Environmental Science and Pollution Research (2023) 30:33181–33194
https://doi.org/10.1007/s11356-022-24426-2

RESEARCH ARTICLE

Improving NH_3 and H_2S removal efficiency with pilot-scale biotrickling filter by co-immobilizing *Kosakonia oryzae* FB2-3 and *Acinetobacter baumannii* L5-4

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Received: 15 June 2022 / Accepted: 23 November 2022 / Published online: 7 December 2022

Abstract

In this study, two NH_4^+-N and S^{2-} removal strains, namely, *Kosakonia oryzae* (FB2-3) and *Acinetobacter baumannii* (L5-4), were isolated from the packing materials in a long-running biotrickling filter (BTF). The removal capacities of combined FB2-3 and L5-4 (FB2-3 + L5-4) toward 100 mg L^{-1} of NH_4^+-N and 200 mg L^{-1} of S^{2-} reached 97.31 ± 1.62% and 98.57 ± 1.12% under the optimal conditions (32.0 °C and initial pH = 7.0), which were higher than those of single strain. Then, FB2-3 and L5-4 liquid inoculums were prepared, and their concentrations respectively reached 1.56×10^9 CFU mL^{-1} and 1.05×10^9 CFU mL^{-1} by adding different resuspension solutions and protective agents after 12-week storage at 25 °C. Finally, pilot-scale BTF test showed that NH_3 and H_2S in the real exhaust gases from a pharmaceutical factory were effectively removed with removal rates > 87% and maximum elimination capacities were reached 136 g (NH_3) m^{-3} h^{-1} and 176 g (H_2S) m^{-3} h^{-1} at 18 °C-34 °C and pH 4.0–7.0 in the BTF loaded with bamboo charcoal packing materials co-immobilized with FB2-3 and L5-4. After co-immobilization of FB2-3 and L5-4, in the bamboo charcoal packing materials, the new microbial diversity composition contained the dominant genera of *Acinetobacter*, *Mycobacterium*, *Kosakonia*, and *Sulfobacillus* was formed, and the diversity of entire bacterial community was decreased, compared to the control. These results indicate that FB2-3 and L5-4 have potential to be developed into liquid ready-to-use inoculums for effectively removing NH_3 and H_2S from exhaust gases in BTF.

Keywords Biotrickling filter · *Kosakonia oryzae* · *Acinetobacter baumannii* · Co-immobilized · NH_3 and H_2S removal · Pilot-scale test

Abbreviations

BTF	Biotrickling filter
NH_3	Ammonia
H_2S	Hydrogen sulfide
VOCs	Volatile organic chemicals
BTF_0	The long-term running BTFs in a pharmaceutical factory
BTF_1	The BTFs without inoculated bacterial agent
BTF_2	The BTFs inoculated bacterial agent
BPM_0	The bamboo charcoal packing materials samples from BTF_0
BPM_1	The bamboo charcoal packing materials samples from BTF_1
BPM_2	The bamboo charcoal packing materials samples from BTF_2
EC	Elimination capacity
ECmax	Maximum elimination capacity
ECcrit	Critical elimination capacity
IL	Inlet load

Responsible Editor: Gerald Thouand

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Introduction

Odor pollution produced during agricultural and industrial activities from landfills, livestock and poultry farms, composting plants, wastewater treatment plants,

pharmaceutical factories, and other pollution sources is a severe problem all around the world (Guo and Gao 2021, Wu et al. 2018). The main components of most odorous gases, H_2S and NH_3, commonly coexist, and they are released in large quantity with unpleasant smell, thus mainly contributing to the odor values in many emission sources (Huan et al. 2021a, b). Both NH_3 and H_2S are irritating gases. NH_3 is a colorless, strongly odorous, toxic, reactive, and corrosive gas with a low odor threshold (about 4 ppmv) (Morral et al. 2021). H_2S is a toxic, flammable, and colorless gas with an unpleasant smell, similar to that of rotten eggs with a low odor threshold (about 0.5 ppb) (García-Pérez et al. 2020; Ren et al. 2019). Both these two odorous gases can negatively affect human health and the environment (Sironi et al. 2010). Therefore, simultaneous removal of H_2S and NH_3 is of great significance for reducing odor pollution.

Nowadays, in order to reduce or eliminate air pollution caused by odorous gases, many technologies have been successfully developed. Among them, the common odor treatment methods can be classified into three categories including chemical (such as chemical scrubbers, thermal oxidation, catalytic oxidation, and ozonation), physical (condensation, adsorption, water scrubbers), and biological (such as biofilters, bioscrubbers, and biotrickling filter (BTFs)) technologies (Ferdowsi et al. 2017; Morral et al. 2022). Biological technologies with the advantages of high efficiency, environment friendliness, relatively low cost, convenient maintenance and management, and no secondary pollution have been regarded as the more feasible methods for treatment of low and moderate concentrations of waste gases (Ryu et al. 2011). As the representative biological technology, BTFs with the advantages of controllable operation conditions (including pH value, salt concentration, and circulating liquid nutrients), low cost, and low-pressure drop during long-term operation can intensively treat acidic degradation products of VOCs and acidic or alkaline odorous gases, have been regarded as the more feasible methods for treatment of low and moderate concentrations of waste gases (De Vela et al. 2021; Lebrero et al. 2012).

Since the microorganisms in the biofilm formed on the surface of packing materials are the major catalysts of biodegradation and gas deodorization, they can also affect gas removal efficiencies of BTFs; thus, they are regarded as the engine of the biotreatment process (Barbusinski et al. 2017; Rybarczyk et al. 2019). Moreover, microbial species composition is the most important controlled parameter for biofilm in BTFs (Rybarczyk et al. 2019; Schiavon et al. 2016). Biofilm formed by inoculated activated sludge is frequently used in BTF systems; however, the long period of acclimation ranging from several weeks to months, that is one of the drawbacks when using them as the inoculum (Watsuntorn et al. 2020). To date, in BTF, formed biofilm by inoculated specific strains including *Paracoccus versutus* MAL 1HM19 (Watsuntorn et al. 2020), *Paracoccus pantotrophus* NTV02 (Juntranapaporn et al. 2019), *Acinetobacter* sp. and *Alcaligenes faecalis* (Potivichayanon et al. 2006), nitrifying bacteria (Xue et al. 2010), and sulfide-oxidizing bacteria(Chen et al. 2014), with the easy-to-cultivate property and good removal performance, has also proven to be feasible method for treatment of odor pollution. Therefore, it is meaningful to develop new specific bacterial inoculum with high efficiency and good environmental adaptation for treatment of odorous pollution by BTFs.

As an important heterotrophic NH_4^+-N and S^{2-} removal strain, *Acinetobacter* sp. can convert NH_4^+-N to N_2 through heterotrophic nitrification and aerobic denitrification and utilize NH_4^+-N by assimilation (An et al. 2020; Yang et al. 2019). Moreover, as the sulfide-oxidizing bacteria, this genus can convert S^{2-} compounds into sulfate (Haosagul et al. 2020). *Acinetobacter* has been reported to be enriched in a pilot-scale biofilter after treatment with odorous gases (main components are NH_3 and H_2S at an inlet concentration of 200 to 210 ppmV) emitted from tanneries (removal efficacy of both NH_3 and H_2S was about 90–99%) (Gandu et al. 2021). Similarly, *Acinetobacter towneri* (MF765755) and sulfide-oxidizing bacteria have been found to play a key role in biogas H_2S removal efficiency and system stability (Haosagul et al. 2020). Therefore, *Acinetobacter* sp. can be considered an effective inoculum for removal of NH_3 and H_2S in BTFs.

In this study, the NH_4^+-N and S^{2-} removal strains named *Acinetobacter baumannii* and *Kosakonia oryzae* were isolated from long-term running BTFs in a pharmaceutical factory. The aim of this work is (1) to examine the effects of initial pH and culture temperature on the NH_4^+-N and S^{2-} removal capacities of isolated strains; (2) to prepare liquid inoculum for NH_4^+-N and S^{2-} removal; and (3) to investigate the NH_3 and H_2S removal efficiencies of liquid ready-to-use inoculums in the pilot-scale BTFs and explore the effects of these liquid inoculums on microbial diversity of packing materials.

Materials and methods

Materials, strains, and media

The odorous gas treatment system in a pharmaceutical factory (Changsha, Hunan, China) combines a washing tower (Fig. 1Aa), a BTF (Fig. 1Ab), a UV-photolysis equipment (Fig. 1Ac), and an air-exhausting equipment (Fig. 1Ad) (continuously running for 18 months). The packing material sample was collected from the long-term running BTF and used for bacterial screening. NH_4Cl (analytical grade)

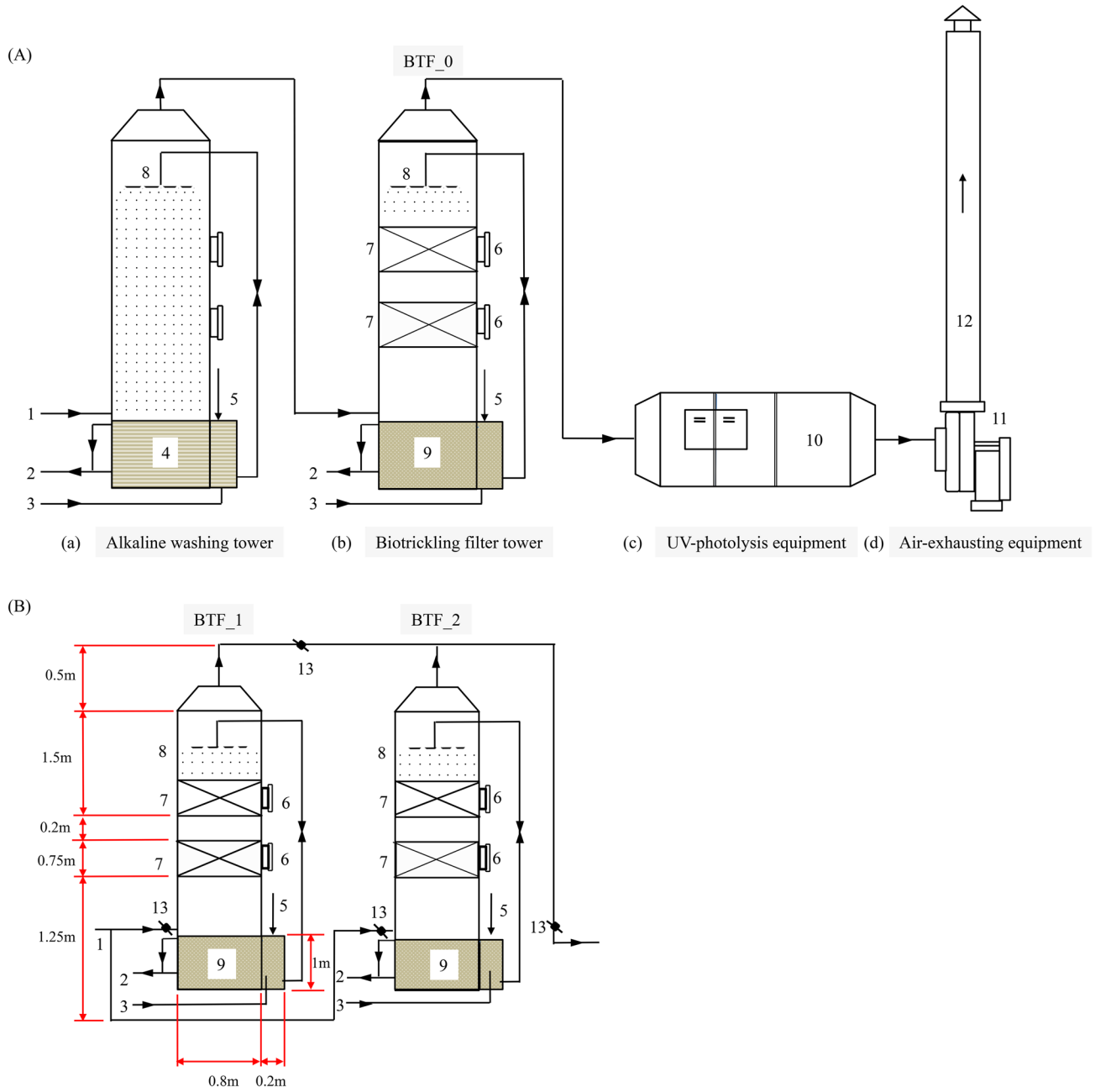

Fig. 1 Schematic diagrams of odorous gas treatment system in a pharmaceutical factory (**A**) and the pilot-scale BTFs in this study (**B**). 1, Factory exhaust gas inlet port. 2, Dewatering port. 3, Supplementary water inlet. 4, Alkaline washing circulating liquid tank. 5, Circulating liquid inlet port, pH detection port. 6, Sample feeding port. 7, Filler layer. 8, Spray port. 9, Biotrickling filter circulating liquid tank. 10, UV-photolysis. 11, Draught fan. 12, Air-exhausting pipe. 13, H_2S, NH_3, temperature detection port

and $Na_2S{\cdot}9H_2O$ (analytical grade) were purchased from Sinopharm Chemical Reagent Co. LTD (Shanghai, China).

The screened *Kosakonia oryzae* FB2-3 was preserved at China Center for Type Culture Collection (CCTCC M 2,020,508) and *Acinetobacter baumannii* L5-4 was preserved at College of Life Science and Technology, Huazhong Agricultural University (Wuhan, Hubei, China). Luria–Bertani (LB) medium (containing10.0 g L^{-1} tryptone, 5.0 g L^{-1} yeast extract, and 10.0 g L^{-1} NaCl; pH 7.0–7.4) was used for bacterial culture. Heterotrophic nitrification medium (containing trisodium citrate 5.0 g L^{-1}, $(NH_4)_2SO_4$ 0.5 g L^{-1}, and vickers salt solution 50 mL L^{-1}; pH 7.2 ~ pH7.4) was used for the determination of bacterial NH_3 removal capacity. Sulfur-removing strain medium (NH_4Cl 0.4 g L^{-1}, $MgCl_2$ 0.2 g L^{-1}, KH_2PO_4 2 g L^{-1}, Na_2CO_3 0.4 g L^{-1}, and $Na_2S{\cdot}9H_2O$

0.75 g L^{-1}; pH 7.2 ~ 7.4) was used for determination of bacterial H_2S removal capacity. The $Na_2S{\cdot}9H_2O$ was dissolved into double distilled water (ddH_2O) and passed through a sterile disposable syringe filter (0.22 μm, Millipore, Shanghai, China) before use. Nutrient solution containing glucose 8.0 g L^{-1}, K_2HPO_4 2.0 g L^{-1}, KH_2PO_4 2.0 g L^{-1}, NH_4Cl 0.4 g L^{-1}, $MgCl_2{\cdot}6H_2O$ 0.4 g L^{-1}, and $CaCl_2{\cdot}2H_2O$ 0.05 g L^{-1} (pH7.0 ~ 7.2) was used for bacterial activation and loading process in pilot-scale test.

Isolation, screening, and identification of NH_4^+-N and S^{2-} removal strains

For enrichment culture, 10 g packing materials sample (obtained from a long-term running BTFs in a pharmaceutical factory) was added into 90 mL sterile water with 20 sterilized glass beads and shaken at 30 °C at 200 r min^{-1} for 1 h. Subsequently, the suspension was inoculated into LB medium at 5% inoculum amount and cultured at 30 °C and shaken at 200 r min^{-1} for 24 h. The single colony bacteria were obtained by dilution coating method for the preliminary screening of NH_4^+-N and S^{2-} removal strains. Then, the NH_4^+-N and S^{2-} removal capacities of these stains were determined for secondary screening.

The colony morphology of FB2-3 and L5-4 single colonies on LB agar plate was observed. The 16S rRNA sequences of FB2-3 and L5-4 strains and those of their similar strains obtained from EzBioCloud's Identify service (http://www.ezbiocloud.net/) (Jeon et al. 2014) were used to identify the bacteria. The phylogenetic tree was constructed by using the construct/test neighbor-joining tree programs of MEGA6 software (Tamura et al. 2013).

Determination of NH_4^+-N and S^{2-} removal capacities of screened strains

NH_4^+-N concentration of the supernatant was determined according to Water Quality-Determination of Ammonium-Nessler's Reagent Colorimetric Method (the State Standard of the People's Republic of China, GB/T 7479–87) with some modified (detail see supporting materials). S^{2-} concentration in the supernatant was determined according to Water Quality-Determination of Sulfide-Methylene Blue Spectrophotometric Method (the State Standard of the People's Republic of China, GB/T 16,489–1996) with some modified (detail see supporting materials). The NH_4^+-N or S^{2-} removal capacity (RC) of screened strain was calculated as follows.

$$NH_4^+ - N \text{ or } S^{2-} \text{ removal capacity (RC)} = \frac{C_0 \times D_0 - C_t \times D_t}{C_0 \times D_0} \times 100\% \quad (1)$$

where C_0 is NH_4^+-N or S^{2-} concentration of initial medium (mg L^{-1}); D_0 is the corresponding dilution ratio during determination process; C_t is NH_4^+-N or S^{2-} concentration of the supernatant after treatment with bacteria (mg L^{-1}); and D_t is the corresponding dilution ratio during determination process. The data presented were the average of at least three assays.

NH_4^+-N and S^{2-} removal curves and specific rate of consumption

To evaluate the bacterial growth tolerance, the single colony was added into LB medium and cultured overnight. Then, the bacterial suspension was inoculated into LB medium at 1.0% (*vol./vol.*) under different stress conditions including pH (from 3.0 to 12.0) and culture temperature (from 10 to 45 °C). After 24-h culture, the $OD_{600\ nm}$ of the bacterial suspension was determined.

To examine NH_4^+-N and S^{2-} removal capacity and specific consumption rate, the single colony was added to LB medium and cultured overnight. Then, the bacterial suspension was inoculated into heterotrophic nitrification medium or sulfur-removing strain medium at 1.0% (*vol./vol.*). NH_4^+-N and S^{2-} removal capacities of the bacterial suspensions and concentration of cells (*N*) under different incubation time were monitored.

$$NH_3 \text{ or } H_2S \text{ specific rate of consumption (Qs)} = -\frac{1}{N}\frac{dC}{dt} \quad (2)$$

where *N* is the concentration of cells (g L^{-1}), *C* was the concentration of NH_4^+-N or S^{2-} (mg L^{-1}), and *t* is cultured time (h) (Arranz and Peinado 2017).

Preparation of liquid inoculum

The single colony was added 5.0 mL LB medium and cultured overnight. Then, the bacterial suspension was inoculated into 1.0 L LB medium at 1.0% (*vol./vol.*) at 28 °C. After 16-h culture, the bacterial suspension was centrifuged at 5000 rpm for 5 min twice. The bacterial precipitate was resuspended in 6 different solutions (0.01 M, 0.10 M, and 0.20 M phosphate buffer (PB); 0.05×LB, 0.1×LB, and 0.2×LB) and in different protective agents (1.0%, 3.0%, and 5.0% of trehalose, sucrose, sorbitol, PEG 6000, and glycerinum) with the resuspension solution volume of 1.0 L. Initial bacterial concentration (C_0) was approximately 2.16×10^9 CFU mL^{-1} for FB2-3 and 2.34×10^9 CFU mL^{-1} for L5-4. After 12-week storage at 4 or 25 °C, the bacterial concentration (C_t) of the liquid inoculums was measured by dilution coating method. Finally, survival rates (*A*) were calculated according to the following formula.

$$\text{Survival rate } A(\%) = \frac{C_t \times V_t}{C_0 \times V_0} \times 100\% \quad (3)$$

where C_0 and V_0 are initial bacterial concentration (CFU mL^{-1}) and initial volume (mL) of liquid inoculums; C_t and V_t are bacterial concentration (CFU mL^{-1}) and volume (mL) of liquid inoculums after storage. The data presented were the average of at least three assays. Liquid inoculum was used directly after preparation and storage.

Pilot-scale BTF tests

The pilot-scale test was carried out at Hangzhou, China; two BTFs with same size were parallel connected (Fig. 1B). One BTF was inoculated with liquid inoculums (BTF_2), and the other BTF without inoculation served as a control (BTF_1). The operation parameters were as follows: two layers of packing materials (bamboo charcoal, purchased from Tianjin Grace Environment Co., LTD, Tianjin, China, size 30 ~ 50 mm), total loading height of 1.5 m, total loading volume of 0.75 m^3, air flow of 300 $m^3\ h^{-1}$, empty bed residence time (EBRT) of 18 s, the volume of circulating spray liquid of 0.5 m^3, and uninterrupted circulating liquid spray flow of 500 L h^{-1}. Every BTF was equipped with a circulating fluid tank containing approximately 400 L loading nutrient solution. The 4.0 L FB2-3 and 4.0 L L5-4 were inoculated into circulating fluid tank for bacterial activization and loading process; in contrast, sterile water was added into the circulating fluid tank of control BTFs. After 14-day circulating spray, the loading nutrient solution was replaced with water. Then, the exhaust gas containing NH_3 and H_2S passed through BTFs, and pH of circulating liquid was monitored by a FE-20 type pH meter (Mettler Toledo Instruments Co., LTD, Switzerland). Temperature was measured by a AS887 type double channel thermocouple thermometer (Smart Sensor Group Co. LTD, Hong Kong, China). NH_3 and H_2S concentration was monitored by a MX-6 iBrid type compound gas detector (Industrial Scientific, USA). Pilot-scale test was performed for 84 days. Finally, removal efficiency (RE), inlet load (IL), and elimination capacity (EC) were calculated according to the following formulas.

$$NH_3 \text{ or } H_2S \text{ removal efficiency (RE)} = \frac{C\text{inlet} - C\text{outlet}}{C\text{inlet}} \times 100\% \quad (4)$$

$$NH_3 \text{ or } H_2S \text{ inlet load (IL)} = \frac{Q}{1000V} C_{\text{inlet}} \quad (5)$$

$$NH_3 \text{ or } H_2S \text{ elimination capacity (EC)} = \frac{Q}{1000V} C_{\text{inlet}} - C_{\text{outlet}} \quad (6)$$

where C_{inlet} is the inlet NH_3 or H_2S concentration (mg $(m^3)^{-1}$); C_{outlet} is the outlet NH_3 or H_2S concentration (mg $(m^3)^{-1}$); Q is air flow ($m^3\ h^{-1}$), and V is the total loading volume (m^3) of packing materials (Fernández et al. 2014).

Bacterial community sequencing and analysis

A total of 200 g bamboo charcoal packing materials (lower layer) were collected for bacterial community analysis after the BTFs tests running for 30 days. Bacterial genomic DNA in bamboo charcoal packing materials was extracted by QIAamp ® Fast DNA Stool Mini Kit (Qiagen, Germany). The hypervariable V3-V4 region of the 16S rRNA gene from bamboo charcoal packing materials was subjected to high-throughput sequencing using the primers 338 F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′) on an Illumina MiSeq platform, and subsequently analyzed by Personalbio Co., Ltd. (Shanghai, China). Each sample was tested in three parallel assays.

Data analysis

Statistical analysis was carried out using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The statistical significance was evaluated through Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results and discussion

Identification of NH_4^+-N and S^{2-} removal strains

The packing materials from the long-term running BTFs were used for isolating strains. As shown in Tables S1 and S2, FB2-3 and L5-4 strains exhibited the highest NH_4^+-N and S^{2-} removal capacity. Both FB2-3 and L5-4 were determined to be gram-negative, non-spore-forming, and rod-shaped bacteria. After culturing on LB agar plates at 28 °C for 48 h, FB2-3 colonies were yellowish, round, and smooth with a bulged surface (Fig. S1A), whereas L5-4 colonies were white, round, and smooth with a bulged surface (Fig. S1B). As shown in Fig. 2, the phylogenetic tree showed that partial 16S rRNA gene sequences of strain FB2-3 (GenBank accession No. MW812239.1) and L5-4 (GenBank accession No. MZ935668) exhibited the highest similarities to the 16S rRNA gene of *Kosakonia oryzae* Ola 51(T) (GenBank accession No. CP014007) (99.86%) and *Acinetobacter baumannii* ATCC 19,606(T) (GenBank accession No. ACQB01000091) (99.73%). Therefore, morphologically and phylogenetically, FB2-3 and L5-4 were identified as *Kosakonia oryzae* strain and *Acinetobacter baumannii* strain, respectively.

Acinetobacter baumannii have been recognized as an environmental pollutant degrading bacterial agent (Zhang et al. 2021). *Kosakonia oryzae* have been recognized as a

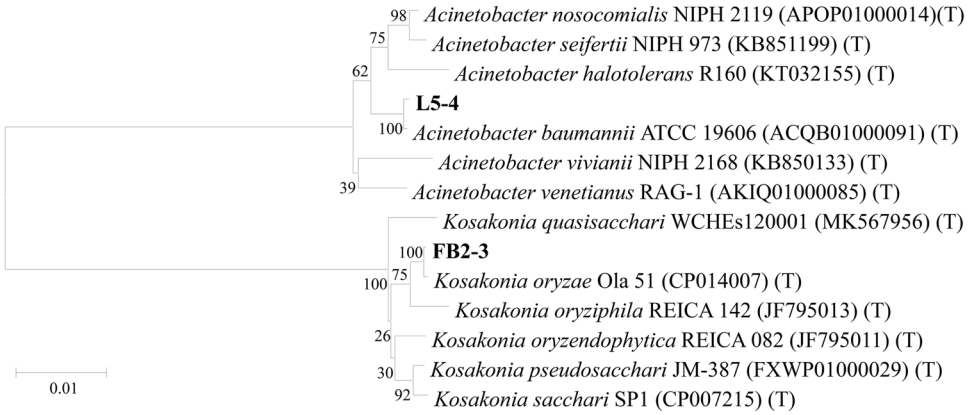

Fig. 2 Phylogenetic tree based on the partial 16S rRNA gene sequences of *Kosakonia* and *Acinetobacter* species

plant endophytic and growth-promoting strain (Preyanga et al. 2021) and have the biodegradation potential for pollutants (Dash and Osborne 2020). To the best of our knowledge, there has been no report on NH_4^+-N or S^{2-} removal capacity of *Kosakonia* sp. Therefore, FB2-3 and L5-4 with highest NH_4^+-N or S^{2-} removal capacity were selected for further investigation.

Effects of initial pH and culture temperature on NH_4^+-N and S^{2-} removal capacities for isolated bacteria

Previously, the autotrophic BTF inoculum *Rhodococcus* sp. zw11 showed growth tolerance at pH 5.5 to 6.5 and 20 to 28 °C (Zhang et al. 2009). The acid-resistant bacterium *Acinetobacter* sp. JR1 from pharmaceutical wastewater showed good adaptabilities toward pH 4.5 to 10.0 and 20 to 40 °C (Yang et al. 2019). By contrast, FB2-3 and L5-4 exhibited good growth tolerance (bacterial suspension $OD_{600\,nm} > 0.6$) at pH 4.0–9.0 and pH 4.0–10.0 (Fig. S2A), 10–37 °C and 15–45 °C (Fig. S2B) in LB medium after 24-h culture, respectively, indicating both FB2-3 and L5-4 have good adaptabilities to the application environment.

Previously, combination used *Enterobacter* sp. Z1 and *Klebsiella* sp. Z2 exhibited remarkable abilities for simultaneous nitrogen removal (Zhang et al. 2019). Moreover, the *Acinetobacter* sp. MU1_03 and *Alcaligenes faecalis* MU2_03 exhibited more than 91% of hydrogen sulfide removal efficiency while a mixture of the two strains was capable of 98% hydrogen sulfide removal in the bioscrubber systems (Potivichayanon et al. 2006). To explore the possibility of combining these two strains, effects of pH and culture temperature on NH_4^+-N and S^{2-} removal capacities and specific consumption rates under the optimal conditions were measured. Interestingly, under the same conditions of initial pH and culture temperature, FB2-3 + L5-4 exhibited higher NH_4^+-N and S^{2-} removal capacities than single strain (FB2-3 or L5-4) (Fig. 3). The highest NH_4^+-N and S^{2-} removal rates reached 96.46 ± 1.68% and 96.62 ± 1.61% at initial pH 7.0, respectively (Fig. 3A and B). The highest NH_4^+-N and S^{2-} removal rates reached 97.31 ± 1.62% and 98.57 ± 1.42% at 32.0 °C, respectively (Fig. 3C and D).

Specific substrate consumption rate for isolated bacteria

As shown in Fig. 4, under the optimal conditions (pH 7.0 and 32.0 °C), FB2-3 + L5-4 exhibited higher maximum specific NH_3 and H_2S consumption rates (243.86 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$ and 509.87 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$) than single strain FB2-3 (217.57 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$ and 277.55 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$) or L5-4 (166.21 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$ and 474.02 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$). Similarly, Wang et al. (2019) reported that *Acinetobacter* sp. JQ1004 exhibited the maximum specific degradation rate of ammonium occurred at the substrate concentration of 13.94 mg L^{-1} with a value of 3.97 g $g_{DCW}^{-1}\ day^{-1}$ (165.41 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$). Moreover, Cho K.S. et al. reported the heterotrophic bacteria *Xanthomonas* sp. strain DY44 exhibited the relatively lower maximum specific H_2S removal rate of 3.92 mmol $g_{dry\ cells}^{-1}\ h^{-1}$ (133.28 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$) at pH 7 and 30 °C (Cho et al. 1992). However, Lee et al. (2006) reported the chemoautotrophic bacteria *Acidithiobacillus thiooxidans* AZ1 exhibited a relatively higher maximum specific sulfur oxidation rate of 21.2 g $g_{DCW}^{-1}\ day^{-1}$ (883.33 $mg_{sub}\ g_{DW}^{-1}\ h^{-1}$), which was observed at pH 1.5.

These results indicated the simultaneous application of these two bacteria has better odorous gas removal effects than the use of one bacterium alone. As a result, we combined FB2-3 and L5-4 to prepare liquid ready-to-use inoculums and further applied in BTFs.

Preparation of FB2-3 and L5-4 liquid inoculums

Since the microbial communities inhabiting the packing materials play a crucial role in the biodegradation of the

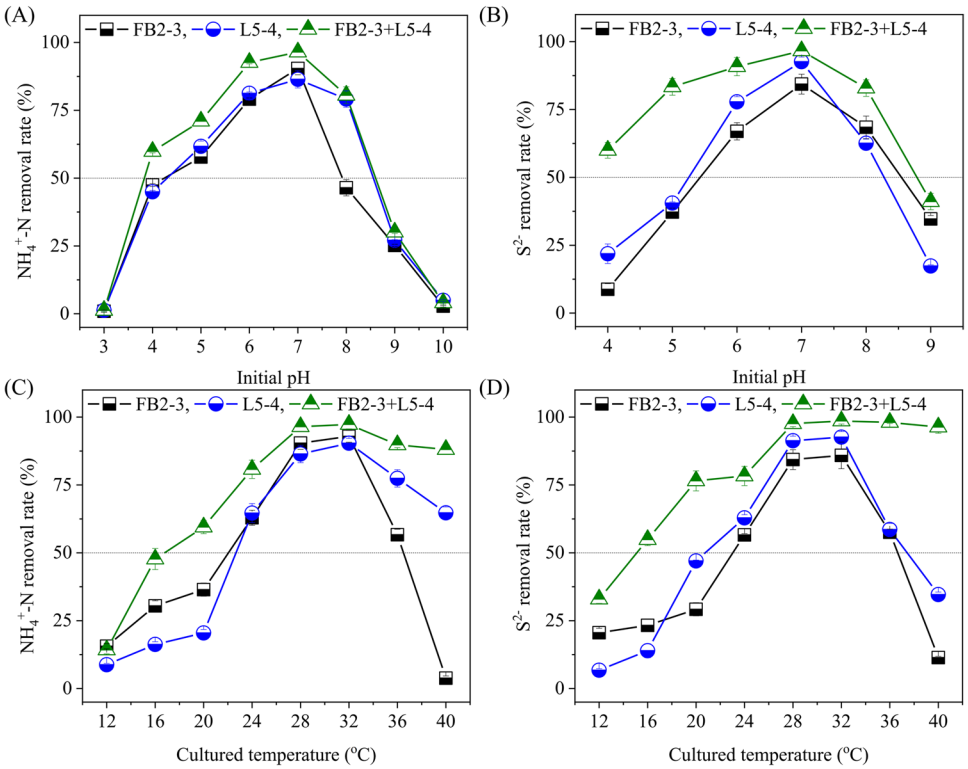

Fig. 3 Effects of initial pH (**A** and **B**) and culture temperature (**C** and **D**) on NH_4^+-N and S^{2-} removal capacities of FB2-3 (inoculation volume, 1.0%), L5-4 (inoculation volume, 1.0%), and FB2-3 + L5-4 (inoculation volume, 0.5% + 0.5%)

pollutants (Wu et al. 2018, 2020), the development of new bacterial agents is of great significance for increasing the removal efficiencies of odorous gases in BTFs. Nowadays, the major commercial bacterial agents usually are prepared by using spray-drying method with main bacteria being spore-forming bacteria, since the dormant spore has the advantages of high resistance to high temperature, hunger, dryness, radiation, and others (Yánez-Mendizábal et al. 2012). In contrast, the drying survival rates of the non-spore-forming strains, especially thin cell-wall gram-negative bacteria including FB2-3 and L5-4, are relatively low; thus, they are not suitable for spray-drying (Wu et al. 2021; Yánez-Mendizábal et al. 2012). In this study, we developed the liquid bacterial inoculum preparation methods specifically for FB2-3 and L5-4 bacterial agents.

As shown in Fig. 5A, the addition of 1.0% polyethylene glycol (PEG) as the protective agent significantly increased the survival rate of FB2-3 during the bacterial agent preparation and storage, which is consistent with the previous reports (Muangchinda et al. 2020; Torres et al. 2003). During the preparation of FB2-3 liquid inoculum, water and 1.0% of PEG were selected as the resuspension solution and the protective agent, respectively, and the storage temperature was set as 25 °C. The survival rate of FB2-3 was 72.13 ± 2.01% with bacterial concentration of 1.56×10^9 CFU mL^{-1} after 12-week storage.

As shown in Fig. 5B, the addition of 0.01 M PB and 1.0% trehalose as the resuspension liquid and the protective agent significantly improved the survival rate of L5-4 during the bacterial agent preparation and storage, which was in agreement with previous findings by Torres et al. (2003) and Sui et al. (2015). Therefore, 0.01 M PB and 1.0% trehalose were selected for preparation of L5-4 liquid inoculum. The survival rates of L5-4 were 44.94 ± 1.24% and 76.46 ± 4.67% after being stored at 25 °C and 4 °C for 12 weeks, respectively.

Considering the cost, the storage temperature was selected as 25 °C, and the bacterial concentration was 1.05×10^9 CFU mL^{-1} after 12-week storage. However, the survival rate of L5-4 in liquid inoculum was lower than that of FB2-3 under 25 °C storage conditions; thus, the addition of PB and trehalose needs to be further optimized in the future study.

Pilot-scale BTF tests

In the long-term running BTF_0, the NH_3 and H_2S removal capacities ranged from 45.2 to 100.0% and from 55.4 to 100% (Fig. 6A) at the NH_3 inlet concentration of 6.8 ~ 228.9 mg $(m^3)^{-1}$ and H_2S inlet concentration of 3.5 ~ 242.0 mg $(m^3)^{-1}$ (Fig. S3A). The daily average environment temperature was from 27.0 to 36.0 °C and running pH was from 5.3 to 7.2. The maximum NH_3 and H_2S EC of the BTF_0 were 52.3 g (NH_3) m^{-3} h^{-1} and 58.4 g (H_2S) m^{-3} h^{-1} with 57.1% and 60.3% removal efficiency, respectively. The critical NH_3 and H_2S elimination capacities (ECcrit) of the

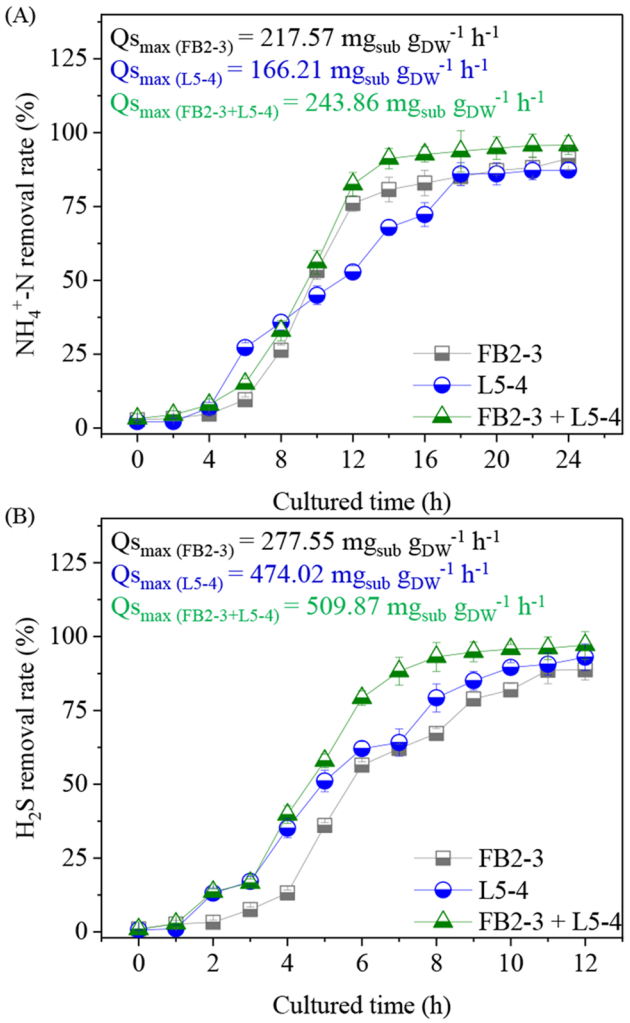

Fig. 4 NH_4^+-N (**A**) and S^{2-} (**B**) removal curves and specific substrate consumption rate of FB2-3 (inoculation volume, 1.0%), L5-4 (inoculation volume, 1.0%), and FB2-3 + L5-4 (inoculation volume, 0.5% + 0.5%) after culture at 28 °C and initial pH 7.0

BTF_0 were 18.2 g (NH_3) m^{-3} h^{-1} and 16.4 g (H_2S) m^{-3} h^{-1} with > 95% removal efficiency, respectively (Fig. 6C and D).

For pilot-scale BTF tests, in the control BTF_1, both the NH_3 and H_2S removal capacities were < 45.0% (Fig. S4) at NH_3 inlet concentration ranging from 0.8 to 345.7 mg $(m^3)^{-1}$ and H_2S inlet concentration from 1.6 to 413.4 mg $(m^3)^{-1}$ (Fig. S3B), respectively. Both the maximum NH_3 and H_2S ECs of BTF_1 were < 10.0%. Interestingly, after 8-day inoculation with FB2-3 and L5-4 liquid inoculums, NH_3 and H_2S removal capacities in BTF_2 respectively ranged from 87.8 to 100.0% and from 79.1 to 94.35% (Fig. 6B) at the NH_3 inlet concentration of 0.9 ~ 380.0 mg $(m^3)^{-1}$ and H_2S inlet concentration of 1.5 ~ 474.0 mg $(m^3)^{-1}$ (Fig. S3C). The daily average environment temperature was from 18.8 to 34.0 °C, and running pH was from 4.3 to 7.0. The maximum NH_3 and H_2S ECs of the BTF_2 were 136 g (NH_3) m^{-3} h^{-1} and 176 g (H_2S) m^{-3} h^{-1} with 89.5% and 93.0% removal efficiency, respectively. The critical NH_3 and H_2S elimination capacities (ECcrit) of the BTF_2 were 40.0 g (NH_3) m^{-3} h^{-1} and 71.4 g (H_2S) m^{-3} h^{-1} with > 95% removal efficiency, respectively (Fig. 6C and D). By contrast, the maximum NH_3 and H_2S EC were ranged from 38.7 to 241 g (NH_3) m^{-3} h^{-1} and 4.6 to 370 g (H_2S) m^{-3} h^{-1} after inoculated with *Acinetobacter* sp., *Alcaligenes faecalis*, *Paracoccus pantotrophus*, *Paracoccus versutus*, nitrifying bacteria, sulfide-oxidizing bacteria, or activated sludge (Table 1) (Aroca et al. 2007; Chen et al. 2014, 2019; Huan et al. 2021a; Juntranapaporn et al. 2019; Potivichayanon et al. 2006; Watsuntorn et al. 2020; Xue et al. 2010). The results indicated that the BTF inoculated with FB2-3 and L5-4 liquid inoculums exhibited excellent NH_3 and H_2S removal capacities.

Bacterial community analysis of bamboo charcoal packing materials after running

Biofilm formed on the packing materials is the key factor affecting the odorous gas removal from BTFs (Mudliar et al. 2010; Wu et al. 2020). As shown in Figure S5, biofilm formation on bamboo charcoal was clearly observed by naked eye and Scanning Electron Microscopy (SEM) after inoculated with FB2-3 and L5-4 liquid inoculums and running for 84 days. Since the high-throughput sequencing is frequently used method to examine the microbial communities, especially the unculturable strains, in the biofilm on the surface of packing materials in BTFs (Wu et al. 2018), the microbial communities of packing materials were analyzed by high-throughput sequencing in this study. A total of 1,749,828 (range from 91,702 to 129,928) valid sequences were extracted from the twelve samples (Table S3). In total, 52 to 2053 operational taxonomic units (OTUs) were detected from the twelve samples at the genus level. The biodiversity of bamboo charcoal packing material samples inoculated with the liquid inoculums was significantly decreased, relative to that of bamboo charcoal packing material samples without inoculation (Fig. S6), indicating that addition of the liquid inoculums could decrease the biodiversity of the microbial community (Sun et al. 2020).

This study explored the similarities and differences in the bacterial communities among the bamboo charcoal packing material samples at the genus levels after BTF running. In the long-term running BTFs, the dominant bacteria (BPM_0) in the packing materials were *Sulfobacillus* (50.33 ± 8.42%), *Mycobacterium* (7.45 ± 1.31%), *Alicyclobacillus* (5.40%), *Novosphingobium* (4.57 ± 1.86%), and *Azospirillum* (1.45 ± 0.59%). In BTF_1, the dominant bacteria in the bamboo charcoal packing materials without liquid inoculum inoculation (BPM_1) were *Brochothrix* (20.94 ± 3.08%), *Bacillus* (1.66 ± 2.62%), *Lactobacillus* (10.76 ± 6.51%), *Aquabacterium* (6.54 ± 2.90%), and *Serratia* (6.80 ± 0.68%), whereas the dominant bacteria in the bamboo charcoal packing materials (BPM_2) inoculated with

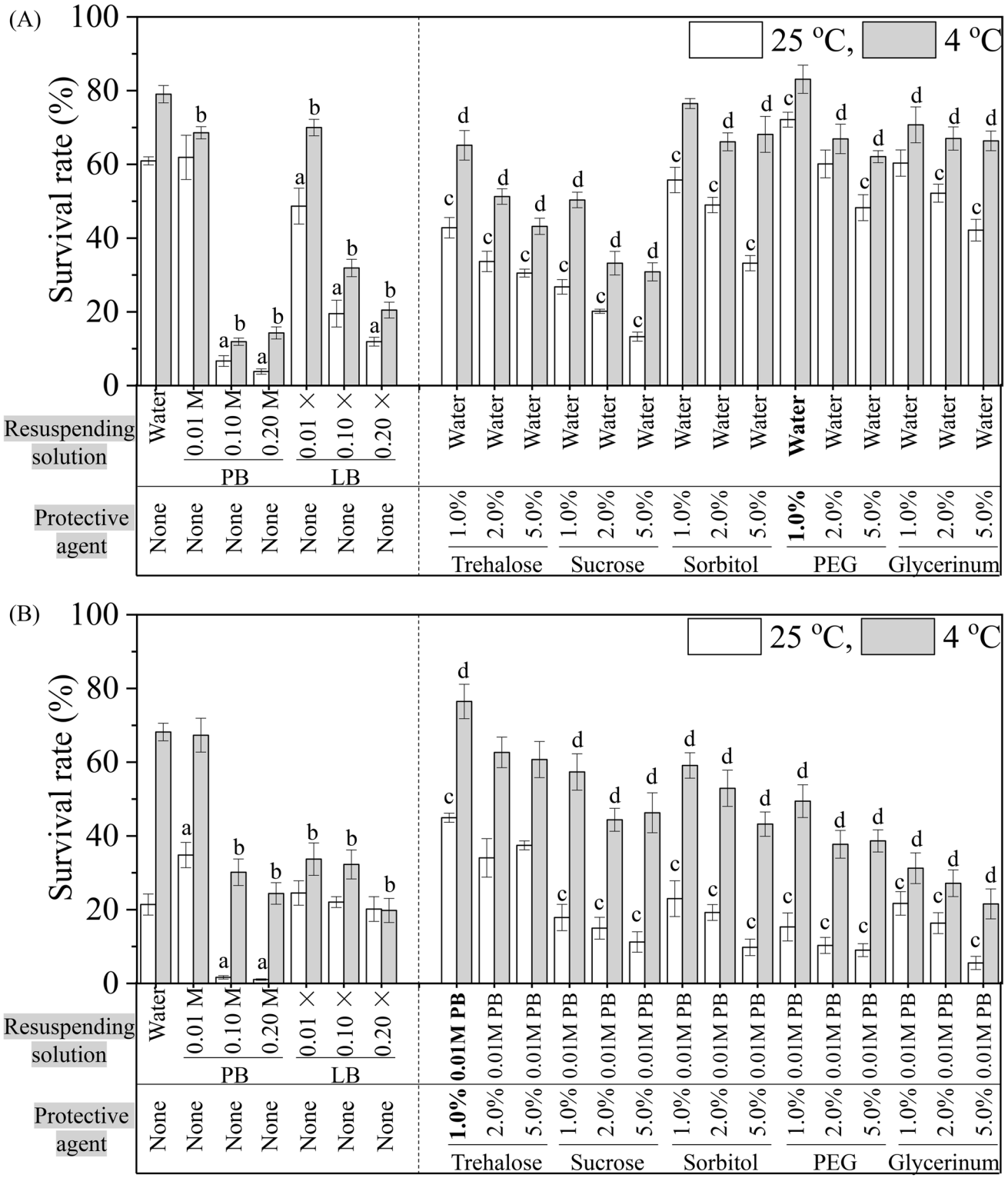

Fig. 5 Effects of different resuspension solutions and protective agents on survival rates for FB2-3 (**A**) and L5-4 (**B**) in liquid inoculums after 12-week storage at 25 °C or 4 °C. [a], Statistically significant compared with the control (water as the resuspension solution) after storage at 25 °C ($P < 0.05$). [b], Statistically significant compared with the control (water as the resuspension solution) after storage at 4 °C ($P < 0.05$). [c], Statistically significant compared with the control (water and 0.01 M PB respectively serving as the resuspension solution of FB2-3 and L5-4, with no protective agent) after storage at 25 °C ($P < 0.05$). [d], Statistically significant compared with the control (water and 0.01 M PB respectively serving as resuspension solution of FB2-3 and L5-4, with no protective agent) after storage at 4 °C ($P < 0.05$)

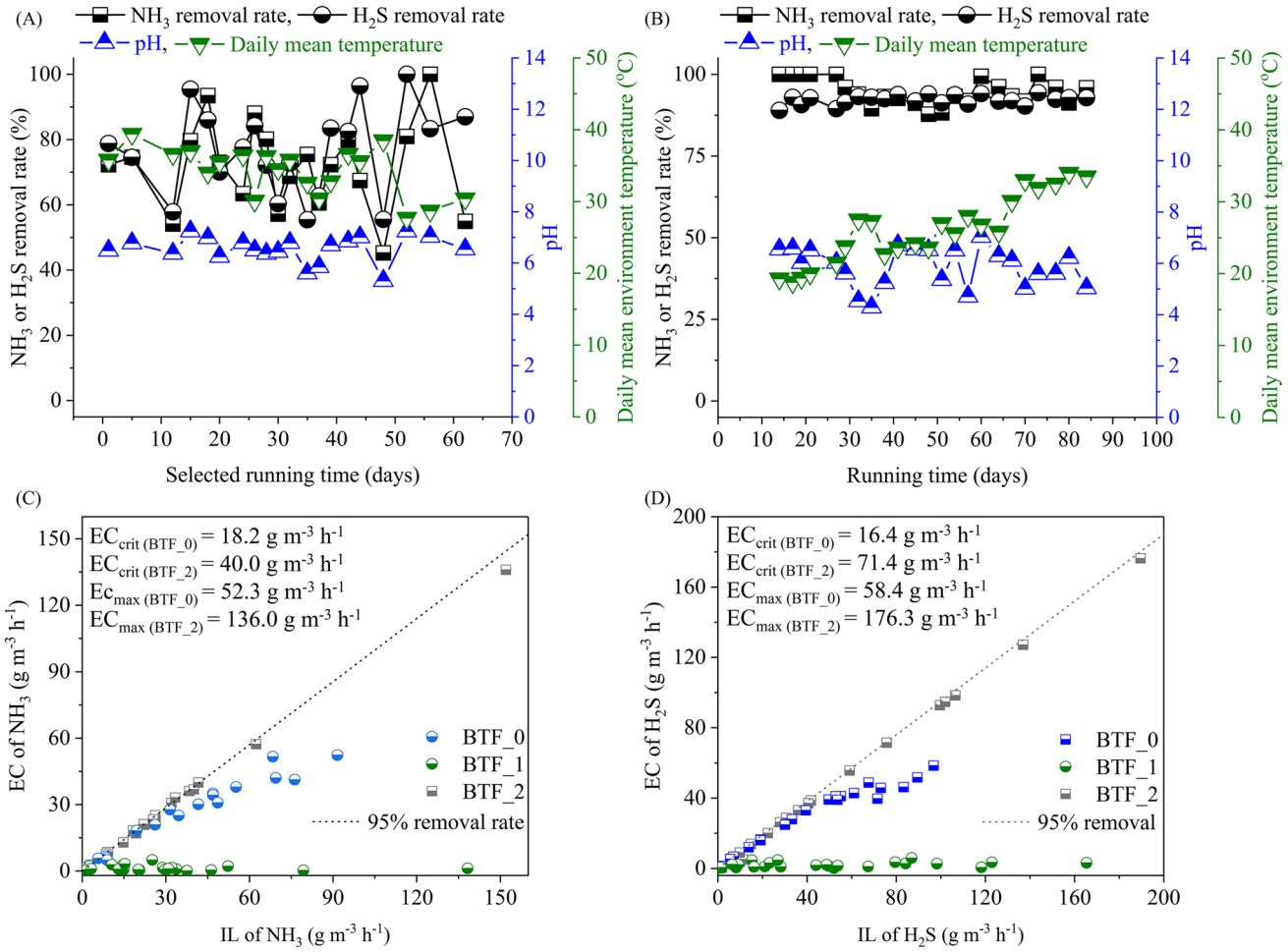

Fig. 6 The operating parameters (pH, environment temperature, removal rates of NH_3 and H_2S) in the BTFs, BTF_0 (**A**) and BTF_2 (**B**). Inlet load (IL) and elimination capacity (EC) of NH_3 (**C**) and H_2S (**D**) in the BTFs

the FB2-3 and L5-4 liquid inoculums were *Acinetobacter* (63.73 ± 6.32%), *Mycobacterium* (17.84 ± 5.93%), *Kosakonia* (4.25 ± 1.78%), and *Sulfobacillus* (9.54 ± 1.21%) (Fig. 7A). *Acinetobacter* genera have been reported to be enriched in a pilot-scale biofilter (Gandu et al. 2021) and have the ability to remove H_2S (Potivichayanon et al. 2006). *Mycobacterium* and *Sulfobacillus* genera were likely the players for H_2S oxidation, based on their capability of the oxidation of sulfur compounds (Bu et al. 2022). At present, there is no relevant report on the removal of NH_3 or H_2S by *Kosakonia* genera. In addition, as shown in Table 1, the composition of BTF_2 microflora is different from that reported previously.

Further principal coordinates analysis (PCoA) based on weighted UniFrac distances (Fig. 7B) showed that the bamboo charcoal packing material samples exhibited high similarities in bacterial community composition after liquid inoculum inoculation in different pilot-scale tests, whereas the bamboo charcoal packing materials samples with or without inoculation and packing materials samples from the long-term running BTFs exhibited differences. Moreover, the comparison of bacterial communities at the genus level showed that the relative abundances of *Acinetobacter* and *Kosakonia* were significantly increased in bamboo charcoal packing materials inoculated with the liquid inoculums in BTF_2 (Fig. S7). However, both the heterotrophic bacteria *Kosakonia* and *Acinetobacter* are not the abundant genus in the long-term running BTFs_0. This result might be due to the growth rate of some dominant autotrophic bacteria in BTFs_0 is relatively lower than that of heterotrophic bacteria under the screening condition. Nevertheless, after inoculation with easily cultivated liquid inoculums, the new microbial community composed of *Kosakonia* and *Acinetobacter* and other genera were formed in the packaging materials also exhibited effectively treat NH_3 and H_2S exhaust gases in BTFs.

Table 1 Comparison of the maximum NH_3 or H_2S elimination capacity and microbial community of current study with previous researches

Air intake				EBRT	Inoculated microorganisms	Packing materials	Microbial community composition	EC ($g\ m^{-3}\ h^{-1}$)		Reference
Source	Composition	Inlet concentration								
		NH_3	H_2S					NH_3	H_2S	
Pharmaceutical factory	H_2S, NH_3 and mixture of VOCs	6.8 ~ 228.9 mg $(m^3)^{-1}$	3.5 ~ 242.0 mg $(m^3)^{-1}$	18 s	*Bacillus* sp., *Lactobacillus* sp., etc	Bamboo charcoal	*Sulfobacillus*, *Mycobacterium*, *Alicyclobacillus*, *Novosphingobium*	58.4	52.3	This study BTF_0
		0.8 to 345.7 mg $(m^3)^{-1}$	1.6 to 413.4 mg $(m^3)^{-1}$	18 s	None	Bamboo charcoal	Brochothrix, Bacillus, Lactobacillus, Aquabacterium	< 10	< 10	This study BTF_1
		0.9 ~ 380.0 mg $(m^3)^{-1}$	1.5 ~ 474.0 mg $(m^3)^{-1}$	18 s	*Kosakonia oryzae* FB2-3 and *Acinetobacter baumannii* L5-4	Bamboo charcoal	*Acinetobacter*, *Mycobacterium*, *Kosakonia*, *Sulfobacillus*	136	176	This study BTF_2
Laboratory-synthesis	$H_2S + N_2$	-	10–100 ppmv	13–32 s	*Acinetobacter* sp. and *Alcaligenes faecalis*	Polypropylene pall ring	Undetected	-	19.2	Potivichayanon et al. (2006)
Laboratory-synthesis	Synthetic biogas	-	100–2000 ppmv	120 s	*Paracoccus pantotrophus*	Random packing media	Undetected	-	83.9	Juntranapaporn et al. (2019)
Wastewater lift station	Polluted air	-	3–20 ppmv	25 s	Sulfide-oxidizing bacteria	Bamboo charcoal and ceramsite	Undetected	-	4.6	Chen et al. (2014)
Wastewater odor	Trimethylamine, H_2S, styrene, methyl mercaptan	-	0–20 ppmv	10.9 s	Activated sludge	Bamboo charcoal	Undetected	-	6.6	Chen et al. (2019)
Biogas residue	$H_2S + NH_3$	0–500 ppmv	0–500 ppmv	31.8 s	Sludge	Polyhedral spheres	*Candidatus_alysiosphaera Thiobacillus Nitrosomonas Dechloromonas Paracoccus versutus*	38.77	84.57	Huan et al. (2021a)
Laboratory-synthesis	$N_2 + H_2S$	-	100–4000 ppmv	180 s	*Paracoccus versutus*	PUF cubes	*Brevundimonas* sp. *Microbacterium* sp. *Ochrobactrum* sp. *Pseudomonas* sp.	-	113	Watsuntorn et al. (2020)
Composting	NH_3	3–197 mg $(m^3)^{-1}$	-	96 s	Nitrifying bacteria	Fiber with spheroidal or ellipsoidal shapes	Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria	241	-	Xue et al. (2010)
Laboratory-synthesis	H_2S	-	-	45 s	*Thiobacillus thioparus* and *Acidithiobacillus thiooxidans*	Polyethylene rings	Undetected	-	370	Aroca et al. (2007)

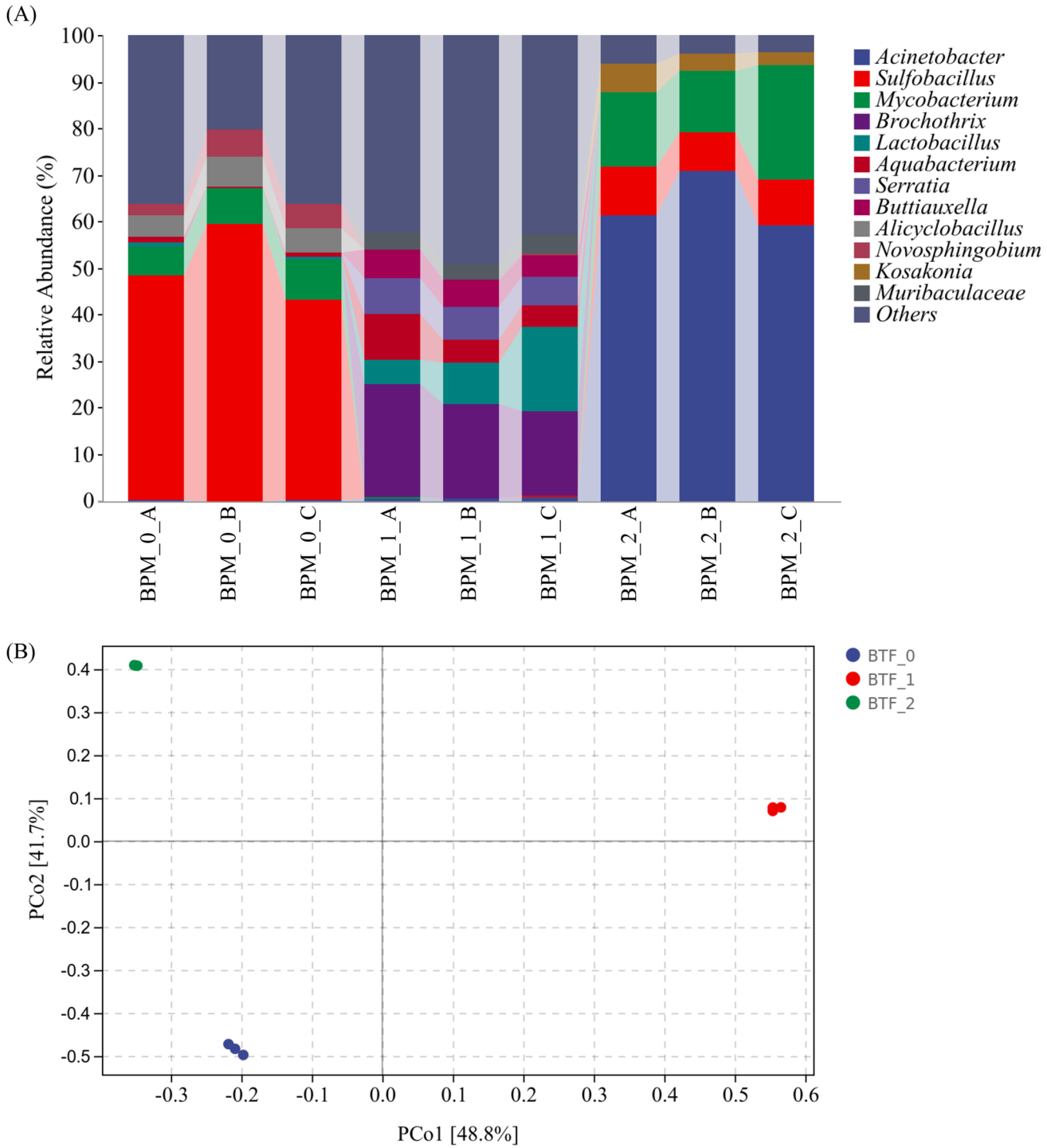

Fig. 7 Principal coordinates analysis (PCoA) of bacterial communities based on weighted UniFrac distances (**A**). The dominant bacterial communities at the genus level in different packing material samples in different BTFs (**B**)

Conclusion

In the present study, the NH_4^+-N and S^{2-} removal strains FB2-3 and L5-4 were successfully isolated from the packing materials in the long-running BTFs. Combined utilization of FB2-3 and L5-4 exhibited high NH_4^+-N and S^{2-} removal capacities of 97.31 ± 1.62% with maximum specific NH_3 consumption rate 243.86 mg_{sub} g_{DW}^{-1} h^{-1} and 98.57 ± 1.12% with maximum specific H_2S consumption rate 509.87 mg_{sub} g_{DW}^{-1} h^{-1} and good tolerance to pH, temperature, and salt.

Water and 0.01 M PB were selected as the resuspension solution, and 1.0% of PEG and 1.0% of trehalose as the protective agent respectively for preparation of FB2-3 and L5-4 liquid inoculums at 25 °C. The pilot-scale test of BTF inoculated with FB2-3 and L5-4 showed that NH_3 and H_2S could be effectively removed (removal rates > 87%, maximum NH_3 and H_2S ECs were reached 136 g (NH_3) m^{-3} h^{-1} and 176 g (H_2S) m^{-3} h^{-1} with 89.5% and 93.0% removal efficiency, respectively) from the exhaust gases, and that the relative abundances of genera *Acinetobacter* (L5-4) and *Kosakonia* (FB2-3) were significantly increased in the packing materials, whereas the diversity of entire bacterial communities was decreased, indicating that these two strains have the potential to be developed into the liquid ready-to-use inoculums for effectively removing NH_3 and H_2S from BTFs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-24426-2.

Acknowledgements Great gratitude goes to linguistics Prof. Ping Liu from Huazhong Agricultural University, Wuhan, China, for her work at English editing and language polishing.

Author contribution All authors contributed to the study conception and design. Investigation, formal analysis, and writing—original draft were performed by QZ. PW, BC, QW, FC, HW, and XS contributed to methodology, resources, and data curation. LX and YM contributed to co-supervision and methodology. The supervision, project administration, funding acquisition, and writing—review and editing were ensured by ZC. All authors read and approved the final manuscript.

Funding The work was supported by the Fundamental Research Funds for the Central Universities of China (Grant No. 2662021FW006) and Key Research and Development Program of Hubei Province, China (Grant No. 2020BAB095).

Data Availability Not applicable.

Declarations

Ethical Approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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