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Characteristics and Mechanisms of Simultaneous Organic and Inorganic Nitrogen Removal by a Robust Bacterium *Pseudomonas stutzeri* H3

Jie Hu [1,*], Bing Xu [1,*], Jiabao Yan [2] and Guozhi Fan [1]

[1] School of Chemical and Environmental Engineering, Wuhan Polytechnic University, Wuhan 430023, China; fgzcch@whpu.edu.cn (G.F.)

[2] Hubei Province Key Laboratory of Coal Conversion and New Carbon Materials, School of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, China

* Correspondence: hujie9231@whpu.edu.cn (J.H.); xubing200806@163.com (B.X.)

Abstract: The discharge of organic and inorganic nitrogenous pollutants in wastewater leads to eutrophication and disrupts the ecological balance. Therefore, the pressing need for an effective treatment method has become increasingly evident. A robust bacterium *Pseudomonas stutzeri* H3 capable of simultaneous organic and inorganic nitrogen removal was isolated from the activated sludge in the coking wastewater treatment system. Strain H3 achieved above 90% of ammonium nitrogen and quinoline removal, exhibiting excellent simultaneous nitrogen removal capabilities compared with other nitrogen removal bacteria such as *Halomonas* sp. and *Acinetobacter* sp. The outstanding nitrogen removal efficiencies in the presence of quinoline and different inorganic nitrogen sources further confirmed the simultaneous organic and inorganic nitrogen removal capability of strain H3. The whole genome sequencing and nitrogen metabolic intermediates determination of strain H3 were performed to elucidate the gene function annotations, nitrogen removal function genes, and nitrogen metabolic pathways. The findings provide a promising pathway to treat the organic and inorganic nitrogenous pollutants in wastewater.

Keywords: simultaneous nitrogen removal; quinoline; inorganic nitrogen; nitrogen metabolic pathway; whole genome sequencing

1. Introduction

With the acceleration of industrialization and the expansion of agriculture, plenty of nitrogenous pollutants are discharged into aquatic environments [1-4], which have become global environmental problems. Nitrogenous pollutants in wastewater are mainly divided into organic and inorganic nitrogen. Among them, organic nitrogenous pollutants mainly include proteins, organic bases, and nitrogenous heterocyclic compounds [5-9], while inorganic ones consist of ammonium nitrogen (NH_4^+–N), nitrite nitrogen (NO_2^-–N), and nitrate nitrogen (NO_3^-–N) [10-12]. The discharge of nitrogenous pollutants will not only lead to eutrophication, affecting water quality and ecological balance, but also threaten the health of humans and aquatic animals [13-16]. Therefore, the effective treatment of nitrogenous pollutants in wastewater is of paramount importance for the stable discharge of wastewater and the protection of the aquatic ecological environment.

Quinoline is a typical toxic and carcinogenic nitrogenous heterocyclic pollutant, which mainly originates from various industrial processes, such as coking, refining, pharmaceutical manufacturing, and dyeing [4,17-19]. Owing to the extreme toxicity and recalcitrant degradability of quinoline, effectively treating quinoline in wastewater has become an intractable problem. Biological nitrogen removal mainly utilizes the metabolism of microorganisms to convert nitrogenous pollutants into harmless substances [7,20,21]. Compared with physicochemical nitrogen removal technology, biological nitrogen removal possesses the advantages of wide application, low cost, and simple

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operation [14,22-24]. Recently, researchers have been exploring efficient biological nitrogen removal processes to remove quinoline from wastewater [17,25-27]. For instance, a membrane aerated biofilm reactor (MABR) was constructed to remove quinoline from wastewater under aerobic conditions. More than 80% of quinoline was effectively removed at a quinoline concentration ranging from 50 to 250 mg/L. Metagenomic analysis indicated that aerobic quinoline-degrading bacteria such as *Rhodococcus*, *Pseudomonas*, and *Comamonas* were highly enriched in the MABR biofilm, potentially enhancing the degradation of quinoline in wastewater [25].

In addition to quinoline, the wastewater discharged from industrial processes also contains large amounts of inorganic nitrogenous pollutants, such as NH_4^+–N and NO_3^-–N [2,5,28,29]. Biological nitrogen removal is extensively applied to remove inorganic nitrogenous pollutants in wastewater [30-33]. At present, the widely studied technologies for removing inorganic nitrogenous pollutants include shortcut nitrification and denitrification [34,35], anaerobic ammonium oxidation [30,36,37], heterotrophic nitrification and aerobic denitrification [10,11,38]. Heterotrophic nitrification and aerobic denitrification are mainly carried out by some novel nitrogen-removing bacteria in the coexistence of dissolved oxygen and organic carbon sources, thereby effectively removing NH_4^+–N and NO_3^-–N simultaneously [39-41]. Very recently, a novel *Klebsiella* sp. TSH15 was isolated and demonstrated the efficient NH_4^+–N and NO_3^-–N removal capability. The whole genome analysis indicated that strain TSH15 contained the assimilatory/dissimilatory nitrate reduction and ammonia assimilation functional genes [29].

Previous studies primarily concentrated on the removal performance of individual organic or inorganic nitrogenous pollutants [7,14,42], with scant reports on the simultaneous removal of organic and inorganic nitrogen. The research on the simultaneous biological removal of NH_4^+–N and quinoline facilitates the solution of the current water pollution problem and promotes the protection of the ecological environment. In addition, organic nitrogenous pollutants in wastewater are usually converted into inorganic nitrogen, which requires removal by other microorganisms [3,14,16,25]. The main challenge of the simultaneous nitrogen removal process is to identify microorganisms capable of efficiently biodegrading organic and inorganic nitrogenous pollutants and to explore the simultaneous nitrogen removal characteristics and mechanism.

In this work, a robust bacterium *Pseudomonas stutzeri* H3 capable of simultaneous organic and inorganic nitrogen removal was isolated from the activated sludge collected from the coking wastewater treatment plant (CWTP). Single-factor experiments were performed to optimize NH_4^+–N and quinoline removal conditions. The excellent simultaneous NH_4^+–N and quinoline removal capabilities of strain H3 were ascertained by comparing with other nitrogen removal bacteria such as *Halomonas* sp. and *Acinetobacter* sp. The nitrogen removal performances for quinoline and different inorganic nitrogen sources were investigated, further confirming the capability of strain H3 to remove both organic and inorganic nitrogen simultaneously. Furthermore, the whole genome sequencing analysis and nitrogen metabolic intermediates determination of strain H3 was performed to elucidate the gene functional annotations and nitrogen metabolic pathways. This study provided new insight into the simultaneous organic and inorganic nitrogen removal by *Pseudomonas stutzeri* H3.

2. Materials and Methods

2.1. Isolation and Identification

The activated sludge samples taken from the CWTP (Wuhan, China) were utilized to isolate the efficient degrading bacteria for simultaneous removal of NH_4^+–N and quinoline. The simultaneous nitrogen removal capabilities of the isolated strain were determined in a mixed nitrogen source (MNS) medium, and its composition is shown in Supplementary Material. Strain H3 with superior simultaneous NH_4^+–N and quinoline removal efficiency was selected for comprehensive assays. Polymerase chain reaction (PCR) was used to amplify the 16S rRNA of strain H3. The PCR-amplified product was sequenced by TSINGKE Biotechnology Co., Ltd. (Wuhan, China). Subsequently, the 16S rRNA were uploaded to the GenBank database and aligned with those of other bacteria via online

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BLAST. A phylogenetic tree was created using MEGA 7.0. The microscopic morphology of strain H3 was observed by scanning electron microscope (SEM) at 3.0 kV (Regulus 8100, Hitachi).

2.2. Effect of Culture Conditions on Nitrogen Removal

The impacts of C/N ratio, initial pH, culture temperature, and shaking speed on NH_4^+–N and quinoline removal performance were investigated. Three milliliters of bacterial suspensions were transferred into MNS medium with initial NH_4^+–N and quinoline concentrations of 200 mg/L and 100 mg/L, and then cultured for 72 h. To investigate the effect of different culture conditions on NH_4^+–N and quinoline removal performance, the C/N ratio was regulated to 5, 10, 15, and 20 by changing the amount of additional carbon source; the initial pH was adjusted to 6, 7, 8, and 9; the culture temperature was regulated at 10, 20, 30, and 40 °C; the shaking speed was varied at 50, 100, 150, and 200 rpm. NH_4^+–N and quinoline concentrations were determined termly.

2.3. Assessment of Simultaneous Nitrogen Removal Capability of Strain H3

To assess the simultaneous NH_4^+–N and quinoline removal capability of strain H3, the bacterial suspensions (3 mL) were inoculated into MNS medium. Both NH_4^+–N and quinoline were independently set in the range of 50-400 mg/L. NH_4^+–N and quinoline removal performances by strain H3 were determined at different concentrations. In addition, *Pseudomonas stutzeri* H3 and other nitrogen removal bacteria *Halomonas* sp. and *Acinetobacter* sp. were cultivated in single NH_4^+–N, single quinoline, and mixed NH_4^+–N and quinoline media for 72 h. The bacterial growth and NH_4^+–N/quinoline removal performance of different bacteria were measured at intervals.

2.4. Removal Performance of Strain H3 for Quinoline and Different Inorganic Nitrogen

To evaluate the nitrogen removal capability of strain H3 in the presence of quinoline and different inorganic nitrogen, the bacterial suspensions were transferred into sterilized media containing different inorganic nitrogen sources (single NH_4^+–N, NO_2^-–N, NO_3^-–N, and mixed NH_4^+–N + NO_2^-–N + NO_3^-–N) and 100 mg/L quinoline. The initial inorganic nitrogen concentration was adjusted to 200 mg/L. Strain H3 was incubated at 30 °C and 150 rpm. The bacterial growth and the concentrations of total nitrogen (TN), NH_4^+–N, quinoline, NO_2^-–N, and NO_3^-–N were measured at intervals, respectively.

2.5. Whole Genome Sequencing and Gene Function Annotations of Strain H3

To clarify the nitrogen metabolic pathway and analyze the gene function annotations of strain H3, the whole genome sequencing was performed using the Illumina Hiseq platform at Majorbio (Shanghai, China). The obtained genome sequences were assembled using SOAPdenovo 2.04 and GapCloser 1.12. The whole genome sequences were submitted and compared with the GO, KEGG, and COG databases to obtain the gene function annotations. The coding DNA sequences associated with nitrogen metabolism were annotated using the KEGG database to identify the nitrogen metabolic pathways present in strain H3.

2.6. Determination of Quinoline Metabolic Intermediates

The metabolic intermediates of quinoline degraded by strain H3 were analyzed by gas chromatography-mass spectrometer (GC-MS) (Agilent, 7890A GC, 5975C MS). The bacterial suspensions (3 mL) were added into sterile medium containing 100 mg/L quinoline. Samples cultivated for 24, 48, and 72 h were collected and centrifuged at 5000 g for 10 min. The metabolic intermediates of quinoline in the supernatant were extracted by ethyl acetate. The extracts were concentrated by vacuum rotary evaporation. The GC-MS equipped with the HP-5MS capillary column was used to determine the metabolic intermediates of quinoline. The column temperature control programs were initial temperature of 40 °C for 2 min, rose to 300 °C with a heating rate of 7 °C/min, and kept at 300 °C for 10 min.

2.7. Analytical Methods

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The bacterial growth (OD_{600}) was estimated by determining the absorbance of bacterial suspension at the wavelength of 600 nm. The concentrations of NH_4^+–N, NO_2^-–N, NO_3^-–N, and TN were measured through the standard methods [43]. The concentration of quinoline was determined by spectrophotometry at the maximum absorbance wavelength of 313 nm. All the experiments were conducted in triplicates and the results were expressed as means ± standard deviation.

3. Results and Discussion

3.1. Identification of the Isolated Strain H3

In this work, ten distinct robust bacteria with simultaneous NH_4^+–N and quinoline removal capabilities were isolated from the activated sludge collected from the CWTP. Among these isolated bacteria, strain H3 with the optimal NH_4^+–N and quinoline removal property was selected for further investigation. The colony of strain H3 was beige, rounded with a diameter around 1 mm, and had a smooth and slightly raised surface (Figure 1A). Moreover, SEM image showed that strain H3 presented as short rods with the size of (0.5-0.8) × (2.0-3.0) μm (Figure 1B).

After PCR amplification and amplified product sequencing, the 16S rRNA sequences (1434 bp) of strain H3 were obtained and submitted to GenBank database (GenBank ID: PQ241653). The homology analysis by BLAST showed that strain H3 was highly similar to *Pseudomonas* sp. (above 99% similarity). The phylogenetic tree of strain H3 was created by neighbor-joining method and further demonstrated that strain H3 was affiliated to *Pseudomonas stutzeri* (Figure 1C). At present, *Pseudomonas* sp. widely exists in the wastewater treatment process [44-46], but few have been reported to possess the simultaneous NH_4^+–N and quinoline removal capabilities.

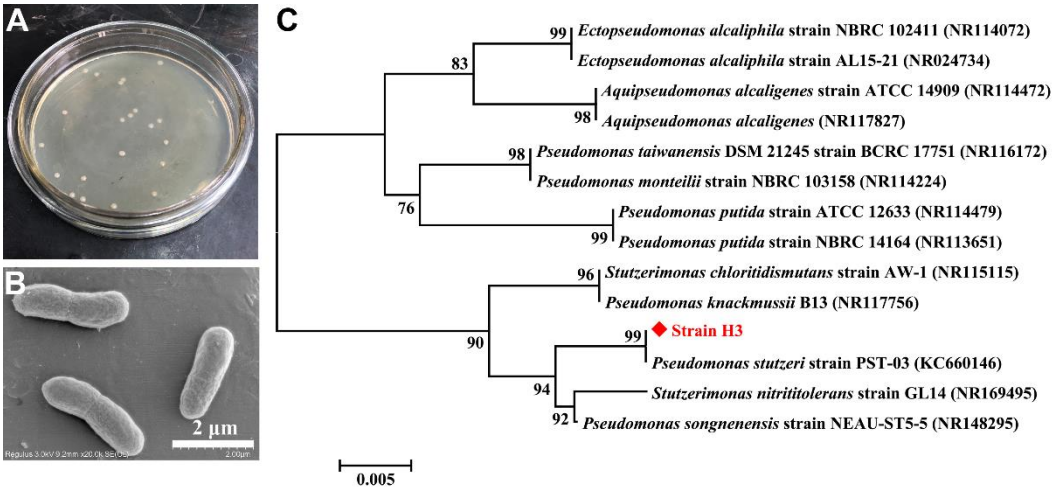

Figure 1. (**A**) The colony morphology of strain H3. (**B**) SEM image of strain H3. (**C**) The phylogenetic tree of strain H3 created by neighbor-joining method.

3.2. Optimization of Nitrogen Removal Conditions

To evaluate the influence of different culture conditions on NH_4^+–N and quinoline removal performance of strain H3, single-factor experiments were conducted. Figure 2A and 2B show the influence of C/N ratio on NH_4^+–N and quinoline removal capability. In the range of C/N ratio from 5 to 20, the removal efficiencies of NH_4^+–N and quinoline were gradually enhanced due to the increase of C/N ratio. Specifically, when the C/N ratio was 5, the removal efficiencies of NH_4^+–N and quinoline were merely 38.5% and 40.8% after strain H3 was cultivated for 72 h. When the C/N ratio was regulated to 10, the removal efficiencies of NH_4^+–N and quinoline were significantly improved to 82.3% and 78.0%. When the C/N ratio was further increased to 15 and 20, the removal efficiencies of strain H3 for NH_4^+–N and quinoline were comparable, both reaching above 95%. In the initial pH range of 6-9, strain H3 exhibited excellent NH_4^+–N removal properties (Figure 2C). When strain H3 was cultured for 72 h, NH_4^+–N removal efficiency was more than 99%. The removal performances of strain H3 for quinoline varied at different initial pH values (Figure 2D). When strain H3 was cultivated for

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72 h, the removal efficiency of quinoline was above 90% at the initial pH of 7-8, which was significantly higher than that of initial pH 6 (78.4%) and 9 (83.1%).

Culture temperature mainly affects the enzyme activity in bacteria, thereby influencing the metabolic activities of bacteria [47-49]. As shown in Figure 2E and 2F, in the culture temperature range of 10-40 °C, the removal performances of NH_4^+−N and quinoline by strain H3 were significantly different. At the culture temperature of 10 °C, the removal efficiencies of NH_4^+−N and quinoline by strain H3 were approximately 60% after cultivation for 72 h. After cultivation at 20 °C for 72 h, NH_4^+−N and quinoline removal efficiencies were improved to 91.4% and 74.0%. The removal performances of NH_4^+−N by strain H3 were nearly identical after cultivation at 30 and 40 °C for 72 h, but the removal performances of quinoline were noticeably different. Specifically, the removal efficiencies of quinoline were 93.3% and 84.3% at 30 and 40 °C. Shaking speed primarily affects the dissolved oxygen (OD) content. Generally, as the shaking speed accelerates, the OD content increases accordingly. As shown in Figure 2G and 2H, strain H3 exhibited more efficient NH_4^+−N and quinoline removal at the shaking speed of 150-200 rpm.

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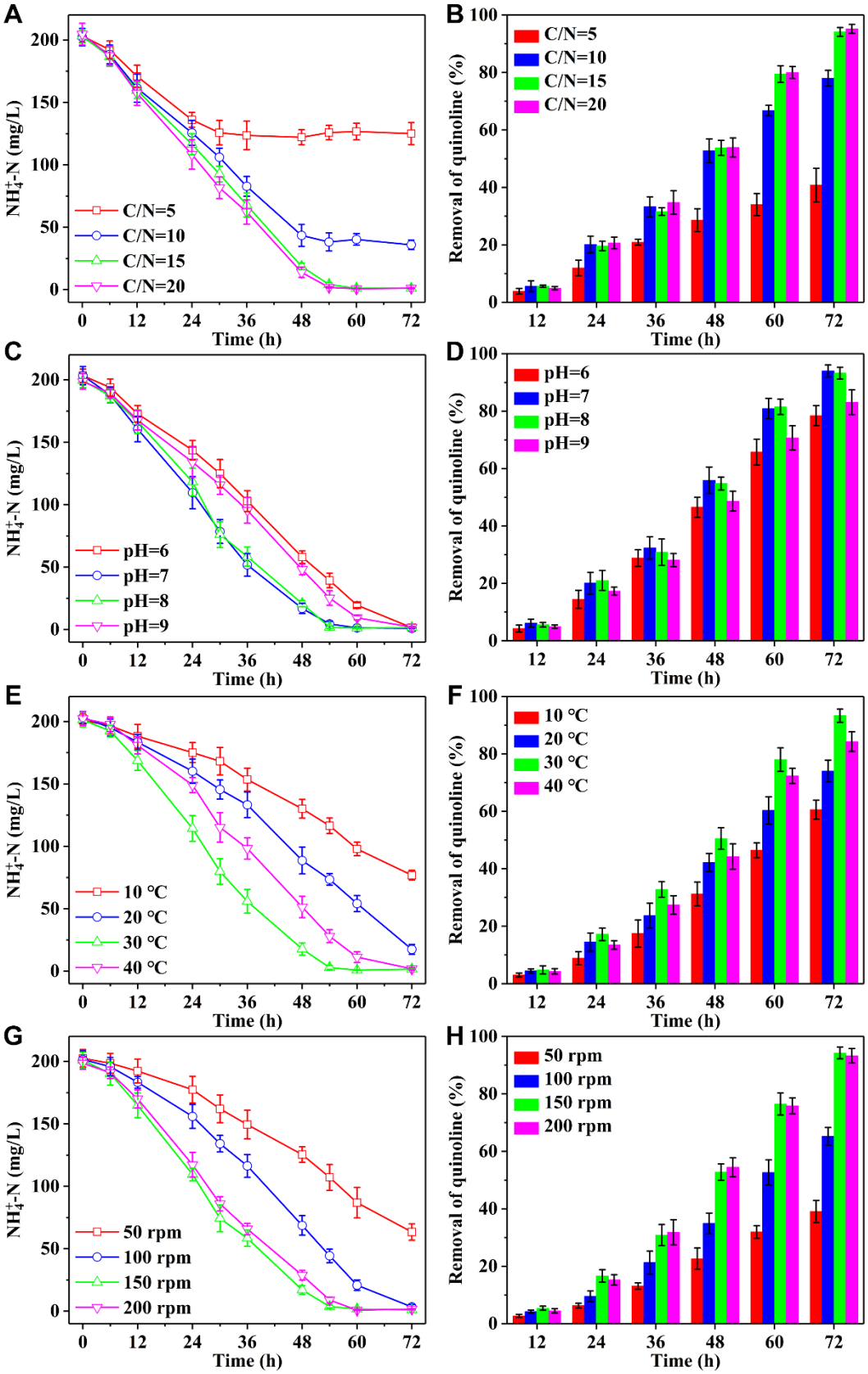

Figure 2. The effects of (**A**, **B**) C/N ratio, (**C**, **D**) initial pH, (**E**, **F**) culture temperature, (**G**, **H**) shaking speed on NH_4^+–N and quinoline removal performance of strain H3.

3.3. Simultaneous Nitrogen Removal Capability of Strain H3

The simultaneous nitrogen removal capability of strain H3 at different NH_4^+–N and quinoline concentrations is shown in Figure S1. At the initial concentrations of 100 mg/L quinoline and 50-400

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mg/L NH_4^+–N, NH_4^+–N was effectively removed by strain H3 after cultivation for 72 h, with a removal efficiency over 95% (Figure S1A). The removal efficiencies of quinoline were enhanced with the increase of initial NH_4^+–N at 50-200 mg/L (Figure S1B). However, when NH_4^+–N was further raised to 400 mg/L, the removal performance of quinoline was comparable to that at the NH_4^+–N of 200 mg/L. In addition, at the initial concentrations of 200 mg/L NH_4^+–N and 50-400 mg/L quinoline, the removal rates of NH_4^+–N gradually declined as the quinoline concentration increased (Figure S1C). The removal efficiencies of quinoline by strain H3 decreased from 98% to 55% as the increase of quinoline concentration from 50 to 400 mg/L after cultivation for 72 h (Figure S1D).

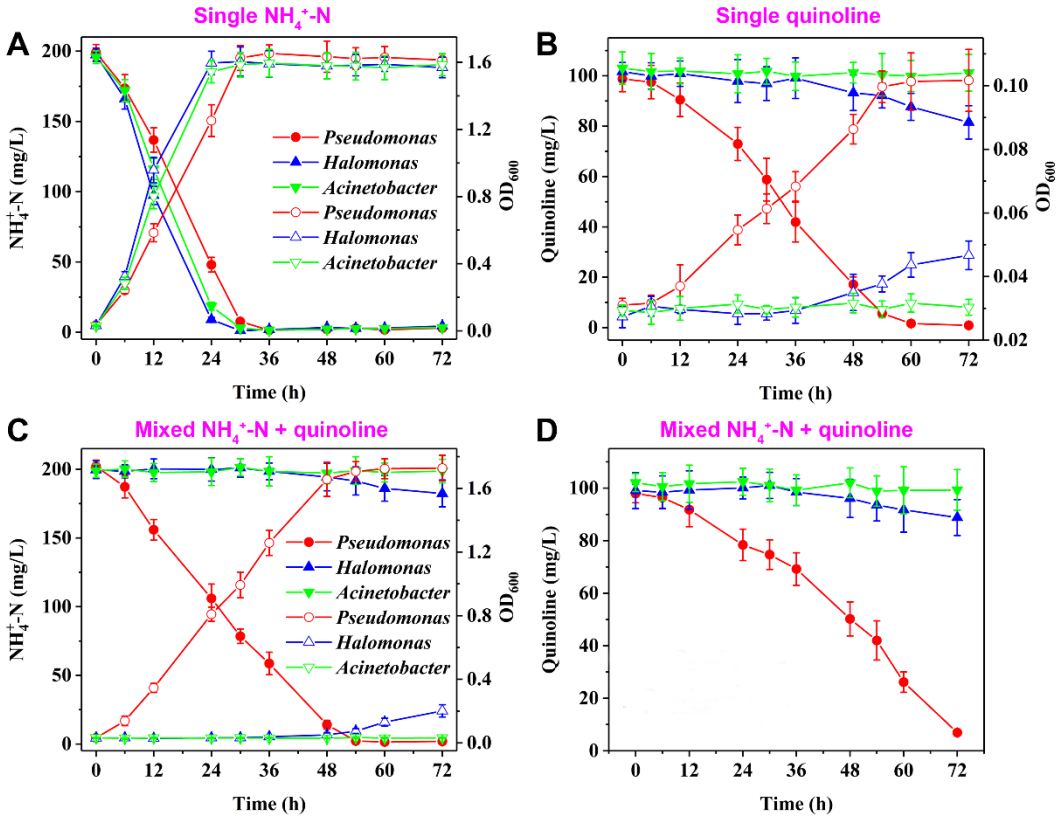

Figure 3. The bacterial growth and nitrogen removal performance of different nitrogen removal bacteria with (**A**) NH_4^+–N as single nitrogen source, (**B**) quinoline as single nitrogen source, (**C**, **D**) NH_4^+–N and quinoline as mixed nitrogen sources.

When *Pseudomonas stutzeri* H3 and other nitrogen removal bacteria *Halomonas* sp. and *Acinetobacter* sp. were utilized to remove single NH_4^+–N, single quinoline, and mixed NH_4^+–N and quinoline, the bacterial growth and nitrogen removal performances of different bacteria were shown in Figure 3. When NH_4^+–N was utilized as single nitrogen source, the removal efficiency of NH_4^+–N by *Halomonas* sp. and *Acinetobacter* sp. was slightly higher than that by *Pseudomonas* sp. (Figure 3A). When quinoline was used as single nitrogen source, the bacterial growth and nitrogen removal performance of *Halomonas* sp. and *Acinetobacter* sp. were significantly inhibited, while *Pseudomonas* sp. exhibited excellent bacterial growth and remarkable quinoline removal capability (Figure 3B). When *Pseudomonas stutzeri* H3 was cultivated for 72 h, quinoline removal efficiency was above 95%. When NH_4^+–N and quinoline served as mixed nitrogen sources, the removal performances of NH_4^+–N/quinoline were different from those of single NH_4^+–N and single quinoline (Figure 3C and 3D). NH_4^+–N and quinoline could not be effectively removed by *Halomonas* sp. and *Acinetobacter* sp. in the presence of mixed nitrogen sources. However, when *Pseudomonas stutzeri* H3 was cultivated for 72 h, more than 90% of NH_4^+–N and quinoline was effectively removed. The result indicates that strain H3 exhibited excellent simultaneous NH_4^+–N and quinoline removal performance.

3.4. Simultaneous Removal Performance of Quinolines and Different Inorganic Nitrogen

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The bacterial growth and nitrogen removal performance of strain H3 in the presence of quinoline and different inorganic nitrogen sources were shown in Figure 4. When NH_4^+–N and quinoline were utilized as mixed nitrogen sources, NH_4^+–N and quinoline were simultaneously removed by strain H3 (Figure 4A). More than 98% of NH_4^+–N and 92% of quinoline were effectively removed by strain H3 after cultivation for 72 h. The accumulation of NO_3^-–N reached a maximum of 1.3 mg/L after cultivation for 54 h, while NO_2^-–N remained undetected throughout the culture process. Figure 4B shows the nitrogen removal performance when NO_2^-–N and quinoline serve as mixed nitrogen sources. Strain H3 achieved removal efficiencies of 99% for NO_2^-–N and 72% for quinoline after cultivation for 72 h. It can be found that the removal performance of quinoline was inferior to that of NH_4^+–N and quinoline as mixed nitrogen sources. NO_3^-–N reached a maximum accumulation of 2.4 mg/L after cultivation for 48 h and a small quantity of NH_4^+–N was detected in the later stage of cultivation.

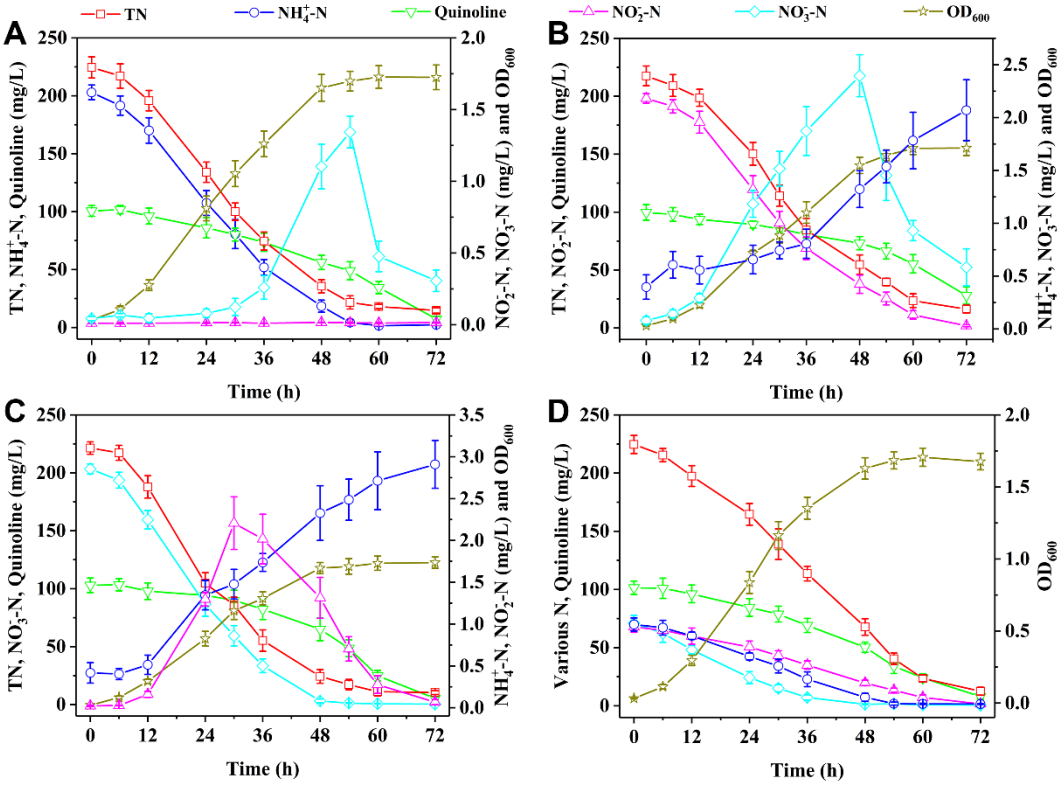

Figure 4. Simultaneous organic and inorganic nitrogen removal performance of strain H3 with (**A**) NH_4^+–N + quinoline, (**B**) NO_2^-–N + quinoline, (**C**) NO_3^-–N + quinoline, and (**D**) NH_4^+–N + NO_2^-–N + NO_3^-–N + quinoline as mixed nitrogen sources.

When NO_3^-–N and quinoline were utilized as mixed nitrogen sources, quinoline removal performance was comparable to that of NH_4^+–N and quinoline as mixed nitrogen sources (Figure 4C). Around 95% of quinoline was effectively removed by strain H3 after cultivation for 72 h. The accumulation of NO_2^-–N reached a maximum of 2.2 mg/L after cultivation for 30 h. Figure 4D shows that NH_4^+–N, NO_2^-–N, NO_3^-–N, and quinoline used as mixed nitrogen sources can be removed simultaneously by strain H3. The nitrogen removal rates decreased in the order of NO_3^-–N > NH_4^+–N > NO_2^-–N > quinoline. When strain H3 was cultivated for 72 h, the removal efficiencies of NH_4^+–N, quinoline, NO_2^-–N, NO_3^-–N, and TN all exceeded 90%, indicating that strain H3 exhibits excellent performance in simultaneously removing organic and inorganic nitrogen.

3.5. Whole Genome Sequencing and Gene Function Annotations

The whole genome was sequenced and assembled to investigate the genomic characteristics of strain H3. The draft genome of strain H3 was 4612156 bp with an average GC content of 64.16% (Figure S2). The whole genome was assembled into 45 scaffolds with N50 coverage of 480929 bp. The

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number of coding genes was 4392, including 68 tRNA genes and 3 rRNA genes. In addition, the number of genes annotated by the databases of GO, KEGG, and COG was 2573, 3373, and 3673 respectively. This variation in numbers implies that different databases have diverse focuses and coverage in gene annotation, which can provide comprehensive insights into the gene functions of strain H3.

Figure 5 shows the gene function annotations of strain H3 by the databases of GO, COG, and KEGG, which are crucial for comprehensively understanding the biological characteristics and metabolic functions of strain H3. The number of gene function annotations by the GO database was 2573, including 1464 in the biological process (BP), 1311 in the cellular component (CC), and 2050 in the molecular function (MF). The top 30 gene function annotations were shown in Figure 5A, which contained 9 in the BP category, 5 in the CC category, and 16 in the MF category. The gene function annotations in the BP category related to nitrogen metabolism included 53 in the nitrogen compound metabolic process and 37 in the nitrogen utilization. The gene function annotations based on the KEGG database were initially classified into 6 categories, including cellular processes, metabolism, environmental information processing, genetic information processing, organismal systems, and human diseases (Figure 5B). The number of gene function annotations related to metabolism was the largest. Specifically, carbohydrate metabolism (281) and amino acid metabolism (260) were the primary metabolic activities. In Figure 5C, the gene function annotations based on the COG database are presented and these annotations are divided into 24 categories. It was observed that the numbers of gene function annotations for amino acid transport and metabolism (336) and signal transduction mechanisms (349) were the largest among all the categories.

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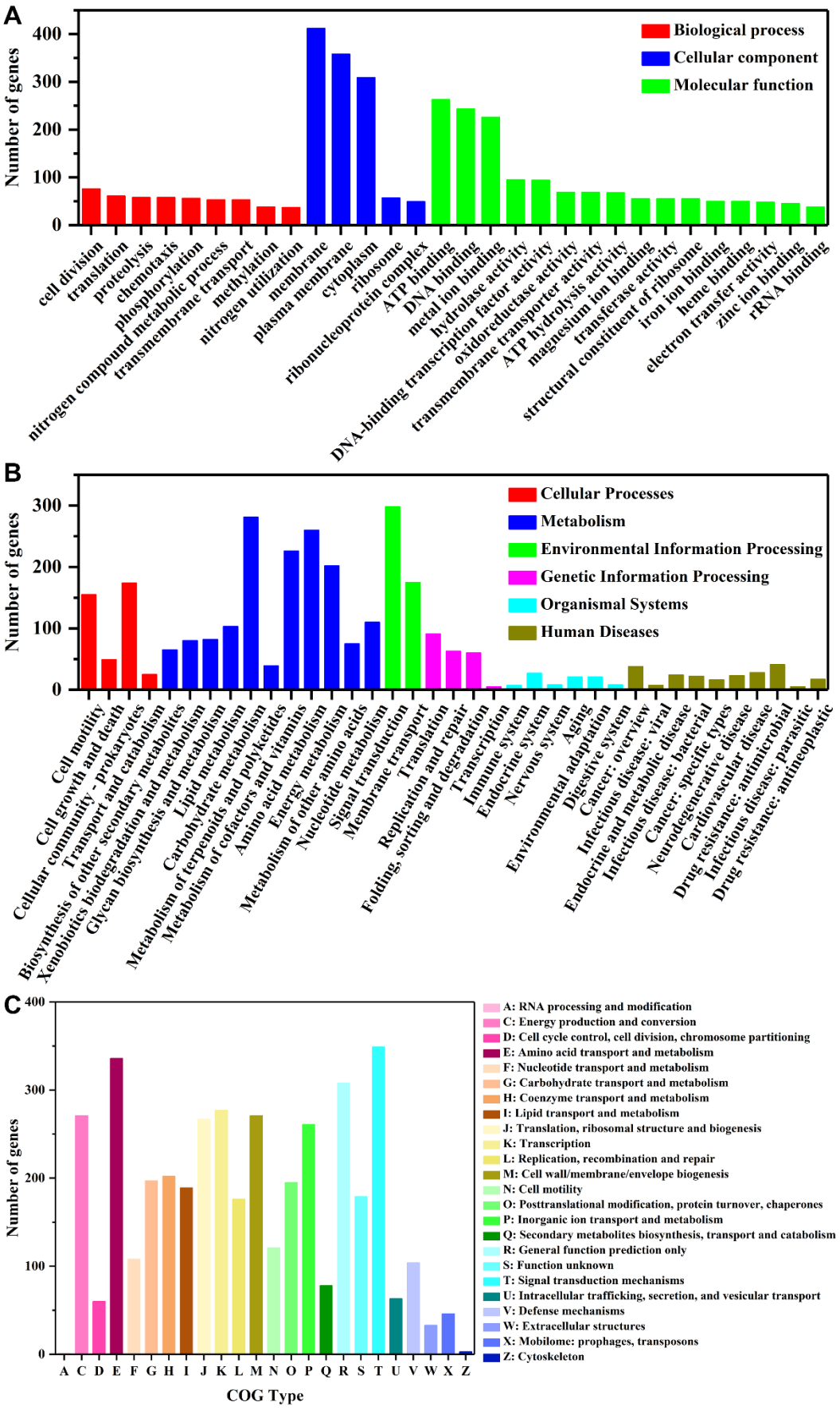

Figure 5. The gene function annotations based on (**A**) GO, (**B**) KEGG, (**C**) COG databases.

3.6. Nitrogen Metabolic Pathways and Mechanisms

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The gene function annotations of strain H3 for the nitrogen metabolic pathway based on the KEGG database were shown in Figure 6A. Approximately 44 genes related to nitrogen metabolic pathways were annotated, including *NarH*, *NarG*, *NasD*, *NapA*, *NirS*, *NorB*, *NosZ*, etc. The identified nitrogen metabolic pathways included dissimilatory/assimilatory nitrate reduction, denitrification, and ammonia assimilation. The *NarGHI*, *NapAB*, and *NirBD* genes were present in strain H3 and involved in the dissimilatory nitrate reduction. The *NasAB* and *NasBDE* genes were primarily responsible for the assimilatory nitrate reduction. The complete denitrification pathways and associated nitrogen removal functional genes including *NapAB*, *NarGHI*, *NirS*, *NorBC*, and *NosZ* were present in strain H3. Among these nitrogen removal functional genes, *NapAB* and *NarGHI* were responsible for reducing NO_3^-–N to NO_2^-–N under aerobic and anoxic conditions, respectively [50-52]. NH_4^+–N was converted into L-Glutamine and L-Glutamate through glutamine synthetase and glutamate synthase encoded by *glnA* and *gltBD* genes. In addition, NH_4^+–N could also be converted into L-Glutamate via glutamate dehydrogenase encoded by the *gdhA* gene.

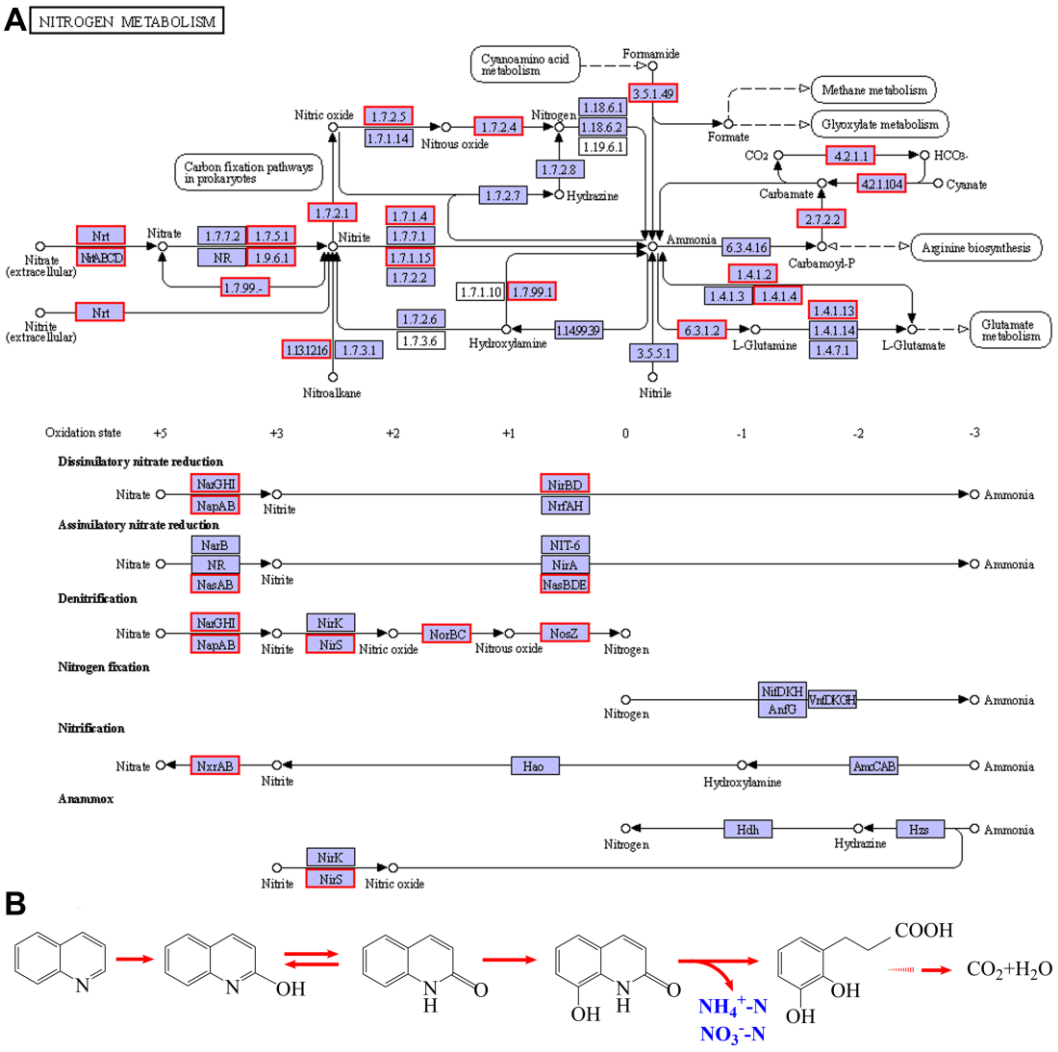

Figure 6. (**A**) The nitrogen metabolic pathway based on the KEGG database with the nitrogen removal function genes present in strain H3 annotated in red boxes. (**B**) The possible metabolic pathway of quinoline degraded by strain H3.

The metabolic intermediates of quinoline degraded by strain H3 were determined by GC-MS analysis. As shown in Figure 6B, the possible metabolic pathway of quinoline degraded by strain H3 was proposed. Quinoline was first converted into 2-hydroxyquinoline by strain H3 under aerobic conditions, which was consistent with the reported quinoline degradation process by other bacteria [3,13,14]. Subsequently, 2-hydroxyquinoline was oxidized to 2,8-dihydroxyquinoline by strain H3. During the biodegradation of quinoline, both NH_4^+–N and NO_3^-–N were simultaneously detected

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and promptly removed. It is speculated that elemental nitrogen in quinoline was converted into NH_4^+–N and NO_3^-–N. The 2,8-dihydroxyquinoline was further converted into 2,3-dihydroxyphenyl-propionic acid and eventually completely mineralized to CO_2 and H_2O. This finding is highly significant for understanding the biodegradation process of quinoline by strain H3, providing a potential solution for the treatment of quinoline in wastewater.

4. Conclusions

A simultaneous organic and inorganic nitrogen removal bacterium was isolated and identified as *Pseudomonas stutzeri* H3. Above 90% of both NH_4^+–N and quinoline were removed by strain H3 after optimizing the nitrogen removal conditions through single-factor experiments. The excellent simultaneous nitrogen removal capabilities of strain H3 were demonstrated by comparing with other nitrogen removal bacteria *Halomonas* sp. and *Acinetobacter* sp. The outstanding removal performances of quinoline and different inorganic nitrogen sources further confirmed the simultaneous organic and inorganic nitrogen removal capability of strain H3. The gene function annotations, nitrogen removal function genes and nitrogen metabolic pathways of strain H3 were identified by the whole genome sequencing analysis and nitrogen metabolic intermediates determination.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: The removal performance of (A) NH_4^+–N and (B) quinoline by strain H3 at the initial concentrations of 100 mg/L quinoline and 50-400 mg/L NH_4^+–N. The removal performance of (C) NH_4^+–N and (D) quinoline by strain H3 at the initial concentrations of 200 mg/L NH_4^+–N and 50-400 mg/L quinoline.; Figure S2: Circle map of the *Pseudomonas stutzeri* H3 genome.

Author Contributions: Conceptualization, Jie Hu, Bing Xu and Jiabao Yan; Data curation, Jie Hu, Bing Xu and Jiabao Yan; Formal analysis, Jie Hu and Guozhi Fan; Funding acquisition, Jie Hu, Bing Xu, Jiabao Yan and Guozhi Fan; Investigation, Jie Hu, Bing Xu and Guozhi Fan; Methodology, Jie Hu, Jiabao Yan and Guozhi Fan; Project administration, Bing Xu and Jiabao Yan; Resources, Bing Xu and Jiabao Yan; Software, Jie Hu; Supervision, Bing Xu, Jiabao Yan and Guozhi Fan; Validation, Jie Hu, Bing Xu and Guozhi Fan; Visualization, Jie Hu and Bing Xu; Writing – original draft, Jie Hu; Writing – review & editing, Jie Hu and Bing Xu. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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