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Denitrification and phosphorus removal efficiency of different pretreated qingke straw carbon sources and microbial response mechanisms

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Abstract: This study aims to explore the efficiency of nitrogen and phosphorus removal in a laboratory-scale SBR reactor under plateau habitat conditions, using different pretreated qingke straw fermentation broths as external carbon sources. It investigates microbial evolution and community structure changes, conducts a correlation analysis between microorganisms and environmental factors, and examines changes in microbial community structure and six nitrogen metabolism functional modules. The results indicate that different pretreatment methods of qingke straw fermentation broth as external carbon sources lead to an increase in the abundance and diversity of microbial communities, influencing the direction of microbial community succession. The addition of acid-base pretreated qingke straw fermentation broth significantly inhibits three

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functional modules: denitrification, dissimilatory nitrate reduction, and complete digestion, while enhancing three functional modules: nitrate assimilatory reduction, nitrification, and nitrogen fixation. In plateau habitat conditions, nitrogen and phosphorus removal processes require enhanced treatment for phosphorus removal.

Keywords: qingke straw, low C/N ratio, external carbon source, plateau habitat, pretreatment method

1 Introduction

The biological treatment process based on nitrification and denitrification is a widely adopted high-efficiency nitrogen and phosphorus removal technique for urban wastewater treatment in China (Zheng et al., 2018). In the wastewater treatment process, nitrification is the process of converting ammonia nitrogen into nitrate, while denitrification is the process of reducing nitrogen pollution, primarily targeting nitrate in wastewater. During this process, nitrate is converted into nitrite, then into nitrous oxide (nitrate ions are converted into nitrite ions), and ultimately into nitrogen gas (Ding et al., 2019; Fang et al., 2021; Wang et al., 2017). Denitrification is a crucial link in the nitrogen cycle in both natural and engineered ecosystems, converting nitrate or nitrite into molecular nitrogen (Wang and Chu, 2016; Wu et al., 2023a). Typically, the reduction of nitrate is accompanied by the utilization of carbon sources, especially in wastewater treatment plant (WWTP) systems (Lee and Welander, 1996; Osaka et al., 2008). Therefore, denitrification is closely related to the carbon and nitrogen cycles (Wu et al., 2023b).

Relevant research indicates that the influent carbon source is a crucial factor in the denitrification process as an electron donor. Due to insufficient influent COD, the influent is persistently under conditions of low carbon to nitrogen (C/N) ratio (3.8-8.5), posing frequent

challenges to municipal wastewater treatment plants in China (Liu et al., 2023a). This limitation hinders nitrogen removal efficiency, often resulting in effluent nitrogen concentrations exceeding mandatory standards.

Numerous studies have attempted to address the limitations of low C/N ratio on wastewater treatment efficiency, including modifications to nitrogen removal technologies (e.g., partial nitrification/anaerobic ammonium oxidation, denitrifying polyphosphate accumulating organisms (DPAO)), optimization of operational parameters, and adjustments to treatment systems (Zekker et al., 2021; Zhao et al., 2016; Zhu et al., 2023). Additionally, a simultaneous anaerobic oxidation/partial nitrification-denitrification process (SAO/PND) has been proposed, which reduces the required COD and lowers costs by minimizing sludge production and energy consumption (Hosseini, 2021). One of the most widely adopted strategies is to add external carbon sources during the anoxic phase based on the influent water quality to achieve the optimal COD concentration (Liu et al., 2016).

The Xizang region is located on the Qinghai-Xizang Plateau, with an average elevation of over 4,000 meters. It is known as the "Third Pole of the World" and serves as China's "ecological protection barrier". It possesses unique environmental characteristics such as high ultraviolet intensity, large diurnal temperature variation, and low water temperature (Chen et al., 2024). In recent years, with the accelerated advancement of urbanization, a large number of wastewater treatment facilities based on nitrification and denitrification processes have been built in the Xizang region. These facilities primarily utilize the anaerobic-anoxic-aerobic (A²O) process and the anoxic-aerobic (AO) process (Hao Kaiyue et al., 2020; Hao Kaiyue et al., 2021a). Field investigations have found that the influent concentration of wastewater treatment plants across various cities in Xizang

has long been in the low carbon to nitrogen (C/N) ratio range (1-5). To enhance wastewater treatment efficiency, some plants have added external carbon sources such as glucose, starch, and sodium acetate to the wastewater treatment facilities. Although this has improved nitrogen and phosphorus removal efficiency, it has significantly increased costs. Therefore, finding a low-cost and environmentally friendly external carbon source is a necessary measure to reduce the cost of external carbon sources for wastewater treatment plants in the Xizang region.

To reduce costs, some studies have attempted to enhance denitrification by utilizing solid slow-release carbon source materials, including natural cellulose materials such as corn cob (Li et al., 2020) and peanut shell (Xiong et al., 2019), as well as synthetic biodegradable polymers such as polycaprolactone (PCL) (Wang et al., 2019) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Sun et al., 2020a). Among these, natural cellulose materials are widely used due to their abundant sources and lower cost compared to biodegradable polymers. In 2022, "Xizang qingke(a type of Xizang barley" was successfully selected into China's National Plan for Cultivating High-quality Agricultural Brands in 2022. In 2023, our region plans to implement a qingke planting area of 2.2 million mu, indicating that qingke straw possesses distinct Xizang characteristics and a wide range of sources. As the main agricultural waste in Xizang, qingke straw has an annual output of 1.3221 million tons. Currently, it is mostly disposed of through incineration, which not only results in resource waste but also releases pollutants and greenhouse gases (CO₂) (Zhu et al., 2025). Research indicates that volatile fatty acids (VFA) produced through anaerobic fermentation of straw-based biomass can serve as high-quality carbon sources for denitrification processes. Additionally, combined pretreatment methods, such as KOH and NH₃·H₂O, can significantly enhance lignin degradation rates and methane production, providing new insights for the development of

carbon sources in wastewater treatment. Furthermore, microbial metabolites (such as acetic acid) present in the fermentation broth of qingke straw may regulate microbial community structure, optimizing denitrification and phosphorus removal pathways. qingke straw is rich in lignocellulose, which can release soluble organic matter (such as acetic acid and lactic acid) through pretreatment methods (such as chemical and biological fermentation). This organic matter, as an external carbon source, can effectively supplement the carbon demand in wastewater treatment while achieving resource utilization of waste materials (Zhang et al., 2022; Ma et al., 2025; Li et al., 2024a).

However, there is currently limited research on the effects of different pretreatment methods on nitrogen and phosphorus removal, as well as changes in microbial community structure, when using qingke straw as a carbon source for wastewater treatment. Therefore, this study selected qingke straw with different pretreatment methods as an external carbon source to treat wastewater with a low C/N ratio. The main objectives of this study are: 1) to investigate the release composition of carbon sources different from that of treated qingke straw; 2) to study the impact of adding qingke straw carbon source on the nitrogen and phosphorus removal performance of SBR; 3) to investigate the effect of adding qingke straw carbon source on changes in the microbial community structure of SBR; 4) to provide theoretical support for the mechanism research of SBR reactors in plateau habitats and support for practical wastewater treatment operations in plateau areas through the obtained patterns.

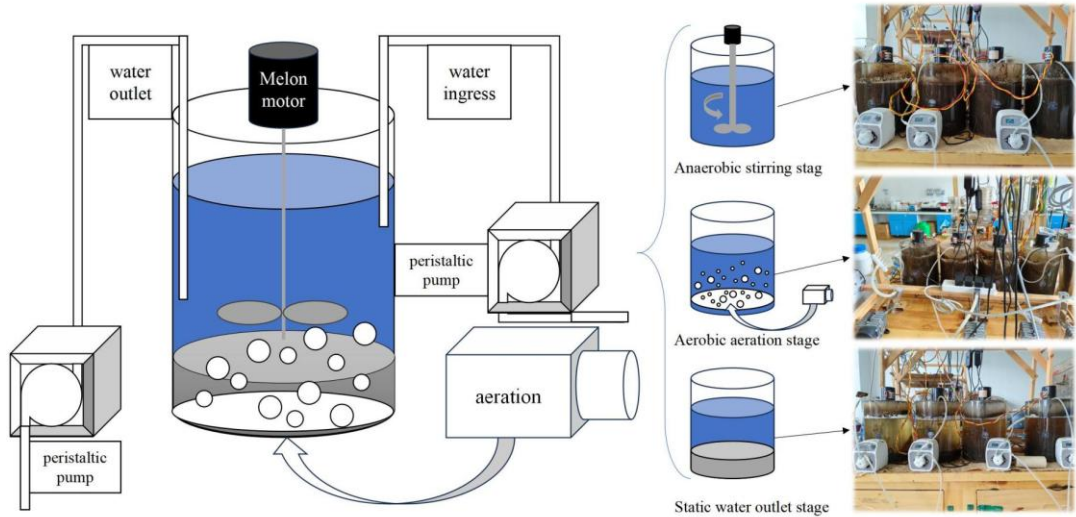
2 Materials and Methods

2.1 Test apparatus and procedure

To investigate the effects of different pretreatments and carbon-to-nitrogen ratios on nitrogen and phosphorus removal, this study set up four parallel experiments using the SBR process. Device

S1 served as the control experiment, while Device S2 was treated with a mixture of untreated qingke straw fermentation broth and sewage. Device S3 was treated with a mixture of qingke straw fermentation broth pretreated with 2% sulfuric acid and sewage, and Device S4 was treated with a mixture of qingke straw fermentation broth pretreated with 2% sodium hydroxide and sewage. The designed daily treatment capacity was 5.66L (2.83L/12h). The device dimensions were a cylindrical barrel with an inner diameter of 19cm and a height of 25cm, with an effective volume of 5.66L. The hydraulic retention time was 12 hours, and the AOA operation mode was adopted. The influent water was introduced for 0.5 hours, followed by anaerobic stirring for 2 hours at a speed of 50 r·min⁻¹, aerobic aeration for 6 hours, anoxic stirring for 2 hours, sedimentation for 1 hour, and effluent water was discharged for 0.5 hours. The drainage rate per cycle was 50%, and the sludge discharge volume was 280mL, with a sludge age of approximately 60 hours. The initial sludge for the experiment was self-cultivated using domestic sewage from Xizang Agriculture and Animal Husbandry University as the influent source. The influent water quality was as follows: COD: 39.898 mgL⁻¹ to 410.29 mgL⁻¹, NH₄⁺-N: 17.84 mgL⁻¹ to 102.22 mgL⁻¹, TN: 24.53 mgL⁻¹ to 131.96 mgL⁻¹, TP: 2.68 mgL⁻¹ to 21.75 mgL⁻¹. During the acclimation period, a 12-hour cycle was adopted, and the operating environmental control conditions were temperature (T): (25±1)°C, pH: 4.58 to 9.36, dissolved oxygen during the aeration phase was between 2.04 mgL⁻¹ and 4.92 mgL⁻¹. The designed acclimation period was 30 days to ensure adaptation to the experimental operation cycle. When the sludge concentration (MLSS) stabilized at 1490 mgL⁻¹ to 1980 mgL⁻¹, the experiment entered the operational phase. The external carbon source devices S2, S3, and S4 controlled the influent water with a C/N ratio between 5 and 9. The experiment was continuously monitored for 100 days, with water quality tested every two days. Based on 50 sets of data, the

130 impact of external carbon sources on nitrogen and phosphorus removal efficiency, as well as their
131 effects on microbial genera, was analyzed. See Figure 1 for details.



132
133 **Figure 1: Device operation diagram**

134 **2.2 External carbon source treatment of qingke straw**

135 For the preparation of exogenous carbon sources, the qingke straw selected was obtained from
136 the farm of the College of Botany at our university. The collected straw was cut into 2-4cm segments,
137 rinsed twice with deionized water, and then dried. Afterwards, the straw segments were crushed into
138 powder using a grinder. 20g of the powder was added to a 1L beaker, followed by the addition of
139 900mL of deionized water, 2% H_2SO_4 solution, and 2% NaOH solution. The mixture was stirred
140 and heated in a 90°C water bath for 1 hour. After cooling, the pH was adjusted to a neutral level.
141 The beaker opening was sealed with plastic wrap for 12 hours of constant temperature fermentation
142 at 30°C . After fermentation, the supernatant was filtered to obtain the exogenous carbon source from
143 qingke straw.

144 **2.3 Activated sludge sampling and high-throughput analysis**

The sequencing experiment first requires the extraction of sample DNA using the Fast DNA Spin Kit (Fast DNA Spin Kit for Soil, MP, USA) (Guan *et al.*, 2018), followed by detection using 1% agarose gel electrophoresis. Then, PCR amplification is performed on the variable region (V₃~V₄) of the 16S rRNA gene using a PCR instrument (ABI GeneAmp® Model 9700) with primers 338F (5'-actcctacgggaggcagag-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The three replicate PCR amplification products of the same sample are mixed and detected using 2% agarose gel electrophoresis.

Based on the preliminary quantification results of electrophoresis, the PCR products were quantified using the QuantiFluor™-ST blue fluorescence quantification system (Promega), and then mixed in appropriate proportions according to the required sequencing amount for each sample. The PCR added Illumina splicing sequences to the outer ends of the target regions, and the gel was cut using the AxyPrep DNA gel Recovery Kit (AXYGEN) to recover the PCR products. The products were eluted with Tris-HCl buffer and detected by 2% agarose electrophoresis. After denaturation in NaOH, single-stranded DNA fragments were obtained, and their ends were immobilized on the chip using complementary primer bases to synthesize target DNA fragments, forming a denatured "bridge". DNA polymerase and dNTP were added, and the surface of the reaction plate was scanned using a laser to read the types of nucleotides polymerized on the template sequence. Finally, the "fluorescent fragments" and "termination fragments" were chemically cleaved to restore the 3' end cohesion, allowing the second nucleotide to continue polymerization. This cycle was repeated, and the sequence of the template DNA fragments obtained in each round was counted, completing the Illumina MiSeq sequencing. The amplicon sequencing of all samples was conducted by Shanghai Meiji Biotechnology Co., Ltd. on the Illumina MiSeq platform. The raw data obtained from MiSeq

sequencing were first assembled based on overlap relationships, and the sequence quality was controlled and filtered. The clustering method used was the USEARCH7-uparse algorithm, and sequence grouping was performed according to operational taxonomic units (genera) and a threshold of 97% (UPARSE (7.0.1090)). Classification was performed using the SILVA bacterial database (Bremen, Germany).

Thirteen microbial samples were collected, including S0D1 as the initial sample from four devices. The remaining samples were collected three times each from the early, middle, and late stages of the experiment, with each stage lasting 30 days and named D1, D2, and D3 according to the time period. A total of 12 samples were collected from the four reactors, making a total of 13 samples. The samples were stored at -80°C. The 13 microbial samples of activated sludge from the SBR device obtained in this experimental study (-80°C environment) were entrusted to Shanghai Meiji Biotechnology Co., Ltd. (<https://www.majorbio.com>) for testing. The activated sludge microbial testing included DNA extraction and 16s rRNA gene sequencing(chen et al., 2024).

2.4 Analytical methods

In this study, Sheng'ao Hua water quality testing instruments (<https://www.sahkeji.com>) were used to detect routine water quality indicators such as COD, NH₄⁺-N, TN, and TP, while Hach instruments (<https://www.hach.com.cn/US>) were employed to measure environmental factors like temperature (T), dissolved oxygen (DO), and pH.

The main instruments for measuring sewage indicators in the experiment and their corresponding methods are detailed in Table 1.

Table 1: Detection Methods for Water Quality Indicators

indicator	Determination method	Instrument model
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T, DO, pH	electrode method	HACH HQ40d
COD	Potassium dichromate method	6B-3000A
NH ₄ ⁺ -N	Nessler's reagent photometric method	6B-3000A
TN	Potassium persulfate ultraviolet spectrophotometry	6B-3000A
TP	Molybdenum-antimony anti-ultraviolet spectrophotometry	6B-3000A

188 The raw data obtained from Miseq sequencing are first assembled based on overlap
 189 relationships, and the sequence quality is controlled and filtered. The clustering method employs
 190 the USEARCH7-uparse algorithm, and sequence grouping is performed according to operational
 191 taxonomic units (genera) and a 97% threshold (UPARSE (7.0.1090)) (Caporaso *et al.*, 2010).
 192 Classification is conducted using the SILVA bacterial database (Bremen, Germany). Information on
 193 species abundance, coverage, and diversity in the community is obtained through diversity index
 194 analysis. To study the differences in community structure among different samples, a cluster analysis
 195 is performed on the sample community distance matrix to construct a sample-level clustering tree.
 196 Beta-diversity is plotted using R language (version 3.3.1). The Qiime software is used to calculate
 197 the *beta-diversity distance matrix* for sample-level clustering. The sample distance Heatmap uses
 198 FastTree (version 2.1.3, <http://www.microbesonline.org/fasttree/>) to construct an evolutionary tree
 199 based on the maximum likelihood method. Then, the FastUniFrac (<http://UniFrac.colorado.edu/>) is
 200 used to analyze and obtain the distance matrix between samples. PCoA analysis is performed using
 201 R language (version 3.3.1) for PCoA statistical analysis and plotting. LEfSe
 202 (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload) performs linear
 203 discriminant analysis (LDA) on samples according to different grouping conditions based on
 204 taxonomic composition, identifying communities or species that significantly affect sample

classification. The PICRUSt functional prediction first standardizes the OTU abundance table using PICRUSt, and then obtains COG family information and KEGG Ortholog (KO) information corresponding to each genus through the greengene id associated with each genus. The abundance of each COG and KO is calculated. For pathways, PICRUSt can obtain three levels of information, including functional modules and enzyme information, for metabolic pathways, and generate abundance tables for each level.

The pretreatment fermentation broth of qingke straw was detected using a Shimadzu GCMS-QP2010 SE instrument for gas chromatography-mass spectrometry. The electron impact (EI) mode was employed, and the fermentation broth was separated by gas chromatography. The mass spectrometry detection utilized EI (70 eV) ionization, with a typical scanning range of 40–600 m/z. The spectral library matching was conducted using the NIST20 spectral library for component identification. The functional groups of the fermentation broth were detected using infrared spectroscopy, employing a Thermo Scientific Nicolet Summit X Fourier transform infrared spectrometer with ATR-FTIR (Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy). The instrument model was Nicolet Summit X, with 16 scans and a scanning range of 4000–525 cm⁻¹.

3 Results and Discussion

3.1 Analysis of water quality results

To explore the impact of different treatment methods on the wastewater treatment capacity of the SBR reactor, an analysis was conducted on the changes in pollutant removal efficiency, and the results are shown in Figure 2.

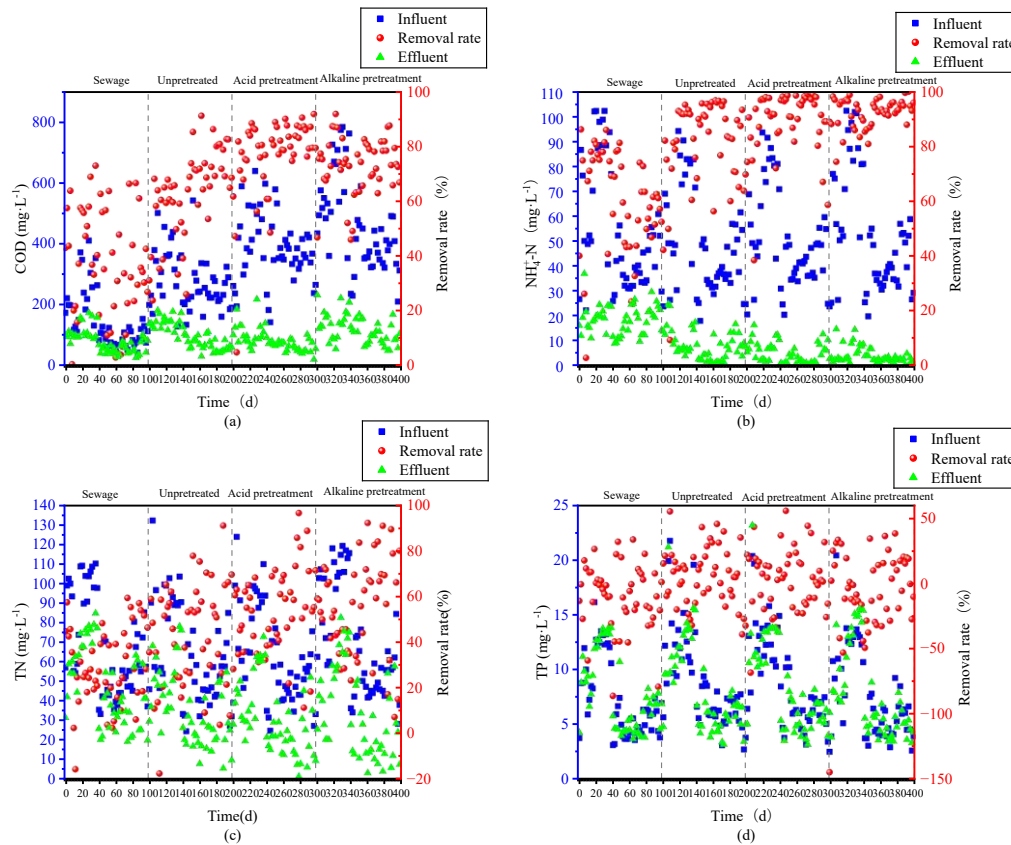


Figure 2 Diagram of pollutant removal efficiency of each device (a: COD, b: $\text{NH}_4^+\text{-N}$, c: TN, d: TP)

As can be seen from Figure 2, there are significant differences in the COD removal rates among the devices. Compared to the raw water control group S1, the COD removal rates of devices S3 and S4, which added acid-base pretreated fermentation broth, were significantly improved. The COD removal rate of device S2, which added untreated qingke straw carbon source fermentation broth, was improved, but the improvement was relatively small. Overall, the average COD removal rate of device S3 with acid pretreatment was the best (76.96%), slightly higher than that of device S4 (73.16%). This result indicates that both acid and alkali pretreatment can significantly enhance the carbon source release ability of qingke straw, suggesting that the cellulose in qingke straw can be hydrolyzed into monosaccharides and other substances as degradation carbon sources (Zhang et al.,

2021; Zou et al., 2021)

After adding fermentation broth with different pretreatment methods, the NH_4^+ removal rates of all devices increased. Among them, the removal rate of NH_4^+ from untreated fermentation broth increased slightly, while the removal rates of S3 and S4 devices increased significantly. Compared with S1, the average removal rate of NH_4^+ increased by 28.79% and 31.75%, respectively, maintaining a high level of 89.30% and 92.26%. This phenomenon indicates that no matter what kind of pretreatment method is used for the fermentation broth, it may significantly enhance nitrification in the system.

Compared to the blank control S1 device, the TN removal rate of the three devices with added fermentation broth showed a certain degree of improvement. Among them, the improvement in S2, which added untreated fermentation broth, was relatively small, with an average removal rate increase of 12.76%. The improvement in S3 and S4 devices, which added acid-base pretreated fermentation broth, was more significant, with an average removal rate increase of over 22.5%. The improvement was greatest in the Xizang barley straw fermentation broth after alkali treatment, reaching 24.79%. Overall, the addition of acid-base pretreated fermentation broth significantly enhanced nitrogen removal in the SBR system.

During the experimental operation period, the TP removal rate of each device exhibited significant fluctuations, generally remaining at a low level, and even showing negative removal rates. Relevant studies have indicated that under different operating conditions, the biological treatment process in plateau habitats may indeed remove some phosphorus-removing microorganisms, leading to poor phosphorus removal efficiency within the system (Chen et al., 2024; Chen et al., 2023a). Simultaneously, the ratio of TP concentration to TN concentration in the influent

is relatively high. At low C/N levels, phosphorus-accumulating organisms compete with nitrogen-removing bacteria for carbon sources, thereby inhibiting the denitrification process and resulting in poor nitrogen and phosphorus removal efficiency (Guerrero et al., 2011). Overall, the addition of different pretreatment methods using qingke straw carbon sources, especially alkali pretreatment fermentation broth, significantly improved the organic matter removal and nitrogen removal efficiency of the experimental operation devices, but the improvement in phosphorus removal efficiency was not significant.

3.2 Analysis of carbon source components in qingke straw with different pretreatments

3.2.1 Composition analysis of fermentation broth from qingke straw with different pretreatments

To investigate the composition of carbon source fermentation broth from qingke straw with different pretreatments, this study employed gas chromatography-mass spectrometry (GC-MS) detection. The detected components and functions for each treatment are presented in Table 2.

Table 2 List of Components Detected by Chromatography-Mass Spectrometry

handling	retention	peak area	name	Carbon source function
method	time			
Untreated fermentati on broth	9.546	1333322	3-Heptanone, 6-methyl-	Methyl ketone, easily degradable
	0.842	978852	L-Alanine ethylamide, (S)-	Amino acid derivatives, easily utilizing carbon and nitrogen sources
	30.396	633575	2,5-Dihydroxybenzoic acid, 3TMS derivative	Aromatic acid derivatives,

			degradable	
acid treatment	14.751	460236	Benzene, 1,2,3,4-tetramethyl-	Aromatic hydrocarbons have an inhibitory effect
	16.011	439601	Benzene, 1,2,4,5-tetramethyl-	Aromatic hydrocarbons have an inhibitory effect
	17.211	371518	Azulene	Inert organic compounds, slightly toxic
	32.087	231676	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) es	Inert organic compounds (plasticizers)
	31.914	225450	2-Pentadecanone, 6,10,14-trimethyl-	Long-chain ketones, which can degrade slowly
	13.972	216256	2-Nonanone	Medium chain length, easily degradable
	14.145	193947	Benzene, 2-ethyl-1,4-dimethyl-	Aromatic hydrocarbons have an inhibitory effect
	8.483	823280	Benzaldehyde	Aromatic carbon source, degradable
	3.049	725273	Hexanal	Lipid carbon source, easily degradable
	14.740	444627	Benzene, 1,2,3,4-tetramethyl-	Aromatic hydrocarbons have an inhibitory effect
	17.210	365882	Azulene	Inert organic compounds,

				slightly toxic
	16.004	360578	Benzene, 1,2,4,5-tetramethyl-	Aromatic hydrocarbons have an
				inhibitory effect
	18.827	304122	3-Pyridinecarbonitrile, 1,6-dihydro-1-methy	Inert organic matter
	18.721	223036	Benzaldehyde, 2,4-dimethyl-	Aromatic carbon source,
				degradable
	27.330	214811	Phenol, 2,6-bis(1,1-dimethylethyl)-	Phenols have an inhibitory
				effect
	14.573	201636	Benzene, 1,2,4,5-tetramethyl-	Aromatic hydrocarbons have an
				inhibitory effect
	30.394	199399	2,6-Dihydroxybenzoic acid, 3TMS derivativ	Aromatic acid derivatives,
				degradable
	5.888	1084504	2-Heptanone	High content, easy to degrade
	31.906	748255	2-Pentadecanone, 6,10,14-trimethyl	Long-chain ketones, which can
				degrade slowly
	9.759	289022	2-Octanone	Medium chain length, easy to
alkali				degrade
treatment	14.758	280956	Benzene, 1,2,3,4-tetramethyl-	Aromatic hydrocarbons have an
				inhibitory effect
	9.638	219524	5-Hepten-2-one, 6-methyl-	Unsaturated ketone, degradable
	16.019	189936	Benzene, 1,2,4,5-tetramethyl-	Aromatic hydrocarbons have an
				inhibitory effect

9.579	146915	3-Octanone	Medium chain length, easily degradable
16.350	107618	3-Isopropylidene-5-methyl-hex-4-en-2-one	Inert organic matter
17.271	95788	Azulene	Inert organic compounds, slightly toxic
14.580	93266	Benzene, 1,2,3,4-tetramethyl-	Aromatic hydrocarbons have an inhibitory effect

274 As can be seen from Table 1, the untreated fermentation broth has a low carbon production,
275 with about 20% being rapidly degradable carbon sources, 70% being slowly degradable carbon
276 sources, and about 10% being toxic substances such as halogenated hydrocarbons and nitrogen-
277 containing aromatic rings. Although the inhibitory effect of untreated fermentation broth is small
278 and the proportion of treatable carbon sources is large, the COD content of untreated fermentation
279 broth is relatively low, only ranging from 300 to 500 mg·L⁻¹, making it difficult to achieve effective
280 carbon source supplementation.

281 Acid treatment releases a large amount of carbon sources, with a COD content that can reach
282 2000 mg·L⁻¹. The composition is relatively complex, with only 25% of the carbon sources capable
283 of being rapidly degraded, 40%-50% degradable but with a slower degradation rate, 20% toxic
284 substances, and a relatively high proportion of inhibitory substances. Under the same conditions,
285 the effect is inferior to that of alkali-treated fermentation broth.

286 Alkali treatment yields the highest carbon production, with a COD content exceeding 6000
287 mg·L⁻¹. However, some carbon sources are difficult to utilize. Only three rapidly degradable carbon
288 sources, namely 2-Heptanone, 2-Octanone, and 3-Octanone, account for approximately 30%.

Degradable carbon sources, albeit with a slower degradation rate, constitute about 30%. Non-degradable substances make up about 20%, toxic and inhibitory substances about 10%, and other substances about 10%. Compared to acid treatment and untreated fermentation broth, alkali-treated fermentation broth has a higher carbon production rate, a higher proportion of utilizable carbon sources, and fewer toxic side effects. Consideration could be given to extending the fermentation time or using adsorption treatment to reduce toxic inhibitory effects.

Based on the analysis of water quality changes, it can be concluded that both acid and alkali treatments can reduce the degree of polymerization of cellulose, separate cellulose, hemicellulose, and lignin, and increase the contact surface area and enzyme accessibility of cellulose (Kumar et al., 2020). Although the denitrification effect of carbon sources treated with acid has improved, it is not stable. This may be because acid treatment produces some soluble organic matter with multi-chain structures that are difficult to degrade. In addition, during the fermentation process, although the hydrolyzates from acid and alkali treatments are neutralized before fermentation, the alkali treatment shows better performance in degrading multi-chain organic matter during anaerobic fermentation, which may be one of the reasons for the superior denitrification and phosphorus removal effect of alkali treatment.

3.2.2 Analysis of functional groups in carbon source fermentation broth from qingke straw with different pretreatment methods

To investigate the changes in functional groups in the carbon source fermentation broth of qingke straw with different pretreatment methods, infrared spectroscopy was used to detect the fermentation broth, and the detection results are shown in Figure 3.

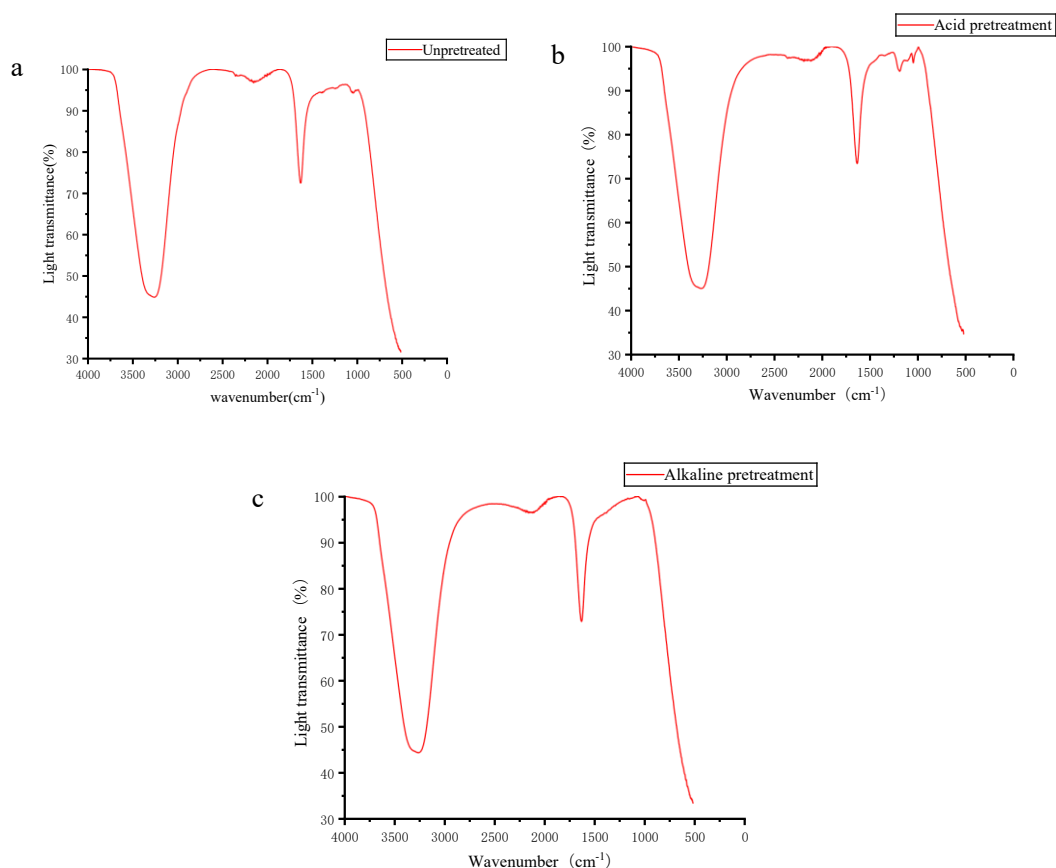


Figure 3 Fourier transform infrared spectrum of hydrolyzate

In the figure, several wave peaks can be clearly observed. The absorption peak near 1636 cm^{-1} appears in all three samples with similar intensities (72.5-73.5). It may correspond to the stretching vibration of $\text{C}=\text{O}$ (carbonyl group), commonly found in compounds such as amides, ketones, and acids. The absorption peak at 2157 cm^{-1} (only appearing in the "untreated" sample) has a very high intensity (96.765), and disappears after treatment in the "acid pretreatment" and "alkali pretreatment" samples. It may correspond to the stretching vibration of $\text{C}\equiv\text{N}$ (nitrile) or $\text{C}\equiv\text{C}$ (alkyne). The disappearance indicates that this functional group may undergo hydrolysis or structural destruction under acid-base conditions. The absorption peak at 1192 cm^{-1} (only appearing in the acid pretreatment sample) has a high intensity (94.413), and may correspond to the stretching vibration of $\text{C}-\text{O}$ (such as alcohols, ethers, and esters). Its appearance may be related to esterification,

dehydration, or structural rearrangement caused by acid treatment. The absorption peak at 3258-3260 cm^{-1} appears in all samples with similar intensities (about 45). It corresponds to the stretching vibration of O-H or N-H (hydrogen bonding region), and may come from water, alcohols, phenols, amines, or amides. Untreated sample: it may be an organic compound containing nitrile or alkyne groups (2157 cm^{-1}). Acid pretreatment: it leads to the disappearance of nitrile/alkyne groups, and the appearance of C-O bond signals (1192 cm^{-1}), indicating possible hydrolysis to generate carboxylic acids or esterification reactions. Alkali pretreatment: it also leads to the disappearance of nitrile/alkyne groups, but no new peaks appear, indicating possible alkaline hydrolysis to generate carboxylates or amines/amines, whose infrared signals may be masked by broad peaks.

3.3 Diversity analysis

3.3.1 Alpha diversity analysis of microbial communities

In the Alpha diversity indices of microbial communities, the Chao index and Sobs index reflect the richness of microbial communities, the Simpson index and Shannon index reflect the diversity of microbial communities, and the Shannon-even index and Simpson-even index reflect the evenness of communities (Hou et al., 2023). Community diversity is a comprehensive result of richness and evenness. After adding different pretreatment methods of qingke straw carbon source fermentation broth, the changes in Alpha diversity indices of samples from each device are shown in Table 3.

Table 3 Alpha diversity index of each device under different pretreatment methods

Sample name	Sobs	Chao	Shannon	Simpson	Shannoneven	Simpsoneven
S0D1	265	265.00	4.14	0.0343	0.7425	0.1100

S1D1	190	190.00	3.27	0.0957	0.6240	0.0550
S1D2	235	235.25	3.63	0.0610	0.6655	0.0697
S1D3	265	265.00	3.63	0.0734	0.6505	0.0514
S2D1	194	194.25	3.23	0.1093	0.6131	0.0471
S2D2	241	241.11	3.56	0.0652	0.6499	0.0636
S2D3	241	241.00	3.44	0.0817	0.6277	0.0508
S3D1	212	212.00	3.64	0.0568	0.6791	0.0831
S3D2	228	228.00	3.67	0.0551	0.6767	0.0796
S3D3	241	241.00	3.85	0.0654	0.7028	0.0634
S4D1	199	199.00	3.58	0.0684	0.6770	0.0735
S4D2	274	274.14	4.23	0.0322	0.7533	0.1132
S4D3	274	274.09	4.08	0.0457	0.7262	0.0798

342 Based on the analysis of Table 3, it can be observed that the microbial abundance in the S1
343 device of the blank control group is relatively high. The addition of different pretreated qingke straw
344 carbon source fermentation broths significantly inhibited the microbial abundance in each device
345 during the initial operation period, significantly affecting the microbial abundance within the device.
346 As the operation time increased, the microbial abundance in each device significantly improved,
347 with the S4 device, which added alkali-treated qingke straw carbon source fermentation broth,
348 showing a notable increase in abundance, reaching its maximum during the middle operation period.
349 The Shannon and Simpson indices indicate that the addition of fermentation broths from different
350 pretreatment methods suppressed the diversity in each device. As the operation time increased, the
351 diversity within the system increased, reaching its maximum in each device during the reaction end

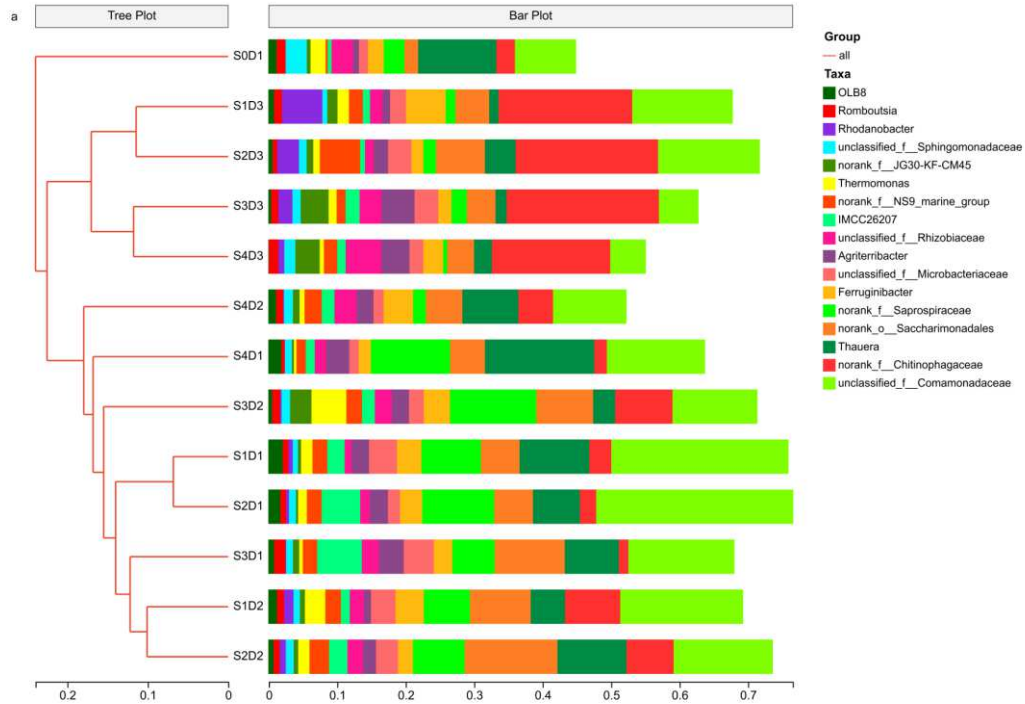
period. A comparison between the Shannon-even index and the Simpson-even index reveals that the uniformity of each device changed slightly, indicating that the addition of qingke straw fermentation broth had a minor impact on the microbial uniformity within the SBR system. Overall, the addition of qingke straw carbon source fermentation broth had a significant impact on the microbial abundance and diversity in each device, inhibiting microbial diversity during the initial operation period and significantly improving it as the operation time increased.

Comparing Alpha diversity with previous experiments and related studies conducted under low C/N ratio operating conditions in non-plateau habitats, the Chao index of the SBBR reactor in plateau habitats ranged from 1206.68 to 1320.13 (Chen et al., 2022a), which was significantly higher than the Chao index of 624 to 922 for the SBR reactor in non-plateau habitats (Sun et al., 2020b), and also significantly higher than the Chao index in this experiment. This indicates that the total species count in the SBR reactor in plateau habitats is lower than that in non-plateau areas, and the increase in packing material is beneficial for enhancing the total species count. However, the Simpson index (0.0343 to 0.1093) in this study was lower than that in the SBR reactor in non-plateau habitats (0.82 to 0.96), while the Shannon index (3.23 to 4.23) was within the range of that in the SBR reactors in non-plateau habitats (4.59 to 6.76) (Sun et al., 2020b; Chen et al., 2022a), suggesting that the SBR reactor in plateau habitats may possess good biodiversity.

3.3.2 Analysis of microbial community β diversity

To investigate the differences in community structure among different samples, a cluster analysis was conducted on the distance matrix of sample communities, and a hierarchical cluster tree of samples was constructed. The hierarchical cluster analysis of samples is based on the β diversity distance matrix for hierarchical clustering analysis, utilizing the UPGMA algorithm to

construct a tree structure and visualize the degree of difference in community distribution among different environmental samples. The hierarchical cluster analysis results of samples at the genus level at each stage, using the Bray-Curtis distance algorithm, are shown in Figure 4a.



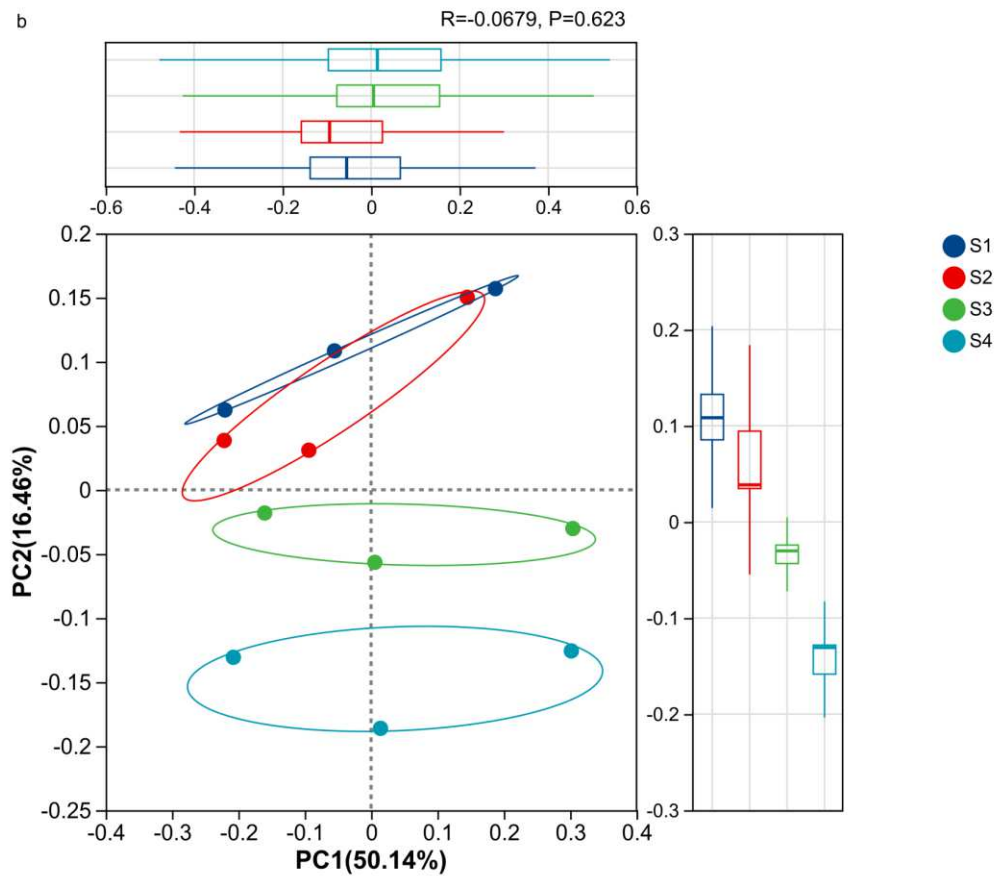


Figure 4 Hierarchical clustering tree of samples at each stage of genus level (a) and PCoA analysis of samples at each stage of genus level (b)

As can be seen from Figure 4a, during the initial stage of operation, the microbial community structures of S3 and S4 devices, which added acid-base pretreated qingke straw fermentation broth, showed significant differences, while the microbial structures of S1 and S2 devices were highly similar. The microbial community evolution in each stage of S1 and S2 devices was relatively distinct, with significant leaps. The microbial communities in S3 and S4 devices exhibited a stepwise evolution, with the S4 device, which added alkali-treated qingke straw carbon source fermentation broth, showing a more rigorous stepwise evolution. As the operation progressed, the microbial structures of all devices became highly similar at the end of the operation. This

phenomenon indicates that acid-base pretreatment fermentation broth and operating conditions have a significant impact on the evolution of microbial communities within the system, a result similar to previous studies (Liu et al., 2022; Chen et al., 2022a).

Principal coordinates analysis (PCoA) can be utilized to investigate the similarity or difference in the composition of sample communities. It employs a two-dimensional scatterplot visualization to display the similarity and dissimilarity between control and treatment groups, with the distance between samples reflecting the degree of clustering or dispersion within the sample communities. Based on the Bray-Curtis algorithm, this study analyzes the genus-level changes in the microbial community structure of various devices for qingke straw carbon source fermentation broth with different treatment methods. The results are presented in Figure 3b. As can be observed from the figure, the two principal coordinates of PCoA account for 66.6% of the variation in all samples. The samples from each device are relatively dispersed, exhibiting a distribution trend from left to right as the operational conditions change. There is a significant distance along the horizontal axis, while the dispersion distance between devices S3 and S4 is relatively small along the vertical axis. This phenomenon may indicate that the addition of acid-base pretreated qingke straw fermentation broth has a significant impact on the microbial community structure of the SBR system.

3.4 Classification and composition of microbial communities

To analyze the impact of qingke straw fermentation broth with different pretreatment methods on the microbial community within the SBR system, high-throughput sequencing was performed on the collected samples. The composition of the microbial community in each sample is shown in the figure below.

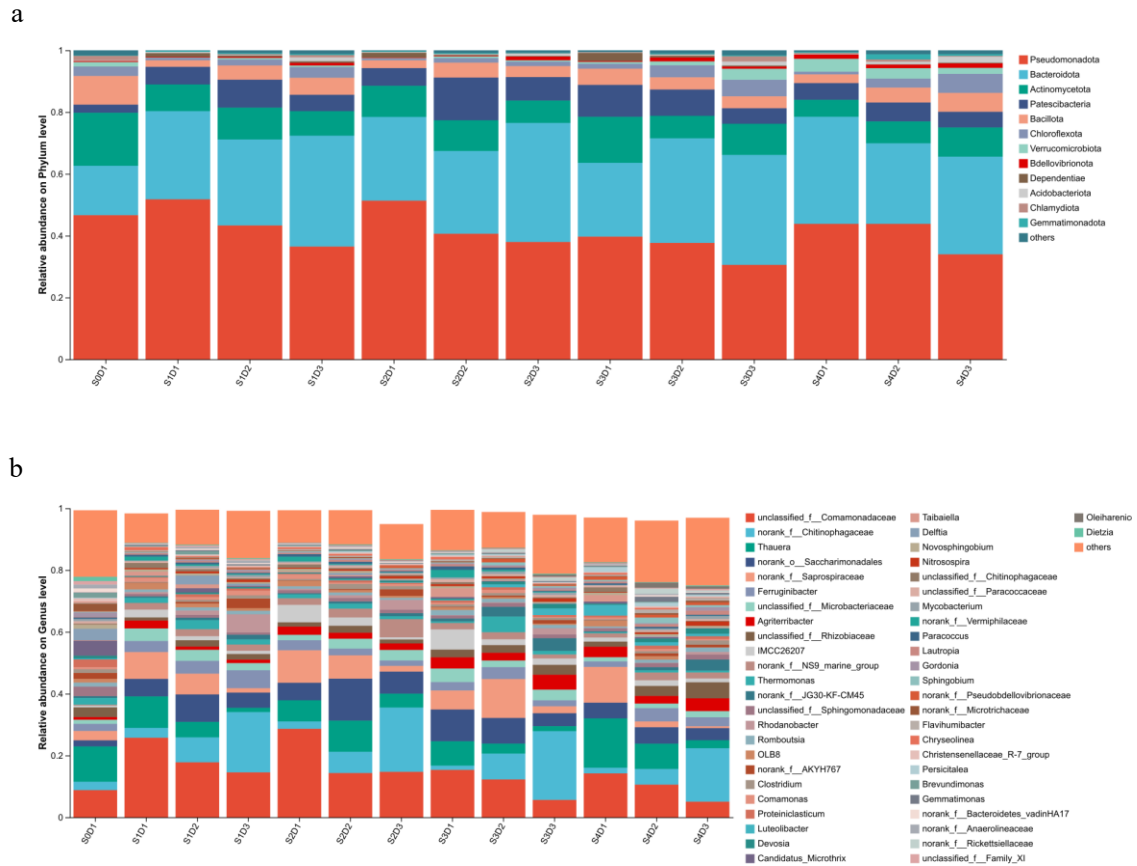


Figure 5: The composition of microbial communities at the phylum level (a) and genus level (b) using different pretreatment methods for qingke straw fermentation broth as external carbon sources

The composition of qingke straw carbon sources at the phylum level for different pretreatment methods is shown in Figure 5a (species with abundance < 1% are classified as "other"). The TOP5 species at the phylum level include *Pseudomonadota*, *Bacteroidota*, *Actinomycetota*, *Patescibacteria*, and *Bacillota*. Their community composition differs to some extent from other related studies in high-altitude habitats (Hao et al., 2022; Li et al., 2022). The sum of their abundances ranges from 85.10% to 96.76%, occupying an absolute dominant position and being sufficiently representative. These variations indicate the impact of different pretreatment methods on the microbial communities in various devices, and the addition of qingke straw as an external

carbon source has similar abundance changes at the phylum level in the SBR system.

Pseudomonadota accounts for the largest proportion in various samples, ranging from 30.56% to 51.77%, and exhibits a gradual downward trend across different devices. This phenomenon suggests that *Pseudomonadota* may be influenced by the operational conditions of the SBR process and the fermentation broth of qingke straw. *Pseudomonadota* is a major degrader of complex organic pollutants (such as aliphatic hydrocarbons, aromatic hydrocarbons, dissolved organic matter (DOM)) in wastewater. Its members hydrolyze macromolecular organic matter into volatile fatty acids (VFA), alcohols, and other small molecules through the secretion of extracellular enzymes, providing electron donors for subsequent denitrification and phosphorus removal. Studies have shown that in a gradient magnetic field-coupled constructed wetland system, the abundance of *Pseudomonadota* is significantly positively correlated with organic matter removal efficiency (Chen et al., 2025; Li et al., 2025). Meanwhile, related research indicates that some microorganisms within *Pseudomonadota* are the main force of heterotrophic denitrifying bacteria, which use nitrate (NO_3^-) or nitrite (NO_2^-) as electron acceptors under anoxic conditions and gradually reduce them to nitrogen gas (N_2) using organic carbon sources, achieving efficient nitrogen removal (Zhong et al., 2024; Naziębło et al., 2025). The high abundance of *Pseudomonadota* indicates that the devices have a good effect on the removal of total nitrogen (TN), which is consistent with the trend of water quality changes.

The abundance of *Bacteroidota* exhibits a wave-like variation, with high levels on both sides and a low level in the middle, maintaining a level above 20% across all devices. This indicates that *Bacteroidota* gradually adapts to the qingke straw carbon source as the operational conditions change. Related studies have shown that *Bacteroidota* possesses high environmental adaptability in

plateau habitats and is the dominant phylum in nitrogen and phosphorus removal processes in such environments (Chen et al., 2024). It can degrade carbohydrates and convert organic matter into acetic acid and lactose, among others (Hill et al., 2007). Additionally, research indicates that ultraviolet radiation has a certain enhancing effect on this phylum (Chen et al., 2023b).

Actinomycetota can secrete various extracellular enzymes (such as protease, lipase, and cellulase) that effectively decompose refractory organic matter in wastewater, including proteins, fats, starches, cellulose, and lignin derivatives, significantly reducing chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (Cai et al., 2022). They often serve as "pioneers" in activated sludge and biofilm systems, initiating the mineralization chain of organic matter and providing available substrates for subsequent nitrification/denitrification bacteria (Li et al., 2024). Some strains possess aerobic denitrification capabilities, converting nitrate (NO_3^-) to nitrogen gas (N_2) under aerobic conditions (Li et al., 2024b; Huang et al., 2021; Boubekri et al., 2023). In the S3 device, this phylum showed a trough variation, while in the S4 device with alkali pretreatment of fermentation broth, it showed a significant upward trend, indicating that different pretreatment methods have a significant impact on this phylum.

Patescibacteria play a role in providing fermentation by-products to microorganisms possessing lactate dehydrogenase (Chung et al., 1985) and formate dehydrogenase (Overkamp et al., 2002). Meanwhile, relevant studies indicate that the *Patescibacteria* population in anaerobic ammonia oxidation reactors may play an ecological role in the production of short-chain fatty acids and the degradation of chitin-related compounds. Their survival may depend on the production of PGA by major anaerobic ammonia-oxidizing bacteria based on metagenomic information (Hosokawa et al., 2021). In the S1 device without the addition of fermentation broth and the S2

device with the addition of untreated fermentation broth, the abundance of this phylum showed a gradual increase. However, in the S3 and S4 devices with the addition of acid-base pretreated fermentation broth, the abundance of this phylum showed a gradual decrease, indicating that it was inhibited by the acid-base pretreated fermentation broth, which may be caused by some toxic substances contained in the broth.

Bacillota primarily serves as the core functional bacterial community in wastewater treatment, playing a pivotal role in the hydrolysis and acidification processes of complex organic matter. It collaborates with methanogens to enhance energy recovery efficiency and strengthens sludge flocculation and system stability by secreting extracellular polymeric substances (EPS) (Chen et al., 2022c; Uysal et al., 2021). In this study, with the exception of the S3 device treated with acid-pretreated fermentation broth, the overall trend exhibited a gradual increase. However, the S3 device treated with acid-pretreated fermentation broth showed a gradual decrease, indicating that the acid-pretreated qingke straw fermentation broth exerts a certain inhibitory effect on this bacterial species.

The species composition at the genus level of each device sample is shown in Figure 5b (species with abundance < 1% are classified as "other" and not shown in the figure). The TOP5 genera in each device include: *unclassified_f__Comamonadaceae*, *norank_f__Chitinophagaceae*, *Thauera*, *norank_o__Saccharimonadales*, and *norank_f__Saprospiraceae*. Among them, *unclassified_f__Comamonadaceae* and *norank_o__Saccharimonadales* have been detected in related studies on plateau habitats. These genera may be dominant in plateau habitats, while the typical plateau habitat dominant genus OLB8 is not among the top ten genera in this study (Chen et al., 2023b; Chen et al., 2024). This result indicates that adding qingke straw as a carbon source has a significant impact on the microbial community structure of the SBR system.

489 *Unclassified_f__Comamonadaceae* (genus from the family *Comamonadaceae*), belonging to the
490 phylum *Proteobacteria*, functions to degrade complex organic matter, participate in nitrogen cycling,
491 and denitrification. Its abundance decreases later, indirectly affecting nitrogen removal efficiency
492 (Chen et al., 2022B; Liu et al., 2023B; Su et al., 2026). *Norank_f__Chitinophagaceae* (genus from
493 the family *Chitinophagaceae*), whose abundance increases with the progress of the experiment,
494 belongs to the phylum *Bacteroidetes* and functions to degrade organic matter. Chitin, also known as
495 chitosan, is a nitrogen-containing polysaccharide (carbohydrate polymer) consisting of N-
496 acetylglucosamine monomers connected by chemical bonds, which can be simply understood as
497 cellulose with amino groups. This substance mainly comes from the qingke straw carbon source.
498 The response of this genus to the added carbon source is reflected in the increase in abundance
499 (Zhong et al., 2023; Sun et al., 2021). *Norank_f__Saprospiraceae* (belonging to the *Saprospiraceae*
500 family) belongs to the *Bacteroidetes* phylum and functions in nitrification. As the experiment
501 progresses, the abundance of this genus decreases. Analysis suggests that this genus is a typical
502 filamentous bacterium, with low abundance during the sludge cultivation stage. The source of
503 sewage for sludge cultivation is domestic sewage, and the concentration of pollutants such as COD
504 is not high. Filamentous bacteria do not proliferate significantly in this environment. During the
505 formal operation period, due to the addition of external carbon sources to the reactor, the COD
506 concentration increases, which may cause sludge bulking and a significant proliferation of
507 filamentous bacteria. As the microbial community adapts to higher concentrations of carbon sources,
508 the abundance of filamentous bacteria, led by *norank_f__Saprospiraceae*, decreases in the later
509 stages (Zhang et al., 2019; He et al., 2021; Huang et al., 2023). *Thauera* belongs to the
510 *Proteobacteria* phylum and primarily functions in denitrification and degradation of aromatic

compounds. The abundance of this genus decreases as the experiment progresses, and the TN removal rate also decreases with the progress of the experiment. Analysis suggests that the decrease in denitrification efficiency is related to the reduction in abundance of this genus (Ren et al., 2021; Ren et al., 2024). *Norank_o__Saccharimonadales* belongs to the *Patescibacteria* phylum, which has a single function and relies heavily on the metabolic products of other microorganisms for survival (Wang et al., 2023; Zhou et al., 2024). The abundance of this genus can reflect the diversity of the microbial community. As the experiment progresses, the abundance of this genus first increases and then decreases, indicating that the external carbon sources are abundant. The abundance of this genus significantly increases during stages D1 and D2, indicating that microorganisms are in an adaptation period to the external carbon sources during these stages. Combined with α diversity analysis, it is believed that the community diversity is at its highest during the experiment.

3.5 Analysis of abundance changes in nitrogen metabolism functional modules

In the wastewater treatment process, nitrogen metabolism is a crucial step. Nitrogen metabolism involves the conversion of various nitrogen-containing substances in wastewater into substances such as N_2 , NO, N_2O , and NH_3 through complex interactions among microorganisms. The nitrogen cycle process involves four reduction pathways and two oxidation pathways, namely nitrogen fixation [MD: M00175], assimilatory nitrate reduction [MD: M00531], dissimilatory nitrate reduction [MD: M00530], denitrification [MD: M00529], nitrification [MD: M00528], complete nitrification reaction [MD: M00804], and anaerobic ammonia oxidation [MD: M00973], totaling seven modules. These pathways are integrated in Kyoto Gene and Genome Encyclopedia (<http://www.genome.jp/kegg/>) to form map00910. To explore the metabolic relationships of

microorganisms involved in nitrogen cycling in the SBR system under the influence of different pretreatment methods and external carbon sources from qingke straw, the changes in the proportion of nitrogen metabolism functional modules in each device were analyzed. The results are shown in Table 4.

Table 4: Changes in the proportion of nitrogen metabolism functional modules in each device

	M00529	M00530	M00804	M00531	M00528	M00175
Functional Description Sampling Example	Dissimilatory		Complete	Nitrate		
	Denitrification, nitrate → nitrogen	nitrate reduction, nitrate → ammonia		assimilation and reduction, nitrate → ammonia	Nitrification reaction, ammonia → nitrite	Nitrogen fixation, nitrogen → ammonia
			ammonia → nitrite → nitrate	reduction, nitrate → ammonia	ammonia → nitrite	nitrogen → ammonia
Related genes	<i>narG/H/I, napA/B, nirK/S, norB/C, nosZ</i>	<i>narG/H/I, napA/B, nirB/D, nrfA/H</i>	<i>pmoA-amoxA, pmoB-amoxB, pmoC-amoxC, hao, narG/H</i>	<i>narB, NR, nasA/B, nirA, NIT-6</i>	<i>pmoA-amoxA, pmoB-amoxB, pmoC-amoxC, hao</i>	<i>nifH/D/K, anfG, vnfD/K/G/H</i>
S0D1	7.91%	8.24%	7.62%	9.40%	3.05%	15.93%
S1D1	11.37%	10.02%	12.58%	5.81%	16.21%	3.87%
S1D2	8.23%	7.73%	8.35%	6.58%	6.75%	4.05%
S1D3	6.29%	7.01%	6.31%	8.04%	8.08%	6.16%

S2D1	10.54%	9.50%	11.25%	5.31%	7.87%	3.26%
S2D2	8.42%	8.01%	8.21%	7.16%	5.44%	11.03%
S2D3	7.03%	7.62%	6.77%	8.14%	7.05%	7.11%
S3D1	7.86%	7.27%	7.95%	5.99%	6.80%	3.37%
S3D2	6.58%	6.46%	6.82%	6.58%	6.39%	7.57%
S3D3	4.07%	5.59%	3.85%	10.12%	9.23%	11.28%
S4D1	9.89%	9.30%	9.10%	7.38%	4.22%	3.77%
S4D2	7.15%	7.27%	6.54%	8.72%	6.47%	8.22%
S4D3	4.66%	5.96%	4.64%	10.78%	12.44%	14.38%
Total						
absolute	1304980.75	1154591.89	351585	263387.5	42502	22228.77
abundance						

539 As shown in Table 4, by adding different pretreated qingke straw external carbon sources to
 540 the SBR system in highland habitats, six out of seven functional modules of nitrogen cycling were
 541 detected, with only the anaerobic ammonia oxidation [MD:M00973] functional module remaining
 542 undetected. This indicates that highland habitats possess certain nitrogen metabolism functions, a
 543 result consistent with other related studies on highland habitats (Hao et al., 2021b; Chen et al., 2024).
 544 Denitrification reaction (M00529) in each device system accounted for the highest proportion
 545 among all nitrogen metabolism functional modules, but it still remained at a relatively low level
 546 among all detected functional modules, ranking only 110th out of all 313 functional modules
 547 detected. This suggests that each device possesses a certain denitrification capacity, but the
 548 denitrification ability needs to be enhanced. This is corroborated by the analysis of water quality

changes, which revealed that the TN removal rate was enhanced compared to the blank control, but the overall removal rate was not high. The abundance variation trend of dissimilatory nitrate reduction reaction (M00530) and complete nitrification reaction (M00804) in each device followed the same trend as the denitrification reaction, showing a gradual decrease. This may be due to the addition of qingke straw external carbon sources, which significantly increased the activity of MLSS and enhanced microbial community diversity with the operation of the system, leading to the loss of dominance of microorganisms related to the three functional modules. The nitrate assimilation reduction reaction (M00531) and nitrification reaction (M00528) showed a gradual increase trend in each device, especially in devices S3 and S4 with acid-base pretreated qingke straw fermentation broth added. The abundance of these two functional modules, as well as the nitrogen fixation reaction (M00175), significantly increased with the operation of the system, with the increase being more pronounced in alkali-pretreated fermentation broth compared to acid-pretreated fermentation broth. This result is consistent with the analysis of water quality changes, indicating that alkali-pretreated qingke straw fermentation broth is more conducive to the operation of the SBR system.

3.6 Correlation between nitrogen metabolism functional modules and microbial genera in qingke straw with external carbon sources added, which differ from the treatment method

To explore the interactions between microbial communities, environmental factors, and nitrogen metabolism functional modules in the SBR reactor, a two-factor microbial community co-occurrence network was constructed using Spearman correlation analysis. To reduce network complexity and improve the accuracy of results, the top 50 bacterial genera with the highest average abundance across all samples were selected. Correlation analysis was performed at the phylum level and genus level, with absolute values of correlation coefficients ≥ 0.6 and p-values < 0.05 . The

results are shown in Figure 6.

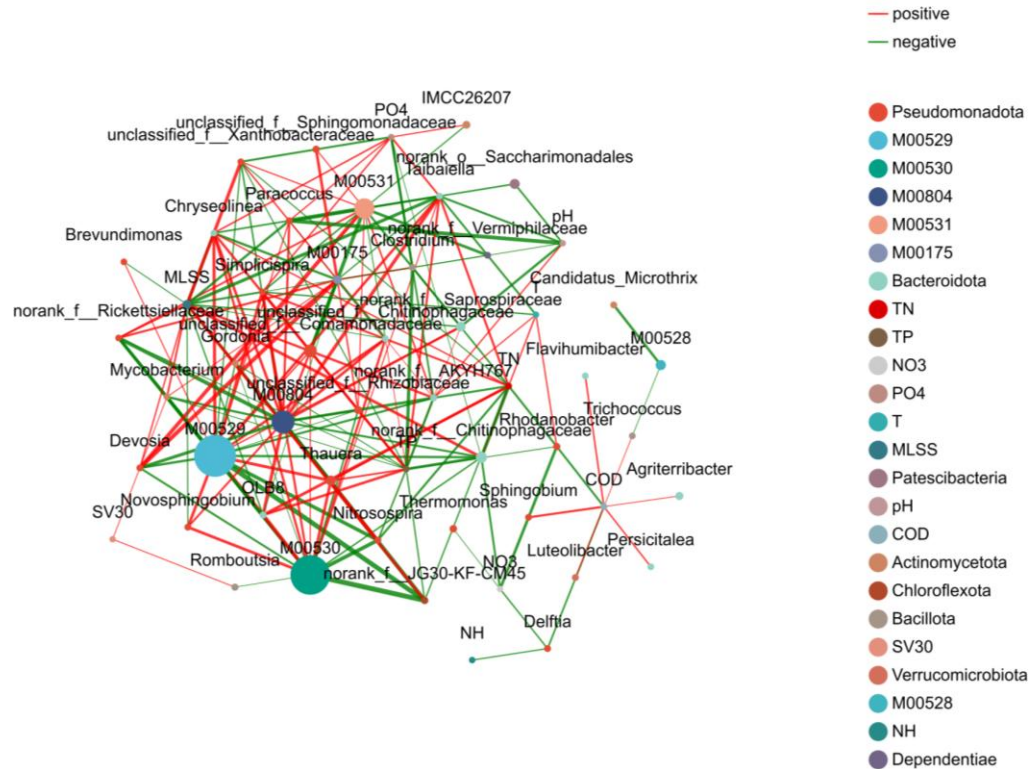


Figure 6 Plot of covariance analysis between functional modules of nitrogen metabolism and microbial communities at genus level (green and red lines indicate negative and positive correlation, respectively)

The two-factor network diagram comprises 53 nodes and 176 edges, with 47.16% being positive correlations and 52.84% being negative correlations. Among them, denitrification reaction (M00529) is the functional module with the most connected nodes, reaching 21, followed by complete nitrification reaction (M00804) with 20 connected nodes. This result indicates that the larger functional modules of microbial communities in this experiment are denitrification reaction (M00529) and complete nitrification reaction (M00804), which is consistent with the analysis of functional module abundance changes. Meanwhile, denitrification reaction (M00529) is significantly influenced by the evolution of microbial communities, which is consistent with

previous studies on SBR processes in plateau habitats (Chen et al., 2024). Among environmental factors, MLSS has the most connected nodes, reaching 19, followed by TP with 17. This result suggests that the addition of qingke straw fermentation broth significantly increases microbial abundance, and MLSS profoundly affects the evolution of microbial communities. Additionally, a higher TP concentration under low C/N conditions also influences the evolution of microbial communities within the system, which is corroborated by the analysis of diversity and water quality changes. Meanwhile, relevant research has shown (Wang et al., 2022) that changes in environmental factors can enhance interactions among core bacterial groups, further strengthening the "linking function" of microbial communities.

4 Conclusion

The results indicated that the addition of external carbon sources promoted the efficiency of nitrogen and phosphorus removal in the SBR process under plateau habitats. When acid pretreatment and alkali pretreatment controlled the same C/N ratio, the alkali pretreatment exhibited a higher carbon source production rate than other treatment methods, and the alkali pretreatment demonstrated the best nitrogen and phosphorus removal effect. The addition of qingke straw fermentation broth significantly enhanced the microbial richness within the SBR system, thereby improving the diversity of the microbial community. The addition of acid-alkali pretreated qingke straw fermentation broth influenced the evolution of the microbial community. There were significant changes in the community composition of dominant phyla and genera in the SBR reactor, and notable differences in the abundance of dominant bacterial genera across different stages in each device. The addition of acid-alkali pretreated qingke straw fermentation broth significantly inhibited three functional modules: denitrification, dissimilatory nitrate reduction, and complete digestion,

while enhancing three functional modules: nitrate assimilatory reduction, nitrification, and nitrogen fixation. Due to the high influent TP and the lack of certain phosphorus-accumulating organisms, the phosphorus removal efficiency of the system was poor. Therefore, nitrogen and phosphorus removal processes in plateau habitats require enhanced treatment for phosphorus removal.

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Author Contributions Xiangyu Chen was responsible for designing the study, interpreting the results, and making major contributions to manuscript writing. Renxu Wang, Yansong Wang, and Yuhao Zheng were responsible for executing the experiments and collecting data. Yongchen Zong was responsible for all tasks. Jiajing Zhang and Shibo Liu participated in the gene expression experiments. All authors read and approved the final manuscript.

Data availability Not applicable

Abide by moral standards

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Consent to participate All authors mentioned in the manuscript have agreed for authorship and

for participation in the manuscript.

Consent to publish All authors have read and agreed to the published version of the manuscript.

Ethics approval This article is a research in the direction of sewage treatment and does not involve ethical codes.

Consent for publication Not applicable

Manuscript not submitted on preprint server.

Declarations

Ethics approval Not applicable This study did not involve human subjects or animal experiments.

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Availability of data and materials The experimental data are included in the article.

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