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Effect of UV irradiation on microbial structure, metabolic pathways and functional genes of nitrogen metabolism in highland wastewater

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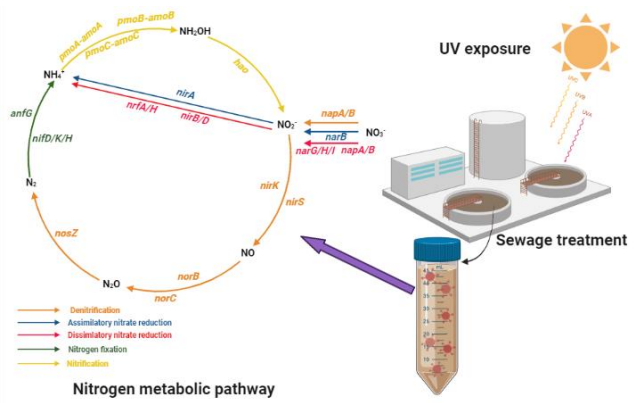
Graphical abstract



Abstract

In this experiment, in the background of the plateau environment, the effects of ultraviolet irradiation on the microbial community structure, metabolic pathways and nitrogen metabolism function genes of sewage were studied at 0 (control group), 5, 10, 30, and 180 min. The results showed that the increase of UV irradiation time promoted the microbial richness of sewage, but decreased the microbial diversity. In terms of community structure, the increase of ultraviolet irradiation time will inhibit or promote some bacterial genera, for example, it has a promoting effect on Bacteroidota, norank_f_ Saprospiraceae, etc., and an inhibitory effect on Phreatobacter. The relative abundance of metabolic pathways changed significantly with the increase of UV irradiation time, and the change trends at different metabolic levels were the same. In nitrogen metabolism pathways, the relative abundance of genes involved in nitrification and denitrification functions was inhibited by the duration of UV irradiation.

Keywords: ultraviolet irradiation; highland sewage; microbial communities; metabolic pathways; functional genes

1. Introduction

In recent years, UV irradiation has been widely used in wastewater treatment processes due to its advantages of low energy consumption and low cost, such as disinfection of treated wastewater by UV irradiation and wastewater treatment using the UV/H_2O_2 process (Anastasi *et al.*, 2013; Vogna *et al.*, 2004). This is because UV disinfection primarily works by damaging dsDNA and forming toxic photooxidation by-products that kill or damage microorganisms prior to effluent discharge (Liang *et al.*, 2012). It has also been shown that UV irradiation has varying degrees of effect on pollutant removal and microbial community structure, metabolic pathways and functional genes in wastewater treatment (Chen and Li, 2020; Hao *et al.*, 2021; Hollman *et al.*, 2020).

Tibet, known as the "roof of the world" and the last piece of pure land in the world, has an average altitude of over 4000 m, with low temperatures, strong ultraviolet rays and low dissolved oxygen (DO) as the main characteristics of the Tibetan region (Hao *et al.*, 2019). The intensity of UV radiation in Tibet is high, for example, the average daily UV radiation dose in Lhasa, Tibet is 1.24 MJ/m^2, which is 1.5 to 2 times higher than inland cities (Yan *et al.*, 2021). Most previous studies have looked at the analysis of the effects on pollutant removal and microorganisms from the perspective of UV irradiation being applied to wastewater treatment processes. For example, (Malvestiti *et al.*, 2019) studied the effect of UV/H_2O_2 process on microbiological indicators of municipal secondary wastewater; (Liu *et al.*, 2019) combined ozone with UV/H_2O_2 to degrade pollutants from different sources. However, few scholars have studied and analysed the effects of UV radiation on wastewater microorganisms from the perspective of UV radiation as an influencing factor in wastewater treatment processes. For areas such as Tibet, which itself has a high level of UV radiation, the removal of pollutants or wastewater micro-organisms in the wastewater treatment process can be affected by

varying degrees of UV radiation (Hao *et al.*, 2021; Fang *et al.*, 2020).

Therefore, this experiment takes the characteristics of strong UV radiation in Tibet as the research, and selects the AAO process used by most of the sewage treatment plants in Tibet as a representative. The effects of different UV irradiation times (0, 5, 10, 30, and 180 minutes) on microbial community structure, metabolic pathways, and nitrogen metabolism functional genes in wastewater were investigated using high-throughput sequencing technology. The purpose is to study the micro-reaction mechanism of microorganisms to ultraviolet irradiation and irradiation time in the process of wastewater treatment in plateau areas, and to provide theoretical reference for wastewater treatment in plateau areas.

2. Materials and methods

2.1. Test device

Using the AAO process, as shown in Figure 1. AAO wastewater treatment process is a common secondary wastewater treatment process, with simultaneous nitrogen removal and phosphorus removal, where anaerobic means DO <0.2 mg/L, anoxic means DO ≤0.5 mg/L, aerobic means DO>2 mg/L. The total volume of the reaction tank is 210 L, of which the anaerobic, anoxic and aerobic tanks are 35, 58 and 117 L respectively, and the volume of the secondary sedimentation tank is 39 L.

Stirring devices are provided in both the anaerobic and anoxic tanks, with a stirring speed set at 110 rpm. A diaphragm type microporous aeration head is provided at the bottom of the aerobic tank to supply oxygen to it. Both the test influent and the sludge return use a magnetic drive circulating pump, and the flow control is carried out by means of a rotameter and a ball valve, nitrifying solution reflux is controlled by peristaltic pump, and the sludge reflux ratio and the nitrifying solution reflux ratio are 100% and 200% respectively. An aerobic tank was used for 35 days of activated sludge incubation at a temperature of 20.0 °C ± 1.0, DO of 2.5 ± 0.5 mg/L and pH of 7 ± 0.5. At the end of the incubation phase, the sludge settling ratio (SV_{30}) was measured to be 28% and the mixed liquor suspended solids mass concentration (MLSS) was 3716 mg/L, at which point the AAO process began to operate. In this experiment, the domestic wastewater from the office building area of Tibet Agricultural and Animal Husbandry College in Tibet, China was directly used as the test water, and the main influent water quality indicators are shown in Table 1.

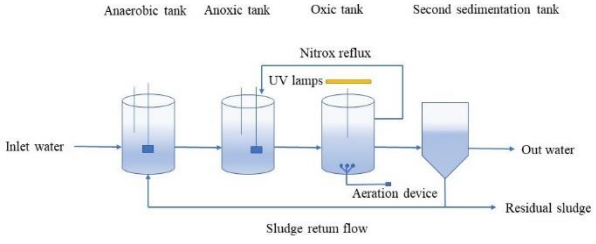


Figure 1 AAO process flow diagram.

2.2. UV settings and data testing

UV was achieved using two 40W UV lamps, the irradiated tank was aerobic tank and the distance between the UV lamps was 20cm, thus the average UV radiation intensity $S = \frac{P}{2\pi\gamma L} = \frac{40}{2\pi \times 0.2 \times 0.53} = 60.05 J(m^2.s)$, for the activated sludge located in the surface layer of the aeration state in this experiment (Wang., 2012). That is to say, when the irradiation time is 0 min, 5 min, 10 min, 30 min, and 180min, the ultraviolet radiation energy received by the surface activated sludge is 0 J, 954 J, 1908 J, 5724 J, 34344 J. The UV irradiation time was controlled from short to long, and each set of conditions was run for 9 days, and the activated sludge from the aerobic tank was taken as the test sample at the end of each set of conditions. To eliminate the effects of condition adjustments, the next test is carried out at 72 h intervals after each operating condition. Take 100ml of the mixture at the end of the aerobic tank, put it into a 100ml measuring cylinder, and after standing for 30min, read the scale line L, and then use L×100%, which is the result of SV30. The calculation method of MLSS is to first take 100ml of the mixture at the end of the aerobic tank. Then use quantitative filter paper to dry at 103–105℃, cool down during the drying period, weigh, repeat until a constant weight was obtained or the loss in weighing was less than 4% of the previous weighing and the weight was recorded as m0. The mixed solution is filtered with dried quantitative filter paper, put into an oven at 103–105°C for drying and taken out, cooled to equilibrium temperature in a desiccator and weighed, and repeatedly dry to make a constant weight or the weight loss is less than 5% of the previous weighing or 0.5 mg (taking the smaller value), the weight is recorded as m1, then MLSS=(m1-m0)/0.1.

2.3. Sample testing and analytical methods

2.3.1. Sample testing

This sample was sent to Shanghai Meiji Biomedical Technology Co., Ltd. For testing (htpp://www.majorbio.com) and sequencing of the 16S rRNA gene. 16S rRNA gene sequencing is generally The V3-V4 variable region of the 16S rRNA gene is sequenced using MiSeq as a way to study microbial diversity (Gantuya *et al.*, 2021). Genomic DNA was extracted using 1% agarose gel electrophoresis assays, and primers 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTW TCTAAT) were amplified on biofilm samples in the V3 and V4 regions, and a preliminary sequence quality screen was performed. Raw sequences that passed the initial quality screen were divided into libraries and samples according to index and Barcode information, and barcode sequences were removed. The de-duplication operation (100% similarity clustering) was performed using the DADA2 method (Callahan *et al.*, 2016), and each de-duplicated sequence generated after quality control was called ASVs (amplicon sequence variants), and finally, with the help of the QIIME2 cloud platform, based on the Silva database (Release 132,

http://www.arb -silva.de) for taxonomic annotation of the species (Quast *et al.*, 2013).

2.3.2. Bioinformatics analysis

All sequences were clustered into OTUs (Operational Taxonomic Units) based on 97% sequence similarity using the RDP (Ribosomal Database Project) database classification method. Based on the number of OTUs, Mothur software analysed the relative abundance and diversity indices, including the Chao1 index, the ACE (relative abundance-based coverage estimator) index Shannon and Simpson indices.

2.3.3. Analysis of community composition and test of significance of differences between groups

Community composition analysis consists of community bar charts (Bar charts), community pie charts (Pie charts), multi-level species Sunburst charts, and community Heatmap charts. In this case, the community bar map (Bar map) is used. The community bar map (Bar map) is based on the results of taxonomic analysis, which can tell the species composition of different groupings (or samples) at each taxonomic level (e.g. domain, boundary, phylum, order, family, genus, species, OTU, etc.). According to the community Bar diagram, two aspects of information can be presented visually: (1) which dominant species each sample contains at a given taxonomic level; and (2) the relative relative abundance (share) of each dominant species in the sample. Plots were made using data tables based on those in the tax_summary_a folder, using the R language (version 3.3.1) tool. Test for significant differences between groups Based on the community relative abundance data obtained, hypothesis testing was applied using rigorous statistical methods to assess the significance level of species differences in relative abundance between different groups (or samples) of microbial communities to obtain significantly different species between groups (or samples).

2.3.4. 16S rRNA gene function prediction

The OTU relative abundance tables were normalised by PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) software to obtain OTU corresponding COG (Cluster of Orthologous Groups of proteins) and KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) Ortholog (KO) information. KO, PATHWAY is available based on information from the KEGG database. The KEGG PATHWAY database includes various metabolic pathways and is graphically plotted using Origin 8.6 software.

3. Results

3.1. Microbial relative abundance and diversity

The alpha-diversity of the microbial community was expressed using Ace and Chao1 estimates and the Shannon and Simpson diversity indices. Higher alpha-diversity values indicate higher bacterial community richness and diversity. Table 2 shows the microbial richness and diversity of five groups of sludge samples with different UV irradiation durations. The number of valid sequences ranged from 121922 to 155179, with OTU ranging from 1531 to 2147, showing that the duration of UV irradiation had a significant effect on microbial community richness and diversity. similar trends were observed in the estimates of Ace, Chao1 and Shannon. The Ace and Chao1 indices reflect the microbial community richness, with higher values indicating higher microbial richness. Ace and Chao1 indices varied in a wave-like pattern with increasing UV irradiation time, with the lowest group being UV0 without UV irradiation and the highest being UV30, which compares with the plain areas where the AAO process is also used (Jinan and Xiamen, China, with Chao1 indices of 3351.16, 3844.05 (Hu *et al.*, 2012; Zhang *et al.*,2020)), indicating that UV irradiation in the plateau region promoted the richness of the wastewater microbial community. Also, previous studies have shown that UV irradiation has a facilitative effect on specific bacterial relative abundances (Kauser *et al.*, 2019). For the diversity index Shannon, it was found that with the increase of UV radiation time, only the UV5 group was greater than UV0, suggesting that UV irradiation had some reducing effect on microbial diversity, in line with the findings of (Kauser *et al.*, 2019). It is noteworthy that the Shannon index was overall lower than that of 5 min at 5 to 180 min of UV irradiation. When UV irradiation increased at 5–10 min, α-diversity decreased, and when considered together, the α-diversity index was highest at 5 min of UV irradiation. Thus, indicating that UV irradiation and the duration of irradiation changes the microbial relative abundance and diversity within the wastewater treatment system.

3.2. Microbial community composition

The microbial community structure was analysed at the phylum level for different UV irradiation durations and the results are shown in Figure 2(a). Proteobacteria (66%), Bacteroidota (19.5%), and Actinobacteriota (5.4%) were the main dominant phylum, which is consistent with (Lan *et al.*, 2018) and (Pan *et al.*, 2020) The results of the study were the same. This indicates that UV irradiation did not change the structure of the dominant phylum of microorganisms in the wastewater treatment system. Proteobacteria play an important role in the removal of carbon and nitrogen as the most abundant group (Ansola *et al.*, 2014), and the study found that the majority of current nitrogen removal bacteria were from Proteobacteria (Wei *et al.*, 2015); the relative abundance of Proteobacteria increased with increasing UV irradiation time in the grouping of 5–180 minutes of UV irradiation. This was followed by Bacteroidota, which was reported by (Shi *et al.*, 2015) as an important group of heterotrophic organisms capable of participating in the cycling of organic carbon and proteins; the relative abundance in the remaining four groups was greater than UV0 compared to the control UV0. It is noteworthy that the relative abundance of both dominant phyla, which play an important role in the removal of pollutants, increased after UV irradiation, which echoes the results of the change in microbial abundance described above. The remaining four dominant phyla, Actinobacteriota and Chloroflexi, decreased in relative abundance with

increasing UV irradiation time, but Firmicutes and Patescibacteria increased in relative abundance, with Actinobacteriota and Firmicutes being associated with nitrogen removal (Kang., 2018). A test for significance of differences between groups was also performed, as shown in Figure 2(b), where the relative relative abundance of Firmicutes, Chloroflexi, and Patescibacteria among the first six dominant phyla was significantly different by the duration of UV irradiation in the five groups of test samples. Combined with Figure 2(a), in which the relative abundance of Chloroflexi decreases by 80–95% when subjected to different lengths of UV irradiation. It can be seen that the degree of inhibition of certain bacteria by ultraviolet radiation is great.

As can be seen from Figure 2(c), the microorganisms were diverse at the genus level, with the main dominant genera being norank_f_ Saprospiraceae (31.9%), Phreatobacter (4.5%), and norank_f_AKYH767 (3.5%), which is different from the results of previous studies (Xu *et al.*, 2021; Zheng *et al.*, 2021), main reason is that previous studies were conducted under plain conditions, whereas the results of the present study are similar to those of the plateau region (Li *et al.*, 2022) and (Zong *et al.*, 2020), which is more indicative of the specificity of the plateau region. Which is more indicative of the specificity of the plateau region. norank_f_ Saprospiraceae is an organic matter degrading bacterium (Fu *et al.*, 2017), and the study by (Zhang *et al.*, 2013) showed that norank_f_ Saprospiraceae was the dominant species in the denitrification phosphorus removal system with nitrite as the electron acceptor, also indicated that denitrification phosphorus removal occurred in this system. In Figure 2(d), it can be seen that the relative abundance of norank_f_Saprospiraceae in the remaining four groups is significantly different from that of the UV0 group, and the relative abundance increases greatly after being irradiated by UV light. The relative abundance of Phreatobacter decreased significantly with increasing UV irradiation, as did that of AAP99, Gemmobacter and Hydrogenophaga. Phreatobacter is currently less described and belongs to the phylum Proteobacteria. It was discovered for the first time in ultrapure water purification systems and is the second most dominant flora in microbial contamination in the Paris water supply network system (Perrin *et al.*, 2019; Tóth *et al.*, 2014), it is evident that this genus survives very easily in nutrient-poor environments (COD/TN in this trial was in the range of 2.7–3.8, which is a low carbon to nitrogen ratio) and is very good at using substrates, which is most likely the main reason for it being the dominant bacteria. Norank_f_AKYH767 belongs to the Bacteroidetes, with the highest relative abundance at UV5. According to (Hao *et al.*, 2020) and (Zong *et al.*, 2020) studies shouw, norank_f_AKYH767 was the dominant genus with the highest relative abundance in the AAO process at DO2mg/L and temperature 10 °C, indicating that norank_f _AKYH767 is fully adapted to this low DO, low temperature and strong UV environment in the highland region, and is also the reason for being the dominant genus. In combination with Figure 2(d) it can be seen that five of the top eight dominant genera had significant inter-group variability in relative abundance, with the main reason for the inter-group differences being the presence or absence of UV irradiation.

3.3. Metabolic pathways

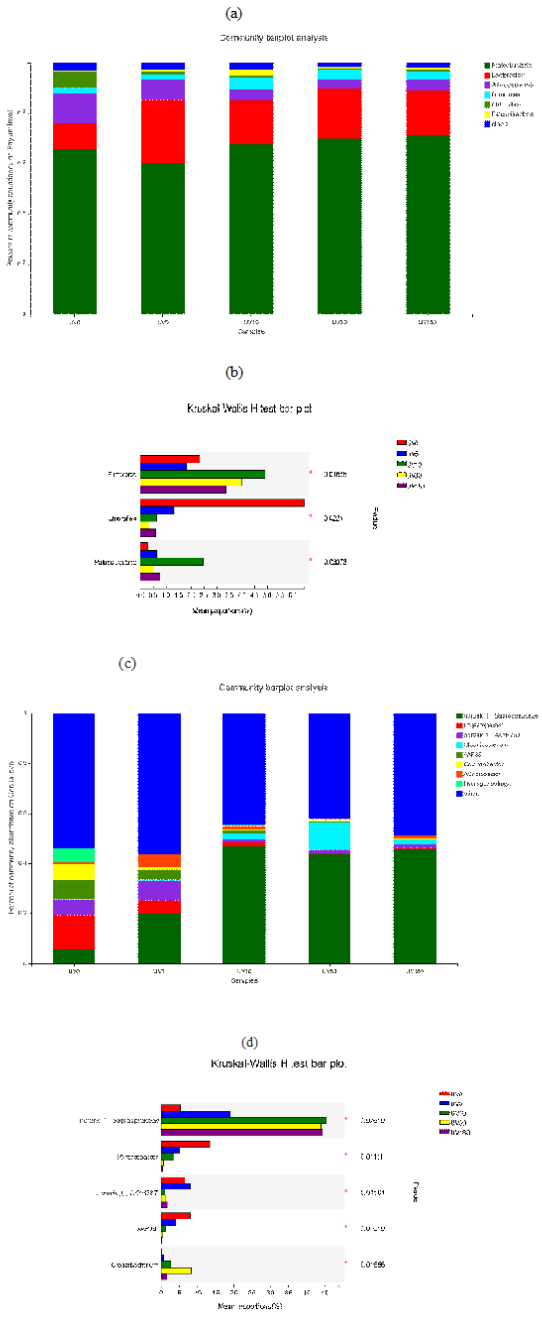


Figure 2 Microbial community composition at different UV irradiation durations. (a) Microbial composition at the Phylum level. (b) Significance test for differences between groups at the Phylum level. (c) Microbial composition at the Genus level. (d) Significance test for differences between groups at the Genus level，* indicates P<0.05.

To further understand the effects of UV irradiation on the functional characteristics of wastewater microbial communities, this study predicted the metabolic pathways of these microbial communities by PICRUSt based on the KEGG pathway database. On KEGG pathway level 1 in Figure 3(a), Metabolism (76.7%), Genetic Information Processing (6.4%), Environmental Information Processing (6.1%) Cellular Processes (4.5%), Human

Diseases (4.2%), and Organismal Systems (2.1%), with Metabolism (61%) being the main functional metabolism, which is the same as the findings of (Yin and Wang., 2021) and (Liu *et al.*, 2021). In Cellular Processes, Human Diseases, the relative abundance of the UV0 group without UV irradiation was higher than the other four groups, indicating that the growth and reproduction of microorganisms were inhibited by UV irradiation, which also corresponds to the microbial communities mentioned above. The relative abundance of the remaining four is highest in UV10.

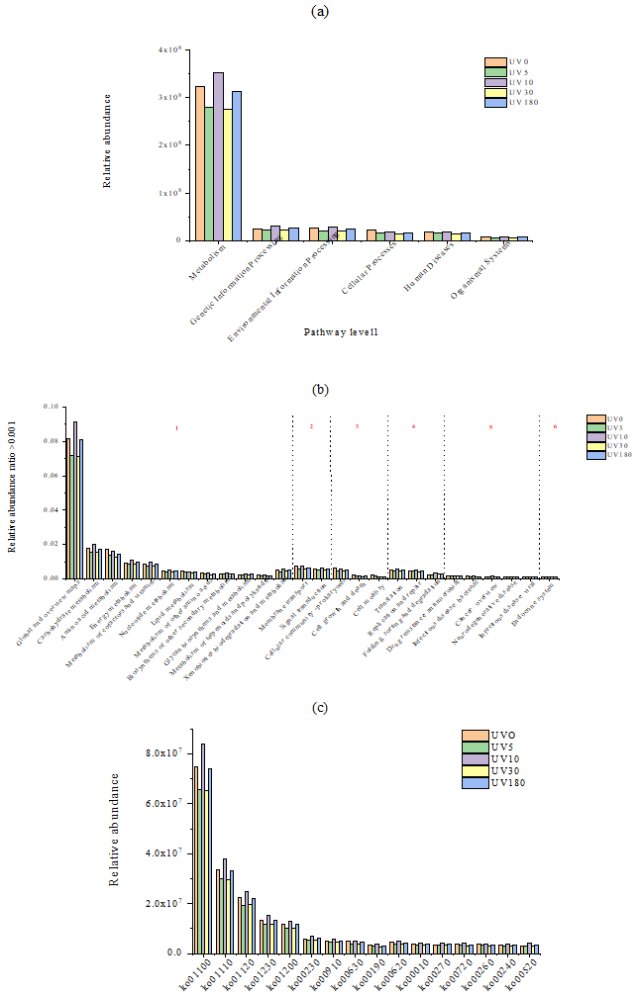


Figure 3 (a) Relative abundances of metabolic pathways on KEGG categories (level 1). (b) Relative abundances of metabolic pathways on KEGG categories (level 2). Note:1: Metabolism, 2: Environmental Information Processing, 3: Cellular Processes, 4: Genetic Information Processing, 5: Human Diseases, 6: Organismal Systems. (c) Relative abundance of different pathways in metabolism.

As shown in Figure 3(b), the KEGG pathway level 2 is further analyzed. Among the functions of Metabolism, the main functional sub-metabolisms are Global and overview maps, Carbohydrate metabolism, and Amino acid metabolism, accounting for 39.7%, 8.6%, and 7.4%, respectively. Carbohydrate metabolism is the main functional sub-metabolism, which is consistent with (Guo *et al.*, 2017) are consistent with the results. Carbohydrate metabolism, which includes glycolysis, carbohydrate transport and the TCA citric acid cycle pathway, is a typical energy production and cellular biosynthetic process in activated sludge systems (Noor *et al.*, 2010). The relative abundance of Amino acid metabolism, Lipid metabolism, Metabolism of other amino acids, Metabolism of terpenoids and polyketides were also found to be inhibited by UV irradiation. This suggests that UV irradiation reduces the source of microbial substrates and nutrient utilisation in the system, which would be detrimental to the degradation of pollutants. In Environmental Information Processing, membrane transport (3.3%) and signal transduction (2.8%) play an important role in microbial growth, metabolism and gene expression. It was also found that the percentage relative abundance of each sub-metabolism varied in a wave pattern with increasing duration of UV irradiation throughout the pathway level 2, and the highest relative abundance percentage was dominated by UV10 and UV0, which was the same as the variation in pathway level 1.

Figure 3(c) shows the relative abundance of different pathways (>0.01) in KEGG pathway level 3 regarding Metabolism, the main pathways being Metabolic pathways (ko01100), Biosynthesis of secondary metabolites (ko01110), and Microbial metabolism in diverse environments (ko01120), all of which belong to global and overview maps, which correspond to the results in KEGG pathway level 2. The presence of Carbon metabolism (ko01200), Nitrogen metabolism (ko00910), Oxidative phosphorylation (ko00190), Carbon fixation pathways in prokaryotes (The presence of ko00720) also indicates that the metabolic pathways for the degradation of carbon, nitrogen and phosphorus pollutants are more active in this system, and this corresponds to the dominant nitrogen and phosphorus removal functional bacteria in the composition of the microbial community mentioned above. It can be seen from Figure 3(c) that the abundance of Glyoxylate and dicarboxylate metabolism (ko00630) and Glycine, serine and threonine metabolism (ko00260) was highest at UV0, but all the other pathways were highest at 10 min of UV irradiation. The trend of the abundance of all pathways with the duration of UV irradiation was the same as that of pathway level l and pathway level 2. It is also found that the metabolic pathways related to acids are the most, such as Biosynthesis of amino acids (ko01230), Glyoxylate and dicarboxylate metabolism (ko00630), Pyruvate metabolism (ko00620), et al, which once again shows that amino acids are the main nutritional substrates in this system material source.

3.4. Functional genes for nitrogen metabolism

Nitrogen as a major pollutant in sewage, it is important to study the response of its functional genes to UV irradiation. Therefore, the PICRUSt and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases based on statistical analysis of 16S rRNA gene relative abundance were used to map the gene map of nitrogen metabolism 4(a) and the relative abundance of each gene involved in nitrogen metabolism 4(b) under different UV irradiation. As shown in Figure 4(a), nitrogen metabolism consists of five nitrogen conversion processes and 24

functional genes. The highest gene relative abundance was found for the Dissimilatory nitrate reduction process regarding nitrate-nitrogen, and the relative abundance of nirB genes belonging to the Dissimilatory nitrate reduction process was again the highest among the 24 functional genes, thus indicating that Dissimilatory nitrate reduction is the main pathway for nitrate-nitrogen removal (Fan *et al.*, 2017). The relative abundance of narG/H/I genes in NO_3^-reduced NO_2^- were all increased by UV irradiation, and the relative abundance of nrfA/H and nirB/D genes fluctuated widely by UV irradiation. The energy generated by this pathway has been reported to be used for the oxidation of organic matter, and in the case of restricted transport of organic substrates, the pathway can provide a carbon source for nitrogen fixation and denitrification (Xiang *et al.*, 2020). In the process of Denitrification, the relative abundance of norB gene was the highest, followed by nosZ and norC; at the same time, it was found that the relative abundance of genes involved in Denitrification decreased with the increase of UV irradiation time, and the relative abundance was the highest at UV0, indicating that UV irradiation and the increase of irradiation time all caused inhibition on the relative abundance of genes involved in denitrification. Also, in the process of Nitrification, due to the low relative abundance of involved functional genes (only 4-265), which could not be shown in Figure 4(b), the gene relative abundance was also inhibited by UV irradiation. The relative abundance of functional genes narB and nirA associated with Assimilatory nitrate reduction were both low, and the relative abundance of nirA gene in NO_2^- reduced NH_4^+ was highest at UV0. The relative abundance of nifD/K/H and anfG genes in Nitrification action were both highest at UV10, which may be due to the fact that nitrogen fixation energy is mainly caused by light irradiation or oxidation of the carbon source (Liu *et al.*, 2021), but nifD/K/H gene relative abundance decreased more slope after 10 min of UV irradiation, which indicates that after a certain UV irradiation duration (>10 min), the radiation dose increased and caused an inhibitory effect on it.

Table 1 Inlet water quality index

pH	COD(mg/L)	BOD(mg/L)	TN(mg/L)	TP(mg/L)	NH_4^+-N(mg/L)
8.36±0.06	258.17±86.39	180.5±80.5	38.5±7.14	5.11±2.34	25.89±8.21

Table 2 Microbial community relative abundance and diversity indices at different UV radiation times

Samples	Amount of effective sequencing	OTUs	Ace	Chao1	Shannon	Simpson
UV0	127509	1732	3933.19	3596.49	13.10	0.13
UV5	121922	2147	4811.15	4386.37	14.00	0.16
UV10	155179	1698	3954.92	3953.61	10.91	0.56
UV30	122442	1531	4841.26	4381.29	9.77	0.74
UV180	134655	1649	4193.99	4204.37	10.68	0.60

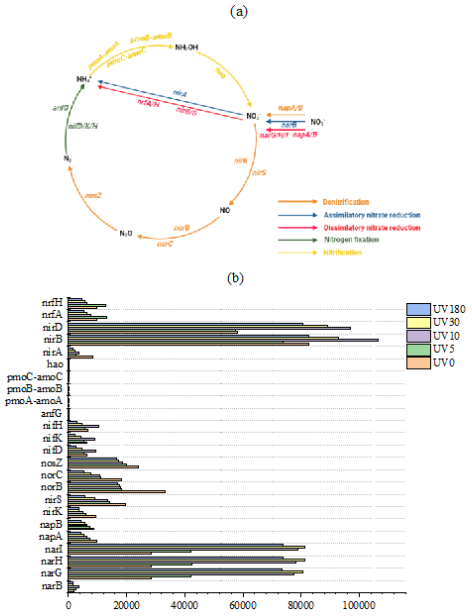


Figure 4 (a) Nitrogen metabolism pathway. (b) Relative abundance of functional genes for nitrogen metabolism.

4. Discussion

UV irradiation significantly affected microbial communities in plateau wastewater treatment. UV irradiation, as a treatment method aimed at inactivating microorganisms (Hijnen *et al.*, 2006), did reduce the overall microbial diversity (Simpson) in the effluent in our study, but the relative abundance (Ace, Chao1) increased after UV irradiation, both compared to the control UV0. This suggests that the effect of UV irradiation on microorganisms is either inhibitory or promotive, and that UV irradiation has an impact on the treatment of highland wastewater. A recent report showed that UV irradiation had little effect on the composition of microbial communities in sewage (Narciso *et al.*, 2018), which is similar to our study. At the phylum level, UV irradiation did not change the dominant bacterial phyla in traditional sewage, which remained the same as our study. Proteobacteria, Bacteroidota and Actinobacteriota were the main ones, but their relative abundance was inhibited and promoted by ultraviolet radiation. Among them, Bacteroidota and norank_f_ Saprospiraceae et al were promoted by UV irradiation, and Actinobacteriota and Phreatobacter et al were inhibited by UV irradiation. However, the change in Bacteroidota relative abundance is contrary to previous studies that showed a significant decrease in relative abundance following UV irradiation (Kauser *et al.*, 2019), probably due to the specific environment and sampling time in the highlands. According to existing studies, the relative abundance of

Bacteroidota increases with decreasing DO (Hao *et al.*, 2019); (Zong *et al.*, 2020) also from the highlands, showed that the relative abundance of Bacteroidota was highest at 10°C, indicating that low DO and low temperature also affect its relative abundance. The dominant genera at the genus level were similarly affected, e. g. norank_f_AKYH767. Also, the timing of sampling had an effect, sampling was done after 24 hours of UV irradiation, during which time it may have occurred that some bacteria underwent dark repair and increased in relative abundance (Hijnen *et al.*, 2006). In conclusion, we confirmed the different degrees of response of microbial community composition and abundance to UV irradiation and irradiation duration in plateau wastewater treatment, especially as the main dominant bacteria for pollutant removal. Therefore, from a microbiological point of view, UV irradiation and irradiation duration can be considered as influencing factors to be controlled in operating conditions for open-air wastewater treatment systems in highland areas. It also shows that the environmental factors of low DO and low temperature in the plateau area also jointly created the unique microbial community structure in the plateau sewage treatment system.

In wastewater treatment systems using activated sludge, the removal of pollutants requires the synergistic action of multiple metabolic pathways and functional genes. In the present study, the metabolic pathways were still dominated by Metabolism, and the relative abundance of the different pathways of Metabolism was found to follow the same trend as the relative abundance of pathway level 1and pathway level 2 by UV irradiation, and the highest relative abundance was mainly found at UV10. This indicated that the responses of the three levels of metabolic pathways to UV irradiation were consistent, and also indicated the interaction among the three levels of metabolic pathways. At the same time, there are also interrelationships with functional genes of nitrogen metabolism. For example, ultraviolet irradiation has an inhibitory effect on functional genes involved in nitrification. The nitrification reaction is to oxidize ammonia nitrogen into nitrite nitrogen and nitrate nitrogen, which belongs to the deamination process, deamination process is a part of amino acid metabolism (Ramsay *et al.*, 2001), but amino acid metabolic relative abundance is also inhibited by UV irradiation. We also found that amino acids, the main source of substrate for the system's microorganisms, were somewhat inhibited by UV irradiation, which would also affect nitrogen degradation. The results also showed that UV irradiation had inhibitory effects on functional genes of Denitrification and Nitrification, which are the main reaction processes of nitrogen removal, which would severely restrict nitrogen removal. Therefore, the results of the study can indicate that UV irradiation has a significant effect on the relative abundance of metabolic pathways and the relative abundance of nitrogen metabolism functional genes. with changes in the relative abundance of metabolic pathways being mainly facilitated by UV irradiation for 10 min, while the relative abundance of functional genes in the main reactions of nitrogen metabolism was inhibited by UV irradiation. The results also showed that there was a correlation between metabolic pathways and nitrogen metabolism functional genes in response to UV irradiation. This further elucidates the mechanism of the effect of UV irradiation on highland wastewater treatment at the microscopic level.

5. Conclusion

The results show that UV irradiation has a significant effect on microbial communities, metabolic pathways and functional genes for nitrogen metabolism in plateau wastewater. UV irradiation promoted the abundance but reduced the diversity of microorganisms in plateau wastewater. In terms of microbial community structure, UV irradiation had an increasing and decreasing effect on the relative abundance of some bacteria. In terms of metabolism, the relative abundance of different metabolic levels changed significantly with increasing duration of UV irradiation. The relative abundance of both nitrification and denitrification genes in nitrogen metabolism was suppressed by UV irradiation and duration of irradiation. These results provide insight into the effects of UV irradiation and irradiation time as influencing factors on microbial communities and metabolic pathways as well as functional genes of nitrogen metabolism in highland wastewater treatment and provide theoretical references for controlling UV irradiation and irradiation time in practical wastewater treatment processes in highland areas.

Acknowledgements

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