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RESEARCH ARTICLE

Bioaerosolization behavior along sewage sludge biostabilization

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HIGHLIGHTS

- Aerosolization behavior during a lab-scale sludge biostabilization was determined.
- Many pathogenic species were identified to be preferentially aerosolized.
- Bioaerosol concentration along the biostabilization ranged from 160 to 1440 cell/m^3.
- Sludge aerosolization behavior was different with that of other biowaste.

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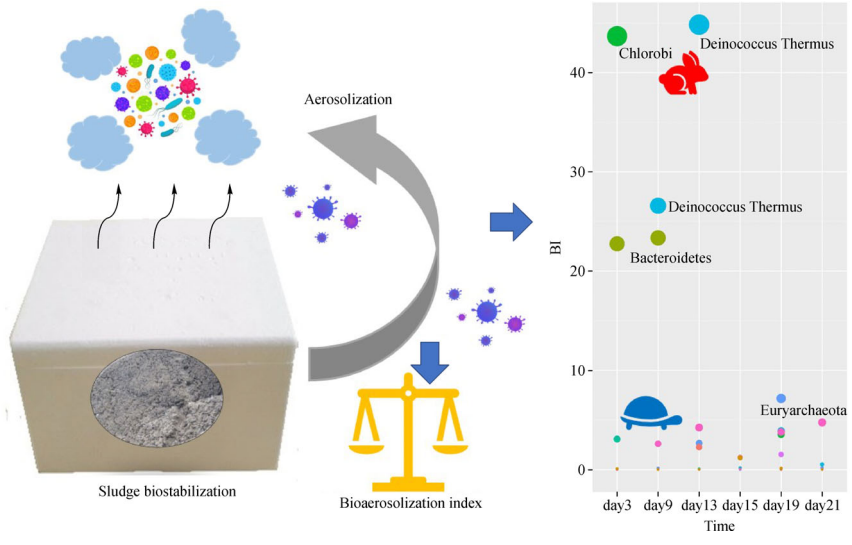
GRAPHIC ABSTRACT



ABSTRACT

Biostabilization is a cost-effective method for the beneficial utilization of sewage sludge. However, during the operation of sludge biostabilization, some microbial species could be released into the atmospheric environment from the solid-phase of sludge easily and present a high risk to human health. This study aimed to evaluate the risk of bioaerosol during sludge biostabilization. We found a total of nine bacterial phyla, one archaeal phylum, and two fungal phyla in the bioaerosol samples. Among them, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes,* and *Ascomycota* were the dominant phyla. In addition, the bioaerosolization indexes (BI) of prokaryotic phyla and fungal phyla ranged 0–45 and 0–487, respectively. *Massilia*, *Pseudarthrobacter*, *Pseudomonas*, *Tremellales spp.,* and *Fusarium* were the preferentially aerosolized microbial genera with maximum bioaerosolization indexes of 19962, 10360, 1802, 3055, and 7398. The bioaerosol concentration during the biostabilization ranged from 160 to 1440 cell/m^3, and we identified species such as *Stenotrophomonas rhizophila* and *Fusarium graminerum* with high bioaerosolization indexes that could be threats to human health. *Euryachaeota*, which belongs to archaeal phyla, had the highest biostabilization index in our study. We also found that *Pseudarthrobacter* was the easiest to aerosolize during the sludge biostabilization process.

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1 Introduction

Municipal and industrial sewage sludge has become a global problem. Composting is a useful measure to reduce and stabilize organics contained in sewage sludge and to

simultaneously obtain nutrients from the by-products for land application (Zittel et al., 2020). According to the Chinese Standard GB 4284-2018, stabilized and heavy metal-free sludge is cleared for agricultural use. Several studies have reported that bacteria and fungi both play an essential role in the degradation of sludge organics (Amir et al., 2008; Zhao et al., 2019). However, the composting process of sewage sludge could also cause the problem of odor emission, which is closely related to microbiological activities, and correspondingly, operational conditions and microbial community (Ding et al., 2019; Jiang et al., 2019; Robledo-Mahón et al., 2019; He et al., 2020). Furthermore, microorganisms could be released into the atmospheric environment from the solid phase during mechanical actions, e.g., shredding and turning the composting pile (Mbareche et al., 2017; Feeney et al., 2018). Some researchers have reported that the bioaerosol emitted from the sewage sludge composting process could reach the concentration of 1.03×10^5 CFU/m^3 without mechanical agitation and thereby could cause adverse health impacts (Chung, 2007; Kim et al., 2012; De Giudici et al., 2013). The aerosolization behavior of microorganisms emission by composting has been investigated for carbohydrate-rich biowaste (He et al., 2019), and manure/pig carcasses with relatively high protein content (Veillette et al., 2018). Nonetheless, there has been little research exploring the preferential aerosolization behavior of airborne microorganisms during sewage sludge composting.

The concept of preferential behavior was first proposed by Parker et al. to describe the aerosolization behavior of specific microorganisms (Parker et al., 1983). In addition, Moletta et al. showed that some species of microbial communities in the anaerobic digestor presented a high level of aerosolization while others were unable to form aerosol (Moletta et al., 2007). Many factors including morphology and hydrophobicity can influence preferential aerosolization and spore formation of microorganisms (Perrott et al., 2017; Lainhart, 2018; Liu et al., 2020a). Many pathogenic microorganisms such as *Actinobacteria* and *Mycobacterium* have shown preferential aerosolization during composting and wastewater treatment (Veillette et al., 2018; Liu et al., 2020a). Other microorganisms such as *Streptococcus suis* and *Pseudomonas aeruginosa* showed preferential aerosolization through bioaerosol generation experiments in the laboratory (Gauthier-Levesque et al., 2016; Perrott et al., 2017). Constant exposure to these bioaerosols could have serious health effects and cause occupational diseases among workers, including asthma, chronic obstructive pulmonary diseases, and lung cancer (Robertson et al., 2019). In previous studies, pathogenic microorganisms, including *Pseudomonas* and *Aspergillus,* were reported to maintain a particular relative abundance throughout the sewage sludge composting process (Guo et al., 2020; Robledo-Mahón et al., 2020). However, it is unknown whether these pathogens performed preferential aerosolization. Therefore, it is necessary to investigate the microbial community and aerosolization potential of the bioaerosol emission from sewage sludge to assess and prevent potential health risks.

To address the current knowledge gaps in the potential aerosolization risk for sewage sludge treatment, in the present study, a laboratory-scale sewage sludge biostabilization was simulated to investigate the microbial community and aerosolization behavior of microorganisms, including bacteria, archaea, and fungi, at different stages of sewage sludge biostabilization. We aimed to distinguish the preferential aerosolization potential of various microorganisms during this process for a protein-rich biowaste.

2 Materials and methods

2.1 Experimental materials and the biostabilization process

Sewage sludge usually can not be composted alone due to its poor gas permeability (Zhou et al., 2014). Therefore, in the present study, feedstock materials comprised a mixture of dewatered sewage sludge and straw. We collected the sludge from Quyang Sewage Treatment Plant located in Shanghai, China and the straw with a grain size of 5 mm from a farm. We performed the experiment with the feedstock mixture of straw and sludge in the wet mass proportion of 1:2. The biostabilization was conducted for 21 days. The reactor used for biostabilization was made of a 3-cm thick white foam box with a removable lid (Fig. 1). The insulation layer made of foam with the thickness of 5 cm was used for the reactor to keep the temperature. Approximately 30 kg feedstock mixture was fed into the reactor and stacked to a height of 25 cm. Two ventilation pipes with diameters of 3 cm were placed approximately 10 cm from the bottom of the reactor and linked with a flowmeter and a blower outside. Aeration was provided at a rate of 2.5–3 m^3/h, and the blower was operated for 15 min and then stopped for the next 15 min in the early 0–7 days and stopped for 45 min in the later period of 8–21 days to alleviate the heat loss. Temperature and oxygen were monitored by the probes placed in the lower part of the reactor and inserted into the middle of the material pile for biostabilization. A total of one oxygen probe and one temperature probe are used. The moisture content was adjusted on the 15th day to promote the metabolic process of microorganisms.

The mixtures of sludge and straw were taken from the middle of the pile on days 3, 5, 9, 13, 15, 19, and 21 in triplicate. The solid samples were labeled S3, S5, S9, S13, S15, and S19, respectively, corresponding to the sampling days. Aliquots of the samples were used for physicochemical analysis, and others were stored at –40°C until DNA extraction.

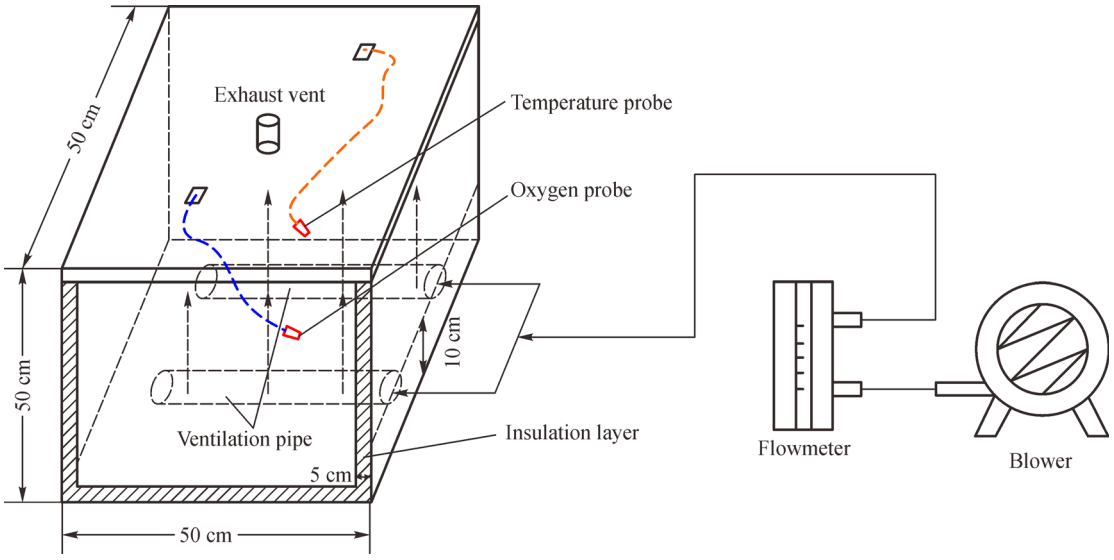

Fig. 1 Reactor for sewage sludge biostabilization.

2.2 Sampling of bioaerosol

The bioaerosol samples were taken on days 3, 5, 9, 13, 15, 19, and 21, corresponding to the bio-stabilized samples. After we opened the lid partly and stopped the blower, the liquid cyclonic impactor Coriolis μ® (Bertin Instruments Co. Ltd., France) was installed at a height of 0.5 m from the ground close to the biostabilization reactor, such that it was directly exposed to the lip opening. This allowed the aspirated air to continuously flush the surface of the pile. Bioaerosol samples were collected at 200 L/min for 2 h, and a total of 7.5 mL sterile water was used as the collection matrix, which was poured into and filled the sampling cones. Each time, the lid was opened partly with an equal opening and the bioaerosol sample was collected at the sample position to guarantee the comparability of samples as accurately as possible. The gaseous samples were labeled as G3, G5, G9, G13, G15, G19, and G21 in chronological order. For each sample collection, three parallels of bio-stabilized samples and two parallels of bioaerosol samples were used for the test. The samples were immediately used for quantitative analysis of microorganisms, and the other bioaerosol samples was stored at –40°C for DNA extraction.

2.3 Physicochemical analysis for the solid samples

We used similar methods for the physicochemical analysis for the solid samples as in our previous study (He et al., 2019) including tests for total solid (TS) and volatile solid (VS). In addition, other physiochemical indexes such as electrical conductivity (EC), pH, NH_4^+-N, dissolved organic carbon (DOC), and dissolved nitrogen (DN) were measured after mixing fresh solid samples with sterile water in a ratio of 1:10 in weight for 10 h.

2.4 DNA extraction and high-throughput sequencing

The collection matrix in the sampling cone was transferred to the centrifuge tube and mixed with the sterile water adjusted to the volume of 8 mL. Hereafter, 7 mL of the mixture was used for DNA extraction and qualitative analysis of bacteria, archaea, and fungi. DNA isolation kit (12888-100, MOBIO Laboratories Inc., Canada) was used for DNA extraction, and the extraction step followed the manufacturer's instructions. As for the solid samples, 0.5 g of each sample was mixed with the sterile water and used for DNA extraction. After extraction, the sample was stored at –20°C until high-throughput sequencing. As described in our previous work (He et al., 2019), the DNA samples were quantified via a UV–vis spectrophotometer (Nanodrop 2000c, Thermo Scientific, USA), and 1% agarose gel electrophoresis was used to test the DNA integrity. The DNA samples were then subjected to high-throughput sequencing using the Illumina MiSeq platform (Majorbio Bio-pharm Technology Co., Ltd, Shanghai, China) using the primers ArBa515F_Arch806R for prokaryotic 16S rRNA gene and SSU0817F_1196R for fungi 18S rRNA gene, respectively.

PCR was performed with TransStart® Fastpfu DNA Polymerase (TransGen AP221-02, TransGen Biotech, China). The reaction system consisted of 4-μL 5 × FastPfu Buffer, 2 μL dNTPs, 0.8 μL of each primer, 0.4 μL FastPfu polymerase, 0.2 μL bovine serum albumin, 10 ng template DNA, and sterile water to make a final volume of 20 μL. PCR reaction parameters were set as follows: one cycle at 95°C for 3 min, followed by cycling times of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. Finally, we cooled the sample to 72°C for 10 min. After the PCR amplification was completed, 3 μL of PCR product was used for quality test check by an electrophoretogram with

2% agarose gel concentration. The microbial diversity of sequences obtained were analyzed using the I-Sanger platform (I-Sanger Inc., China).

2.5 Quantitative analysis of microorganisms in the bioaerosol sample

For the quantitative analysis of microorganisms, 1 mL of bioaerosol sample in the sampling cone was treated by immobilization and hybridization. The experimental process was the same as in our previous work (He et al., 2019). The bioaerosol samples were mixed with the immobilization agent at first. Then, immobilized samples were placed into the hybridization tube again and incubated at room temperature in the dark. After eluting the hybridization buffer and DAPI, we counted the number of fluorescent spots under an epifluorescent confocal microscope.

2.6 Bioaerosolization Index (BI)

In the following sections, Bioaerosolization Index (BI) was used to quantify the aerosolization behavior of microorganisms in our study. The calculation of BI is presented in Eq. (1), which is the concentration factor (Moletta-Denat et al., 2010).

$$\mathrm{BI} = \frac{\mathrm{RA}_{\mathrm{aerosol}}}{\mathrm{RA}_{\mathrm{biostabilization}}}. \quad (1)$$

In the calculation, $\mathrm{RA}_{\mathrm{aerosol}}$ refers to the relative abundance of microorganisms in the bioaerosol sample. $\mathrm{RA}_{\mathrm{biostabilization}}$ refers to the relative abundance of microorganisms in the bio-stabilized sample corresponding to the bioaerosol sample. Under the same condition, the microorganisms with a high level of BI are easier to aerosolize than others (Moletta-Denat et al., 2010). Laboratory-scale biostabilization eliminated interference to the aerosolization from other factors, including mechanical operations and meteorological factors. Microorganisms with higher BI could be released to the air more easily.

3 Results and discussion

3.1 Temporal changes in the physicochemical properties of bio-stabilized products

The physiochemical properties of the solid samples along the 21 days of the biostabilization process are shown in Table 1 and Fig. A1. In general, the concentration of DOC and DN presented an increasing trend initially, up to 2334±153 and 1017±52 mg/L, respectively. This was caused by the degradation of organic compounds. After nine days, a continuous decrease was observed, which indicated the metabolic processes of microorganisms. Finally, the concentration of DOC and DN decreased to 1040±33 and 599±10 mg/L on the 21st day. Intermediate metabolites which can be detected in Water Extractable Organic Matter (WEOM) like water soluble C/N ratio indicated the biological metabolism. (Lu et al., 2018) The ratio of DOC/DN was calculated as shown in Table 1. It increased rapidly within the first five days and reached the maximum of 2.97. Then it decreased continuously and stabled at around 1.7 on the 21st day which indicated the reduction of biological metabolism. The concentration of NH_4^+-N showed a rising trend to 410±37 mg/L, followed by a decreasing one, and a rising trend again to 290±8 mg/L on the 21st day. This was due to the adjusted moisture content of the bioreaction material on the 15th day, which promoted ammonification. The temperature reached a maximum of 60°C on the 5th day because of the severe decomposition of easily biodegradable organic matter. From the 5th day, the temperature decreased because of the weak decomposition of recalcitrantly biodegradable organic matter which was indicated by the ratio decline of DOC/DN and excessive ventilation. After 21 days, the oxygen content increased to 20.9%, which was close to the oxygen content of the surrounding air

Table 1 Basic properties of the bio-stabilized products along the biostabilization process

Time (d)	pH	EC (mS/cm)	DOC (mg/L)	DN (mg/L)	DOC/DN ratio	NH_4^+-N (mg/L)	TS (%)	VS (%, DM)
Day 3	7.05±0.10	1.44±0.01	1142±11	551±6	2.07	249±2	48.8±2.0	85.7±3.9
Day 5	5.97±0.04	1.88±0.07	2065±87	695±19	2.97	253±13	56.4±1.4	83.3±0.2
Day 9	6.92±0.03	2.74±0.02	2334±153	1017±52	2.29	410±37	67.5±0.6	82.4±0.1
Day 13	7.01±0.04	2.76±0.01	2301±22	983±6	2.34	349±147	68.5±0.1	80.1±7.1
Day 15	7.07±0.02	2.52±0.04	1879±135	888±39	2.12	310±2	70.5±0.8	79.8±7.8
Day 19	7.30±0.01	1.65±0.05	993±0	582±11	1.71	186±44	50.3±0.7	81.3±0.8
Day 21	7.26±0.00	1.86±0.00	1040±33	599±10	1.74	290±8	52.6±0.6	81.8±0.1

environment, as shown in Fig. A1. It indicated the process of biostabilization.

3.2 Microbial diversity and uniformity of the bioaerosol and bio-stabilized samples

A total of seven sludge bio-stabilized samples and six bioaerosol samples were subjected to high-throughput sequencing for prokaryotes and fungi. We obtained 764849 prokaryotic sequences and 877365 fungal sequences. The Rank-Abundance Curve of prokaryote and fungi (Figs. A2 and A3) show that the richness of prokaryote was about an order of magnitude higher than that of fungi. As for uniformity, only the prokaryotes in the bio-stabilized sample showed a general uniformity (Figs. A2 and A3).

3.3 Microbial community composition at the phylum level

As shown in Figs. 2(a) and 2(b), we detected 30 prokaryotic phyla and 17 fungal phyla in the solid sample. There were nine dominant phyla among prokaryotes, including Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Planctomycetes, Saccharibacteria, Euryarchaeota, and Verrucomicrobia. The dominant fungi phylum was Ascomycota. In terms of bioaerosol, there were twenty-five prokaryotic phyla, including Proteobacteria, Actinobacteria, Bacteroidetes, Deinococcus Thermus, Firmicutes, etc., and two fungal phyla, namely Ascomycota and Basidiomycota.

3.3.1 Prokaryotic community composition at the phylum level

The predominant prokaryotic phylum found among the bio-stabilized products was Actinobacteria. The relative abundance of Actinobacteria increased and reached the maximum (64.6%) on the 13th day. Actinobacteria was widely found in the compost of municipal solid waste, agricultural residues, and manure (Liu et al., 2020b; Tang et al., 2020; Wang et al., 2020). Su et al. reported that Actinobacteria accounted for over 50% during the maturation phase (Su et al., 2015). However, the relative abundance of Actinobacteria decreased continuously to 43.5% on the 21st day. This reduction may have been caused by the decomposition of lignocellulosic-like organics at the early stage of the biostabilization process. Actinobacteria at the cooling phase of composting play an important role in the decomposition of lignocelluloses (Steger et al., 2007). The other dominant phylum was Proteobacteria; it presented an abundance trend similar to that of Actinobacteria during biostabilization. The relative abundance reached the maximum (31.3%) value on the 9th day and decreased to 20.7% on the 21st day. Proteobacteria was also found as a dominant phylum detected in the

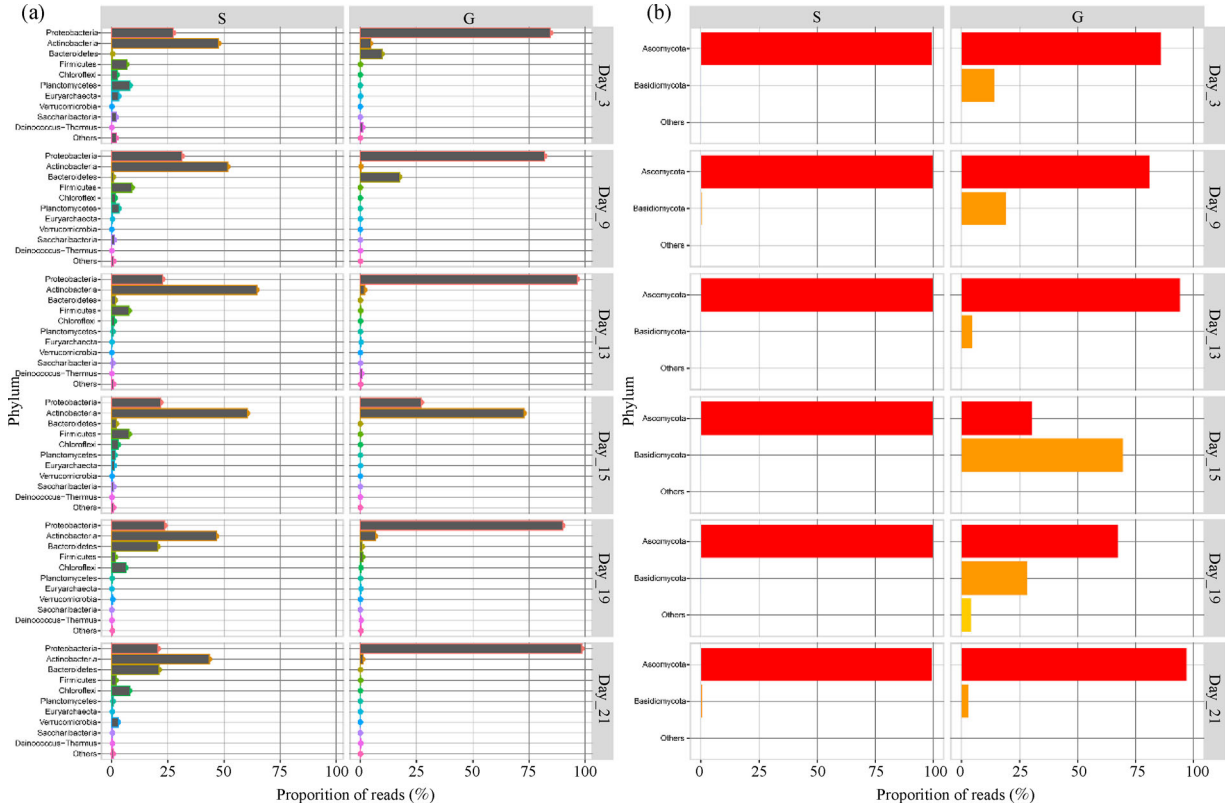

Fig. 2 The relative abundances of prokaryotic phylum (a) and fungal phylum (b) during the sludge biostabilization.

biogas residue composting covered with a molecular membrane. It is considered to play a key role in accelerating the aerobic ammonium oxidation process (Li et al., 2020). Besides the aforementioned prokaryotic phyla, the other phyla could be divided into three groups according to the trend of relative abundance. Firmicutes were included in the first group with the relative abundance reaching the maximum (7.8%) both on the 13th and 15th day and minimum (1.6%) on the 19th day. The relative abundance of phyla in the second group increased gradually throughout the biostabilization process. This group included Bacteroidetes and Chloroflexi, which reached their maximum (21.1% and 8.0%) on the 21st day. The third group has the opposite trend from the second group including phyla such as Planctomycetes, Saccharibacteria, and Euryarchaeota, reaching their minimum (8.1%, 2.0%, and 3%) on the 21st day. With regard to archaeal phyla, Euryarchaeota accounted for 3.1% on the 3rd day and was on the decrease throughout the whole process.

In terms of bioaerosol, the predominant prokaryotic phylum was Proteobacteria. The relative abundance of Proteobacteria remained in a range from 82.0% to 98.5% throughout the whole biostabilization process, except for the 15th day (27%). The emission of Proteobacteria from green compost and dispersion into the surrounding environment has been previously reported (Pankhurst et al., 2012). Other phyla such as Actinobacteria reached the maximum relative abundance (72.8%) on the 15th day and was undetected on the 9th day. Actinobacteria generally exist in soil, air, and water in the form of spores or hyphae. It especially enriches the soil in conditions of low moisture and can provide rich organic matter. Many genera of Actinobacteria could also form spores (Cahyani et al., 2002). Bacteroidetes was another dominant phylum in the bioaerosol samples, with an initially increasing and then a decreasing trend at the end of the process, presenting the maximum (17.5%) proportion on the 9th day.

3.3.2 Fungal community composition at the phylum level

The predominant fungal phylum in the bio-stabilized products was Ascomycota. The relative abundance of Ascomycota was above 98% throughout the biostabilization process, except for the 21st day (86.5%). In contrast, the relative abundance of Basidiomycota accounted for less than 1% throughout the whole process. A similar result was reported by Robledo-Mahón et al. (2020). In their study, Ascomycota was the dominant fungal phylum at the early stage, and Basidiomycota was dominant at the end of the biostabilization process. Ascomycota was found in the compost of agricultural waste (Zhang et al., 2016b; Wang et al., 2020) and was considered to be the main contributor to holocellulose (Glass et al., 2013), which was consistent with the presence of straw as the bulking agent in our study.

The same result was observed regarding bioaerosol, the lowest relative abundance of Ascomycota was present on the 15th day (30.2%), and the maximum abundance was attained on the 21st day (97%). The fungal phylum second to Ascomycota in terms of abundance was Basidiomycota, with a maximum relative abundance (69.1%) on the 15th day and the minimum (3%) on the 21st day.

3.3.3 Aerosolization behavior of microorganisms at the phylum level

The aerosolization behavior of microorganisms is shown in Figs. 3(a) and 3(b). Prokaryote was easier to aerosolize in the first 13 days of the biostabilization process. There were nine prokaryotic phyla with a BI of above 1, including Deinococcus–Thermus, Chlorobi, Bacteroidetes, Chloroflexi, Proteobacteria, Euryachaeota, Acidobacteria, Gemmatimonadetes, and Actinobacteria. From day 13 to day 15, fungi were easier to aerosolize; there were two fungal phyla with a BI of above 1, including Basidiomycota and Neocallimastigomycota, as shown in Fig. 3(b). Neocallimastigomycota is a well-known anaerobic fungi with a key role in lignocellulosic fiber in the gastrointestinal tract of mammalian herbivores (Gruninger et al., 2014).

As for prokaryotic phyla, Chlorobi first exhibited preferential aerosolization on the 3rd day of the biostabilization process, with a BI of 44. The BI of Bacteroidetes and Chloroflexi were 28 and 3 on the 3rd day, respectively. Deinococcus–Thermus was aerosolized the easiest on the 9th day, with a BI of 26, followed by Bacteroidetes and Proteobacteria with BIs of 23 and 3, respectively. These three phyla also showed a high level of aerosolization ability on the 13th day. Similar results were found in the bioaerosol sample of green compost and at the downwind site (Pankhurst et al., 2012). Among them, Proteobacteria was reported as a key player in the degradation of sewage sludge mixed with wheat-straw (Awasthi et al., 2017). Deinococcus–Thermus had the highest BI of 45 on the 13th day; it has been considered to be present at the late stage of compost (De Gannes et al., 2013). The aerosolization behavior of all microorganisms showed no significant differences on the 15th day, with the BI maintained around 1 or below. The anaerobic archaeal Euryachaeota was aerosolized the easiest on the 9th day with a BI of 7, followed by Proteobacteria with a BI of 5.

As for fungal phyla, Basidiomycota had the most preferential aerosolization throughout the biostabilization process, except for the 13th day. It had the highest BI of 487 on the 15th day, followed by 202, 120, 75, and 46 on the 19th, 3rd, 9th, and 13th day, respectively. This was probably due to the adaptability of Basidiomycota to high temperature and low moisture conditions (Gu et al.,

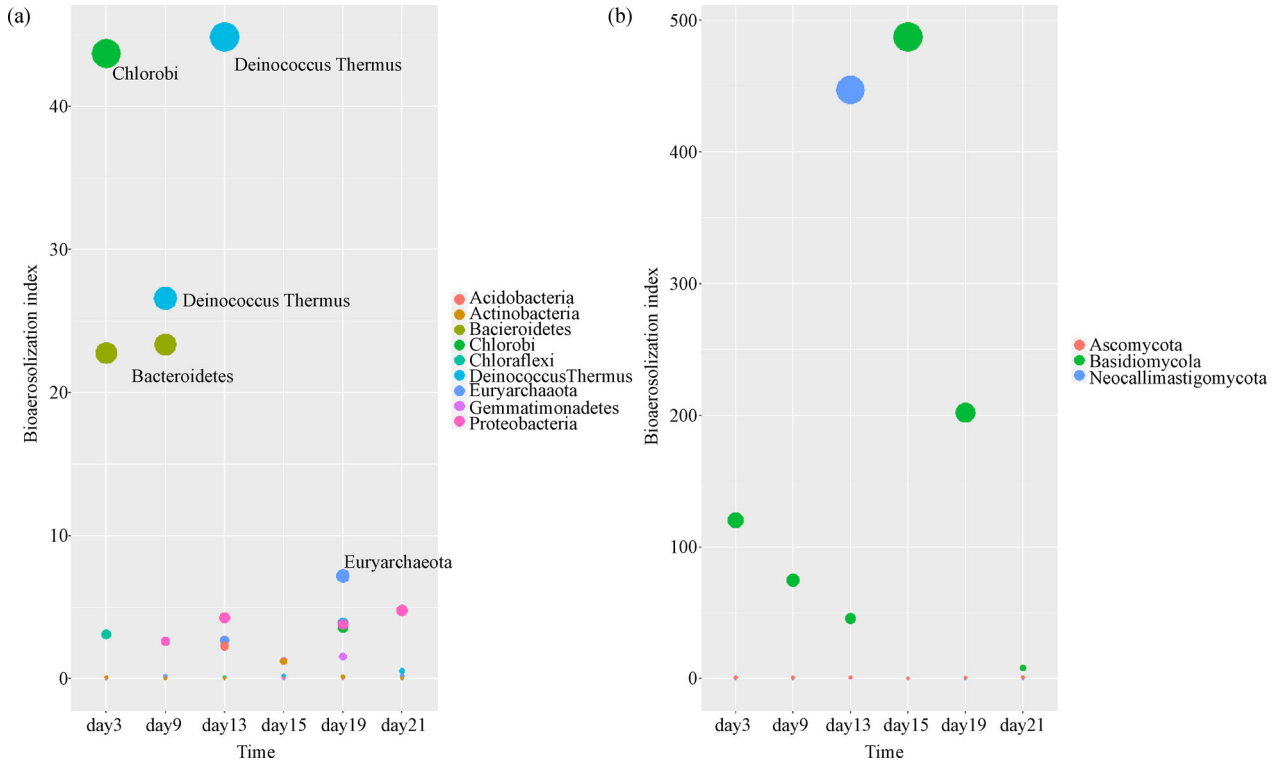

Fig. 3 The bioaerosolization indexes of prokaryotic phylum (a) and fungal phylum (b) during sludge biostabilization.

2017b). Moisture was an influencing factor on the succession of the fungal community in the swine manure composting process (Peng et al., 2019). The preferential aerosolization of Basidiomycota was consistent with our previous study (He et al., 2019). Anaerobic fungi Neocallimastigomycota was aerosolized the easiest on the 13th day, with a BI of 447. Neocallimastigomycota are regarded as obligate anaerobic fungi for the degradation of lignocellulosic biomass (Vinzelj et al., 2020) and are used for the production of biogas because of their unique metabolic pathways (Da Silva et al., 2017). However, Ascomycota was the dominant fungal phylum in the biostabilization process. It showed weak aerosolization behavior throughout the biostabilization process with a BI of below 1.

3.4 Microbial community composition and aerosolization behavior at the genus level

3.4.1 The relative abundance of prokaryote at the genus level

After including the prokaryotic genera, which accounts for less than 5%, in the group of "Others", 20 prokaryotic genera could be detected, as shown in Fig. 4(a). Among them were 15 prokaryotic genera, including *Acinetobacter*, *Pseudarthrobacter*, *Pseudomonas*, *Massilia*, *Saccharomonospora*, *Streptomyces*, *Chryseobacterium*, *Actinomadura*, *Parapedobacter*, *Sphingobacterium*, *Bordetella*, *Promicromonospora*, *Stenotrophomonas*, *Brevundimonas,* and *Hymenobacter*. *Saccharomonospora*, *Streptomyces,* and *Actinomadura* were the dominant genera in the biostabilized products, with a relative abundance range of 6.6%–27.9%, 7.9%–17.1%, and 3.1%–11.7%, respectively. These three genera belong to Actinobacteria. *Saccharomonospora* has been reported to have an excellent ability to degrade lignin and cellulose (Wang et al., 2019) and to be tolerant to high temperatures (Zhou et al., 2019). Therefore, this genus was initially abundant at the early stage of biostabilization and maintained the largest relative abundance from day 3 to 15. In terms of bioaerosol, the dominant prokaryotic genera were *Acinetobacter*, *Pseudarthrobacter*, *Comamonadaceae spp.*, *Pseudomonas,* and *Massilia* with a relative abundance range of 0%–76.1%, 0%–71.9%, 0%–60.6%, 0%–42.8%, and 0%–34.2%, respectively. Among them, *Acinetobacter*, *Pseudomonas,* and *Massilia* belong to the Proteobacteria phylum and were found in the bioaerosol emitted during composting and wastewater treatment (Breza-Boruta and Paluszak, 2009; Pahari et al., 2016; He et al., 2019). In the previous study, *Acinetobacter* was demonstrated to degrade the organic matter and produce acid; then, the organic acid was volatilized into the air (Wei et al., 2016; Li et al., 2019). This was consistent with the increase of pH from day 5 to 13 in our study (Table 1). It could be assumed that acid gas is a carrier of microorganisms, and

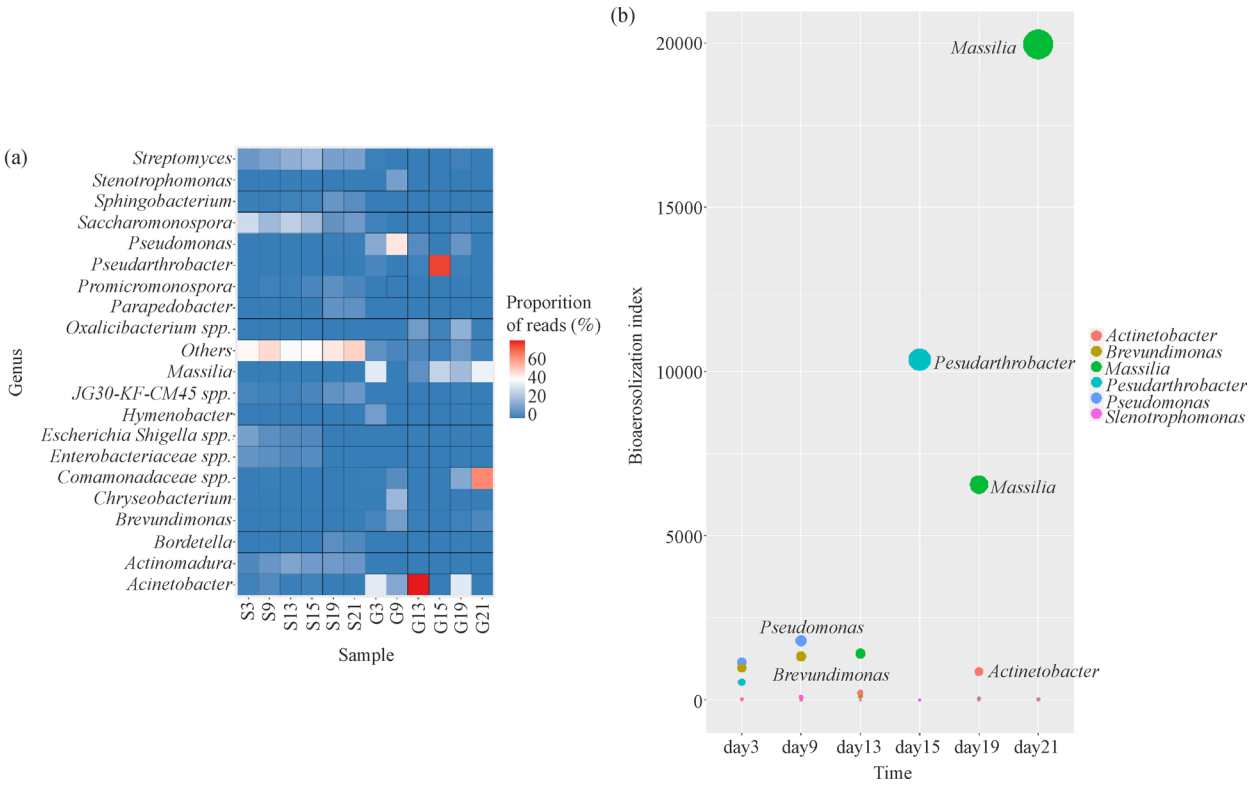

Fig. 4 The relative abundances (a) and bioaerosolization index (b) of prokaryotic genera during sludge biostabilization.

with the mechanical agitation of acid volatilization, the relative abundance of *Acinetobacter* in the bioaerosol reached its maximum on the 13th day.

3.4.2 The aerosolization behavior of prokaryotes at the genus level

There were 10 prokaryotic genera with a BI higher than 1 identified from the analysis of approximately 20 genera. Overall, the BI of prokaryotic genera ranged from 0 to 19961, as shown in Fig. 4(b). Among them, six prokaryotic genera could be identified with an average value of BI, including *Massilia*, *Pseudarthrobacter*, *Pseudomonas*, *Brevundimonas*, *Acinetobacter* and *Stenotrophomonas*, suggesting that these prokaryotic genera could aerosolize easily at the late stage of biostabilization. At the early stage of the biostabilization, namely on the 3rd and 9th day, *Pseudomonas* was the dominant aerosolized genera with BIs of 1145 and 1801, respectively, followed by *Brevundimonas* with a BI of 972 and 1325. *Pseudarthrobacter* was the easiest aerosolized genera on the 15th day, with a BI of 10360. Several studies have reported that *Pseudarthrobacter* plays a key role in the degradation of Polycyclic Aromatic Hydrocarbons (PAHs), which are micropollutants in sewage sludge (Zhang et al., 2016a; Yang et al., 2020). At the late stage of biostabilization, namely the 13th, 19th, and 21st days, *Massilia* showed a preferential aerosolization behavior with a BI of 1407, 6560, and 19961, respectively. *Massilia* was demonstrated to be able to synthesize Polycyclic Aromatic Hydrocarbons (PHAs) in the condition of excess carbon source, and it was isolated from sewage sludge compost (Rodriguez-Diaz et al., 2014). *Massilia* was also found to be dominant in the atmospheric environment of rural areas and dairies (Ravva et al., 2011; Wei et al., 2019). The BI of *Stenotrophomonas* and *Acinetobacter* ranged 0–80 and 0–861, respectively, throughout the whole process.

3.4.3 The relative abundance of fungi at the genus level

There were 12 fungal genera with a relative abundance of higher than 5% (Fig. 5(a)), including *Sordariales* spp., *Eurotiales* spp., *Cryptococcus*, *Fusarium*, *Agaricomycetes* spp., *Boeremia*, *Sordariomycetes* spp., *Cladosporium*, *Tremellales* spp., *Sporidiobolus*, *Magnaporthe,* and *Aureobasidium*. Among them, *Sordariales* spp. and *Eurotiales* spp. were the dominant genera in the biostabilization with the total relative abundance ranging from 94.6% to 99.0%. Langarica-Fuentes et al. reported a similar result (Langarica-Fuentes et al., 2014). Some species of *Sordariales* and *Eurotiales* play a key role in degradation activities at a high temperature. In the aerosol, 3 fungal genera could be identified, namely, *Cryptococcus*, *Fusarium,* and *Cladosporium,* with a relative abundance range of 0%–68.0%, 0%–55.6%, and 0%–18.9% presented on the 15th day, 9th day, and 3rd day, respectively. *Cryptococcus* was demon-

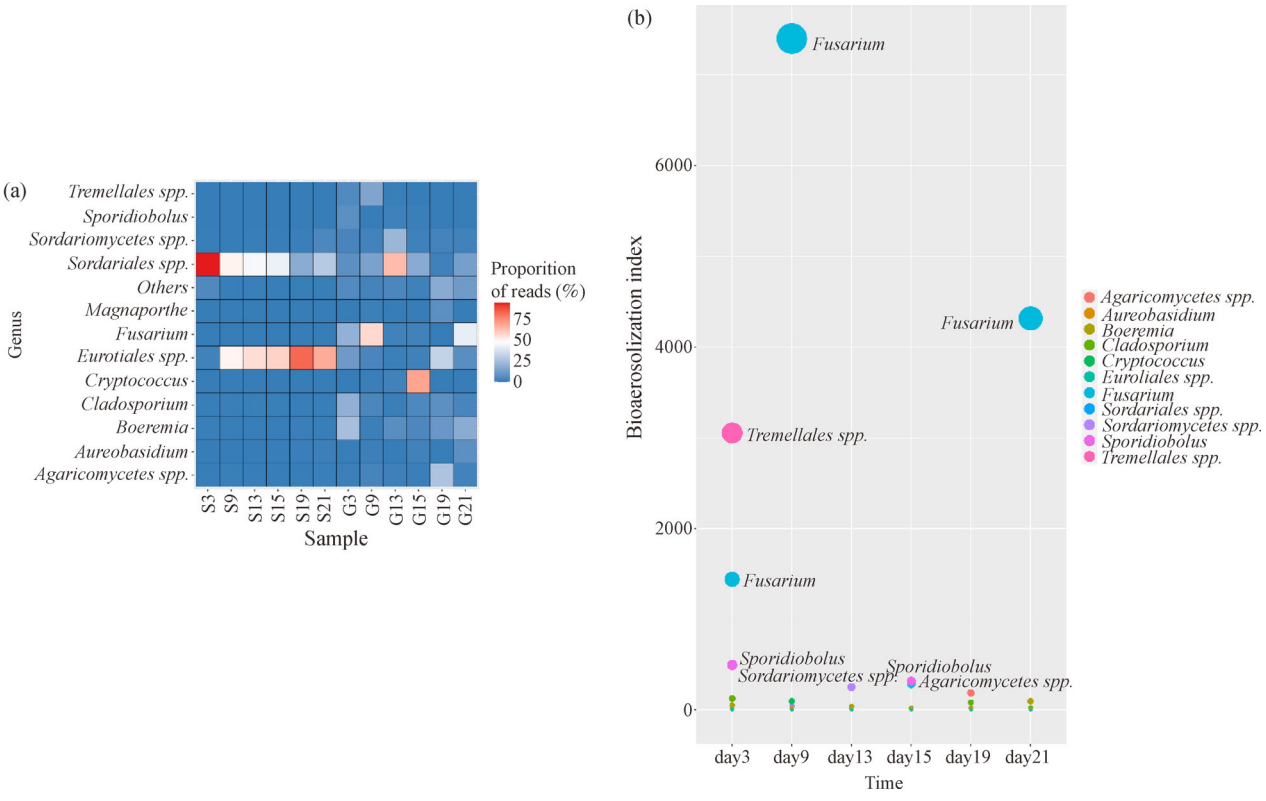

Fig. 5 The relative abundance (a) and bioaerosolization index (b) of fungal genus during sludge composting.

strated to be aerosolize easier than primary bacterial pathogens because of its ability to survive harsh conditions for a long time (Springer et al., 2013). Spore formation is another aerosolization pathway for fungi. The size of spores formed by *Fusarium* is below 1 μm (Lainhart, 2018), and these microorganisms could be more easily transported into the atmosphere (Han et al., 2019). The other three genera, *Sporidiobolus*, *Magnaporthe,* and *Aureobasidium,* all showed a lower level of relative abundance range of 0% to 7%.

3.4.4 The aerosolization behavior of fungi at the genus level

In this study, 11 fungal genera could be found with a BI higher than 1, and most fungal genera could be easily aerosolized at the early stage of the biostabilization, as shown in Fig. 5(b). *Tremellales* spp. showed a preferential aerosolization behavior on the 3rd day with a BI of 3055, followed by *Fusarium* and *Sporidiobolus* with BIs of 1442 and 496, respectively. The BI of other fungal genera ranged from 0 to 125. *Fusarium* was the easiest aerosolized genera on the 9th day with a BI of 7398, others ranged from 0 to 95. On the 13th, 15th, and 19th day, *Sordariomycetes* spp., *Sporidiobolus,* and *Agaricomycetes* spp. showed a preferential aerosolization behavior with BIs of 253, 319, and 188, respectively. On the 21st day, *Fusarium* showed a preferential aerosolization behavior with a BI of 4315. In general, the BI of fungal genera was lower than that of prokaryotic genera. However, this may be due to hydrophobicity. The hydrophobic surface of microorganisms could contribute to the aerosolization behavior and enrichment in the water–air surface (Michaud et al., 2018). Furthermore, most fungi are considered to be hydrophilic (Kohlmeier et al., 2005; Wick et al., 2007; Gu et al., 2017a), and this property of theirs explains the decrease of BI after the adjustment of moisture content in our study.

3.5 Aerosolization behavior of major microorganisms at the species level and health risk assessment

3.5.1 The aerosolization behavior of prokaryotes at the species level

In this study, 12 prokaryotic species could be identified with a relative abundance higher than 1%, including *Brevundimonas nasdae, Stenotrophomonas rhizophila, Brevundimonas bullata, Chryseobacterium sp., Saccharomonospora viridis, Actinomadora hellensis, Saccharomonospora viridis*, and so on. Among them, *Brevundimonas nasdae* showed a preferential aerosolization behavior at the early and late stages of the biostabilization with a BI of 282, 2154, and 858 on the 3rd, 9th, and 21st day, respectively (Fig. 6(a)), followed by

Stenotrophomonas rhizophila and *Brevundimonas bullata* with a BI of 456 and 124, respectively. *Brevundimonas nasdae* and *Brevundimonas bullata* belong to *Brevundimonas*, which are considered to be widespread pathogens (Ryan and Pembroke, 2018), and they could cause respiratory diseases (Madamarandawala et al., 2019). Violetta et al. identified two strains of *Stenotrophomonas rhizophila* as the potential biomarkers of an infection of the airways (Shestivska et al., 2015). As a pathogen, *Stenotrophomonas* could cause respiratory infections in patients with cystic fibrosis and chronic lung diseases (Brooke, 2012).

3.5.2 The aerosolization behavior of fungi at the species level

There were 14 fungal species identified with a relative abundance higher than 1%. Overall, fungi showed more preferential aerosolization behavior than prokaryotes at the species level (Fig. 6(b)). Among them, there were nine fungal species with a BI of over 10, including *Fusarium graminerum*, *Thermomyces langinosus*, *Sporidiobolus pararoseus*, *Torula herbarum*, *Cladosporium herbarum*, *Boeremia exigua var exigua,* and *Aureobasidlum herbarum*. Moreover, *Fusarium graminerum* was the easiest aerosolized fungal species both at the early and late stages of biostabilization, followed by *Sporidiobolus pararoseus* with a BI of 4960 on the 15th day. *Fusarium graminerum* belongs to the *Fusarium* genus, which is a filamentous *Ascomycete* that can produce toxins (Abbasian et al., 2020). Furthermore, extensive exposure to mycotoxins produced by *Fusarium*, especially for workers, may cause respiratory symptoms (Niculita-Hirzel et al., 2016).

3.5.3 Bioaerosol concentration quantification and health risk assessment

The concentration of microorganisms detected by DAPI fluorescence microscopy ranged from 160 to 1440 cell/m^3 (Table 2). The maximum concentration was detected on the 15th day, exceeding the limit of 1000 CFU/m^3 proposed by the Occupational Safety and Health Administration (OSHA), indicating contamination (OSHA, 1994). By comparing the trend of moisture content (Table 1) and bioaerosol concentration (Table 2), a negative correlation between the bioaerosol concentration and moisture content was observed. As mentioned above, the aerosolization of hydrophilic microorganisms (e.g., fungi) may be eliminated by the increase in moisture content. Therefore, the adjustment of moisture content to control bioaerosol emission from sewage sludge biostabilization is proposed.

Bioaerosol concentrations of the samples on other days were within safety limits. Pathogenic microorganisms, e.g., *Cryptococcus*, *Brevundimonas,* and *Pseudomonas,* which could cause allergic reactions, asthma, and wound infections, presented a high level of relative abundance and

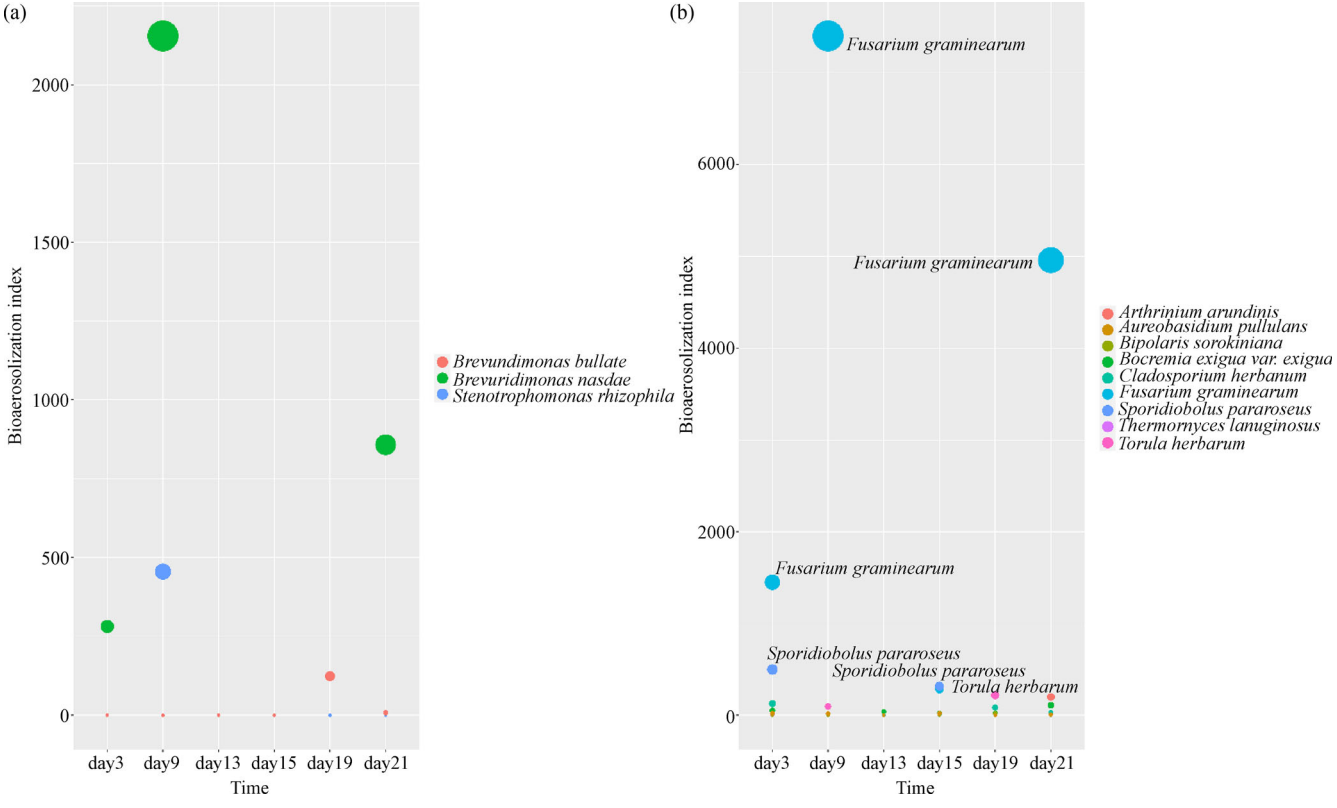

Fig. 6 The bioaerosolization index of prokaryotic species (a) and fungal species (b) during sludge biostabilization.

Table 2 The concentration of bioaerosol of sewage sludge

Time (d)	Concentration (cell/m^3)
3	320
5	160
9	320
13	640
15	1440
19	320
21	320

BI in our study (Barrera et al., 2018; Ryan and Pembroke, 2018). The dominant microorganisms with a high level of BI, such as *Massilia*, have been demonstrated to be pathogens (Ravva et al., 2011), and some species of *Massilia* may cause infectious diseases (Lindquist et al., 2003). Based on the results of our laboratory-scale biostabilization, workers in the sludge composting facilities may be exposed to health threats due to prolonged occupational exposure.

3.6 Comparison of the aerosolization behavior of vegetable waste and manure biodegradation

At the phylum level, Deinococcus–Thermus and Chlorobi were identified to be preferentially aerosolized in our study, even with a comparatively lower relative abundance in the microbial community of sewage sludge mixture. It is different from the results of green-waste composting (Pankhurst et al., 2012), in which the Deinococcus–Thermus was reported to be less abundant in the bioaerosol than in the compost. Firmicutes and Actinobacteria, which were both found in the bioaerosol and the biostabilization products, did not have significant preferential aerosolization. This result was consistent with the study of biogas emission by sludge digestion reported by Moletta et al. (Moletta et al., 2007). There were some discrepancies with the preferential aerosolization of Actinobacteria during the green waste and manure composting (Veillette et al., 2018). As for archaeal phyla, Euryachaeota was aerosolized the easiest on the 9th day in our present study. In our previous study, Thaumarchaeota was aerosolized easier than Euryachaeota during vegetable waste composting (He et al., 2019).

At the genus level, *Pseudarthrobacter* presented a preferential aerosolization behavior on the 15th day, while this prokaryotic genus was rarely found to be aerosolized in the green waste and manure composting processes. Compared with our previous study (He et al., 2019), *Massilia* showed a high level of aerosolization ability at the end of the sludge biostabilization process, rather than at the early stage. In addition, *Fusarium* and *Tremellales spp.* were the fungal genera with the highest level of BI during the different stages of biostabilization. It was reported that *Fusarium* was specific to the type of waste that was treated. This pathogenic fungal genus was found in the bioaerosol of animal composting, whereas it was not found during green waste composting (Mbareche et al., 2017).

4 Conclusions

This study systematically discussed the relative abundance and aerosolization behavior of bacteria, archaea, and fungi at different stages of sewage sludge biostabilization. Bioaerosolization index was used to quantify the strength of aerosolization behavior of microorganisms including pathogens during the whole process. The bioaerosol concentration and aerosolization behavior of microorganisms were found not only to be related to the nature of raw material but also preliminarily thought to be related to operational conditions including moisture content and pH. Based on the bioaerosol concentration and preferentially aerosolized pathogens investigated in this study, we provided the reliable data for further health risk assessment. Further research is necessary to provide detailed information for reducing bioaerosol emissions in the process of sewage sludge biodegradation.

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