactant on the toxicity of Cd^{2+} was related to the effect of surfactant on the outer membrane permeability and the bioavailability of Cd. For SDBS, it enhanced the toxicity of Cd^{2+} with concentrations higher than 1 mg/L. SDBS could react with Cd^{2+} to form SDBS-Cd^+ and $(SDBS)_2$-Cd, which could adsorb to the cell membrane much easily than SDBS, and further increased the outer membrane permeability and the bioavailability of Cd. Different from SDBS, Tween 80 could not react with Cd^{2+}, but Tween 80 could increase the outer membrane permeability, which enhanced the toxicity of Cd^{2+} slightly. (2) Surfactant with concentrations ranging from 0 to 100 mg/L didn't enhance the toxicity of nano-TiO_2 (100 mg/L). In contrast, the bacterial survival increased slightly with increasing surfactant concentrations for both SDBS and Tween 80. This could be explained by the agglomeration of nano-TiO_2 promoted by surfactants.

(3) The toxicity of Cd^{2+} could be reduced by nano-TiO_2, which was mainly due to the adsorption of Cd^{2+} to nano-TiO_2. The effect of surfactant on the combined toxicity of Cd^{2+} and nano-TiO_2 mainly depended on the effect of surfactant on the toxicity of Cd^{2+}. In the presence of SDBS, the effects of SDBS and nano-TiO_2 on the toxicity of Cd^{2+} were opposite, and also SDBS binding to Cd^{2+} could adsorb to nano-TiO_2 and thus decreased the toxicity of Cd^{2+}. Therefore, the combined toxicity was reduced at Cd^{2+} lower than 4 mg/L and enhanced at Cd^{2+} higher than 4 mg/L in our study. As a whole, in most cases, the order of the toxicities was SDBS + Cd^{2+} > Tween 80 + Cd^{2+} > Cd^{2+} > SDBS + nano-TiO_2 + Cd^{2+} > Tween 80 + nano-TiO_2 + Cd^{2+} > nano-TiO_2 + Cd^{2+}.

In natural polluted water, it could be common for the coexistence of surfactants, nanoparticles, and heavy metals. The concentrations of pollutants in real water were possibly much less than those we used in this study. The high concentrations used in tests were advantageous for the observation of toxicity and mechanism based on the present experiment methods. The evaluation of the combined toxicity of mixtures of three or more pollutants was scarce and necessary. This study gives a tentative investigation and some valuable information for the combined toxicity of nanoparticles and heavy metals to bacteria in the presence of surfactants. The combined toxicity in natural water may be more complicated, and further studies are necessary to get more precise data and deeper theoretical support in the future.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.02.016.

REFERENCES

Adams, L.K., Lyon, D.Y., Alvarez, P.J., 2006. Comparative eco-toxicity of nanoscale TiO_2, SiO_2 and ZnO water suspensions. Water Res. 40, 3527–3532.

Amiano, I., Olabarrieta, J., Vitorica, J., Zorita, S., 2012. Acute toxicity of nanosized TiO_2 to *Daphnia magna* under UVA irradiation. Environ. Toxicol. Chem. 31, 2564–2566.

Balbi, T., Smerilli, A., Fabbri, R., Ciacci, C., Montagna, M., Grasselli, E., et al., 2014. Co-exposure to n-TiO_2 and Cd^{2+} results in interactive effects on biomarker responses but not in increased toxicity in the marine bivalve *M. galloprovincialis*. Sci. Total Environ. 493, 355–364.

Calamari, D., Marchetti, R., 1973. The toxicity of mixtures of metals and surfactants to rainbow trout (*Salmo gairdneri rich.*). Water Res. 7, 1453–1464.

Canesi, L., Ciacci, C., Balbi, T., 2015. Interactive effects of nanoparticles with other contaminants in aquatic organisms: friend or foe? Mar. Environ. Res. 111, 128–134.

Chen, J., Qian, Y., Li, H., Cheng, Y., Zhao, M., 2015. The reduced bioavailability of copper by nano-TiO_2 attenuates the toxicity to *Microcystis aeruginosa*. Environ. Sci. Pollut. Res. 22, 12407–12414.

Clemente, Z., Castro, V.L., Jonsson, C.M., Fraceto, L.F., 2012. Ecotoxicology of nano-TiO_2-an evaluation of its toxicity to organisms of aquatic ecosystems. Int. J. Environ. Res. 6, 33–50.

Della Torre, C., Balbi, T., Grassi, G., Frenzilli, G., Bernardeschi, M., Smerilli, A., et al., 2015. Titanium dioxide nanoparticles modulate the toxicological response to cadmium in the gills of *Mytilus galloprovincialis*. J. Hazard. Mater. 297, 92–100.

Fan, W.H., Cui, M.M., Liu, H., Wang, C., Shi, Z.W., Tan, C., et al., 2011. Nano-TiO_2 enhances the toxicity of copper in natural water to *Daphnia magna*. Environ. Pollut. 159, 729–734.

Fan, W.H., Cui, M.M., Shi, Z.W., Tan, C., Yang, X.P., 2012. Enhanced oxidative stress and physiological damage in *Daphnia magna* by copper in the presence of nano-TiO_2. J. Nanomater. https://doi.org/10.1155/2012/398720.

Fan, W., Peng, R., Li, X., Ren, J., Liu, T., Wang, X., 2016. Effect of titanium dioxide nanoparticles on copper toxicity to *Daphnia magna* in water: role of organic matter. Water Res. 105, 129–137.

Fekete-Kertesz, I., Piszman, D., Molnar, M., 2017. Particle size and concentration dependent ecotoxicity of nano- and microscale TiO_2-comparative study by different aquatic test organisms of different trophic levels. Water Air Soil Pollut. 228:245. https://doi.org/10.1007/s11270-017-3394-5.

Hartmann, N.B., Legros, S., Von der Kammer, F., Hofmann, T., Baun, A., 2012. The potential of TiO_2 nanoparticles as carriers for cadmium uptake in *Lumbriculus variegatus* and *Daphnia magna*. Aquat. Toxicol. 118, 1–8.

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.C., Kahru, A., 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71, 1308–1316.

Ince, N., Dirilgen, N., Apikyan, I., Tezcanli, G., Üstün, B., 1999. Arch. Environ. Contam. Toxicol. 36, 365–372.

Jiang, W., Mashayekhi, H., Xing, B.S., 2009. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. Environ. Pollut. 157, 1619–1625.

Li, M., Zhu, L.Z., Lin, D.H., 2011. Toxicity of ZnO nanoparticles to Escherichia coli: mechanism and the influence of medium components. Environ. Sci. Technol. 45, 1977–1983.

Li, M., Lin, D.H., Zhu, L.Z., 2013. Effects of water chemistry on the dissolution of ZnO nanoparticles and their toxicity to Escherichia coli. Environ. Pollut. 173, 97–102.

Li, S., Pan, X., Wallis, L.K., Fan, Z., Chen, Z., Diamond, S.A., 2014. Comparison of TiO_2 nanoparticle and graphene-TiO_2 nanoparticle composite phototoxicity to Daphnia magna and Oryzias latipes. Chemosphere 112, 62–69.

Lin, X., Li, J., Ma, S., Liu, G., Yang, K., Tong, M., et al., 2014. Toxicity of TiO_2 nanoparticles to Escherichia coli: effects of particle size, crystal phase and water chemistry. PLoS One 9, 1–8.

Marchetti, R., 1965. Critical review of the effects of synthetic detergents on aquatic life. Stud. Rev. Gen. Fish. Coun. Mediterr. 26, 1–32.

Neal, A.L., 2008. What can be inferred from bacterium–nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? Ecotoxicology 17, 362–371.

Oleszczuk, P., Jośko, I., Skwarek, E., 2015. Surfactants decrease the toxicity of ZnO, TiO_2 and Ni nanoparticles to Daphnia magna. Ecotoxicology 24, 1923–1932.

Rosenfeldt, R.R., Seitz, F., Zubrod, J.P., Feckler, A., Merkel, T., Luderwald, S., et al., 2015. Does the presence of titanium dioxide nanoparticles reduce copper toxicity? A factorial approach with the benthic amphipod Gammarus fossarum. Aquat. Toxicol. 165, 154–159.

Srivastava, S., Kumar, A., 2017. Comparative cytotoxicity of nanoparticles and ions to Escherichia coli in binary mixtures. J. Environ. Sci. 55, 11–19.

Tan, C., Wang, W.X., 2014. Modification of metal bioaccumulation and toxicity in Daphnia magna by titanium dioxide nanoparticles. Environ. Pollut. 186, 36–42.

Tan, C., Fan, W.H., Wang, W.X., 2012. Role of titanium dioxide nanoparticles in the elevated uptake and retention of cadmium and zinc in Daphnia magna. Environ. Sci. Technol. 46, 469–476.

Tovell, P.W.A., Newsome, C., Howes, D., 1974. Effect of water hardness on the toxicity of an anionic detergent to fish. Water Res. 8, 291–296.

Wang, D., Lin, Z., Yao, Z., Yu, H., 2014. Surfactants present complex joint effects on the toxicities of metal oxide nano-particles. Chemosphere 108, 70–75.

Yang, W.W., Li, Y., Miao, A.J., Yang, L.Y., 2012. Cd^{2+} toxicity as affected by bare TiO_2 nanoparticles and their bulk counterpart. Ecotoxicol. Environ. Saf. 85, 44–51.

Yang, W.W., Wang, Y., Huang, B., Wang, N.X., Wei, Z.B., Luo, J., et al., 2014. TiO_2 nanoparticles act as a carrier of cd bioaccumulation in the ciliate Tetrahymena thermophila. Environ. Sci. Technol. 48, 7568–7575.

Zhang, D., Zhu, L.Z., Li, F., 2013. Influences and mechanisms of surfactants on pyrene biodegradation based on interactions of surfactant with a Klebsiella oxytoca strain. Bioresour. Technol. 142, 454–461.