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# Effect of pH on antibiotic resistance genes removal and bacterial nucleotides metabolism function in the wastewater by the combined ferrate and sulfite treatment

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| Keywords:<br>Antibiotic resistance genes<br>Bacterial nucleotides metabolism<br>Ferrate<br>Sulfite<br>pH | The variations of the antibiotic resistance genes removal and the bacterial nucleotide metabolism function in the wastewater by the combined ferrate and sulfite treatment at different pH levels were investigated. The total DNA as well as the genes <i>sul2</i> , <i>sul3</i> and <i>int11</i> removal by the combined 10 mg/L ferrate and 28.5 mg/L sulfite treatment and the contributions of the sulfate radicals and the hydroxyl radicals produced by the reaction of ferrate with sulfite to the DNA removal decreased as the pH increase from 6.0 to 9.0. Bacterial nucleotide metabolism level changes affected the DNA removal, and the highest nucleotide degradation metabolism level and the lowest level of the nucleotide biosynthesis and salvage metabolism were found at pH 6.0. The reduction of the genes <i>sul2</i> , <i>sul3</i> and <i>int11</i> removal. This study provides novel insights into the antibiotic resistance genes removal by the combined ferrate and sulfite treatment at different pH levels. |

# 1. Introduction

Antibiotic resistance genes (ARGs) are a kind of emerging environmental pollutant that can persist in the environment for a long time, and are mainly derived from the response of bacteria to antibiotic pressure [1–3], and their long-term existence will severely threaten to public health [4]. World Health Organization has proclaimed that if effective action is not taken, more than 10<sup>7</sup> people will die from the infections of antibiotic resistant [5]. Wastewater treatment plants (WWTPs) receive antibiotics emissions from hospitals, residents and agriculture, and the presence of antibiotics in WWTPs can induce bacteria to produce ARGs [6]. This has led to WWTPs becoming a stockroom of ARGs, indicating that WWTPs will become an important shield to discourage the ARGs release into the aquatic environment [7]. Nevertheless, traditional WWTPs could not remove ARGs effectively [6]. Therefore, it is urgent to explore the method on the ARGs removal to avoid the excessive ARGs emissions from WWTPs into the aquatic environment.

The treatment system of combined ferrate ( $Fe^{6+}$ ) and sulfite ( $SO_3^2$ ) as a novel advanced oxidation process (AOP) has been widely used to remove some persistent emerging pollutants, as it is more environmental-friendliness, lower cost, and more stable production of free radicals than other AOPs (e.g., ozone/peroxide and ozone/ ultraviolet) [8,9]. The  $Fe^{6+}$  activation with  $SO_3^{2-}$  can rapidly undergo a single electron transfer reaction to generate pentavalent iron (Fe<sup>5+</sup>) and sulfite radical (SO $_3^{\bullet}$ ) (Eq. (1)), and the generated Fe<sup>5+</sup> and SO $_3^{\bullet}$  experience a series of chain reactions to further generate peroxymonosulfate radicals  $(SO_5^{\bullet-})$  and sulfate radicals  $(SO_4^{\bullet-})$ , hydroxyl radicals  $(HO_{\bullet})$  and hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>) (Eqs. (2)-(5)) [10-12]. Subsequently,  $H_2O_2$  can reduce  $Fe^{6+}$  to tetravalent iron ( $Fe^{4+}$ ) (Eq. (6)). Among the above active oxides, the oxidation activities of  $SO_5^{\bullet}$  and  $SO_3^{\bullet}$ are relatively low, and the reaction rate of  ${\rm Fe}^{6+}$  with  $H_2O_2$  is much lower than that of  $Fe^{6+}$  with  $SO_3^{2-}$ , resulting in the negligible production of  $Fe^{4+}$  [13,14]. In addition, the changes in the  $SO_3^{2-}/Fe^{6+}$  molar ratio can also affect the generation of active oxidizing species in the combined  $Fe^{6+}/SO_3^{2-}$  system. Shao et al. [15] found that when the molar ratio of  $SO_3^2$ /Fe<sup>6+</sup> was in the range of 1.5 to 10.0, the production of Fe<sup>5+</sup> could be negligible due to the promotion of  $Fe^{5+}$  to  $SO_4^{\bullet-}/HO_{\bullet}$  transfer by a large amount of  $SO_3^2$ . The results suggest that  $Fe^{6+}$ ,  $SO_4^{\bullet}$  and  $HO_{\bullet}$  are always the main active oxidizing substances in the system of combined  $\mathrm{Fe}^{6+}$  and  $\mathrm{SO}_3^2$ . It is worth noting that the alkalinity condition was conducive to the conversion of  $SO_4^{\bullet}$  to HO $\bullet$  according to Eq. (4), while at acid conditions, the conversion of  $SO_4^{\bullet-}$  to HO• would be slowed (Eq. (7)) [7]. In addition, many studies found that  $SO_4^{\bullet}$  was a more selective oxidant than HO• [16,17], which would lead to the difference in the

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removal of tested pollutants in the treatment system of combined  $Fe^{6+}$  and  $SO_3^{2-}$  at different pH levels. Notably, the effect of pH on the ARGs removal in the wastewater by the combined  $Fe^{6+}$  and  $SO_3^{2-}$  treatment has been not evaluated.

$$\mathrm{Fe}^{6+} + \mathrm{SO}_3^{2-} \to \mathrm{Fe}^{5+} + \mathrm{SO}_3^{6-} \tag{1}$$

$$\mathrm{SO}_3^{\bullet-} + \mathrm{O}_2 \to \mathrm{SO}_5^{\bullet-} \tag{2}$$

$$SO_5^{\bullet-} + SO_3^{2-} \rightarrow SO_4^{\bullet-} + SO_4^{2-}$$
(3)

$$SO_4^{\bullet-} + OH^- \rightarrow SO_4^{2-} + HO_{\bullet}$$
 (4)

$$Fe^{5+} + H_2O \rightarrow Fe^{5+} + H_2O_2$$
 (5)

$$Fe^{6+} + H_2O_2 \rightarrow Fe^{4+} + O_2$$
 (6)

$$SO_4^{\bullet-} + H_2O \rightarrow SO_4^{2-} + HO\bullet + H^+$$
(7)

$$\mathrm{Fe}^{6+} + \mathrm{H}_{2}\mathrm{O} \to \mathrm{Fe}^{3+} + \mathrm{O}_{2} \uparrow \tag{8}$$

$$Fe^{3+} + OH^- \rightarrow Fe(OH)_3$$
 (9)

Bacterial nucleotide metabolism actively participates in the formation of ARGs [18], and many studies have reported that the changes in the levels of the bacterial nucleotide metabolism could affect the ARGs formation, abundance, and transfer, etc. [19-21]. Zhong et al. [19] investigated the relationship of the ARGs removal with the bacterial nucleotide metabolism during the biogas residues composting, and the results showed that the addition of the bioaugmentation in the composting process caused the increase in the average bacterial nucleotide metabolism level and the decrease of the ARGs abundance compared to the composting process without bioaugmentation. Huang et al. [20] explored the endogenous and exogenous regulations of anammox consortia in responding to lincomycin, and found that bacteria maintained the formation and transmission of ARGs by regulating the nucleotide metabolism. Wu et al. [21] estimated the regulating resistome and metabolome of anammox consortia at the non-antibiotic drug stress, and found that the bacterial metabolism was one of the key driving factors for the ARGs transfer in the anammox systems. Currently, the information on the effects of the bacterial nucleotide metabolism on ARGs mainly focuses on some biological treatment systems. However, few works have been carried out to evaluate the relationship of the ARGs removal in the wastewater treated by combined  $Fe^{6+}$  and  $SO_3^{2-}$  at different pH levels with the bacterial nucleotide metabolism changes.

This study took the genes *sul2*, *sul3* and *int11* which were common genes in some WWTPs [22,23] as the target genes, and mainly aimed to assess the effect of the pH change in the wastewater treatment system of combined  $Fe^{6+}$  and  $SO_3^{2-}$  on (a) the removal of the total DNA and the genes *sul2*, *sul3* and *int11*, (b) the key contributors to the DNA removal, (c) the levels of the bacterial oxidative stress, and (d) the relationship of the ARGs removal with the bacterial nucleotide metabolism function.

# 2. Materials and methods

### 2.1. Wastewater and experiments setup

Three 1 L conical flasks were used to carry out the experiments of DNA removal in the wastewater by the combined  $Fe^{6+}$  and  $SO_3^{2-}$  treatment, and the three flasks were named N1, N2 and N3, respectively. In order to make the raw wastewater (named N0) in N1, N2 and N3 have the same characteristics, the raw wastewater was concurrently taken from the same effluent in one sequencing batch reactor (SBR) managed for longer than 30 days at 15 mg/L sulfamethoxazole in our laboratory. The raw wastewater contained 97.17 mg/L chemical oxygen demand, 5.97 mg/L ammonia nitrogen, 0.97 mg/L nitrate nitrogen, 0.28 mg/L nitrite nitrogen and 18 mg/L sludge (estimated by mixed liquor suspended sludge (MLSS)), and the levels of dissolved oxygen and pH in the raw wastewater were 2.2 mg/L and 6.7, respectively. The SBR was

operated three cycles in one day, and one cycle included 3 min influent, 330 min aerobic step, 90 min anoxic step, 54 min deposition and 3 min drainage effluent. The initial sludge in the SBR was obtained from a municipal wastewater treatment plant aerobic tank (Shenyang City, China), and the MLSS value of the initial sludge in the SBR was about 2000 mg/L. The volume of raw wastewater in N1, N2 and N3 was all 0.5 L. Acetic acid/sodium acetate buffer (0.2 mol/L) was used to regulate the initial pH of the raw wastewater in N1 to 6.0, and borax buffer (0.05 mol/L) was used to regulate the initial pH of the raw wastewater in N2 and N3 to 7.0 and 9.0, respectively. Subsequently, 5 mg Fe<sup>6+</sup> and 14.25 mg SO<sub>3</sub><sup>2-</sup> were immediately added into every conical flask (N1, N2 and N3) to assess the DNA removal by the combined 10 mg/L  $Fe^{6+}$  and 28.5 mg/L  $SO_3^{2-}$  at different pH levels. After the addition of coupled Fe<sup>6+</sup> and  $SO_3^{2-}$  in the raw wastewater, the three conical flasks (N1, N2 and N3) at room temperature were instantly stirred at 600 revolutions per minute (rpm) for 2 min and then beat up at 100 rpm for 20 min and finally settled for 30 min.

# 2.2. Determining methods

The wastewater samples before and after the mixed  $Fe^{6+}/SO_3^2$ treatment were obtained to measure the levels of total DNA, sul2, sul3, intI1, catalase (CAT), superoxide dismutase (SOD), reactive oxygen species (ROS) and lactate dehydrogenase (LDH) in the wastewater, to analyze the key contributors to the DNA removal, and to evaluate the changes in the microbial community. The extraction and determination of DNA in the wastewater was carried out via Bacterial Genomic DNA Extraction Kit (Phygene Biotechnology Company, China) according to Zhao et al. [24], and the DNA concentration was evaluated by spectrophotometry according to Ni et al. [25]. DNA purity was determined by calculating the ratio of absorbance at 260 nm and 280 nm (A260/A280) [26], and the DNA purity in the study was 1.83. The levels of ROS, LDH, CAT and SOD in the wastewater were measured via ROS kit (Beyotime Biotechnology Company, China), LDH kit (Beijing Solarbio Science and Technology Company, China), CAT kit (Beijing Solarbio Science and Technology Company, China) and SOD kit (Beijing Labgic Technology Company, China), respectively. The contributions of SO<sub>4</sub><sup>•</sup> and HO• to the DNA removal were investigated by the scavengers of tert-butyl alcohol (TBA) and ethanol (EtOH) in accordance with Zhang et al. [27]. The wastewater samples used for the determination of the microbial community changes and the levels of genes sul2, sul3 and intl1 were intercepted by 0.22  $\mu m$  membranes, and then these filters were preserved at -80 °C for their analyses [25]. The microbial community variations as well as the levels of genes sul2, sul3 and int11 in the wastewater were evaluated by high-throughput sequencing and by quantitative polymerase chain reaction, respectively, in Personalbio Company (Shanghai, China). The primers for genes sul2, sul3 and intI1 are shown in table S1. The network analysis was carried out by the software of Gephi 9.0 according to Wang et al. [28]. Potential pathways and functions of nucleotide metabolism were predicted by the software of PICRUSt2 based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp).

#### 3. Results and discussion

### 3.1. Effect of pH on the total DNA removal

Fig. 1a displays the changes in the total DNA concentration in the wastewater treated by jointly  $Fe^{6+}$  and  $SO_3^{2+}$  at different pH values. The total DNA concentration in the N0 (raw wastewater), N1 (treated wastewater at pH 6.0), N2 (treated wastewater at pH 7.0) and N3 (treated wastewater at pH 9.0) were 71, 8.5, 13.5 and 19 ng/µL, respectively. The total DNA removal efficiencies at pH 6.0, 7.0 and 9.0 were 87.84 %, 81.08 % and 72.97 %, respectively (Fig. 1b), suggesting that acid condition was more conducive to the total DNA removal by the joint treatment of  $Fe^{6+}$  and  $SO_3^{2-}$ . Alkalinity condition was conducive to



**Fig. 1.** Effect of pH on the total DNA concentration (a) and removal (b) in the wastewater treated jointly by  $Fe^{6+}$  and  $SO_3^{2-}$ . (c) Relative contributions of free radicals in the system of coupled  $Fe^{6+}/SO_3^{2-}$  to the total DNA removal from the wastewater at different pH levels. N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.

the conversion of  $SO_4^{\bullet}$  to HO• according to Eq. (4) [12], while at acid condition, the  $SO_4^{\bullet-}$  to HO• conversion would be slowed (Eq. (7)). Previous studies found that the redox potentials of SO<sub>4</sub><sup>-</sup> and HO• at alkalinity condition were 2.5 V and 1.9 V, respectively, and they at acid condition were 3.1 V and 2.8 V, respectively [29]. Generally, the higher redox potential of free radicals suggests greater oxidizability for pollutants [30], and it has been confirmed that SO<sub>4</sub><sup>•</sup> has a stronger ability to oxidize DNA than HO• [31]. These findings suggested that the promotion of  $SO_4^{\bullet}$  to HO• conversion at the alkalinity condition could adversely affect the total DNA removal, while the slowed conversion of SO<sup>4</sup> to HO• at acid condition would positively affect the total DNA removal. This explained why the total DNA removal in the wastewater treated jointly by  $Fe^{6+}$  and  $SO_3^{2-}$  at pH 9.0 was the lowest among the three pH levels, while the highest removal of total DNA was found at pH 6.0. The result was similar to the previous studies in which the  $k_{obs}$  of the iopamidol degradation by  $SO_4^{\bullet}$  and HO• was the highest at pH 6.0 in the pH range from 6.0 to 9.0 [32]. The contributors of  $SO_4^{\bullet}$  and HO• to the total DNA removal in the wastewater were assessed through quenching experiments, and the results are shown in Fig. 1c. At pH 6.0, 7.0 and 9.0, the relative contribution rates of  $SO_4^{\bullet-}$  to the total DNA removal were 50.86 %, 43.41 % and 29.79 %, respectively, and those for HO• were 30.51 %, 28.30 % and 27.65 %, respectively. The contribution ratios of SO<sub>4</sub><sup>•</sup> to HO• at pH 6.0, 7.0 and 9.0 were 1.67, 1.53 and 1.08, respectively, which also confirmed the promotion of SO<sub>4</sub><sup>•</sup> to HO• conversion by the alkalinity condition and the slowed conversion of SO4 to HO• at acid condition.

It is worth noting that  $Fe^{6+}$  could also clear DNA through oxidizing guanine and thymine bases [25], and  $Fe^{6+}$  has a stronger oxidant

activity in acidic media [33]. The changes also resulted in the promotion of total DNA removal in an acid environment compared to neutral and alkaline conditions. Suyamud et al. [34] found similar results that Fe<sup>6+</sup> caused more significant reductions of antibiotic resistant bacteria at pH 6.0 than those at pH 7.5. Additionally, at alkalinity conditions, Fe<sup>6+</sup> could be converted to Fe(OH)<sub>3</sub> (Eqs. (8) and (9)), which would reduce the performance of total DNA removal by  $SO_4^{\bullet-}$ , HO• and Fe<sup>6+</sup>. The changes might also be one of the reasons that the total DNA removal at pH 6.0 was the highest among the pH 6.0, 7.0 and 9.0. Although  $Fe^{6+}$ , SO<sup>4</sup> and HO• are the primary active oxidizing species in the system of combined  $\rm Fe^{6+}$  and  $\rm SO_3^{2-}$  with an SO\_3^2-/Fe^{6+} molar ratio of 2.0 [15], there is little information on the quenching experiments of Fe<sup>6+</sup> in the combined  $Fe^{6+}/SO_3^{2-}$  system in the previous studies. As a result, the contributions of  $SO_4^{\bullet}$ , HO• and other factors to the total DNA removal were mainly analyzed. Interestingly, the relative contribution rates of HO• to the total DNA removal theoretically seemed to be decreased with the reduction of pH level according to the above conversion of SO4 to HO. at different pH, while the relative contribution rates of HO• to the total DNA removal were actually increased as the decline of pH value. The changes in the relative contribution rates of HO• to the total DNA removal suggested that the contribution of other factors (such as the oxidation of Fe<sup>6+</sup> as well as the adsorption of Fe(OH)<sub>3</sub>) to total DNA removal as the decrease of pH value reduced more significantly than HO•, resulting in the "false increase" in the relative contribution rates of HO• to the removal of total DNA as the decreased pH value. The contributions of other factors at pH 6.0, 7.0 and 9.0 were 18.63 %, 28.29 %and 42.56 %, respectively. The increasing contributions of other factors to the total DNA removal as the incremental pH level were related to the

easier formation of Fe(OH)<sub>3</sub> at the alkalinity condition, suggesting the role of Fe(OH)<sub>3</sub>-adsorption DNA was enhanced as the increasing pH level in the treatment of combined Fe<sup>6+</sup> and SO<sub>3</sub><sup>2-</sup>. Li et al. [35] found similar results in which the DNA removal was efficiently achieved by the coagulation of ferric chloride. At pH 6.0 and 7.0, SO<sub>4</sub><sup>4-</sup> contributed the highest DNA removal rate, while other factors had the relatively highest contribution to DNA removal at pH 9.0. The results confirmed that the effect of pH on the total DNA removal treated jointly by Fe<sup>6+</sup> and SO<sub>3</sub><sup>2-</sup> was achieved by affecting the production of free radicals.

# 3.2. Effect of pH on the removals of antibiotics resistance genes and mobile genetic element genes

Fig. 2 displays the effect of pH on the removals of genes *sul2*, *sul3* and *int11* in the wastewater treated jointly by Fe<sup>6+</sup> and SO<sub>3</sub><sup>2-</sup>. The *sul2* and *sul3* genes belong to ARGs, and the *int11* gene belongs to mobile genetic element genes. The gene copy numbers of *sul2*, *sul3* and *int11* in the raw wastewater (N0) from N1, N2 and N3 were 75507, 189,561 and 562,394 copies/mL, respectively (Fig. 2a). At pH 7.0, the removal efficiencies of genes *sul2*, *sul3* and *int11* were 55.58 %, 87.27 % and 80.46 %, respectively, and they at pH 9.0 were 50.69 %, 73.08 % and 77.69 %, respectively (Fig. 2b). The removal rate of genes *sul2*, *sul3* and *int11* at pH 7.0 were higher than those at pH 9.0. The second-order rate constant of



**Fig. 2.** Effect of pH on the removals of genes *sul2*, *sul3* and *intl1* in the wastewater treated jointly by  $Fe^{6+}$  and  $SO_3^{2-}$ . (a) was genes contents. (b) was genes removal changes. N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.

HO• with purines and pyrimidines in DNA was lower than  $SO_4^{\bullet}$  [36], suggesting that SO<sub>4</sub><sup>•</sup> had higher abilities of breach to DNA than HO•. At alkalinity conditions, the SO<sub>4</sub><sup>•-</sup> to HO• conversion could be promoted (Eq. (4)), resulting in the removal rates of genes sul2, sul3 and intl1 at pH 7.0 were higher than at pH 9.0. At pH 6.0, the removal rate of genes sul2, sul3 and intl1 were 81.40 %, 92.67 % and 87.78 %, respectively, and they were the highest at the three treatment processes. The changes could be explained by the slow-moving conversion of SO<sub>4</sub><sup>-</sup> to HO• at the acid condition (Eq. (7)) resulting in more contents of  $SO_4^{\bullet-}$  in the treatment process at pH 6.0 than at pH 7.0 and 9.0. The gene sul3 removal at pH 6.0 and 7.0 was the highest among the genes of sul2, sul3 and intI1, while the highest removal rate of tested genes at pH 9.0 was gene intl1. Previous studies [36,37] found that ARGs in the wastewater could be directly inactivated via indirect damage of the DNA structure, and the reaction rate constant of pyrimidine with  $SO_4^{\bullet-}$  or HO• at some pathways (such as radical adduct formation, single electron transfer reactions or H atom abstraction) was higher than purine, meanwhile the reaction rate constant could be affected by the change in the structure of purine and pyrimidine. The results suggested that the difference in the genes *sul2*, sul3 and intI1 removals might be interrelated to the composition of purine and pyrimidine in their DNA fragments. Previous studies [38,39] found that gene sul3 had more pyrimidines than gene sul2, and pyrimidines were destroyed by SO<sub>4</sub><sup>•-</sup> and HO• more easily than purines. The results could explain why the removal rate of the gene sul3 was higher than the gene sul2. The composition of purine and pyrimidine in the gene intl1 has not been reported in the literature, and it needs to be further studied to better understand the effect of pH on the removals of DNA treated by the combined  $Fe^{6+}$  and  $SO_3^{2-}$ .

# 3.3. Effect of pH on the bacterial oxidative stress

Fig. 3 displays the effect of pH on the bacterial oxidative stress changes in the wastewater by the joint treatment of  $Fe^{6+}$  and  $SO_3^2$ . Compared to the raw wastewater (N0), the relative CAT level in the treated wastewater at pH 6.0 (N1), 7.0 (N2) and 9.0 (N3) decreased to 74.94 %, 78.59 % and 85.66 %, respectively, and the relative SOD content decreased to 83.67 %, 91.67 % and 94.34 %, respectively. CAT and SOD were antioxidant enzymes produced by bacteria, and they catalyzed the decomposition of strong oxidative matters [40,41]. When the cells were healthy, there would be a balanced relationship of oxidizing substances (such as ROS) with CAT and SOD [42]. If the balanced relationship was disrupted, the bacterial oxidative stress



**Fig. 3.** Effect of pH on the bacterial oxidative stress changes in the wastewater by the joint treatment of  $\text{Fe}^{6+}$  and  $\text{SO}_3^{2-}$ . N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.

would be triggered [43]. The decreased degree of CAT and SOD relative levels in the treated wastewater at pH 6.0 was the highest among pH 6.0, 7.0 and 9.0. The changes were related to the less conversion of  $SO_4^{\bullet}$ (with a relatively high DNA removal ability) to HO• (with a relatively low DNA destruction ability) at acidic conditions compared to neutral and alkaline conditions (Eq. (7)). The decrease of CAT and SOD levels would cause bacterial oxidative stress changes, resulting in the excessive produce of ROS in cells [44]. The changes would increase the ROS levels in the wastewater. Compared to the raw wastewater, the relative ROS levels in the treated wastewater at pH 6.0, 7.0 and 9.0 were 164.27 %, 153.35 % and 148.91 %, respectively. The changes in the ROS levels at different pH values could be explained by the decrease of CAT and SOD levels as the reduction of pH value. The variations in the relative levels of CAT, SOD and ROS in the treated wastewater suggested that the joint treatment of  $Fe^{6+}$  and  $SO_3^{2-}$  at different pH levels disrupted the balance of bacterial oxidative stress, and the disruption at pH 6.0 was more significant. High ROS levels would increase the cell membrane permeability, and the high cell membrane permeability resulted in increasing LDH levels [45]. Compared to the raw wastewater, the relative LDH levels in the treated wastewater at pH 6.0, 7.0 and 9.0 were 148.56 %, 131.09 % and 113.61 %, respectively. The results suggested that the joint treatment of  $Fe^{6+}$  and  $SO_3^{2-}$  at different pH levels could increase the cell membrane permeability due to the bacterial oxidative stress imbalance, and the cell membrane permeability increased more obviously at pH 6.0.

# 3.4. Effect of pH on the nucleotide metabolic pathways in bacteria

As the DNA fragments in the wastewater were mainly derived from bacteria, the bacterial metabolic function changes would alter the DNA levels in the wastewater [46,47]. To understand the effects of bacterial metabolic function on the DNA levels in the wastewater by the combined Fe<sup>6+</sup> and SO<sub>3</sub><sup>2-</sup> treatment at different pH values, the levels in the metabolic pathways of bacteria and in the enzymes involved into these metabolic pathways were analyzed based on the KEGG database. 397 PICRUSt2 predicted metabolic pathways and 2000 enzymes were found in different samples, and the number of pathways and enzymes involved in the nucleotide metabolism were 36 and 44, respectively (Fig. 4). The numbers of metabolic pathways associated with the biosynthesis, degradation and salvage of nucleotide were 23, 8 and 5, respectively, and the numbers of enzymes involved into the nucleotide metabolic pathways were 29, 9 and 6, respectively. In the raw wastewater, the total relative abundance of metabolic pathways associated with the biosynthesis, degradation and salvage of nucleotide was 18.10 %, and the sum of the relative abundance of enzymes involved into nucleotide metabolism was 5.15 % (Fig. S1). They in the raw wastewater were lower than those in the treated wastewater, suggesting the raising DNA metabolism levels (especially increasing biosynthesis and salvage of nucleotide) were the protective response of bacteria to the DNA damaged by the combined  $Fe^{6+}$  and  $SO_3^{2-}$  at different pH levels. In addition, the results also suggested that the bacterial metabolism function changes could affect the DNA levels in the wastewater [46,47]. In comparison with the raw wastewater, the relative level of nucleotide biosynthesis pathways and enzymes involved into these metabolic pathways in the treated wastewater at different pH values were always higher, which could be interrelated to the response of bacteria to the DNA breakdown in the combined Fe<sup>6+</sup> and SO<sub>3</sub><sup>2-</sup> treatment system. When DNA was damaged or even cleared, bacteria produced DNA based on a bacterial self-protection mechanism [48]. This might cause more active nucleotide biosynthesis metabolism. In the treated wastewater at different pH levels, the most relative abundance of nucleotide biosynthesis pathways (14.24 %) and the highest relative levels of corresponding enzymes (4.68 %) were both found at pH 6.0, which was consistent with more DNA being broken down in the wastewater treated by the combined  $\mathrm{Fe}^{6+}$  and  $\mathrm{SO}_3^{2\text{-}}$  at pH 6.0. The relative abundance of nucleotide salvage pathways and enzymes involved into these metabolic

pathways in the raw wastewater were always lower than those in the treated wastewater at pH 6.0, while not always lower than those in the treated wastewater at pH 7.0 and 9.0. The changes were similar to the DNA removal by the combined  $Fe^{6+}$  and  $SO_3^{2-}$  treatment at different pH levels, suggesting that the DNA removal at pH 6.0 would cause more nucleotide fragments. The relative abundance of the nucleotide degradation pathways and the levels of the enzymes involved into these metabolic pathways in the treated wastewater at different pH levels were always lower than the raw wastewater, and those at pH 6.0 were the lowest. The changes might be related to the bacterial self-protection mechanism that allowed bacteria to reduce their own degradation of nucleotides to maintain the balance of DNA levels. The changes in the levels of pathways and enzymes involved in the bacterial nucleotide metabolism confirmed that the pH changes in the combined Fe<sup>6+</sup> and  $SO_3^{2-}$  treatment system had significant effects on the bacterial function on nucleotide metabolism, and the changes in the bacterial nucleotide metabolism function could affect the levels of DNA in the wastewater.

# 3.5. Effect of pH on the relationship of antibiotics resistance genes and mobile genetic element genes with bacterial nucleotide metabolism function

To understand further the effect of bacterial nucleotide metabolism function on the levels of DNA in the wastewater by the combined Fe<sup>6+</sup> and  $SO_3^{2-}$  treatment at different pH levels, the potential genes sul2, sul3 and intI1 hosts (genus level) were analyzed via a network analysis on the basis of the correlation of Pearson (|r| > 0.8) (Fig. 5a). There were 196 genera in the raw and treated wastewater at the different pH values, of which 25 genera accounted for about 90 % of total abundance in every sample (Fig. 5b). In the 25 genera, 7 genera were positive relations with genes sul2, sul3 and intI1, implying that these genera were the potential hosts of genes sul2, sul3 and intI1 [28]. The relative abundance of potential genes sul2, sul3 and intl1 hosts in the treated wastewater at pH 6.0, 7.0 and 9.0 were always lower than those in the raw wastewater, which might cause the decrease in the levels of genes sul2, sul3 and intI1 after the treatment. The cluster analysis of bacterial communities from the raw and treated wastewater showed that the bacterial community in the raw wastewater was similar to that in the treated wastewater at pH 9.0, and the microbial community in the treated wastewater at pH 7.0 and 6.0 had similarities. The results suggested that the combined Fe<sup>6+</sup> and  $SO_3^{2-}$  treatment had more significant effects on the bacterial community at pH 9.0 than those at pH 6.0 and 7.0, which might be one of the reasons that the DNA removal at pH 9.0 was lower than at pH 6.0 and 7.0.

The relationship of genes sul2, sul3 and intl1 with enzymes involved into the bacterial nucleotide metabolism pathways was analyzed via a network analysis on the basis of the Pearson correlation  $(|\mathbf{r}|>0.8)$ (Fig. 6a). The positive relation of genes sul2, sul3 and intl1 with enzymes involved into the metabolic pathways of nucleotide suggested that the inhibition of these enzymes metabolism might be one of the ways for the genes sul2, sul3 and intI1 removal, while the negative relations suggested a bacterial repair response to the genes sul2, sul3 and intl1 breach. Dihydroorotate dehydrogenase (quinone) (EC: 1.3.5.2) and guanine deaminase (EC: 3.5.4.3) were the enzymes involved into the pathways of pyrimidine biosynthesis and purine salvage, respectively, and these enzymes levels were positively related to the changes of genes sul2, sul3 and intI1 levels at different pH values. The results suggested that the inhibition of the dihydroorotate dehydrogenase (quinone) of pyrimidine biosynthesis and the guanine deaminase of purine salvage by the combined treatment of Fe<sup>6+</sup> and SO<sub>3</sub><sup>2-</sup> at different pH levels might be one of the ways for the removal of genes sul2, sul3 and intI1. The relative levels of dihydroorotate dehydrogenase (quinone) were always higher than guanine deaminase at different pH levels (Fig. 4b), suggesting that the pyrimidine biosynthesis was damaged by the combined treatment of  $Fe^{6+}$  and  $SO_3^{2-}$  more easily than purine salvage. In the raw wastewater, the sum of pyrimidine biosynthesis and salvage enzymes (the negative relationship with the changes of genes sul2, sul3 and intI1) relative levels

| S  | Function description   |  |  |   |  | e (%)   | Metabolism pathways                        |  |  |
|--|--|--|--|---|--|---|--|--|--|
| C  | Superpathway of histidine, purine, an  | d pyrimidine biosynthesis  | 0.38   | 0.36  | 0.39   | 0.39  |  |  |  |
| Superpathway of  | f pyrimidine deoxyribonucleotides de   | novo biosynthesis (E. coli)  | 0.63   | 0.70  | 0.66   | 0.66  | (a)  |  |  |
| 1000   | Pyrimidine deoxyribonucleotic  | es de novo biosynthesis III  | 0.18   | 0.15  | 0.20   | 0.18  | ()   |  |  |
|  | Pyrimidine deoxyribonucleot  | ides de novo biosynthesis I  | 0.64   | 0.71  | 0.67   | 0.68  |  |  |  |
|  | Pyrimidine deoxyribonucleoti<br>Pyrimidine deoxyribonucleoti   | des de novo biosynthesis II<br>les hiosynthesis from CTP   | 0.46   | 0.52  | 0.45   | 0.45  |  |  |  |
| Superpar   | thway of pyrimidine deoxyribonucleo  | tides de novo biosynthesis   | 0.60   | 0.60  | 0.60   | 0.60  |  |  |  |
|  | Superpathway of arginine a   | nd polyamine biosynthesis  | 0.14   | 0.10  | 0.13   | 0.12  |  |  |  |
|  | L-arginine bios  | nthesis I (via L-ornithine)  | 0.58   | 0.66  | 0.63   | 0.61  |  |  |  |
|  | Superpathway of purine nucleoti  | des de novo biosynthesis II  | 0.52   | 0.57  | 0.51   | 0.50  | N. I. of the state of the                  |  |  |
|  | 5-aminoimidazole ril   | onucleotide biosynthesis I   | 0.68   | 0.75  | 0.72   | 0.71  | Nucleofide biosynthesis metabolisi         |  |  |
|  | Superpathway of guanosine nucleoti   | des de novo biosynthesis II  | 0.68   | 0.73  | 0.69   | 0.69  |  |  |  |
|  | Superpathway of adenosine nucleoti   | des de novo biosynthesis II  | 0.70   | 0.77  | 0.74   | 0.73  |  |  |  |
| S  | Superpathway of 5-aminoimidazole r   | ibonucleotide biosynthesis   | 0.68   | 0.76  | 0.72   | 0.71  |  |  |  |
|  | Adenosine ribonucle  | tides de novo biosynthesis   | 0.74   | 0.81  | 0.79   | 0.77  |  |  |  |
|  | Guanosine ribonucleou  | des de novo biosynthesis il  | 0.00   | 0.71  | 0.09   | 0.69  |  |  |  |
|  | Guanosine deoxyribonucleoti  | des de novo biosynthesis II  | 0.66   | 0.71  | 0.69   | 0.69  |  |  |  |
|  | Superpathway of guanosine nucleot  | ides de novo biosynthesis I  | 0.69   | 0.74  | 0.70   | 0.70  |  |  |  |
|  | Superpathway of adenosine nucleot  | ides de novo biosynthesis I  | 0.73   | 0.79  | 0.76   | 0.76  |  |  |  |
|  | Superpathway of purine nucleot   | ides de novo biosynthesis I  | 0.68   | 0.75  | 0.71   | 0.71  |  |  |  |
| 2  | Sum of nucleotide biosynthesis meta  | bonucleosides degradation  | 0.10   | 0.07  | 0.08   | 0.09  |  |  |  |
|  | Pyrimidine deoxyribor  | ucleotide phosphorylation  | 0.59   | 0.65  | 0.62   | 0.61  |  |  |  |
|  | Purine nucleobase  | degradation I (anaerobic)  | 0.13   | 0.09  | 0.11   | 0.11  |  |  |  |
|  | Purine ri  | bonucleosides degradation  | 0.15   | 0.10  | 0.12   | 0.13  | Nucleotide degradation motabolism          |  |  |
|  | Superpathway of purine deoxyri   | bonucleosides degradation  | 0.25   | 0.24  | 0.23   | 0.21  | restruction and a second second            |  |  |
|  | Purine nucleotic   | es degradation II (aerobic)  | 0.49   | 0.46  | 0.45   | 0.47  |  |  |  |
|  | Guanosine  | nucleotides degradation III  | 0.52   | 0.46  | 0.53   | 0.55  |  |  |  |
|  | Sum of nucleotide degradation meta   | bolism relative abundance  | 2.74   | 2.51  | 2.66   | 2.71  |  |  |  |
|  | Superpathway of pyrimidi   | ne ribonucleosides salvage   | 0.34   | 0.31  | 0.28   | 0.29  |  |  |  |
|  | Pyrimidine des   | xyribonucleosides salvage  | 0.24   | 0.24  | 0.23   | 0.20  |  |  |  |
|  | Superpathway of pyrimidine de  | oxyribonucleoside salvage  | 0.35   | 0.37  | 0.35   | 0.31  | Nucleotide salvage metabolism              |  |  |
|  | Superpathway of pyrin  | idine nucleobases salvage  | 0.72   | 0.79  | 0.75   | 0.75  |  |  |  |
|  | Adenin   | e and adenosine salvage ill  | 0.47   | 0.50  | 0.50   | 0.50  |  |  |  |
| -  | oum of nucleotide salvage meta   | ousin relative abundance   | 2.13   | 2.20  | 2.10   | 2.04  | Market Inc. 12                             |  |  |
|  | Module level 3   |  | NU   | NI  | N2   | NS  | Module level 2                             |  |  |
| Entry of   | Relative abundance (%)   | Functio  | n desc   | ription   | apres  | Nu  | icleotides metabolism nathways             |  |  |
| enzymes  | ( 0 0 21 ( 0 0 21 ( 0 0 21 ( 0 0 22  | Pihonuclassida dinharnhata   | raductors  |   |  |   |  |  |  |
| C:1.3.1.14   | 0 0.07 0 0.10 0 0.09 0 0.05  | Dihydroorotate dehydrogena   | se (NAD(+  | -11   |  |   |  |  |  |
| C:1.3.5.2  | 0.07 0 0.06 0 0.06 0 0.07  | Dihydroorotate dehydrogena   | se (quinor   | 143   |  |   | (b)  |  |  |
| C-1.3.98.1   |  | Dihydroorotate oxidase (fun  | arate)   |   |  |   | (0)  |  |  |
| C:2.1.1.45   | 0 0.17 0 0.21 0 0.18 0 0.20  | Thymidylate synthase   |  |   |  |   |  |  |  |
| C:2.1.3.2  | 0 0.14 0 0.16 0 0.15 0 0.15  | Aspartate carbamovitransfer  | ase  |   |  |   |  |  |  |
| C-2.4.2.10   | Co12 Co14 Co15 Co15  | Orotate phosphoribosyltran   | ferase   |   |  | Perintidia  |  |  |  |
| C:2.7.4.22   | 0 0.14 0 0.16 0 0.15 0 0.15  | UMP kinase   |  |   |  | - /   | -  |  |  |
| C:2.7.4.6  | 0 0.14 0 0.16 0 0.15 0 0.15  | Nucleoside-diphosphate kins  | ise  |   |  |   |  |  |  |
| 0.16133  | Colla Colle Colls Colls  | dTMP kinase  |  |   |  |   |  |  |  |
| C:4.1.1.23   | 0 0.14 0 0.16 0 0.15 0 0.15  | Orotidine-5'-photphatate deca  | rboxvlate  |   |  |   | 1  |  |  |
| C:6.3.4.2  | @ 0.14 @ 0.16 @ 0.15 @ 0.15  | CTP synthase (glutamine hy   | drolvnng)  |   |  |   | Nucleotide biosynthesis                    |  |  |
| C:6.3.5.5  | 0.30 0.33 0.31 0.31  | Carbamovi-phosphate synth  | ase (glutar  | nine-hvdrol   | lvzing)  |   | metabolism                                 |  |  |
| C-111205   | 0 0.19 0 0.20 0 0.15 0 0.18  | TMP dehydrogenere  |  | sum of reli   | nive abund   | ance  | ine thousan                                |  |  |
| C2122  | 0 0.16 0 0.17 0 0.17 0 0.16  | Phosphoribosviglycinamide  | formyltra  | asferase  |  |   |  |  |  |
| C24214   | 0 0.14 0 0.16 0 0.15 0 0.15  | Amidophosphoribosvitransf  | erase  |   |  |   |  |  |  |
|  | 0.15 0.17 0.16 0.16  | Adenvlate kinase   |  |   |  |   |  |  |  |
| C:1.7.4.3  | 0 0 14 0 0 16 0 0 15 0 0 15  | I CONTRACTOR AND A |  |   |  |   |  |  |  |
| C:2.7.4.3<br>C:2.7.4.8<br>C:4.3.2.2  | 0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.14 0 0.16 0 0.15 0 0.15   | Adenvlosuccinate lyace   |  |   |  |   |  |  |  |
| C 2.7.4.3<br>C 2.7.4.8<br>C 4.3.2.2<br>C 6.3.2.6   | 0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.19 0 0.23 0 0.21 0 0.22  | Adenvlosuccinate lvase<br>Phosphoribosvlaminoimidaz  | olesuccino   | carboxamie  | ie synthase  | Purine  |  |  |  |
| C 2.7.4.3<br>C 2.7.4.8<br>C 4.3.2.2<br>C 6.3.2.6<br>C 6.3.3.1  | 0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.19 0 0.23 0 0.21 0 0.22<br>0 0.14 0 0.16 0 0.15 0 0.15   | Adenvlosuccinate lvase<br>Phosphoribosylaminoimidae<br>Phosphoribosylaminoimidae   | olesuccino<br>amidine c  | carboxamie<br>velo-ligase   | de svuthase  | Purine  |  |  |  |
| C-2.7.4.3<br>C-2.7.4.8<br>C-4.3.2.2<br>C-6.3.2.6<br>C-6.3.3.1<br>C-6.3.4.13<br>C-6.3.4.14  | 0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.19 0 0.23 0 0.21 0 0.22<br>0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.14 0 0.16 0 0.15 0 0.15<br>0 0.14 0 0.16 0 0.15 0 0.15   | Adenvlosuccinate lvase<br>Phosphoribosylaminoimidar<br>Phosphoribosylaminoimidar<br>Phosphoribosylamine-glycin<br>Solarbayyaminoimidar   | olesuccino<br>amidine cu<br>te ligave  | carboxamie<br>velo-ligase   | le svuthase  | Purine  |  |  |  |
| C2743<br>C2748<br>C4322<br>C6326<br>C6331<br>C63413<br>C63418<br>C6344   | 0         0.14         0         0.16         0         0.15         0         0.15           0         0.14         0         0.16         0         0.15         0         0.15           0         0.14         0         0.16         0         0.15         0         0.15           0         0.14         0         0.16         0         0.15         0         0.15           0         0.14         0         0.16         0         0.15         0         0.15           0         0.14         0         0.16         0         0         0         0         0.15         0         < | Adenviate innase<br>Adenviosucinate ivase<br>Phosphoribosviaminoimidaz<br>Phosphoribosviformvigivcin<br>Phosphoribosviamine-givcii<br>5-(carboxvamino)imidazole r<br>Adenviosucinate svathase  | olesuccino<br>amidine cu<br>le ligave<br>ibonucleo   | carboxamie<br>vclo-ligase<br>tide synthas   | de svuthase<br>æ   | Purine  |  |  |  |
| C1743<br>C1748<br>C4322<br>C6326<br>C6331<br>C63413<br>C63413<br>C63418<br>C6344<br>C6352  | $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$   | Connyine cinate lyase<br>Adenviouccinate lyase<br>Phosphoribosylaminoimidaz<br>Phosphoribosylamine-zlyci<br>5-(carboxyamino)inidazole r<br>denviouccinate synthase<br>GMP synthase (glutamine-hy   | olesuccino<br>amidine cu<br>te ligate<br>ibonucleo   | carboxamic<br>velo-ligase<br>tide synthas   | de svuthase<br>ie  | Purine  |  |  |  |
| C1743<br>C2748<br>C4322<br>C6326<br>C6331<br>C63413<br>C63418<br>C6344<br>C6352<br>C6352<br>C6353  | 0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.14         0.16         0.15         0.15         0.15           0.15         0.15         0.15         0.15         0.15           0.15         0.16         0.15         0.15         0.15           0.15         0.16         0.15         0.15         0.15           0.15         0.16         0.15         0.15         0.15           0.20         0.20         0.20         0.20         0.20         0.20         0.20           0.20         0.20         0.20         0.30         0.30         0.30         0.30   | Gunnyian cinais<br>Phosphoribosylaminoimidar<br>Phosphoribosylaminoimidar<br>Phosphoribosylamine-zlyci<br>5-(carboxyamino)imidarole r<br>Adenylosuccinate synthase<br>GMP synthase (edutamine-h<br>Phosphoribosylformylglycin  | olesuccino<br>amidine cr<br>ie ligave<br>ibonucleo<br>rdrolvring<br>amidine st   | carboxamis<br>vclo-ligase<br>tide synthas<br>)<br>ynthase   | de svuthase<br>se  | Purine  |  |  |  |
| C:1743<br>C:2748<br>C:4322<br>C:6326<br>C:6331<br>C:63413<br>C:63418<br>C:6344<br>C:6352<br>C:6353<br>C:6353   | 0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.14         0.16         0.15         0.15           0.15         0.15         0.15         0.15           0.15         0.15         0.15         0.15           0.15         0.15         0.15         0.15           0.20         0.20         0.19         0.19           0.20         0.22         0.30         0.30           1.16         2.37         2.23         2.23           0.80         0.80         0.80         0.80  | Guantine tinate<br>Adentioucinate Vase<br>Phosphoribotylaminoimidaz<br>Phosphoribotylamino-givin<br>5-(carboxyamino)inidazole r<br>Adentioucinate synthase<br>GAP synthase (caltamine)-<br>Phosphoribotylforawigiytin<br>Dibydronesell Adentica  | olesuccino<br>amidine c<br>ie ligate<br>ibonucleo<br>adrohving<br>amidine s  | carboxamis<br>velo-ligase<br>tide tynthas<br>ynthase<br>Sum of rels   | de synthase<br>æ<br>ntive abund  | Purine  |  |  |  |
| C2.7.4.3<br>C2.7.4.8<br>C4.3.2.2<br>C6.3.2.4<br>C6.3.4.13<br>C6.3.4.13<br>C6.3.4.14<br>C6.3.5.2<br>C6.3.5.3<br>C1.3.14<br>C3.5.1.10  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guanviae timore<br>Adenviouciante Vasse<br>Phosphoribovylaminoimida<br>Phosphoribovylamine-advin<br>Phosphoribovylamine-advin<br>S-(carboxyaminoimidanole r<br>Adenviouciante vynthase<br>GMP synthase (glutamine-br<br>Phosphoribovylforawigivin<br>Dihvdrourscil dehvdrogenas<br>Peroxymeidoacytals uraido   | olesuccino<br>amidine cu<br>ibonucleo<br>adrohving<br>amidine su<br>e (NAD(+)  | carboxamic<br>vclo-ligase<br>tide tynthas<br>ynfhase<br>Sum of rela<br>)<br>nidohydrol  | de synthase<br>æ<br>stive abund  | Purine  |  |  |  |
| C2.7.43<br>C2.7.45<br>C4.3.2.2<br>C5.3.2.6<br>C5.3.2.6<br>C5.3.413<br>C5.3.413<br>C5.3.415<br>C5.3.415<br>C5.3.42<br>C5.3.5.2<br>C5.3.53<br>C1.3.11<br>C3.5.110<br>C3.5.2.2  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Gunning innie<br>Adenviouciansk Pass<br>Phosphoriboviloruvigivin<br>Phosphoriboviloruvigivin<br>Phosphoriboviloruvigivin<br>Scarboxyaminojinidarole r<br>Adenviouciansk vathase<br>GMP vynthase (glutamine-hy<br>Phosphoriboviloruvigivin<br>Dihvdropracii dehvdrogenas<br>Peroxyureidoactylais ureido<br>Dihvdroprimidinase   | olesuccino<br>amidine c<br>ie ligave<br>ibonucleo<br>odrolvning<br>amidine s<br>e (NAD(+)<br>acrylate an                   | carboxamic<br>vclo-lizate<br>tide tynthas<br>ymthase<br>Sum of rela<br>()<br>midohydrol   | de synthase<br>e<br>ative abund<br>ase   | Purine<br>ance<br>Pyrimidir   |  |  |  |
| C2.7.43<br>C2.7.43<br>C2.7.45<br>C4.3.2.2<br>C6.3.2.6<br>C6.3.3.1<br>C6.3.4.13<br>C6.3.4.13<br>C6.3.4.13<br>C6.3.4.4<br>C6.3.5.2<br>C6.3.5.3<br>C1.3.1.1<br>C3.5.1.110<br>C3.5.2.2   | $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$   | Guanviae cinaxe<br>Adewstouccinate Yaxse<br>Phosphoribovylaminoimida<br>Phosphoribovylforuvtdytion<br>Phosphoribovylforuvtdytion<br>Phosphoribovylforuvtdytion<br>CAE yvanhase (guanaime-br<br>Fhosphoribovylforuvtdytion<br>Dihvdrouracii dehvdrogenas<br>Peroxyureidoscrylate ureido<br>Dihvdropyrimidinase  | olesuccino<br>amidine cr<br>se ligave<br>ibonucleot<br>rdrolvzing<br>amidine sr<br>e (NAD(+)<br>acrylate an                | carboxamic<br>velo-ligase<br>tide tynthas<br>ynthase<br>Sum of rela<br>nidohydrol<br>Sum of rela                                  | de synthase<br>e<br>stive abund<br>ase<br>stive abund                                | nnce Pyrimidiz  | 1  |  |  |
| C27.43<br>C27.43<br>C27.45<br>C43.22<br>C43.24<br>C63.26<br>C63.41<br>C63.413<br>C63.44<br>C63.52<br>C63.53<br>C13.11<br>C3.51.110<br>C3.522<br>C3.52.17<br>C3.52.17<br>C3.52.17   | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guinvine timore<br>Adews/outcome by your<br>Phosphoribov/four-tdytwin<br>Phosphoribov/four-tdytwin<br>Phosphoribov/four-tdytwin<br>Phosphoribov/four-tdytwin<br>GMP synthase (clutamine-ln<br>Phosphoribov/formvigtytin<br>Dihvdrourscil dehvdrogenas<br>Peroxymeidoacr/lais-urido<br>Dihvdropvrimidinase<br>Hvdropvrimidinase<br>Hvdropvrimidinase  | olesuccino<br>amidine (t<br>se ligate<br>ibonucleol<br>rdrolvzing<br>amidine (t<br>e (NAD(+)<br>acrylate an                | carboxamic<br>vclo-ligase<br>itide tynthat<br>)<br>withase<br>Sum of rela<br>nidohydrol<br>Sum of rela                            | de synthase<br>e<br>ative abund<br>ase<br>ative abund                                | Purine<br>ance<br>pyrimidiz   | n<br>Nucleotide degradation                |  |  |
| C2.7.4.3<br>C2.7.4.8<br>C2.7.4.8<br>C4.3.2.2<br>C6.3.3.1<br>C6.3.4.13<br>C6.3.4.13<br>C6.3.4.145<br>C6.3.5.3<br>C6.3.5.3<br>C1.3.1.1<br>C.3.5.1.10<br>C.3.5.2.17<br>C3.5.2.6<br>C3.5.2.17<br>C3.5.2.6  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guanviae cinave<br>Adewstouccinate Yasse<br>Phosphoribovylaminoimida<br>Phosphoribovylamine-alvcin<br>Phosphoribovylamine-alvcin<br>S-(carboxynamio)imidanole r<br>Ademstouccinate synthase<br>GAP synthase (clutamine-hr<br>Phosphoribosylforawigivein<br>Dihvdrouracil dehvdrogenas<br>Peroxynreidoacyvlate ureido<br>Dihvdrouracil dehvdrogenas<br>Peroxynreidoacyvlate ureido<br>Dihvdropyviimidinase<br>Hvdroxyviiourate hvdrolase<br>Allantoicase  | olesuccino<br>amidine (t<br>se ligate<br>ibonucleol<br>rdrolvzing<br>amidine (t<br>e (NAD(+)<br>acrylate an                | carboxami<br>vclo-ligase<br>tide ivnthas<br>)<br>vnthase<br>Sum of rela<br>nidohydrol<br>Sum of rela                              | de synthase<br>a<br>ative abund<br>ase<br>ative abund                                | Purine<br>Ance<br>Pyrimidir   | se<br>Nucleotide degradation<br>metabolism |  |  |
| C27.43<br>C27.43<br>C27.48<br>C43.22<br>C43.22<br>C43.24<br>C63.413<br>C63.413<br>C63.413<br>C63.413<br>C63.42<br>C63.52<br>C63.53<br>C13.110<br>C3.522<br>C3.52.17<br>C3.52.45<br>C3.5.24<br>C3.5.24<br>C3.5.24<br>C3.5.24  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guanviate Einkve<br>Adewstouccinste Frase<br>Phosphoribovylforurdytfor<br>Phosphoribovylforurdytfor<br>Phosphoribovylforurdytfor<br>Phosphoribovylforurdytfor<br>Carbovynamiopilidadou<br>GMP vynafase (Photomyrffyria<br>Phosphoribovylforurdytfi<br>Dihydrourscil dehydrogenas<br>Peroxyneidoacryfas ursido<br>Dihydrourscil dehydrogenas<br>Peroxyneidoacryfas ursido<br>Dihydropyrianidinase<br>Hydroxyriouras hydrolase<br>Allantoinase<br>JAP cyclohydrolase   | olesuccino<br>amidine (<br>te ligate<br>ibonucleot<br>odrobving<br>amidine (<br>e (NAD(+)<br>acrylate an                   | carboxamis<br>vclo-ligase<br>tide synthat<br>)<br>wnthase<br>Sum of rela<br>)<br>nidohydrol<br>Sum of rela                        | de swuthase<br>æ<br>ative abund<br>ase<br>ative abund                                | nnce Pyrimidir<br>ance Pyrimidir                                    | s<br>Nucleotide degradation<br>metabolism  |  |  |
| C1743<br>C2743<br>C2743<br>C4322<br>C4322<br>C4331<br>C6343<br>C6344<br>C6354<br>C6354<br>C35110<br>C3522<br>C35217<br>C3524<br>C3544<br>C3544   | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guanviate Einste<br>Adewsfouctante Frase<br>Phosphoribov/fourwidytion<br>Phosphoribov/fourwidytion<br>Phosphoribov/fourwidytion<br>Phosphoribov/fourwidytion<br>GMP vurhase (glutamine-br<br>Phosphoribov/forwidytion<br>Dihvdrouracii dehvdrogenas<br>Peroxvureidoactvlate ureido<br>Dihvdrouracii dehvdrogenas<br>Peroxvureidoactvlate ureido<br>Dihvdrourste hvdroiase<br>Allantoiase<br>Allantoiase<br>IMP cyclohvdrolase<br>Adenosine deaminaxe   | olesuccino<br>amidine (<br>te ligate<br>ibonucleot<br>rdrolvzing<br>amidine (<br>e (NAD(+)<br>acrylate an                  | carboxamis<br>vclo-ligase<br>tide synthat<br>)<br>wnthase<br>Sum of rela<br>)<br>nidohydrol<br>Sum of rela                        | de swuthase<br>æ<br>ative abund<br>ase<br>ative abund                                | Purine<br>Pyrimidia<br>Purine                                       | ae<br>Nucleotide degradation<br>metabolism |  |  |
| C2743<br>C2743<br>C2743<br>C4322<br>C4322<br>C4332<br>C4343<br>C63413<br>C63413<br>C6344<br>C6343<br>C6344<br>C6353<br>C1341<br>C35110<br>C3522<br>C3524<br>C3524<br>C3524<br>C3544<br>C3544<br>C3544<br>C41197  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guintine Einese<br>Adewstouccinne Frase<br>Phosphoriborylaminoimida<br>Phosphoriborylamina-chvii<br>Phosphoriborylamina-chvii<br>S-Garboxynamioimidanole ri<br>Adenstouccinate synthase<br>GMP synthase (clutamine-lu<br>Phosphoriborylformvigivein<br>Dihvdrourscii dehvdrogenas<br>Peroxyneidoacrvlase urido<br>Dihvdropvrimidinase<br>Hvdrozvisourale hydrolase<br>Allantoiase<br>Allantoiase<br>JaBr cylohydrolase<br>Allantoiase<br>Jacos-4-hydroxy-4-arboxy-4  | olesuccino<br>amidine cu<br>se ligate<br>ibonucleot<br>rdrolvzing<br>amidine sy<br>e (NAD(+)<br>acrylate an<br>5-ureidoim  | carboxamic<br>vdo-lizzse<br>tide tynthat<br>)<br>rathase<br>Sum of rela<br>iddazoline d<br>Sum of rela                            | de svuthase<br>æ<br>stive abund<br>ase<br>ative abund<br>ecarboxylasi<br>stive abund | Purine<br>Anne<br>Pyrimidia<br>Purine                               | *<br>Nucleotide degradation<br>metabolism  |  |  |
| C27.43<br>C27.43<br>C27.43<br>C23.43<br>C43.22<br>C53.26<br>C63.413<br>C63.413<br>C63.415<br>C63.415<br>C63.53<br>C63.53<br>C63.53<br>C13.11<br>C3.5110<br>C3.522<br>C3.5217<br>C3.524<br>C3.534<br>C3.5410<br>C3.544<br>C4.11.97<br>C1.51.3   | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guantiate Einixe<br>Adewstouccinate Yasse<br>Phosphoriborylaminoimida<br>Phosphoriborylamine-alvcin<br>Phosphoriborylamine-alvcin<br>S-(carboxynamio)imidatole r<br>Adewstouccivforwrigtvein<br>Dihvdrourscil dehvdrogenas<br>Phosphoriborylforwrigtvein<br>Dihvdrourscil dehvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Peroxynreidod adhvdrogenas<br>Dihvdropyriamidinase<br>Hvdroxyriourate hydrolase<br>Allantoicase<br>IAP erolohvdrolase<br>2-oxo-4-hvdroxy-4-carboxy-4-<br>Dihvdrofolate reductase   | olesuccino<br>amidine cu<br>se ligate<br>ibonucleof<br>rdrolvzing<br>amidine st<br>e (NAD(+)<br>acrylate an<br>5-ureidoim  | carboxamic<br>vdo-lizzse<br>tide tynthase<br>ynathase<br>Sum of reli<br>nidohydrol<br>Sum of reli<br>sum of reli                  | de synthase<br>e<br>ative abund<br>ase<br>ative abund<br>ecarboxylas                 | Purine<br>Annee<br>Pyrimidin<br>Purine                              | a<br>Nucleotide degradation<br>metabolism  |  |  |
| C27.43<br>C27.43<br>C27.43<br>C43.22<br>C43.22<br>C43.23<br>C63.43<br>C63.44<br>C63.52<br>C63.52<br>C35.22<br>C35.22<br>C35.217<br>C35.22<br>C35.24<br>C35.24<br>C35.44<br>C41.197<br>C15.13<br>C27.121  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guanviae tinkie<br>Adewstoucchae Fasse<br>Phosphoribovylaminoimida<br>Phosphoribovylaminoimidae<br>Phosphoribovylamine-tycii<br>S(carboxynamioimidaube)<br>S(carboxynamioimidaube)<br>Adewsteuccinate synthase<br>GMP vynhase (glutamine-th<br>Phosphoriboxylforwrdfytun<br>Dihydrouracii dehydrogenar<br>Peroxyneidoacrylate ursido<br>Dihydropyrimidinase<br>Hydroxyrisourale hydrolase<br>Allantoirase<br>IAP cyclohydrolase<br>Adenoine deaminase<br>2-oxo-4-hydroxy-4-carboxy-i<br>Dihydrofolate reductase<br>Thymdine kinnos   | olesuccino<br>amidine (t<br>ie ligave<br>ibonucleot<br>rdrolvring<br>amidine (t<br>e (NAD(+))<br>acrylate an<br>5-ureidotm | carboxamii<br>yclo-ligase<br>iide synthas<br>yrathase<br>Sum of rela<br>nidohydroli<br>Sum of rela                                | de svuthase<br>e<br>ative abund<br>ase<br>ative abund<br>ecarboxylas                 | Purine<br>Pyrimidia<br>ance<br>Purine<br>Pyrimidia                  | a<br>Nucleotide degradation<br>metabolism  |  |  |
| C27.43<br>C27.43<br>C27.43<br>C43.22<br>C63.26<br>C63.413<br>C63.413<br>C63.44<br>C63.52<br>C63.53<br>C13.11<br>C3.52.110<br>C3.52.2<br>C3.52.17<br>C3.52.5<br>C3.52.4<br>C3.52.4<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3.54<br>C3 | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guantine Ennie<br>Guantine Ennie<br>Phosphoribovilaminoimida<br>Phosphoribovilaminoimida<br>Phosphoribovilamine-dvin<br>Phosphoribovilamine-dvin<br>S-(carboxynamiojimidane) er<br>Adamiouccinate synthase<br>GMP synthase (clutamine-lu<br>Phosphoribovilaruvickyrin<br>Dihvdrourzeit dehvdrogena<br>Peroxyureidoacylate/ureido<br>Dihvdropyrimidinase<br>Hydroxyrisourale hydrolase<br>Allantoiase<br>IMP cyclohydrolase<br>Adenosine deaminase<br>2-oxo-laydroxy-4-arboxy-i<br>Dihvdrofolate reductase<br>Thwmdfine kinase<br>Uridime kinase  | olesuccino<br>amidine (t<br>ligate<br>ibonucleo)<br>rdrolvzing<br>amidine (t<br>e (NAD(+)<br>acrylate an<br>5-ureidoim     | carboxamii<br>yclo-lizzse<br>tide tynthas<br>ynthase<br>Sum of reli<br>nidohydrol<br>Sum of reli<br>nidazoline d<br>Sum of reli   | de svuthase<br>æ<br>ntive abund<br>ase<br>ecarboxylas<br>trive abund                 | Purine<br>ance<br>ance<br>ance<br>ance<br>Pyrimidiz                 | a<br>Nucleotide degradation<br>metabolism  |  |  |
| C2743<br>C2743<br>C4322<br>C4322<br>C6326<br>C6331<br>C63413<br>C63413<br>C6344<br>C6352<br>C6353<br>C6353<br>C1311<br>C35217<br>C3522<br>C3524<br>C3524<br>C3544<br>C41197<br>C1513<br>C27148<br>C2421  | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Gunning Einste<br>Adewsloutenne Frase<br>Phosphoriborylaminoimida<br>Phosphoriborylamine-edvin<br>Phosphoriborylamine-edvin<br>S-Garboxynminoimidanole r<br>Adewsloutenia synthase<br>GMP synthase (clutamine-lu<br>Phosphoriborylformvigiven<br>Dihvdrourseil dehvdrogenas<br>Peroxynreidoatrylamine-<br>Phosphoriborylformvigiven<br>Dihvdropyrimidinase<br>Hvdroxyriourale hydrolase<br>Allantoiase<br>Allantoiase<br>Allantoiase<br>JaBe cylohydrolase<br>Adenoim deaminase<br>J-oxo-4-hydroxy-4-arboxy-i<br>Dihvdrofolate reductase<br>Thymidine kinase<br>Unifane linase   | olesuccino<br>amidine (<br>ligate<br>ligate<br>amidine y<br>e (NAD(+)<br>acrylate an<br>5-ureidoim                         | carboxamii<br>victo-lizzse<br>iide synthat<br>yrathase<br>Sum of rela<br>midohydrol<br>Sum of rela<br>sidazoline d<br>Sum of rela | de synthase<br>e<br>ative abund<br>ase<br>ecarboxylas<br>ative abund<br>ative abund  | Purine<br>ance<br>ance<br>pyrimidiz<br>Purine<br>pyrimidiz          | a<br>Nucleotide degradation<br>metabolism  |  |  |
| C2743<br>C2743<br>C4322<br>C4322<br>C4322<br>C4324<br>C6343<br>C6343<br>C6344<br>C6344<br>C6352<br>C6352<br>C3522<br>C3522<br>C3524<br>C3524<br>C3544<br>C3544<br>C3544<br>C3544<br>C3544<br>C3544<br>C3544<br>C2121<br>C1513<br>C27121<br>C2145   | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Gunnine Einste<br>Adewsiouciane Passe<br>Phosphoriborylaminoimida<br>Phosphoriborylamine-alvcin<br>Phosphoriborylamine-alvcin<br>S-(carboxynamio)imidatole r<br>Adewsiouciane synthuse<br>GMP synthuse (clutamine-lu<br>Phosphoriborylformylgivin<br>Dihydropurscil dehydrogenas<br>Percywreiddactvlafe ursid<br>Dihydropyriamidinase<br>Hydroxyriaurate hydrolase<br>Allantoicase<br>IMP erchhydrolase<br>IMP erchhydrolase<br>Allantoicase<br>IMP erchhydrolase<br>IAP erchhydrolase<br>Allantoicase<br>IMP erchhydrolase<br>Jacheotine deminase<br>2-oxo-4-hydroxy-4-carboxy-4<br>Dihydrofolate reductase<br>Thymidine Einase<br>Uridine Einase   | olesuccino<br>amidine ci<br>se ligave<br>libonucleon<br>drohvzing<br>amidine s<br>e (NAD(+)<br>acrylate an<br>5-ureidoim   | carboxamie<br>vyclo-lizase<br>itide tynthas<br>)<br>ynthase<br>Sum of reli<br>nidohydrol<br>Sum of reli<br>Sum of reli            | de synthase<br>e<br>ative abund<br>ase<br>ecarboxylas<br>ative abund                 | Purine<br>Pyrimidia<br>Purine<br>Purine<br>Purine                   | e<br>Nucleotide degradation<br>metabolism  |  |  |
| C27.43<br>C27.43<br>C43.22<br>C43.22<br>C43.24<br>C63.34<br>C63.413<br>C63.413<br>C63.44<br>C63.52<br>C13.11<br>C3.5217<br>C3.522<br>C3.524<br>C3.524<br>C3.524<br>C3.544<br>C41.197<br>C1.513<br>C2.71.121<br>C2.71.121<br>C2.71.23<br>C3.543   | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | Guantiale Einkie<br>Adewstouccinale Fasse<br>Phosphoribov/fours/dytien<br>Phosphoribov/fours/dytien<br>Phosphoribov/fours/dytien<br>Phosphoribov/fours/dytien<br>S(carboyrumiojimidaube)<br>Ademsteine (guantamine-br<br>Fhosphoribov/form/dytien<br>Dihvdroursail dehvdrogenas<br>Peroxyvaridoactvlate ursido<br>Dihvdroursail dehvdrogenas<br>Peroxyvaridoactvlate ursido<br>Dihvdroursail dehvdrogenas<br>Peroxyvaridoactvlate ursido<br>Dihvdroursail dehvdrogenas<br>Peroxyvaridoactvlate<br>Allantoinsae<br>Allantoinsae<br>Allantoinsae<br>Jahr exclohvdrolase<br>Adenotine deaminate<br>Uridiane Einase<br>Uridiane Einase<br>Parine-buckeside phosphor<br>5-aucleotdase   | olesuccino<br>amidine ci<br>ligane<br>ibonucleol<br>cdrolvzing<br>amidine st<br>e (NAD(+)<br>acrylate an<br>S-ureidoim     | carboxamie<br>vyclo-ligase<br>itide tynthas<br>ymthase<br>Sum of reli<br>nidohydrol<br>Sum of reli<br>Sum of reli                 | de synthase<br>e<br>ative abund<br>ase<br>ative abund<br>ecarboxylai<br>ative abund  | nnce<br>Annce<br>Annce<br>Annce<br>Pyrimidiz<br>Pyrimidiz<br>Purine | a<br>Nucleotide degradation<br>metabolism  |  |  |

**Fig. 4.** Changes in the pathways (a) and enzymes (b) involved in the nucleotides metabolism of bacteria in the wastewater treated jointly by  $Fe^{6+}$  and  $SO_3^{2-}$  at different pH levels. N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.



**Fig. 5.** The potential genes *sul2, sul3* and *intl1* hosts based on the network analysis (a) and the changes in the bacterial community (b) at the genus level in the wastewater treated by combined  $Fe^{6+}$  and  $SO_3^{2-}$ . The edge thickness in the network analysis was proportional to Pearson's correlation coefficients. N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.

and the total relative levels of purine biosynthesis and salvage enzymes negatively correlated with genes sul2, sul3 and intI1 were 2.087 % and 1.788 %, respectively (Table 1). In these metabolic enzymes of nucleotide negatively correlated with genes sul2, sul3 and intI1, the relative levels sum of pyrimidine biosynthesis and salvage enzymes in the treated wastewater at pH 6.0, 7.0 and 9.0 were 2.365 %, 2.218 % and 2.204 %, respectively, and the total relative levels of purine biosynthesis and salvage enzymes were 1.991 %, 1.894 % and 1.878 %, respectively (Table 1). The relative levels of above these enzymes (the negative correlation with the changes of genes sul2, sul3 and intI1) in the treated wastewater were higher compared to the raw wastewater, suggesting that the bacterial response to the destruction of genes sul2, sul3 and intI1 by the combined treatment of  $Fe^{6+}$  and  $SO_3^{2-}$  was the activation of these enzymes. Among pH 6.0, 7.0 and 9.0, the relative levels of biosynthesis and salvage enzymes of pyrimidine and purine in the treated wastewater at pH 7.0 were higher than at pH 9.0, while were lower than those at pH 6.0. The results suggested that there might be more levels of genes sul2, sul3 and int11 damaged by the combined treatment of  $Fe^{6+}$  and  $SO_3^{2-}$  at acid conditions, which were coincident with the changes in the removal of genes sul2, sul3 and int11 by the combined treatment of  $Fe^{6+}$  and  $SO_3^{2-}$ at different pH levels. After the combined treatment of  $Fe^{6+}$  and  $SO_3^{2-}$ , the increasing degree in the total levels of pyrimidine biosynthesis enzymes (the negative correlation with the changes of genes sul2, sul3 and int11) involved in the bacterial response to the destruction of genes sul2, sul3 and intl1 at pH 6.0, 7.0 and 9.0 were 13.729 %, 6.385 % and 6.048 %, respectively, and the increased degree about pyrimidine salvage increased by 10.936 %, 5.812 % and 3.157 %, respectively (Table 2). The increasing levels of pyrimidine biosynthesis enzymes at different pH values were higher than the pyrimidine salvage enzymes. However, the purine change was the opposite result in which the increasing levels of purine biosynthesis enzymes at different pH values were lower than the purine salvage enzymes. This change could be caused by the greater availability of purine fragments in the wastewater than pyrimidines, suggesting that there might be more thorough damage of the pyrimidines bases by the combined treatment of  $Fe^{6+}$  and  $SO_3^2$  compared to the purine bases. Gmurek et al. [39] found similar results in which the bases of pyrimidines had a faster reaction with free radicals than purine.

After the treatment of combined  $Fe^{6+}$  and  $SO_3^{2-}$ , the decreasing degree in the total levels of pyrimidine metabolic enzymes (the positive relationship with the changes of genes sul2, sul3 and intI1) at pH 6.0, 7.0 and 9.0 were 22.761 %, 13.537 % and 11.309 %, respectively, and the reducing levels of purine metabolic enzymes were 20.897 %, 30.592 % and 23.803 %, respectively (Table 2). The decreasing degree of pyrimidine biosynthesis metabolic enzymes at pH 6.0 was higher than purine, while the opposite changes were found at pH 7.0 and 9.0. The changes suggested that SO<sub>4</sub><sup>•</sup> might be more likely to destroy pyrimidines than HO•, since the conversion of SO<sub>4</sub><sup>•-</sup> to HO• was more under acid while less in alkalinity. Zhang et al. [38] found that gene sul3 had more pyrimidines than gene sul2. This might be one of the reasons that the removal efficiency of gene sul2 was lower than gene sul3 at different pH levels. The relationship between the potential genes sul2, sul3 and intl1 hosts and enzymes involved into the bacterial nucleotide metabolism pathways was analyzed via a network analysis on the basis of the correlation of Pearson (|r| > 0.8) (Fig. 7b). Potential genes sul2, sul3 and int11 hosts were found to be inversely associated with all nucleotide metabolic enzymes (the negative correlation with the changes of genes sul2, sul3 and intI1), which could confirm that these enzymes changes were the bacterial repair response to the nucleotide breach. Fu et al. [49] found similar results that a high level of glutamine synthetase (GS) could promote nucleotide synthesis and DNA repair, and the changes in the GS level were negatively correlated with cancer treatment outcomes.

# 3.6. Hypothetic mechanisms

Fig. 7 shows the hypothetic mechanisms of the ARGs removal in the wastewater by the combined  $Fe^{6+}$  and  $SO_3^{2^-}$  treatment. The activation of the pyrimidine biosynthesis and salvage enzymes and the purine biosynthesis and salvage enzymes in the wastewater by the treatment of combined  $Fe^{6+}$  and  $SO_3^{2^-}$  was found at pH 6.0, 7.0 and 9.0 (Fig. 4b), and this activation was more significant at pH 6.0 compared to that at pH 7.0



**Fig. 6.** Network analysis on the relationship of enzymes involved into the bacterial nucleotides metabolism pathways with the genes *sul2*, *sul3* and *intl1* (a), and with the potential genes *sul2*, *sul3* and *intl1* hosts (b) in the wastewater treated by combined  $Fe^{6+}$  and  $SO_3^{2-}$ . The edge thickness was proportional to Pearson's correlation coefficients. The red edges and the blue edges represented positive and negative relations between two nodes, respectively. \* was the enzymes with negative relations with genes *sul2*, *sul3* and *intl1*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## Table 1

The relative levels of nucleotides metabolic enzymes with negative and positive relation with genes sul2, sul3 and int/1.

| Relation | Sample | Pyrimidine metabolic enzymes relative levels (%) |         |             |       | Purine metabolic enzymes relative levels (%) |         |             |       |
|----------|--------|--|---------|-------------|-------|--|---------|-------------|-------|
|          |        | Biosynthesis                                     | Salvage | Degradation | Sum   | Biosynthesis                                 | Salvage | Degradation | Sum   |
| Negative | NO     | 1.787  | 0.300   | /           | 2.087 | 1.650  | 0.138   | /           | 1.788 |
|          | N1     | 2.032  | 0.333   | /           | 2.365 | 1.837  | 0.154   | /           | 1.991 |
|          | N2     | 1.901  | 0.318   | /           | 2.218 | 1.748  | 0.146   | /           | 1.894 |
|          | N3     | 1.895  | 0.310   | /           | 2.204 | 1.732  | 0.146   | /           | 1.878 |
| Positive | NO     | 0.075  | /       | /           | 0.075 | /  | 0.034   | /           | 0.034 |
|          | N1     | 0.058  | /       | /           | 0.058 | /  | 0.027   | /           | 0.027 |
|          | N2     | 0.065  | /       | /           | 0.065 | /  | 0.024   | /           | 0.024 |
|          | N3     | 0.066  | /       | /           | 0.066 | /  | 0.026   | /           | 0.026 |

/: no found.

N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.

and 9.0. In consideration of the DNA removal performance in the wastewater at different pH values (Fig. 1b), it could be inferred that the activation of these enzymes mentioned above is the bacterial response to

the destruction of DNA, and the response of bacteria could be enhanced at the acidic condition. In addition, the DNA removal treated by the combined  $Fe^{6+}$  and  $SO_3^{2-}$  was achieved by disrupting the pyrimidines and Table 2

| The reaction of the bound of the bar | The relative levels of nucleotides metabolic enz | ymes with negative and | positive relation with | genes sul2, sul3 and intI1. |
|--|--|------------------------|------------------------|-----------------------------|
|--|--|------------------------|------------------------|-----------------------------|

| Relation | Sample | Change rate of pyrimidine metabolic enzymes relative levels (%) |         |             |         | Change rate of purine metabolic enzymes relative levels (%) |         |             |         |  |
|----------|--------|---|---------|-------------|---------|---|---------|-------------|---------|--|
|          |        | Biosynthesis  | Salvage | Degradation | Sum     | Biosynthesis  | Salvage | Degradation | Sum     |  |
| Negative | N1     | 13.729  | 10.936  | /           | 13.327  | 11.290  | 11.915  | /           | 11.338  |  |
|          | N2     | 6.385   | 5.812   | /           | 6.302   | 5.894   | 6.359   | /           | 5.930   |  |
|          | N3     | 6.048   | 3.157   | /           | 5.633   | 4.959   | 5.810   | /           | 5.025   |  |
| Positive | N1     | -22.761   | /       | /           | -22.761 | /   | -20.897 | /           | -20.897 |  |
|          | N2     | -13.537   | /       | /           | -13.537 | /   | -30.592 | /           | -30.592 |  |
|          | N3     | -11.309   | /       | /           | -11.309 | /   | -23.803 | /           | -23.803 |  |

/: no found.

N0 was the raw wastewater, and the pH value of N0 was 6.7 (unadjusted). N1, N2 and N3 were the treated wastewater at different pH levels, and the pH values of N1, N2 and N3 were 6.0, 7.0 and 9.0, respectively.



Fig. 7. Hypothetic mechanisms of the ARGs removal in the wastewater by the combined  $Fe^{6+}$  and  $SO_3^{2-}$  treatment.

purines of DNA by SO<sub>4</sub><sup>-</sup> and HO• (produced by the reaction of Fe<sup>6+</sup> with SO<sub>3</sub><sup>-</sup>). Pyrimidines were damaged by SO<sub>4</sub><sup>-</sup> and HO• more easily than purines, and SO<sub>4</sub><sup>-</sup> had better abilities to destroy purines and pyrimidines compared to HO•. The promotion of SO<sub>4</sub><sup>-</sup> to HO• conversion by the alkalinity condition (Eq. (4)) and the slowed conversion from SO<sub>4</sub><sup>+</sup> to HO• at the acid condition (Eq. (7)) resulted in higher removal rates of total DNA at pH 6.0 compared to those at pH 9.0. The contributors of SO<sub>4</sub><sup>-</sup> to the total DNA removal were higher than HO•, and the contributions of other factors to the total DNA removal increased with the increase of pH value from 6.0 to 9.0 (Fig. 1c).

## 4. Conclusions

In the wastewater treatment system of coupled  $Fe^{6+}/SO_3^{2-}$ , the decreasing pH level enhanced the DNA removal, the contributions of SO<sub>4</sub><sup>-</sup> and HO• to DNA removal, the permeability of cells, the metabolism levels of nucleotide biosynthesis and salvage in bacteria, and microbial community changes. SO<sub>4</sub><sup>-</sup> were more likely to cause the changes in the pyrimidine biosynthesis metabolism of nucleotide than HO•. The reduction in the level of genes *sul2*, *sul3* and *int11* host contributed to the

removal of genes sul2, sul3 and intI1.

## CRediT authorship contribution statement

**Changwei Niu:** Data curation, Methodology, Software, Writing – original draft. **Bing Wang:** Data curation, Investigation. **Zichao Wang:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing. **Huaibin Zhang:** Investigation.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Data availability

The authors do not have permission to share data.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2023.148042.

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