Study on microbial metabolic mechanism of activated sludge denitrification by A2O process under strong solar radiation in highland habitat

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Abstract

The effects of plateau solar radiation on microbial abundance and diversity and metabolism during the formal operation of the A²O reactor were analyzed by running three parallel A²O units with solar radiation, constant temperature and room temperature laterally. The results showed that when the formal operation reached the 180th day, the nitrogen removal effect of the solar radiation A²O reactor decreased significantly compared with that of the constant temperature and room temperature units in general; the number of operational taxonomic units (OTUs) in the solar radiation, constant temperature and room temperature A²O systems were 1140, 972 and 1000, and the solar radiation reactor showed an increasing trend throughout the whole process, and the metabolic function of the dominant genera of microorganisms was dominated by organic matter degradation, denitrification and denitrogenation, and the metabolic pathways were dominated by carbon and nitrogen metabolism, and it was also found that the relative abundance of denitrifying bacteria (DNB) increased with the increase in the duration of solar radiation exposure. The bacterial diversity of the solar A²O system was lower than that of the constant and room temperature systems up to 90 days, and the relative abundance of some common dominant genera in the sludge was suppressed by solar radiation, but the abundance of some denitrification-related genera and their functional genes were instead strengthened in the solar radiation reactor.

1 INTRODUCTION

Linzhi of Tibet is a region with an average altitude of about 3000 meters and has a special high-altitude ecosystem. The microbial community structure, diversity, energy metabolic pathways and the abundance of related genes in the water are significantly affected due to the lower water temperature, lower atmospheric pressure, higher intensity of solar radiation at high altitude, and the microbial characteristics of wastewater are influenced by the natural environment such as temperature and dissolved oxygen. More than 20 large-scale sewage treatment plants have been built in Tibet so far, and their secondary treatment is mainly based on the biological method of A²O or AO. Due to the fluctuation of the incoming water quality and the influence of the special habitat of the plateau, the effect of the secondary treatment is not good, which leads to the serious problem of secondary pollution, and ultimately, the effluent quality of the water can not reach the level of Class I A as stipulated by the state.

A²O (anaerobic-anoxic- aerobic) process is in the 1970s, by the United States of America, some wastewater treatment experts in the anaerobic- aerobic (Anaerobic-Oxic) method of denitrogenation process, based on the Wuhrmann process [1], the modified Ludzack-Ettinger process [2], Bardenpho [3] process and the Phoredox [4] process. A²O[5] process were developed on the basis of several stages with the aim of developing a wastewater treatment process capable of simultaneous nitrogen removal. Qinghai-Tibet Plateau from 2020 only began to A²O process of biological nitrogen and phosphorus removal of sewage for a variety of conditions of small pilot studies [6], and in recent years has been gradually applied to urban wastewater treatment, Lhasa River South Bank Artificial Wetland Wastewater Treatment Plant is this year's more successful application of the technology case, mainly through the
planting of lotus, reeds and other aquatic plants and paving of volcanic rocks and other aquatic plants, to remove nitrogen and phosphorus in the water, further purification of water quality, so that the final effluent of the nitrogen and phosphorus ratio, and to make the water quality, and to make the final effluent of the water quality. Further purification of water quality, so that the final water to meet the surface 5 water discharge standards. Nowadays, a lot of successful practical experience has been accumulated. There has been a team of researchers in the previous study to explore the impact of high altitude conditions of A²O treatment process, found that by optimizing the treatment conditions can significantly improve the efficiency of wastewater treatment, but there are still a number of indicators such as nitrogen and phosphorus can not be synchronized with the effective removal of the problem [7]. At present, in the existing research reports related to the effect of radiation on the wastewater treatment process, Most of the research direction is the effect of artificial UV on the treatment effect and microbial community structure of sewage treatment process [8, 9], and there is very little research on the effect of natural strong solar radiation on sewage treatment process, and even in the national scope has not seen relevant reports.

Based on the above research and application of the A²O process, this experiment takes the characteristics of strong solar radiation in Tibet as the research background, selects the A²O process used in most of the sewage treatment plants in Tibet as a representative, and uses high-throughput sequencing technology to study the influence of different solar radiation under different solar radiation influence times during the official operation stage (50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180 days) on microbial community structure, metabolic pathways and functional genes of nitrogen metabolism in the reactor. The aim of this study was to investigate the effects of solar radiation on activated sludge and water quality indicators, microbial community structure, functional profiles and metabolomics in a laboratory-scale A²O wastewater treatment bioreactor in plateau area, which can provide some theoretical references for wastewater treatment in plateau area.

2 MATERIALS AND METHODS

2.1 Test device and operation

The components of the A²O process under solar radiation, constant temperature and room temperature conditions for this test were divided into an anaerobic tank, an anoxic tank, an aerobic tank and a secondary sedimentation tank. The plant is first fed by the anaerobic tank, then flows from the anaerobic tank to the anoxic tank, and finally flows through the secondary sedimentation tank. A mixed liquor return device is installed between the anoxic tank and aerobic tank, and a sludge return device is installed between the anaerobic tank and the secondary sedimentation tank, with a sludge return ratio and a mixed liquor return ratio of 100% and 200%, respectively.

2.2 Experimental water quality
The experimental water was taken from the domestic wastewater in the building of the College of Water Resources and Civil Engineering of Tibet Agriculture and Animal Husbandry College, which was firstly transported to the water tank by the pumping machine, and then entered into the experimental device through the water tank. The range of COD of the influent water was 81.598 ~ 226.930 mg/L, the range of ammonia nitrogen of the influent water was 29.36 ~ 81.38 mg/L, the range of total nitrogen of the influent water was 30.56 ~ 94.18 mg/L, and the range of nitrate nitrogen of the influent water was 0.463 ~ 3.616 mg/L. The temperature of the thermostat device was controlled at 21 ± 1℃, Total HRT 24h, mixture reflux ratio of Ri = 200%, sludge reflux ratio of R = 100%.

2.3 Solar radiation settings and data testing

TBQ-2W portable solar radiometer was selected to collect instantaneous solar radiation and daily accumulation of radiation, and aerobic tank was selected as the irradiation tank. The solar irradiation time was progressive time by time, and each set of conditions was run for 10 days, and the activated sludge of the aerobic tank was taken as the test sample. At the end of the aerobic tank, 100 ml of the mixture was taken and put into a 100 ml measuring cylinder, after standing for 30 min, the scale line L was read, and then L/100%, which is the result of SV30. The MLSS was calculated by taking 100 ml of the mixture at the end of the aerobic tank first. It was then dried with quantitative filter paper at 103–105°C, cooled during the drying period, weighed, and repeated until the weight was constant or the loss of weighing was less than 4% of the previous weighing, noting the weight as m0.

The mixed solution was filtered through dried quantitative filter paper, dried in an oven at 103–105°C and then removed, cooled to equilibrium temperature in a desiccator and weighed, and dried repeatedly until the weight was constant or the weight loss was less than 5% of the previous weighing or 0.5 mg (whichever was smaller), and the weight was noted as m1, then MLSS = (m1 - m0) / 0.1.

2.4 Experimental Methods

2.4.1 Bioinformatics analysis

The RDP (Ribosomal Database Project) database classification method[10] was used to cluster all sequences into OTUs (Operational Taxonomic Units) based on 97% sequence similarity. Based on the number of OTUs, relative abundance and diversity indices were analyzed by Motherur software, including Chao1 index, relative abundance based coverage estimation (ACE) indices Shannon and Simpson indices.

2.4.2 Study of the effect of solar radiation on water quality during the formal operation phase

The experiments were carried out under three parallel conditions of solar radiation, constant temperature and room temperature, the water temperature was measured to be 5 ~ 26 ℃ under the solar radiation condition, the water temperature was constant at 21 ℃ under the constant temperature condition, and the water temperature was measured to be 12 ~ 22 ℃ under the room temperature condition. The
average dissolved oxygen of the three parallel conditions was 2.50 ~ 5.55 mg/L, pH was 7.9 ~ 9.0, and the operation mode was continuous water intake. The activated sludge indicators in the three reactors were measured on day 0 (D0), day 10 (D10), day 20 (D20), day 30 (D30), day 40 (D40), and on days 50 (D50), 60 (D60), 70 (D70), 80 (D80), 90 (D90), 100 (D100), 110 (D110), 120 (D120), day 130 (D130), day 140 (D140), day 150 (D150), day 160 (D160), day 170 (D170), day 180 (D180) to collect the inlet and outlet water samples, determination of effluent COD, BOD$_5$, ammonia nitrogen, total nitrogen, nitrate nitrogen, nitrite nitrogen and removal rate, until the effluent water content and removal rate. The content and removal rate of pollutants in the effluent water is relatively stable until the content of pollutants in the effluent water is relatively stable, so as to determine the formal operation of A$^2$O reactor sludge.

### 2.4.3 16S-rRNA gene sequencing analysis

The activated sludge samples were collected from three sets of parallel A$^2$O reactors at the same time, loaded into sterilized centrifuge tubes, centrifuged at 3000 r/min for 15 min, concentrated in a bench-top freezing centrifuge (Hypertrophic cluster), and then placed into an ultra-low temperature storage refrigerator at -80°C for 24 h. The samples were then sent to Maggie Biology Co. Ltd. (Maggie Biology) for 16 S rRNA gene sequence determination. The extracted genomic DNA was detected by 1% agarose gel electrophoresis[11], and the activated sludge samples were amplified by using primers 338 F and 806 R in V3 and V4 [12], and quality control was carried out, and the original sequences were divided into libraries and samples according to the index and Barcode information, and the Barcode sequences were removed [13], and finally the data were analyzed by using the Maggie Biology BioTrust cloud platform.

### 3 RESULTS AND DISCUSSION

#### 3.1 Changes in activated sludge indicators under different operating conditions

After 40 days of sludge domestication, no sludge expansion (80<SVI<150) occurred in the reactors under all conditions, and the SVI of the A$^2$O reactor under direct solar radiation was more stable than that of the constant temperature and room temperature. Overall from the overall data, it is obvious that the sludge growth of the three sets of comparison devices (Table 1) is room temperature > constant temperature > solar radiation, and from the table, it can be intuitively seen that the growth rate of the solar radiation A$^2$O device is slower compared to the constant temperature and room temperature, which can be seen that solar radiation has a certain inhibitory effect on the domestication of the A$^2$O sludge, but the sludge did not expand, so the effect of solar radiation on the whole process of domestication is not significant. The effect of solar radiation on the whole process of domestication was not significant.

<p>| Table 1 Changes in indicators of activated sludge domestication stage |</p>
<table>
<thead>
<tr>
<th>Mrder number</th>
<th>D0</th>
<th>D10</th>
<th>D20</th>
<th>D30</th>
<th>D40</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV30(ML)</td>
<td>TY</td>
<td>4.2</td>
<td>10.0</td>
<td>15.5</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>HW</td>
<td>4.5</td>
<td>10.2</td>
<td>16.2</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>SW</td>
<td>4.3</td>
<td>10.5</td>
<td>16.7</td>
<td>21.7</td>
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<tr>
<td>MLSS(g)</td>
<td>TY</td>
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<td>0.0968</td>
<td>0.1566</td>
<td>0.2058</td>
</tr>
<tr>
<td></td>
<td>HW</td>
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<td>0.0923</td>
<td>0.1606</td>
<td>0.1931</td>
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<tr>
<td></td>
<td>SW</td>
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<td>0.1927</td>
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<tr>
<td>SVI(ML/g)</td>
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<td>103.3</td>
<td>98.9</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>HW</td>
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<td>110.5</td>
<td>100.9</td>
<td>109.8</td>
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<tr>
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<td>SW</td>
<td>88.1</td>
<td>115.4</td>
<td>102.1</td>
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</tbody>
</table>

### 3.2 Pollutant Removal Effect under Different Duration of Direct Solar Radiation

The formal operation phase started from the 50th day (day 0 in the figure), and the irradiation time of solar radiation was used as an influencing factor for the three parallel A²O units under fifteen different operating time points on the 40th, 50th, 60th, 70th, 80th, 90th, 100th, 110th, 120th, 130th, 140th, 150th, 160th, 170th, and 180th days. BOD₅, COD, ammonia NH₄⁻-N, total nitrogen TN and effects on activated sludge microorganisms were investigated. The three parallel units were operated for 10 days each stage and the sludge and water quality indicators were tested every 10 days, and the 16 S rRNA sequence of activated sludge samples in the reactor was determined after the completion of each operation stage.

From Figure 1, it can be seen that the removal rate of BOD₅ in the solar radiation reactor showed an increasing and then decreasing trend with the elevation of solar radiation, and the average removal rates were 69.23%, 83.24%, and 94.63% at D110, D120, and D130. The increase in the cumulative daily value of radiation with time indicates that the frequency of sunny weather is rising, which in turn caused the increase in the concentration of BOD₅ in the feed water to the reactor, but the BOD₅ removal rate of the reactor showed a significant overall upward trend with the increase in the time of the solar radiation, which indicates that the A²O reactor under the solar radiation still has a better performance of BOD₅ removal in the formal operation of the project. At the same time, the content of BOD₅ in the influent water fluctuated greatly, but the content in the effluent water was relatively stable, basically maintained below 40.00 mg/L, indicating that the load resistance of the reactor was still relatively stable under the influence of solar radiation. With the enhancement of solar radiation, the average COD removal rate decreased from 67.72% in D50 to 50.43% in D60, then increased to 87.57% in D85, and finally fluctuated to 88.1% in D160, with a large variation during the period, but the removal rate has been stable at about 65%. The reason for this trend may be due to the fact that when the solar radiation in the reactor is too...
high, some functional bacteria in the device can not adapt to the strong solar radiation, which affects the growth and reproduction of the genus itself, thus affecting the treatment effect of the reactor, but the reactor has been able to stabilize and be able to achieve a better removal effect after the sludge domestication under solar radiation. In previous studies, many people have used BOD$_5$/COD (B/C) as an indicator of the biochemical degradation characteristics of wastewater, and it is generally believed that when the ratio of the two is greater than 0.45, it indicates that it is easily biodegradable, between 0.3 and 0.45 indicates that it is biodegradable, between 0.2 and 0.3 indicates that it is difficult to biodegrade, and less than 0.2 indicates that it is extremely difficult to biodegrade\[14,15]. However, we found that with increasing time of solar radiation, the mean values of BOD$_5$/COD were 0.398, 0.61, 0.362, 0.394, 0.27, 0.474, 0.477, 0.413, 0.79, 0.625, 0.365, 0.347, 0.351, 0.423, 0.373, 0.544, 0.553, 0.508, 0.356, 0.382, 0.447, 0.287, 0.445, 0.327, 0.588, 0.471, 0.328, 0.386, 0.357, and it is obvious that the average COD and BOD removal rates were extremely unstable during the period of D140-D180, which is obviously related to the variation of the daily cumulative solar radiation and the maximum daily maximum transient radiation maxima were somewhat negatively correlated. This suggests that the increase in BOD$_5$/COD in the presence of increasing solar radiation time makes the degradation efficiency of organic matter in the effluent more unstable.

When the solar radiation device started to operate officially on the 50th day, the average value of NH$_4^-$-N in the effluent of solar radiation reactor was 11.02mg/L, and the average removal rate was 64.52%, which was due to the fact that these nine days were mostly cloudy and rainy days, and the average value of NH$_4^-$-N in the influent water was lower, and at the same time, it could be seen that the plateau activated sludge added to the device was already more mature from the removal rate, which could also be reflected from the removal rate (HW60.17%, SW59.34%) of the constant and room temperature devices; when the solar radiation device reached the 60th day, the effluent NH$_4^-$-N could also be reflected. removal rate (HW60.17%,SW59.34%) can also be reflected; when the solar radiation device reached the 60th day, the mean value of effluent NH$_4^-$-N was 26.48 mg/L, and the mean removal rate increased to 65.24%, which may be due to the fact that the domestication of the original sludge has been completed, and the denitrifying functional bacteria can already play a role in a stable manner; when the solar radiation device reached the 70th day, the NH$_4^-$-N in the reactor shows an increasing trend, the average removal rate is 80.83%, and the average value of effluent NH$_4^-$-N is 13.24mg/L; when the solar radiation device reaches the 90th day, the removal rate of NH$_4^-$-N in the reactor reaches the maximum of the whole treatment process, the average removal rate is 91.63%, and the average value of effluent NH$_4^-$-N is 3.76 mg /L; when the solar radiation device reached the 110th day, the average value of effluent NH$_4^-$-N reached 4.392mg/L, and the removal effect of the reactor on NH$_4^-$-N decreased significantly, and the average removal rate decreased to 54.29%. It can be seen that in the formal operation stage, the average removal rate of NH$_4^-$-N can reach the highest when the solar radiation device reaches the 90th day, and it can be seen that there exists a certain positive relationship between the removal effect of NH$_4^-$-N and the irradiation time of
solar radiation as well as BOD$_5$ and COD. Chaoyun Wang et al. [16] also used a modified AAO-MBR process to treat low C/N domestic wastewater, and their results showed that the highest average removal rate of total inorganic nitrogen (TIN, including NO$_3^-$-N, NH$_4^+$-N, and NO$_2^-$-N) could reach 84.94%. In comparison, it was hypothesized that the main reason for the slightly lower NH$_4^+$-N removal rate in this experiment might be that the solar radiation inhibited some functional bacteria that could play the role of nitrification under the conventional conditions, so that they could not play the role of nitrification completely to convert ammonia into nitrous acid. Meanwhile, the study showed that when the solar radiation device was operated to the 90th day, the concentration of MLSS obviously reached a peak in the whole formal operation (TY 12.1, HW 11.1, SW 11.4), and the higher the concentration of MLSS, the higher the nitrogen source can be provided to more bacterial groups to improve the removal capacity of the reactor for ammonia nitrogen, so as to make the ammonia nitrogen removal efficiency reach the highest.

The average removal rate of TN showed a general upward trend throughout the operating cycle. Meanwhile, the results showed that the trend of the average removal rate of total nitrogen and ammonia nitrogen was basically the same more fluctuating, only in the middle of the formal operation of the total nitrogen removal process was relatively stable. From the removal effect, the TN removal effect of the reactor at TY_D70 and TY_D90 was significantly better than that of the solar radiation TY_D50, TY_D60 and TY_D80, and analyzing the reasons for this, the reason for the poorer TN removal effect of the reactor at TY_D50, TY_D60 and TY_D80 might be that it might have an effect on the denitrification process with denitrifying bacteria competing for the carbon source. Although the carbon source could meet the nitrification requirements, the high intensity of internal solar radiation caused some denitrifying bacteria that had dominated in the normal constant temperature or room temperature A$^2$O reactor to be inhibited and thus could not perform the function of nitrogen removal, and the reason for the poor TN removal effect of the three parallel reactors with constant temperature, room temperature, and solar radiation from D100 to D120 was the low C/N(C/N)/C/N(C/N)/C/N(C/N) of the influent water. The reason for the poor TN removal between D100 and D120 in the three parallel reactors with constant temperature, room temperature and solar radiation was due to the low C/N of the influent water source (C/N<3). It has been demonstrated that achieving simultaneous removal of nitrogen and phosphorus in municipal wastewater with low C/N ratios is a conflict of interest [17,18].

Comprehensive consideration of the analysis of the plateau region constant temperature, room temperature and solar radiation A$^2$O reactor in each time period of the removal efficiency of various water quality indicators, the reactor organic matter degradation efficiency with the increase in the time of solar radiation in general showed a trend of the first rise and then decline, the nitrogen removal efficiency change trend and the trend of the cumulative daily changes in the solar radiation is consistent with the D90 when the reactor has the most comprehensive and efficient pollutant removal effect in the formal operation stage.
3.3 Effects of solar radiation on microbial community composition

In this study, the Shannon index was also used to assess the diversity of the samples, and the larger the value, the higher the biodiversity in the samples and the more evenly distributed the species. As shown in Figure 2, the Shannon index SW_D50>HW_D50>TY_D50, HW_D90>SW_D90>TY_D90, HW_D180>SW_D180>TY_D180, which shows that the microbial diversity of the room and thermostat devices was higher compared to that of the solar radiation device at 90 days of exposure to solar radiation but was higher than that of the room and thermostat devices at 110 days of exposure to solar radiation. 110 days, the solar radiation device had higher microbial diversity than the room temperature and thermostat devices. However, in relation to the results of the pollutant removal effects under different solar radiation time periods in 2.2 above, the average removal rates of water quality indicators such as BOD$_5$ and COD did not reach the highest at D90, but reached the highest at D80 and decreased at D90, while nitrogen-related indicators such as NH$_4^+$-N and TN reached the highest at D90, which is supposedly due to the decrease in the daily cumulative amount of solar radiation. The growth of microorganisms can be accelerated and the diversity of microorganisms can grow normally, but when the solar radiation exceeds the tolerance of microorganisms, it will cause irreversible damage to the cell structure of some bacteria, and it may also strengthen the growth of some bacteria adapted to the strong solar radiation, and this destructive effect has been significantly reflected in the artificial ultraviolet light condition[19,20].

Based on previous studies[21,22], it has been found that although there is a general positive correlation between microbial diversity and ecological activities, the relationship between microbial diversity and pollutant biotransformation in biologically treated wastewater remains to be investigated. Biodiversity is positively correlated when facilitative interactions occur between microorganisms of different ecological niches, but when different taxonomic units perform the same function (i.e., functional redundancy), an increase in biodiversity may not necessarily have a positive effect on biotransformation. Meanwhile, it is obvious that the Simpson index TY_D90>HW_D90>SW_D90, which is consistent with the expression of the Shannon index, shows that when all three parallel A$^2$O units were operated up to the 90th day, the colonies of the room temperature and thermostat units presented a more diverse range of microbial species compared to those of the solar radiation unit. While the colony uniformity index shannoneven was HW_D90>SW_D90>TY_D90, the room temperature and thermostat devices showed a higher species distribution degree compared to the solar radiation device colonies on day 90, i.e., the distribution was more uniform, but this also reveals that there are some functional bacteria affected by the specialization of solar radiation in the solar radiation device from another point of view. The evolutionary changes in the microbial community structure of this system under three different working conditions were analyzed by using Hierarchical clustering (HCl) and Principal coordinates analysis (PCoA) methods for the principal component analysis of the different samples based on the OTU level (Fig. 3). As far as the principal coordinates analysis is concerned, the closer the samples are to each other, the more similar the species composition is, so samples with high similarity in community structure tend to be clustered.
together, and samples with great differences in community structure composition will be farther apart. For this experiment, the PC1 and PC2 axes could explain 25.71% and 18.75% of the differences in microbial community structure, respectively, and the two principal components cumulatively explained 44.46% of the variation in microbial community composition in the samples.

From Fig. 3(a), it can be seen that the microbial community structure of the samples of TY_50, TY_60, TY_70, TY_80, TY_90 is more different from that of the samples of TY_120, TY_130, TY_140, and the microbial community structure of the samples of TY_100, TY_110 is closer to that of the samples of HW_100, SW_120. In connection with Fig. 3(b), the differences in microbial communities of samples with different working conditions were mainly on PC2, from the horizontal axis, the samples of TY_50-TY_90, HW_50-HW_130, SW_50-SW_150 were located in the positive half-axis of PC1, the samples of TY_100-TY_180, HW_140-HW_180, SW_160-SW_180 were located in the negative half-axis of PC1. samples were located in the negative half-axis of PC1, and TY_110 was located in the constant axis; at D50 to D90, the dispersion of the samples of TY, HW, and SW was not very large, while at D120 to D180, the three showed a great dispersion; from the longitudinal axis, the samples of HW, and SW were basically located in the lower half-axis of PC2, while the samples of TY were basically located in the upper half-axis; the biomes of samples at different working conditions The dispersion of the structural differences of the samples under different working conditions is still large on the vertical axis, but with the increase of solar radiation, the dispersion of the samples of TY_120, TY_130 and TY_140 is not large. The results of the analysis of microbial community composition at different time points for different work conditions also echoed the results of pollutant removal rates at different time points for the three parallel work conditions. Such similarity and dissimilarity reveal that long-term solar radiation has a significant effect on microbial diversity, abundance and evenness.

### 3.4 Effects of solar radiation on the composition of microbial communities

In order to explore the abundance of dominant species in each sample and the proportion of distribution of dominant species in different samples, the software RDP classifier was used to analyze the dominant species in the samples. Taxonomic statistics were performed at two taxonomic levels, Phylum and Genus, under five time points of solar radiation working conditions and showed the species with microbial abundance greater than 1%. The results of the study showed that the bacterial domain was the only microbial taxon in the samples from the three sets of solar radiation, thermostatic and room temperature installations, with a percentage of 100% in all of them.

#### 3.4.1 Analysis of colony compositional structure at the phylum level

The solar radiation of the five time points was analyzed at the phylum level of bacterial colonization, see Fig. 4. At the level of microbial colonization phylum, the samples from the five different time points under the influence of solar radiation showed similar diversity but different abundance. Bacteroidota (TY
39.65%, HW 39.63%, SW 40.38%), Proteobacteria (TY 26.67%, HW 29.19%, SW 24.60%), Actinobacteriota (TY 10.38%, HW 10.73% SW 12.12%), Chloroflexi (TY 7.25%, HW 8.33%, SW 8.96%), and Firmicutes (TY 4.28%, HW 3.49%, SW 3.50%) were the main dominant phyla in the three parallel installations and were in the same order of precedence, which was in contrast to the findings differed from Zhang et al [23], in whose study Proteobacteria, a sludge metazoan phylum, was always the first dominant phylum and Bacteroidota, a bacteriophage, was always the second dominant genus in relative abundance in the SBR reactor at either 15°C, room temperature, or 30°C, whereas Bacteroidota, a bacteriophage, was consistently dominant in all three parallel A²O systems in the present study. The first absolute dominance, but the common point is that the top five dominant phyla are all common phyla in these five sludge systems. the microbial community structure of Ascomycetes Proteobacteria, and Bacteroidota of Bacteroidota in the solar radiation of the five time points is the same in general, but there are some differences in the relative abundance of the phyla. Both Proteobacteria and Bacteroidota play a key role in nitrification, and their dominance in the reactor confirms the steady improvement of ammonia and nitrogen removal, further reflecting the gradual maturation of activated sludge in the reactor. Currently, the majority of denitrifying bacteria are from Proteobacteria, followed by Bacteroidota, a group of chemotrophic organic nutrients that dominate nitrifying reactors[24],Its relative abundance showed an increasing and then decreasing trend along with the increase of solar radiation time. Chen XY et al.[25] showed that the relative abundance of Bacteroidota of the phylum Bacteroidota was affected by the overall environmental factors in the plateau environment, which might be the reason for the change of the relative abundance of the phylum Bacteroidota.

Proteobacteria are important components of activated sludge, and previous studies have shown that the most dominant phylum of Proteobacteria is Betaproteobacteria, which contains more aerobic or parthenogenetic bacteria, and is believed to play a key role in sludge denitriﬁcation [26], most of the nitrogen-related functional bacteria such as aerobic ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), and nitrogen oxidizing bacteria (NOB) were found to have a high degree of inhibition. oxidizing bacteria (NOB) and denitrifying bacteria (DNB) belong to this class [27], which is also a Gram-negative bacterial group, the lipopolysaccharides on its outer surface can help microorganisms to attach to the surface and are important for the removal of carbon, nitrogen and organic matter. By comparing the results of euent treatment eciency, the increase in relative abundance of Proteobacteria during formal operation greatly improved the nitrogen removal from the reactor. The relative abundance of Proteobacteria showed a decreasing and then increasing trend with increasing solar radiation, and the highest relative abundance was D70 (32.06%). The differences in relative abundance between the middle two groups (TY_ D70, TY _D80) were very small, but the differences with D50 and D180 at the beginning and end of the formal run were larger, which indicated that the growth of solar radiation time Proteobacteria gradually adapted to the direct effect of solar radiation and could continue to grow under strong solar radiation, and lower (radiant daily radiation is lower than or higher than the solar radiation will lead to a decrease in their relative abundance. decrease in its relative abundance.

The relative abundance of Actinobacteriota can also be seen in Fig. 5, which shows that the relative abundance of Actinobacteriota increases, then decreases, then increases again with the increase of
solar radiation, and reaches a minimum of 7.75% at D90. Actinobacteriota include some of the most common soil, freshwater and marine organisms that play an important role in the decomposition of organic matter[28,29], and thus in the turnover of organic matter and carbon cycling in the AAO process under the influence of solar radiation. In contrast, Figure 5 shows that there are not many significant differences in the gates between solar radiation, constant temperature and room temperature conditions at the gate level.

Chloroflexi are a group of bacteria that produce energy through photosynthesis, they are parthenogenetic anaerobic organisms that do not produce oxygen during photosynthesis and do not fix nitrogen. Organic matter degradation, denitrification and biofilm aggregation were demonstrated to play different roles depending on the environmental conditions in the study by Patricia Bovio-Winkler et al[30]. The relative abundance of Chloroflexi also showed a trend of increasing and then decreasing during the increasing time of solar radiation. Abundance also showed a trend of increasing, then decreasing and then increasing during the lengthening of solar radiation time. The reason for this trend may be that Chloroflexi, although better adapted to solar radiation, cannot compete with other dominant genera for more carbon sources when the intensity of solar radiation decreases. Firmicutes can survive in some extreme environments and some species in the genus Firmicutes are capable of heterotrophic nitrification, and it has been shown that Firmicutes are indeed extremely important for denitrification using solid carbon sources in existing systems[31,32]. Indeed, the presence of Firmicutes thicketii in swine manure-contaminated water and its denitrification capacity triggered speculations about the synergistic effect of the externally added carbon source of the bio-manure and heterotrophic denitrification, which followed a similar trend to that of the relative abundance of the actinobacteriophage Actinobacteriota, which was at its lowest level when the solar irradiation of the A^2O device was up to the 90th day (2.65%), which was analyzed in comparison with the relative abundance of the constant temperature (3.65%) and room temperature (3.53%) devices on the same day, which might be due to the fact that Firmicutes were gradually unable to compete for more carbon sources by being suppressed by solar radiation as the time of solar radiation influence increased. From the perspective of Average Variation Degree (AVD) at the gate level: HW 0.7270<SW 0.7273<TY 0.7487, which is calculated as the deviation from the mean of the relative abundance of OTUs with a normal distribution, the lower the value of AVD represents the higher stability of the microbiota [33], it can be seen that the community structure of the solar radiation reactor at the gate level is more unstable in comparison.

3.4.2 Analysis of colony compositional structure at the genus level

In deeper analysis of microbial community composition at the genus level, the structure of the flora at the genus level comparing solar radiation at five time points is shown in Fig. 6.

Analyzing the genus level with the removal of relative abundance < 0.01, it can be seen that the microorganisms are diverse at the genus level, and a total of 36 genera were detected. By classifying these 36 genera according to different functions, a total of 17 genera were classified as denitrifying bacteria, nitrifying bacteria, nitrogen fixation, and organic matter degradation, and most of these genera
belonged to the phylum Proteobacteria and the phylum Bacteroidota, and the genus Anaerobic ammonia oxidizing bacteria was not detected among these 36 genera. Among them, five dominant genera, norank_f__AKYH767, unclassified_f__Comamonadaceae, OLB8, IMCC26207, norank_f__JG30-KF-CM45, dominated the solar radiation reactor, of which the remaining four genera except IMCC26207 were all denitrifying bacteria. All belong to denitrifying functional bacteria, while IMCC262075 plays the role of degrading organic matter. These four genera can represent the core of denitrifying function in the corresponding solar radiation samples, which ensures a better ammonia and nitrogen removal rate in the reactor. From Figure 6, it can be seen that the first dominant bacterial genus norank_f__AKYH767 [25] in the solar radiation system as a whole decreases with the increase of the solar radiation time, and its specific trend is to first decrease, then increase and then decrease, and has the largest relative abundance of 39.80%, 13.25% at TY_D50, HW_D110, SW_D110, 7.97% in TY_D110, HW_D50, SW_D60, and has the smallest relative abundance of 9.58%, 3.59%, 2.54% in TY_D110, HW_D50, SW_D60. In connection with the above shows that there is a positive correlation between the relative abundance of this genus and the average removal of nitrogen pollutants, which may be caused by the competition with other denitrifying bacteria for nitrogen and carbon sources in low C/N wastewater. The relative abundance of norank_f__AKYH767 in the solar radiation device among the five bacterial genera with the highest scale abundance has been occupying the first dominant genus status before D120 (see Fig. 6 for details), and this genus is also the unique dominant genus in the plateau, apparently compared with other dominant genera, norank_f__AKYH767 is more adaptable to solar radiation, and has the ability to compete for more carbon sources in a high-C/N wastewater. The study on community structure and nitrogen metabolism mechanism in A2O process under different hydraulic retention time conditions at high altitude region [34,35]. The first dominant genus in the constant room temperature device was also norank_f__AKYH767, but its abundance at the genus level was significantly different from that of the solar radiation device. Obviously, this difference reveals that the organic matter degradation ability of the A2O process in these two parallel conditions is stronger than that of the solar radiation condition. The variability of the dominant bacteria between the three conditions at the gate and genus levels was strongly influenced by external factors, and compared with the microbial differences at the gate level, it is obvious that the microbial differences between the solar radiation, constant temperature and room temperature conditions at the genus level were more significant, not only in terms of which functional bacteria dominated in the three parallel units, but also in terms of the inhibitory or reinforcing effect of solar irradiation on microorganisms (Fig 7, 8, and 9, respectively). The relative abundance of the top five dominant genera in the solar radiation device under good direct sunlight throughout the day was affected to different degrees by different transient radiation in the morning, midday and evening, whereas the relative abundance of the top five dominant genera in the solar radiation device under good direct sunlight throughout the day, the relative abundance of the top five dominant genera in the device were all affected to varying degrees with different intensities of transient radiation during the day. Apparently the first dominant genus norank_f__AKYH767 in the solar radiation setup showed a decrease followed by an increase with morning to mid-evening, whereas the second dominant genus unclassified_f__Comamonadaceae likewise showed a decrease followed by an increase with morning to mid-evening and with the time of exposure to solar radiation according to a previous study that The
Unclassified_f__Comamonadaceae are essential for maintaining the activity of anaerobic ammonia-oxidizing bacteria and have been reported to be endogenous denitrifying bacteria that can denitrify by using sodium acetate to produce polyhydroxybutyric acid (PHB) and are typically functional bacteria that can adapt to low C/N environments [36,37], the relative abundance of unclassified_f__Comamonadaceae in the solar radiation reactor was determined to exceed that of norank_f__AKYH767 on day 120. AKYH767, which indicates that at a C/N of about 0.5, appropriate solar radiation accumulation does not have a strong inhibitory effect on the genus unclassified_f__Comamonadaceae, whereas inhibition begins to occur when the intensity of solar radiation exceeds the threshold value, i.e., about 1,000 W/m². The results of this study showed that suitable solar radiation is beneficial to stabilize the dominant genera of norank_f__AKYH767 and unclassified_f__Comamonadaceae, but when the C/N of the influent water source is lower than a certain threshold, unclassified_f__Comamonadaceae will be more effective than norank_f__AKYH767 is more adapted to the reactor environment.

Unclassified_f__Saprospiraceae, a functional denitrifying bacterium that belongs to Chloroflexi and is involved in carbon and nitrogen transformations, occupied the top six dominant genera in each of the three parallel systems [38], with Zhao et al. [39,40] of biofilm samples at 114th, 132nd, and 168th T. The relative abundance of biofilm-forming and oxidation-resistant bacteria at the genus level unclassified_f__Saprospiraceae was the first dominant bacterial genus was similar to the results. This genus exhibited a highly significant abundance difference under solar radiation conditions compared to constant and room temperature conditions, being the sixth most dominant genus in the solar radiation A²O system, while rising to the second and fifth most dominant genera in the constant room temperature conditions. This difference suggests that longer periods of solar radiation favorably inhibit the uptake of carbon sources and growth of unclassified_f__Saprospiraceae. It is well known that N removal in the AAO process relies mainly on nitrification and denitrification reactions. In the microbial composition at the genus level (Fig. 6) denitrifying bacteria genera were relatively more abundant in the solar radiation device, such as norank_f__AKYH767 (TY16.19%, HW12.99%, SW17.24%) and unclassified_f__Comamonadaceae (TY6.84%, HW3.30%, SW4.02%), while organic matter-degrading functional bacteria such as norank_f__JG30-KF-CM45 (TY2.66%, HW7.63%, SW3.66%), a genus itself belonging to Chloroflexi, which is greatly affected by anaerobic and anoxic conditions, dominated the thermostatic room temperature device [41], the degradation of organic matter by this group of genera provided a carbon source for the growth and metabolism of nitrogen-metabolizing functional bacteria, which further enhanced the nitrogen removal capacity. There were fewer species of denitrifying bacteria genera in this study, whereas a variety of denitrifying functional bacteria genera were found to be present in the A²O reactor in the study of Yu Z et al. [42] The reason for this is that the higher intensity of solar radiation in the plateau can damage the nucleoprotein and DNA of microorganisms, and some microorganisms are inhibited or even eliminated under the selection of strong solar radiation conditions [43,44]. And this inhibitory or destructive effect is extremely significant for unclassified_f__Saprospiraceae. Comprehensive analysis of the overall community structure, denitrifying bacteria and their metabolism are the main body of the top ten functional genera, which is consistent
with the trend of the average TN removal rate, which is because norank__f__AKYH767, a group of plateau-specific denitrifying bacteria can adapt to the strong solar radiation and be able to adapt to the strong solar radiation, and be able to grow and metabolize more rapidly under the long-term influence of solar radiation on the plateau as compared with the constant and room temperature conditions, which will in turn This improved the TN removal rate and provided conditions for the growth of nitrifying bacteria, which ultimately improved the performance of the system for nitrogen removal. From the perspective of the average variation degree (AVD) at the genus level: HW0.6760<SW0.6798<TY0.6820, it is evident that the community structure of the solar radiation reactor is more unstable at the genus level.

In Fig. 8, the effect of temperature on the genera was excluded to a certain extent by comparing the solar radiation device with the constant temperature and room temperature devices, and it can be seen from the figure that apparently strong solar radiation for a long period of time at low C/N has a significant inhibitory effect on the genera of norank__f__AKYH767 and OLB17, compared to the constant temperature device in the relative abundance of the same two genera firstly rising, then falling and then rising. Compared with the trend of the same two genera in the thermostatic device, these two genera in the solar radiation device have been showing a decreasing trend, from which it can be inferred that it is the role of solar radiation, while the strengthening effect of solar radiation on the genera was found to be more significant in the trend of the first dominant genus norank__f__AKYH767, which shows that the solar radiation has a different degree of inhibition or strengthening of different genera, and it can be speculated that compared to other dominant genera, the first dominant genus norank__f__AKYH767 is better adapted to or resistant to solar radiation, and has the ability to compete with functional bacteria such as phosphorus polymerizing bacteria Paos, organic matter degrading bacteria, and even nitrifying bacteria and denitrifying bacteria to obtain more carbon sources while adapting to solar radiation, but in the relative abundance of norank__f__AKYH767 could not continue to rise due to the restriction of the low C/N conditions.

During the 140 days of official operation, the daily accumulation of solar radiation was obviously highly variable, which is due to the fact that the experimental site receives more rainfall and rainfall in summer. The daily accumulation of solar radiation on a cloudless day can even reach four times of that on a completely cloudy day. We chose to measure the transient solar radiation in the morning, midday and evening of September 14th because the solar radiation on this day was relatively good for the whole day, and the daily accumulation of radiation and the maximum transient radiation on this day were both at high levels, which could be representative of the changes in relative abundance of the dominant microorganisms inside the reactor with clear weather throughout the day (Fig. 8a in detail). The relative abundance of dominant microorganisms in the reactor under sunny weather conditions throughout the day varied with the instantaneous radiation intensity (see Fig. 8a for details). From Fig. 8(b), it is obvious that solar radiation has a significant inhibitory effect on the two genera, unclassified__f__Comamonadaceae and OLB8, because compared with the decreasing and then increasing relative abundance of the same genera in the thermostatic device, these two genera have been showing a decreasing trend in the solar radiation device, which is deduced to be the result of the long-term effect of solar radiation on these microorganisms. These microorganisms, while the
strengthening effect of solar radiation on the genera is only found in the first dominant genera trend is more significant, it can be seen that solar radiation on different genera have different degrees of inhibition or strengthening effect, it is presumed that compared with the other dominant genera, norank__f__JG30-KF-CM45 is more able to adapt to, or resistance to, solar radiation, and has the ability to adapt to the solar radiation while competing for more carbon sources. Meanwhile, from the figure, we can see that the relative abundance of norank__f__JG30-KF-CM45 increases and then decreases, and the relative abundance of unclassified_f__Comamonadaceae decreases and then increases. Due to the delayed effect of solar radiation, it can be seen that the short-term solar radiation within one day has an inhibitory effect on norank__f__JG30-KF-CM45, and on the second dominant genus, unclassified_f__Comamonadaceae, it has an inhibitory effect on the second dominant genus, unclassified_f__Comamonadaceae. Although the relative abundance of unclassified_f__Comamonadaceae and norank__f__JG30-KF-CM45 has been on the rise in the overall change within 40 days, there is an upward trend in the short-term change within one day. In the short-term changes within a day, it is possible that solar radiation suppresses the abundance of genera in the short term, and it is even possible to judge that the relative abundance of dominant genera does not increase every day but fluctuates, and at the same time there are obviously genera that are not significantly affected by solar radiation within a day, such as OLB17[45] and unclassified_f__Saprospiraceae[46], it is hypothesized that this group of genera, even though they are not tolerant to strong solar radiation, can compete for a certain amount of carbon to maintain their short-term abundance during the day, while genera such as norank__f__AKYH767 and norank__f__JG30-KF-CM45 are not only tolerant to strong solar radiation on the plateau, but also have a certain degree of resistance to strong solar radiation on the plateau. The relative abundances of genera such as norank__f__AKYH767 and norank__f__JG30-KF-CM45 are not only resistant to the strong solar radiation on the plateau, but also have the ability to compete for carbon and nitrogen sources.

In summary, we confirmed that microbial community composition and abundance in plateau wastewater treatment responded differently to solar radiation exposure and irradiation time, especially for pollutant removal by the main dominant bacteria. Therefore, from a microbiological point of view, solar radiation intensity and irradiation time are influential factors to be controlled in the operating conditions of open and semi-shaded wastewater treatment systems in the plateau. The low carbon to nitrogen ratio and low temperature in the plateau region also contribute to the unique microbial community structure in the plateau wastewater treatment system in conjunction with the strong solar radiation, whereas some common functional bacteria in the plain wastewater system, such as unclassified__f__Saprospiraceae, OLB17, etc., which have the function of degrading organic matter, were significantly inhibited by the strong solar radiation in the plateau.

4 CONCLUSION

In this paper, the effects of different solar radiation on pollutant removal efficiencies as well as microbial communities and metabolism of the A²O process under solar radiation were investigated in a plateau
habitat. The experimental results showed that the removal efficiencies of various pollutants under the direct influence of solar radiation from the 10th day to the 40th day of the process showed an overall obvious trend of decreasing, then increasing and then decreasing during the domestication stage, and more than reached the highest at the 30th day of solar radiation. Considering the removal effects of each pollutant, it can be concluded that the nitrogen removal efficiency of \(A^2O\) was more unstable under the influence of long-term plateau solar radiation. Increased solar radiation promoted microbial abundance and diversity. Different solar radiation affected the microbial community structure and relative abundance at both phylum and genus levels to different degrees. The relative abundance of the first dominant bacteria at both phylum and genus levels showed a tendency of increasing and then decreasing by the enhancement of solar radiation. The increase in the number of solar irradiation days not only inhibited or promoted the related functional bacterial genera, respectively, but also significantly affected the relative abundance of nitrogen metabolic pathways and related functional genes.

Based on the experimental results and mechanistic analyses, this study will help to design better wastewater treatment schemes in different regions according to different solar radiation intensities in each place and provide ideas for reactor optimization of the improved \(A^2O\) process.

**Declarations**

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[Statement of novelty and industrial relevance statement ] This study is an original research that investigates the effects of different times of influence of solar radiation in the plateau on the operational effectiveness, microbial abundance and nitrogen metabolism during the domestication of sludge in an A2O system, which is used for the treatment of domestic wastewater in the plateau region. The study also explains the differences between solar radiation, constant temperature and room temperature reactors and how these differences alter the reactor response. By analyzing the relative abundance and functional genes of functional genera such as denitrifying bacteria, phosphorus-polymerizing bacteria, and organic matter-degrading bacteria, which are unique to the plateau region, the reasons for the changing patterns of water quality pollutant removal rates were revealed.

**Author Contribution**
PENG Xianpai wrote the main manuscript text. ZONG Yongchen and PENG Xianpai prepared figures 5-6. All authors reviewed the manuscript.

References


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Figure 5

Plot of absolute and relative abundance and significant differences of dominant genera at phylum level (top 10 relative abundance)
Figure 6

Plot of absolute and relative abundance and significant differences of dominant genera at phylum level (top 10 relative abundance)
Figure 7

Effects of solar radiation on dominant microorganisms