Simultaneous high-efficiency removal of sulfamethoxazole and zinc (II) from livestock and poultry breeding wastewater by a novel dual-functional bacterium, *Bacillus* sp. SDB4

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Abstract
The complex mixtures of antibiotics and heavy metals are commonly existed in livestock and poultry breeding wastewater. Effective and simultaneous removal of these toxic compounds by microorganisms, especially single strains, remains a considerable challenge. In this study, a novel functional strain SDB4, isolated from duck manure and identified as *Bacillus* sp., has been shown to possess high removal capabilities for both sulfamethoxazole (SMX) and Zn$^{2+}$. The maximum removal efficiency achieved 73.97% for SMX and 84.06% for Zn$^{2+}$ within 48 h in the single pollution system. It has great potential for eliminating SMX along with Zn$^{2+}$, 78.45% of SMX and 52.91% of Zn$^{2+}$ were removed in the 20 mg·L$^{-1}$ SMX and 100 mg·L$^{-1}$ Zn$^{2+}$ binary system. Furthermore, the SMX-biotransformation capability of SDB4 was enhanced at low concentrations of Zn$^{2+}$ (below 100 mg·L$^{-1}$). The SMX biotransformation and Zn$^{2+}$ adsorption data fitted well with the pseudo-first-order kinetic model, indicating that the two pollutants were in accordance with the same removal rule. N$^4$-acetyl-SMX was identified as the main stable transformation product during SMX removal. FTIR analyses revealed that OH, NH$_2$, C=O, C-N/N-H, and C-O-C played major roles in the adsorption of Zn$^{2+}$. Our study of the dually functioning strain SDB4 provides a potential application for the simultaneous biological removal of antibiotics and heavy metals.

Keywords Combined contaminants · Antibiotic · Heavy metal · *Bacillus paramycoides* bacteria · Bioremediation · Simultaneous removal

Introduction
With the dietary evolution of humans, the actual scale of global animal rearing and meat production has increased, resulting in intensification of livestock farming (Tullo et al. 2019). In contrast with the traditional feeding methods, livestock diseases are increasingly complex in the intensive and large-scale feeding industry. These diseases spread rapidly and cause huge economic losses once outbreak becomes endemic. Consequently, antibiotics such as sulfonamides and tetracyclines have been widely used to prevent livestock diseases (Shao et al. 2020). At the same time, heavy metals including zinc and copper have also been extensively used as feeding additives to increase production (Li et al. 2019a). However, only partial antibiotics/heavy metals can be metabolized/absorbed by livestock, causing a large number of residues in the excreta (Lin et al. 2021; Xu et al. 2016). These elements are readily accessible to the land and easily entered the water through leaching and direct runoff, thereby spreading drug and heavy metal contamination (Pan et al. 2018; Trenouth and Gharabaghi 2015). The commonly coexistence of antibiotics and heavy metals in high concentrations in livestock farming wastewater generate combined contamination (Riaz et al. 2020). Over time, high levels of complex contaminants also contribute to the accumulation of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) (Christou et al. 2017). The compound pollution of antibiotics and heavy metals in aquatic environments has

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become an international problem, causing harm to human life and ecological health (Hou et al. 2019). Therefore, it is urgently needed to remove the combined contaminants from aquaculture wastewater.

The removal of antibiotics and heavy metals from wastewater is a challenging issue, since the complicated characteristics of combined contamination. Different sorts of composite pollutants have complicated properties (hydrophobic/hydrophilic, negative/positive surface charge characteristics, etc.), which makes the treatment of wastewater even more difficult (Ma et al. 2014). Several physical-chemical methods have been adopted to simultaneously remediate antibiotics and heavy metals, such as adsorption, advanced oxidation, and flocculation (Hou et al. 2019; Deng et al. 2020; Ma et al. 2020). However, the application of traditional physical and chemical methods is limited due to the high cost, consumption of material resources, and even the introduction of new pollutants.

Microbial remediation, a competitor in environmental engineering with the advantages of cost-effectiveness and environmental friendliness for simultaneous treatment of various chemical pollutants, could be an alternative to traditional chemical and physical technologies (Shah and Shah 2020). In recent years, various methods have been reported to remediate antibiotic or heavy metal contaminated wastewater. The Bacillus subtilis LMB-A strain isolated from the wastewater treatment plant could degrade 92.69% lincomycin with the initial concentration of 1117.55 mg·L⁻¹ in 144 h (Li et al. 2020). Al-Dhabi et al. isolated a novel strain Bacillus velezensis Al-Dhabi 140, and the degradation ratio reached 60.52% with the concentration of 143.75 mg·L⁻¹ (Al-Dhabi et al. 2021). Besides, strain Bacillus cereus RC-1 showed the maximum removal efficiencies of 16.7%, 38.3%, 81.4%, and 40.3% for Cu²⁺, Zn²⁺, Cd²⁺, and Pb²⁺, respectively, with initial concentrations of 10 mg·L⁻¹ at pH 7.0 (Huang et al. 2018). Sharma and Shukla observed that the maximum removal efficiency of Pb²⁺ by the Bacillus cereus BPS-9 strain was 79.26% in the culture medium supplemented with 100 mg·L⁻¹ Pb²⁺ (Sharma and Shukla 2021). The removal of antibiotics or heavy metals has been extensively studied in the literature. However, some researchers found that livestock wastewater may containing a variety of organic and inorganic pollutants (including antibiotics, heavy metals, etc.), and the removal process of one pollutant may be affected by the other pollutants (Shao et al. 2020). Though SMX and Zn²⁺ was the typical antibiotic and heavy metal contaminants in livestock and poultry wastewater (Li et al. 2020), limited information was available about their simultaneous removal, especially by single strain. Meanwhile, the differences of bioremoval mechanisms between SMX/Zn²⁺ single and SMX-Zn²⁺ binary systems are still unclear. Hence, it is essential to isolate pure bacterium with SMX and Zn²⁺ simultaneous removal capability and study its further use in aquaculture wastewater microbial remediation.

In the present study, Bacillus sp. SDB4 was applied in treating combined polluted wastewater. The main purposes of this study were to (1) isolate a dual-functional strain from the duck manure, identify the novel aerobic strain based on its physiological characteristics and 16S rRNA sequence subsequently, (2) access the removal characteristics of SMX or/and Zn²⁺ by strain SDB4 in the single and binary pollution systems with various environmental factors, (3) investigate the SMX and Zn²⁺ removal kinetic models, as well as the variations of kinetics under different conditions, (4) study the removal mechanisms of SMX and Zn²⁺ by analyzing the transformation products and the alterations of functional groups on the microbial surface, and (5) provide theoretical basis for strain SDB4 in treating actual aquaculture wastewater.

Materials and methods

Medium and chemicals

Luria-Bertani (LB) medium was prepared by mixing 10 g·L⁻¹ tryptone, 5 g·L⁻¹ yeast extract and 10 g·L⁻¹ NaCl in 1000 mL deionized water, adjusted to 7.0 pH using NaOH. In total, the solid medium was prepared by adding 1.5–2.0% (w/v) agar. All media were autoclaved for 30 min at 121 °C before use. SMX was purchased from Aladdin Company with purity above 98% (China). Zinc sulfate heptahydrate and other reagents were all analytical grade purchased from Tianjin Damao Chemical Reagent Factory, China.

Screening SMX-Zn²⁺ resistant and bioremoving bacteria

In order to enrich SMX and Zn²⁺ resistant strain, which was isolated from the duck manure collected from a farm in Shandong, China, an aliquot of 10 g duck droppings was added to 100 mL of sterile growth LB medium containing 5 mg·L⁻¹ SMX and 5 mg·L⁻¹ Zn²⁺ in a 250-mL conical flask. After 5 days of incubation at 30 °C and 150 rpm in the dark, 10-mL enrichment suspensions were transferred to 100 mL fresh LB containing 10 mg·L⁻¹ SMX and 10 mg·L⁻¹ Zn²⁺. The enrichment process was continued 9 times using the same methods, with both SMX and Zn²⁺ concentrations increased by 5 mg·L⁻¹ during each inoculation period until the concentrations both reached 50 mg·L⁻¹ in LB. After 45 days, the concentrated suspensions were coated on LB agar plates and grown at 30 °C in constant temperature and dark environment. Ten isolated colonies were extracted and purified by repeated streaking on the fresh LB agar plates. The purified strains with SMX and Zn²⁺ resistance were cultivated in fresh LB to test the removal efficiency of SMX/Zn. Finally, SDB4 showed the best SMX removal capability.
Identification of dual-functional strain

According to the manufacturer’s instructions, genomic DNA was extracted with the Bacterial Genome DNA Extraction Kit (Tiangen Biotech Co., Ltd.). The following primers were used to amplify the PCR of the gene encoding 16S rRNA: 27f (5’- AGAGTTTGATCCTGCTCAG- 3’) and 1492r (5’- GGTTACCTTGTTACGACTT- 3’) was the forward and reverse primer, respectively (Pan et al. 2018). The PCR products were sequenced in MajorBio (Shanghai, China).

The IDENTIFY tool was used for comparing the sequence with the available DNA sequences in EzTaxon (http://www.ezbiocloud.net/eztaxon). MEGA version 7.0 software was used to analyze the phylogeny, and the phylogenetic tree was constructed with the neighbor-joining method.

Experiments design

To determine SMX/Zn\textsuperscript{2+} removal capability in the single pollution system (single contaminant of SMX/Zn\textsuperscript{2+} in the liquid medium), SDB4 was firstly grew in LB medium at 30 °C and shook with a speed of 150 rpm. The cells were harvested 24 h later and centrifuged for 15 min at 8000 rpm and 4 °C. After centrifugation, the SDB4 cells were then washed three times with 0.9% sterilized sodium chloride solution. The suspensions of SDB4 were inoculated (10%, v/v) into LB liquid medium containing SMX/Zn\textsuperscript{2+} and incubated with a rotary shaking speed of 150 rpm at the given temperature for the removal of SMX/Zn\textsuperscript{2+}. The removal experiments were performed in a batch of 250-mL flasks. The samples were collected at 6-h intervals and filtered by 0.45-μm membranes for determining the SMX/Zn\textsuperscript{2+} concentrations.

Harmful effects were caused by the accumulation and persistence of antibiotics in various environmental conditions (such as initial antibiotic concentration, pH, and temperature). The heavy metals are commonly coexisted with antibiotics in livestock and poultry wastewater, and they would affect the antibiotics biotransformation through interference with the physiology and ecology of antibiotics degrading microorganism (Shao et al. 2020; Zhang et al. 2017). These heavy metals and antibiotics could accumulate to high levels in water and then discharged into public bodies of water with multiple environmental factors (Hou et al. 2019). The differential antibiotics removal efficiencies can be attributed to various environmental factors (Luo et al. 2014). So, a wide range of environmental factor and the effect of heavy metals on SMX removal have been explored during the study. Based on the previous preliminary experiments of SMX removal, four groups of experiments were designed: various SMX concentrations (20–800 mg·L\textsuperscript{-1}), pH (4.0–10.0), temperature (15–40 °C), and Zn\textsuperscript{2+} concentrations (20–250 mg·L\textsuperscript{-1}).

The experiments were carried out under sterile conditions and all flasks were wrapped in aluminum foil for SMX photodegradation preventing.

Zn\textsuperscript{2+} removal experiments were performed in flasks containing 100 mL LB liquid medium supplemented with single metal (20–250 mg·L\textsuperscript{-1}).

In order to investigate the removal efficiencies of complex contaminants by SDB4 in the binary pollution system (combined contaminants of SMX and Zn\textsuperscript{2+} in the liquid medium), the resuspended cell suspensions were inoculated (10%, v/v) into LB liquid medium with 20 mg·L\textsuperscript{-1} SMX and 100 mg·L\textsuperscript{-1} Zn\textsuperscript{2+}. The culture temperature was 30 °C and the shaking speed was 150 rpm. The biomass, SMX, and Zn\textsuperscript{2+} concentrations were detected at interval of 6 h.

Kinetics model

The characters of SMX and Zn\textsuperscript{2+} removal at different conditions were explored by fitting the temporal residual concentration to a pseudo-first-order kinetic model (Eq. (1)) (Liang and Hu 2019). The concentrations of SMX and Zn\textsuperscript{2+} were measured at intervals of 6 h to confirm the specific removal rates.

\[
\ln C_t = kt + \ln C_0
\]  

Where \( t \) refers to the reaction time, \( C_0 \) and \( C_t \) are the SMX/Zn\textsuperscript{2+} concentrations at initial “0 (h)“ and time “\( t \) (h)”, respectively, and \( k \) represents the specific removal rates. The validity of these calculations is measured by calculating the standard deviation and determining the coefficient \( R^2 \).

Analytical methods

The changes of bacterial concentration were monitored by a UV–1100 ultraviolet spectrophotometer (Mapada in China) at 600 nm and expressed as OD value.

Briefly, 2-mL samples were extracted from each flask and passed through 0.45-μm filters to remove impurities and biomass. Then, the samples were stored at 4 °C for further measurement. SMX was tested by high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS-MS) (Agilent 1200 Series). The determination conditions and the mobile phase composition were set as reported previously (Wang and Wang 2018b).

The supernatants from SDB4 culture medium with single contaminant (20 mg·L\textsuperscript{-1} SMX) and combined contaminants (20 mg·L\textsuperscript{-1} SMX and 100 mg·L\textsuperscript{-1} Zn\textsuperscript{2+}) were collected, and immediately filtered via 0.22-μm membrane three times to remove impurities. Then, the supernatants were analyzed by ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-MS-MS, Ultimate 3000/Q-Exactive, USA) for determination of the SMX biotransformation metabolites. Acquity® UPLC® BEH C18, 1.7 μm, 50 × 2.1 mm column (Waters Corporation) was used. The flow rate was 600 uL-
min$^{-1}$ and the volume of injection was 5 uL. The mobile phases were (A) water-methanol (95:5%, v/v) containing 0.1% acetic acid and (B) acetonitrile-methanol (50:50%, v/v) containing 0.1% acetic acid. The chromatographic analysis was performed in gradient mode, starting at 90% A, holding for 1 min, and then rising to 90% B within 6 min. The electrospray interface (ESI) was provided on the mass spectrometer, and the ESI source was operated in positive ionization mode, with the ionspray voltage of 4.0 kV and temperature of 300 °C. Full scans from m/z 50–600 were acquired for identification of the metabolites. Metabolites in the single SMX system and SMX-Zn$^{2+}$ binary system were identified by comparing with standard compounds.

The residual Zn$^{2+}$ concentration in the solution was determined using the inductively coupled plasma optical emission spectrometry (ICP-OES) method.

Fourier transform infrared spectroscopy (FTIR) was used to analyze the changes of functional groups in control sample (without adding pollutants), 100 mg·L$^{-1}$ Zn$^{2+}$ contaminated samples, 20 mg·L$^{-1}$ SMX, and 100 mg·L$^{-1}$ Zn$^{2+}$ co-contaminated samples. The scanning range was 500–4000 cm$^{-1}$, resolution of 4 cm$^{-1}$, and scanning frequency of 32.

Statistical analysis

All the experiments were repeated three times and the results were averaged. The removal efficiency (%) was calculated by the following equations:

$$\text{Removal efficiency} \% = \frac{(C_0 - C_t) \times 100}{C_0} \quad (2)$$

where $C_0$ (mg·L$^{-1}$) and $C_t$ (mg·L$^{-1}$) are SMX or Zn$^{2+}$ concentration in solution at initial and time $t$, respectively.

Results and discussion

Isolation and identification of the dual-functional strain

The dual-functional strain SDB4, isolated from the duck manure, could grow on solid LB medium with both SMX and Zn$^{2+}$. SDB4 was an aerobic, rod-shaped, and gram-negative strain, forming waxy, circular, and non-translucent colonies on LB plates (Fig. S1 a, b). The partial 16S rRNA gene sequence analysis of strain SDB4 (1426 bp, GenBank accession number: MT647568) showed a close relationship to the genus Bacillus and the similarity to Bacillus paramycoides (GenBank accession number MAO101000012, Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus) was 98.59%.

Based on the morphological characteristics and phylogenetic analysis, SDB4 was identified as Bacillus paramycoides. According to the 16S rRNA gene sequences and other close phylogenetically relatives, the phylogenetic tree was constructed (Fig. S1c). Bacillus paramycoides is a novel specie of the Bacillus cereus group, originally isolated from a diverse marine environment (Liu et al. 2017). There are few studies on its resistance to antibiotics/heavy metals. It is the first manuscript of a strain belonged to Bacillus sp., which could simultaneously remove SMX and Zn$^{2+}$.

Removal performance of SMX by strain SDB4

Effect of initial concentration on SMX removal

Six initial SMX concentrations (20, 50, 100, 200, 400, and 800 mg·L$^{-1}$) were adopted to investigate the effect of SMX concentration on SMX removal efficiency by strain SDB4. The inoculation dose was 10% (v/v), the initial pH was set as 7 and the temperature was adjusted to 30 °C. In the control group without cells inoculation, no significant removal of SMX was observed, indicating that no volatilization, hydrolysis, or photodegradation occurred under the tested conditions (Figure S2a). When SDB4 was added to LB, the bacteria growth and SMX removal efficiency gradually decreased with the increase of SMX concentration. Figure 1 a and b show the remaining concentration ranged from 6 to 310 mg·L$^{-1}$ when the average initial concentration of SMX ascended from 20 to 800 mg·L$^{-1}$, of which the SMX removal efficiency decreased from 72.96 to 61.62% within 48 h. In the meantime, the maximal value of OD$\text{OD}_{600}$ decreased from 15.01 to 11.03. Therefore, the SMX removal efficiency showed strikingly positive relationship with the growth of strain SDB4. As presented in Fig. 1 c and Table S1, the decline of SMX followed pseudo first-order kinetics and the highest removal rate constant of SMX was 0.270 h$^{-1}$ at the lowest initial SMX concentration of 20 mg·L$^{-1}$. The removal rate constant was negatively correlated with the initial concentration of SMX. The reason might be the ecotoxicity of high doses of antibiotics would inhibit the activity and proliferation of antibiotic bioremoving bacteria, which was consistent with the findings of a previous study (Ren et al. 2018). This phenomenon is similar to “hormetic effect” (two-phase dose response relationship between low dose stimulation and high dose inhibition), which has been proved to occur in the biological removal of antibiotics, heavy metals, disinfectants, and nanoparticles under multiple environmental pressures (Zhang et al. 2020).

The maximum removal rate of SDB4 was 10.38 mg·L$^{-1}$·h$^{-1}$ occurred in the initial SMX concentration of 800 mg·L$^{-1}$, which was much higher than that of previous studies (5.0 mg·L$^{-1}$·h$^{-1}$) (Wang and Wang 2018a, b). The related reasons may refer to that tryptone and yeast extract provided sufficient co-metabolic substrates for SDB4 growing and reproducing, resulting in a larger bacterial population. Thus, SDB4 had a strong advantage with an excellent removal capability in a
A wide range of initial SMX concentration and can withstand the high selection pressure of 800 mg·L⁻¹ SMX.

Effect of pH on SMX removal

pH is an important factor affecting microbial physiology and ionic state of organic pollutants, thereby affecting the capability of microorganisms to degrade organic pollutants (Zhang et al. 2017). Figure 2a and b depict the effect of different initial pH on the removal of SMX by strain SDB4. The effects of pH value on SMX removal and bacterial growth were investigated under the conditions of pH 4–10. When the pH was maintained at 4, 5, 6, 7, 8, 9 and 10, the removal efficiency obtained was 45.25, 47.88, 63.42, 73.97, 67.58, 65.02, and 51.60%, respectively, within 48 h under conditions of 20 mg·L⁻¹ initial concentration and 30 °C temperature. The maximal biomass of SDB4 also experienced significant fluctuation. The strain obtained the best growth ability and highest SMX removal efficiency of 73.97% at pH 7. However, lower removal efficiencies of SMX and biomass of SDB4 were detected under acidic or alkaline conditions. Meanwhile, the SMX removal profiles were well fitted to the pseudo-first-order kinetic model. As shown in Fig. 2c and Table S2, the SMX removal rate constant gradually increased and then decreased when pH value increased from 4 to 10, and the maximum removal rate constant was 0.0274 h⁻¹ at the pH of 7.

Compared with acidic environment (pH 4–6), neutral and alkaline environment (pH 7–10) were more conducive to the removal of SMX. The behavior caused by pH might be due to the negative effects of certain extreme pH values on microbial growth (Lin et al. 2014). Acidic or alkaline environments will affect the microbial growth rates and enzymatic activities, thus affecting the removal efficiency and removal rate of SMX (Luo et al. 2018). Similar results have also been reported in the other biodegradant Achromobacter sp. S-3 (Huang et al. 2012). SMX has three different forms, namely cation form, presented in acid environment with pH lower than 1.6; anion form with pH higher than 5.7; and neutral form with the pH between 1.6 and 5.7. The anion form is more easily degraded than the other two forms in the process of AOPs (Qi et al. 2014). Similar information is available for the biodegradation process.
of SMX. Wang’s research showed that *Acinetobacter* sp. was able to degrade SMX better in neutral and alkaline environment than acidic environment (Wang and Wang 2018a, b). To sum up, SMX can be removed effectively by SDB4 at a wide pH range, indicating that this strain might have a good application prospect.

Effect of temperature on SMX removal

The temperature also significantly affects the biological removal of harmful substances by organisms (Zhang et al. 2017). In this study, the SMX removal efficiency by SDB4 was studied at different temperatures from 15 to 40 °C. Figure 3 a shows the decrease in SMX concentration over time at all tested temperatures. The strain could propagate rapidly with temperatures from 30 to 35 °C, and the removal efficiency of SMX reached 73.96 and 66.74%, respectively (Fig. 3b). As shown in Fig. 3 c and Table S3, SMX removal profiles at different temperatures were well correlated with the pseudo-first-order kinetic model ($R^2 > 0.90$). The removal rate constants of SMX were 0.0064, 0.0101, 0.0139, 0.0274, 0.0216, and 0.0106 h$^{-1}$ at temperatures of 15, 20, 25, 30, 35, and 40 °C, respectively. When cultures were incubated at a higher or lower temperature, the growth of SDB4 was slow, and the corresponding removal efficiency and rate constant decreased rapidly. When the temperature rose to 40 °C, the final SMX efficiency was only 41.47%. As for the comparison of bacterial growth trend, the final cell concentration at 40 °C accounted for approximately half of that at 30 °C. The SMX removal efficiency, removal rate constant, and bacterial biomass all decreased to the lowest value when the temperature was 15 °C.

Low temperature retards the combination of enzymes and substrates, thus slows the bacterial growth (Cao et al. 2019). Other researchers also found the temperature has a significant impact on bacterial growth during the process of microbial removal of antibiotics (Pan et al. 2018). Therefore, low or high temperature will inhibit the growth of bacteria and activities of enzymes, resulting in a decrease in SMX removal efficiency. It is generally believed that each enzyme has an optimal temperature range that varies greatly among different microorganisms. The researchers found that the optimal temperature for the degradation of SMX by *Acinetobacter* was 25 °C, while that of *Pseudomonas cryophilus* HA-4 was 10 °C (Jiang et al. 2014; Wang and Wang 2017). *Bacillus paramycoides* is a...
facultatively anaerobic bacterium with an optimal growth temperature of 30 °C (Liu et al. 2017). The discrepancy of SMX removal efficiency at different temperatures indicates the influence of temperature on the enzymes involved in the transformation of SMX. The effect of temperature on the enzyme activity is mainly reflected in the expression of related genes. Wu et al. found that the abundance of ammonia monooxygenase gene, which plays an important role in the degradation of organic pollutants, was lower at 4 °C than that at 25 °C (Wu et al. 2013).

In the actual environment, the dependence of the strain on the ambient temperature will affect the removal capability of SMX, which may cause the bioremediation effect be lower than expected. Fortunately, the strain SDB4 was able to grow and remove SMX at all test temperatures, though the removal efficiency was reduced at low or high temperature.

Effect of Zn$^{2+}$ on SMX removal

To investigate the effect of the coexistence of Zn$^{2+}$ on SMX removal, 20–200 mg·L$^{-1}$ Zn$^{2+}$ were added to the SMX solution with initial concentration of 20 mg·L$^{-1}$. The effects of initial Zn$^{2+}$ concentrations on SMX removal were estimated by the SMX removal efficiencies as shown in Fig. 4 a. It was obvious to notice that SMX removal efficiency varied with Zn$^{2+}$ concentration. The presence of Zn$^{2+}$ (under 100 mg·L$^{-1}$) provided a slight stimulating effect on SMX removal. Compared to single SMX pollution system, SDB4 showed a more outstanding SMX removal capability in SMX-Zn$^{2+}$ combined pollution system, whereas a higher concentration (100–200 mg·L$^{-1}$) exerted inhibition influence. Meanwhile, the concentration of bacteria decreased with the increase of Zn$^{2+}$ addition (Fig. 4b). Figure 4 c shows that the SMX removal data fitted well with the pseudo-first-order kinetic model (Table S4). The SMX removal rate constant reached 0.294 h$^{-1}$ when the coexisting Zn$^{2+}$ concentration was 100 mg·L$^{-1}$. As the Zn$^{2+}$ concentration increased from 20 to 100 mg·L$^{-1}$, the removal efficiency of SMX in the medium increased from 74.09 to 78.06%, which was higher compared to that without Zn$^{2+}$ in 48 h reaction time. A similar result was also reported in which the addition of Cu$^{2+}$ ions stimulated the biodegradation of BDE-209 (Lu et al. 2013). At low concentrations of Zn$^{2+}$ coexistence, SMX removal efficiency could be enhanced by accelerating the transportation of SMX from extracellular...
solution to inside cells and the formation of enzyme-metal-substrate complexes (Liu et al. 2019). Zn\textsuperscript{2+} accelerated SMX removal through uptaking more pollutants inside cells; meanwhile, a low concentration of Zn\textsuperscript{2+} might stimulate the release of the functional enzymes of removing SMX (Liu et al. 2015; Tang et al. 2016). The SMX removal efficiency gradually decreased from 78.06 to 41.74\% with the increase of Zn\textsuperscript{2+} concentration (100 to 200 mg·L\textsuperscript{-1}). Meanwhile, the SMX removal rate constants also showed a downward trend. The inhibition of SMX removal efficiency with the present of Zn\textsuperscript{2+} probably referred to the restrain of enzymatic production process, which modified the enzyme composition, concentration, and activity (Liu et al. 2015). Additionally, superabundant Zn\textsuperscript{2+} competed with other nutrients such as Mg\textsuperscript{2+} and Ca\textsuperscript{2+}, which inhibited the formation of enzyme-metal-substrate complexes (Sherameti and Varma 2010).

**Removal performance of Zn\textsuperscript{2+} by strain SDB4**

The effect of initial concentration on Zn\textsuperscript{2+} removal by SDB4 was investigated under the temperature of 30 °C and pH of 7. There was no significant change in Zn\textsuperscript{2+} concentration during incubation period in control group (Fig. S3), indicating that the removal of Zn\textsuperscript{2+} was mainly caused by microbial adsorption. Figure 5 a and b show the Zn\textsuperscript{2+} removal efficiency decreased with the increase of initial Zn\textsuperscript{2+} concentration. As the initial concentration of Zn\textsuperscript{2+} was 20 mg·L\textsuperscript{-1}, the Zn\textsuperscript{2+} removal efficiency and strain biomass reached the maximum, which were 84.06\% and 16.51, respectively. The removal efficiencies were all above 70\% with the initial Zn\textsuperscript{2+} concentration increased from 20 to 150 mg·L\textsuperscript{-1}, while the bacterial growth was decreased. However, the Zn\textsuperscript{2+} removal efficiencies decreased sharply to 67.40\% and 55.36\% when the initial Zn\textsuperscript{2+} concentration increased to 200 mg·L\textsuperscript{-1} and 250 mg·L\textsuperscript{-1}, respectively. The strain biomass slowly grew of OD\textsubscript{600} 10.03 and 7.98. This result may be due to high initial metal ion concentration leading to heavy metal toxicity that inhibited bacterial growth. Figure 5 c and Table S5 show the pseudo-first-order kinetic models and fitting parameters for Zn\textsuperscript{2+} adsorption by SDB4 at various initial Zn\textsuperscript{2+} concentrations.
removal efficiency and removal rate constant may be due to the lack of sufficient free radicals for metal microbial adsorption. It is possible that all metal ions can have good interaction with the binding sites at lower concentrations, resulting in higher removal efficiencies of Zn$^{2+}$. Similar conclusions have been summarized by other researchers (Jin et al. 2013; Xu et al. 2019). Therefore, the removal efficiency of SDB4 as adsorbent treating low concentration zinc pollution is relatively better, and we can adjust the injection amount of SDB4 based on the concentration of pollutants in the actual wastewater.

**Simultaneous removal SMX and Zn$^{2+}$ by strain SDB4**

In order to explore the interaction between SMX and Zn$^{2+}$ removal, the simultaneous removal by SDB4 was conducted in a binary system under the conditions of initial SMX concentration 20 mg·L$^{-1}$ with adding 100 mg·L$^{-1}$ of Zn$^{2+}$. No significant removal of SMX and Zn$^{2+}$ was observed in the control group (Fig. S4). The changes of pollutants concentration and OD$_{600}$ are shown in Fig. 6 a, b, and c. Within 0–6 h, SDB4 started to fit the medium environment. With OD$_{600}$ increasing from 1.95 to 6.86 during 6–18 h, organism biomass displayed an exponential phase. However, the cell started to grow slowly in the stationary period (18–48 h), and the reduction of carbon substrate may be the reason for this phenomenon.

In the process of simultaneous removal of SMX and Zn$^{2+}$ by SDB4, the concentration of SMX and Zn$^{2+}$ both decreased while the OD$_{600}$ increased. As the organic carbon sources coexisted with SMX, the initial concentration of SMX dropped from 18.98 to 4.09 mg·L$^{-1}$, while the removal efficiency was 78.45% within 48h. The concentration of Zn$^{2+}$ was decreased from 97.47 to 45.90 mg·L$^{-1}$ with the removal efficiency of 52.91% in 48 h. During the simultaneous removal of SMX and Zn$^{2+}$, the corresponding removal rates of SMX and Zn$^{2+}$ were 0.310 mg·L$^{-1}$·h$^{-1}$ and 1.074 mg·L$^{-1}$·h$^{-1}$, respectively.

A relationship between the removal of SMX and Zn$^{2+}$ has been proposed as follows: Firstly, SMX was preferentially removed by SDB4 as a carbon source. Meanwhile, the concentration of Zn$^{2+}$ was slightly removed in the first 6 h. SDB4
can rapidly remove SMX and a small amount of Zn^{2+} in the adaptive period, which indicates that SDB4 can adapt to the new liquid culture environment quickly. Compared with other strains, SDB4 has a short adaptation period (Su et al. 2019). Secondly, the decline of SMX concentration and the exponential increase of OD_{600} promoted Zn^{2+} to be removed at a high rate in the 6–18 h period, while SMX was also rapidly removed. These results show that strain SDB4 can adapt to SMX and Zn^{2+} combined pollution environment well; meanwhile, it grows at high speed in the complex contaminants system. The biomass reached a high level, which could remove the compound pollutants effectively. Both SMX and Zn^{2+} can be removed at a faster rate in the biomass logarithmic growth period, indicating that high concentration of biomass was able to improve the efficiency of various biochemical reactions. Dong has obtained similar conclusion (Dong and Hu 2021). Thirdly, when the SMX and Zn^{2+} concentration reduced to about 4 mg·L^{-1} and 46 mg·L^{-1}, respectively, the cells of SDB4 started to increase slowly. Subsequently, the SMX and Zn^{2+} removal rate became slower, and the removal reaction reached balance. Consistent with the study of Shao et al., when the biomass of SDB4 increased slowly in the stationary period, the pollutants were no longer removed rapidly (Shao et al. 2018). The removal patterns of SMX and Zn^{2+} could be well described using first-order kinetic models with regression coefficients (R^2) all above 0.90 (Fig. 6d and Table S6). In the binary pollution system, the removal rate constants of SMX and Zn^{2+} were 0.03 h^{-1} and 0.0177 h^{-1}, respectively. SMX was removed by SDB4 at a faster rate. Consequently, compared to the strains in previous research, SDB4 can not only remove SMX effectively, but also has excellent performance in removing SMX and Zn^{2+} simultaneously.

Possible removal mechanisms

SMX biotransformation pathway

The main mechanism of SMX removal was considered to be through biotransformation. The possible biotransformation metabolites of SMX in single and binary system were explored for further clarification of SMX removal process. An intermediate product of SMX biotransformation was found in both systems. The intermediates were identified by detection and analysis of
samples at 48 h with UHPLC-MS-MS. Extracted ion chromatogram (EIC) in the full scan mode in the single system indicated that the retention time of the intermediate was 3.46 min and 3.45 min, respectively (Fig. 7a, c). The MS-MS full-scan spectrums of the intermediates in the binary system were acquired in the positive ion mode (Fig. 7b, d). More biotransformation metabolites were produced in the binary system (the peak area of binary system was larger than that of sole system), indicating that low Zn$^{2+}$ concentration could promote SMX removal. The dominant ion peaks of the intermediates were observed at m/z 134 and 198 in both single and binary pollution systems, indicating the consistence of intermediates with N$^4$-acetyl-SMX (Ac-SMX).

Biodegradation and biotransformation are expected to be effective ways for eliminating SMX (Yang et al. 2016). Previous study indicated that N-hydroxy-SMX and N$^4$-acetyl-SMX represented the main stable metabolites in SMX biotransformation process when the antibiotic was used as the carbon source (Zhang et al. 2016). HO-SMX is more stubborn than SMX in the environment. Ac-SMX is less toxic than the parent compound; meanwhile, acetylation is an important way to eliminate many therapeutic drugs (Reis et al. 2018). In this study, only one product N$^4$-acetyl-SMX was detected. According to the structure of metabolite compound, arylamine N-acetyltransferases might be involved in the biotransformation of SMX by SDB4. As demonstrated in the previous study, SMX could be transformed into Ac-SMX by arylamine N-acetyltransferase (Zhang et al. 2016). Previous studies have found that the functional arylamine N-acetyltransferase enzymes encoded by gene banatC in the pathogen Bacillus anthracis were able to acetylate sulfonamide antibiotic SMX (Pluvinage et al. 2007). The arylamine N-acetyltransferases Nat-a and Nat-b, which purified and characterized from Bacillus cereus 10-L-2, was able to convert various aniline compounds into corresponding acetanilides and played a role in detoxification (Takenaka et al. 2009). In the process of SMX biotransformation by SDB4, a metabolite N$^3$-acetyl-SMX was found, whose toxicity was around 10% of SMX (Majewsky et al. 2014). This pathway could detoxify SMX effectively and environmentally friendly.

**FTIR analysis**

FTIR analysis was carried out to determine which functional groups of SDB4 were involved in Zn$^{2+}$ adsorption. The uptake of heavy metal ions by functional groups will lead to higher or lower frequency shift of the spectrum (Rahim et al. 2020). In the single and binary pollution systems, the FTIR
spectrums of SDB4 were both shifted in peaks after adsorption of contaminants. Both of the FTIR spectrums of SDB4 in the single and binary pollution systems were shifted in peaks after adsorption of the contaminants (Fig. 8). The peak value corresponded to OH moved from 3294 to 3298 cm\(^{-1}\) after zinc biosorption in both systems. The peaks observed at 1630–1680 cm\(^{-1}\) corresponded to C=O in amide I band, and the peaks at 1530–1630 cm\(^{-1}\) were for C-N/N-H component in amide II band. The red shifts of amide bands I and II show that C=O, C-N/N-H play significant roles in the biosorption of Zn\(^{2+}\), which is consistent with the results obtained by other researchers (Li et al. 2019a, b). The polysaccharide groups were determined by vibrations at 1050–1090 cm\(^{-1}\), the red shift (from 1071 to 1051 cm\(^{-1}\) and 1054 cm\(^{-1}\), respectively) indicates that C-O-C is also involved in Zn\(^{2+}\) biosorption. In the single and binary pollution systems, the characteristic peaks associated with hydroxyl groups on the carboxylic acids were disappeared, indicating that OH were involved in Zn\(^{2+}\) adsorption. The bending at 600–750 cm\(^{-1}\) represented amino groups, the shift and disappearance of amino group peaks, indicating the interaction of NH\(_2\) in Zn\(^{2+}\) bioaccumulation. Previous studies also found that amino groups could accumulate Cu\(^{2+}\), Zn\(^{2+}\), and Pb\(^{2+}\) through displacement of protons (Yin et al. 2019). All the above implied the hydroxyl, amino, amide, and polysaccharides played important roles in the biosorption of Zn\(^{2+}\) by SDB4. The effective Zn\(^{2+}\) adsorption reduced the interference of Zn\(^{2+}\) on strain SDB4, so that SDB4 could remove SMX effectively. When the strain was treated with combined contaminants of SMX and Zn\(^{2+}\), the peaks of OH and C=O shifted from 3294 cm\(^{-1}\) and 1655 cm\(^{-1}\) to 3298 cm\(^{-1}\) and 1652 cm\(^{-1}\), respectively. The small red-shift showed the two groups might be involved in the adsorption of SMX (Prasannamedha et al. 2021).

In conclusion, SDB4 can not only biotransform SMX/ biosorp Zn\(^{2+}\) in the sole pollution system, but also remove the two pollutants simultaneously in the binary pollution system. We proposed the mechanism of simultaneous removal of SMX and Zn\(^{2+}\) by SDB4 (Fig. 9).

**Conclusions**

A simultaneous SMX biotransformating and Zn\(^{2+}\) bioremoving *Bacillus* sp. strain SDB4 was isolated from duck manure in a farm, Shandong Province, China. In the single system, the strain showed effective removal performance to SMX and Zn\(^{2+}\), respectively. In the SMX-Zn\(^{2+}\) binary system, 20 mg·L\(^{-1}\) SMX and 100 mg·L\(^{-1}\) Zn\(^{2+}\) were removed simultaneously by strain SDB4 in 48 h, with SMX biotransformation

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**Fig. 8** FTIR spectra of strain SDB4. (a) FTIR spectra before Zn\(^{2+}\) removal. (b) FTIR spectra after Zn\(^{2+}\) removal in the single pollution system. (c) FTIR spectra after SMX and Zn\(^{2+}\) simultaneous removal in the binary pollution system.

**Fig. 9** Possible pathway for simultaneous biotransformation of SMX and biosorption of Zn\(^{2+}\) by strain SDB4.
rate of 0.310 mg·L⁻¹·h⁻¹ and Zn²⁺ removal rate of 1.074 mg·L⁻¹·h⁻¹. Low concentrations of Zn²⁺ (<100 mg·L⁻¹) have a positive effect on the removal of SMX due to the acceleration of SMX transportation from extracellular solution to inside cells caused by Zn²⁺ during the reaction, while high concentrations of Zn²⁺ inhibited SMX removal. Furthermore, the removal mechanisms were investigated by UHPLC-MS-MS, strain SDB4 possessed the same biotransformation route of SMX in both SMX single and SMX-Zn²⁺ binary pollutants removal systems. The functional groups of OH, NH₂, C=O, C-N/N-H, and C-O-C were mainly involved in Zn²⁺ removal. This study indicates that Bacillus sp. strain SDB4 has an important potential for microbial remediation of SMX and Zn²⁺ contaminants and the applied experiments in practical complex polluted wastewater treatment will be carried out.

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