Science of the Total Environment 797 (2021) 149194

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

A novel green substrate made by sludge digestate and its biochar: Plant growth and greenhouse emission

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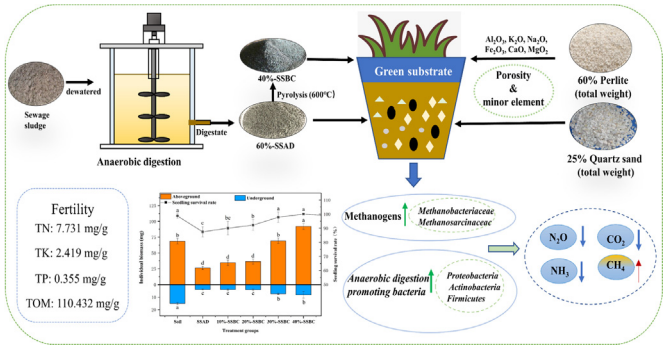
HIGHLIGHTS

- Green substrate based on sewage sludge anaerobic digestate was prepared.
- The growth of ryegrass in green substrate was obviously better than that in soil.
- Emission of N_2O, CH_4 and NH_3 in green substrate was approximate to that in soil.
- Fermentative bacteria/methanogenic archaeal was the dominant group in green substrate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 February 2021
Received in revised form 7 July 2021
Accepted 18 July 2021
Available online 21 July 2021

Editor: Baoliang Chen

Keywords:
Digestate
Greenhouse gas
Green substrate
Biomass
Microbial community

ABSTRACT

Anaerobic digestion of sludge produces a large amount of sewage sludge anaerobic digestate (SSAD) that can be reused. A novel green substrate was prepared by mixing SSAD and its biochar (SSBC) filled with perlite and quartz sand for plant growth, as a replacement of soil. We carried out pot experiment, measured ryegrass biomass, seedling survival rate, and evaluated the emission of greenhouse gas (GHG), NH_3 volatilization. The results showed that the seedling survival rate and individual biomass of ryegrass in green substrate were 100% and 100.02 mg, which were 14.4% and 231.4% higher than those in only SSAD, but were 1.3% and 19.6% higher than those in soil. SSBC significantly reduced N_2O and CO_2 emission, inhibited the NH_3 volatilization, but increased CH_4 emission. However, the cumulative emission of N_2O and CH_4 was approximation to that in soil. Global warming potential of CH_4 and N_2O ($GWP_{(CH4+N2O)}$) green substrate was 11,842.01 kg $CO_2 \cdot hm^{-2}$, which was 1.35-fold higher than that of soil. Microbial community structure analysis showed that fermentative bacteria and methanogenic archaeal had a higher abundance in green substrate than in soil, which caused the different gas emission. This study will provide an effective and economical way to dispose excessive SSAD.

1. Introduction

Sewage sludge is a byproduct of wastewater treatments. The disposal of sewage sludge is one of the most critical environmental challenges in the world (Qin et al., 2017). In China, the amount of dewatered sewage sludge has reached 40 million tons each year (Chen et al., 2020). Sewage sludge usually contains a lot of harmful compounds, i.e., parasites, eggs and pathogenic microorganisms, which is an impediment to its subsequent disposal (Eid et al., 2017). Anaerobic digestion (AD) is a key technology of treating sewage sludge for energy recovery, in which anaerobic digestate (SSAD) is generated as main

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https://doi.org/10.1016/j.scitotenv.2021.149194
0048-9697/

waste (Carrosio, 2013; Cristina et al., 2019). SSAD has great agronomic value due to appreciable nutrient content (Antonkiewicz et al., 2018). Agronomic application is the common way for SSAD in Europe (Alvarenga et al., 2015). However, the amount of SSAD that was reused as soil fertilizer was very poor (Cristina et al., 2019). Direct land use of SSAD may bring other environmental issues, i.e., the emission of greenhouse gas (GHG) and nitrogen (Verdi et al., 2019). Previous research reported that the use of SSAD as a fertilizer increased the content of macro- and microelements in soil and plants (Cristina et al., 2019; Yogev et al., 2020), but lead to an increase of emission of GHG, e.g., nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) (Alburquerque et al., 2012; Chiew et al., 2015).

During the past decades, biochar has attracted much attention due to carbon storage and adsorption capacity (Zhang et al., 2021; Zhu et al., 2017). It can also influence the properties of soil including pH, electric conductivity, and organic matter content (Eid et al., 2017; Xiao et al., 2017); Thus, biochar prepared from sewage sludge (SSBC) may be a potential approach for agriculturally reusing sewage sludge (Schulz and Glaser, 2012). Some works have investigated the phytotoxic effect and the GHG emission when SSBC was added into soil (Chu et al., 2020; Verdi et al., 2019). It is reported that SSBC as an additive significantly increased biomass and yield of rice when it was used in rice plant pot experiment, and it reduced N_2O emission (Khan et al., 2013). SSBC reduced the yield-scale NH_3 volatilization by 20.3% and increased soil N retention when it was added into paddy soil (Chu et al., 2020). States thus, SSAD could provide nutrients, whereas SSBC could help reduce the GHG emission and NH_3 volatilization. At present, most studies focused at using SSBC as a remediation additive of soil or during composting (Tu et al., 2019; Xiao et al., 2017). We found that a combination of SSAD and SSBC may be complementary, which produces a different synthetic substrate compared to soil for plant growth.

The main goal of this research is to prepare a green substrate made by SSAD and SSBC. The low porosity is one of the main factors limiting the growth of ryegrass according to our previous research (Gu et al., 2020). Therefore, quartz and perlite were added into SSAD to improve the porosity of SSAD. This kind of green substrate will be directly used for plant planting. In this research, the green substrate was used to grow ryegrass to explore its phytotoxicity. The seedling survival rate and biomass was recorded. In order to evaluate the effect of the green matrix on the environment, the GHG emission and microbial diversity of the matrix were also detected. This study may provide a new idea for the utilization of SS or SSAD.

2. Materials and methods

2.1. Soil and sewage sludge anaerobic digestate

SS used in this study comes from Shanghai Anting domestic sewage treatment plant. The SS was first put into an anaerobic digestion reactor at medium temperature (35 °C) for 20 days. The 6 L anaerobic digestion reactor used in this study was agitated by a pump driven agitator (60 r/min) and was heated by water bath. After anaerobic digestion, SSAD was produced. Soil used in this study comes from a farmland in Jinze Town, Qingpu District, Shanghai. The properties of soil and SSAD were laboratory determined (Table 1).

2.2. Preparation of SSBC

Green substrate was prepared by mixing SSAD and SSBC. After obtaining SSAD, a part of SSAD was put into muffle furnace for pyrolysis to prepare SSBC. The preparation process was performed according to modified method from Khan et al. (2013). In brief, SSAD was dried by air and sieved through a 10–40 mesh sieve. Then put it into muffle furnace for pyrolysis. The temperature rose to 600 °C at rate of 4–5 °C from initial temperature 25 °C, and retention time was 2 h. The physical and chemical properties of SSBC were tested after obtaining SSBC (Table 2).

2.3. Preparation of green substrate

2.3.1. Exploration of the appropriate porosity on plant growth

After sieving, the SSAD after watering is easy to form solid block with the evaporation of water, and its porosity is too poor thus inhibiting plant growth. Therefore, the porosity and high contents of nutrients in SSAD need to be modified at first. In this experiment 25% quartz sand was added into SSAD to prepare mixed substrate. Then perlite is added into SSAD to modify its porosity. The addition of perlite accounted for 0%, 20%, 40%, 60% and 80%. After mixing evenly, making the moisture content was 30% by watering and putting them into the seedling tray. The seeds of ryegrass were bought from flower seedling wholesale station. Ryegrass seedlings germinating to 2 cm were transplanted into each substrate. Then the seedling tray was put in the greenhouse. After 20 days, seedling survival rate and biomass were recorded to analyze the effect of porosity on the plant growth.

Table 1
Physical and chemical properties of SSAD and soil.

Parameters	Unit	Soil	SSAD	Permissible limits		
				CEC (sludge)	USEPA (sludge)	SEPA (soil)
pH		4.60 ± 0.01	8.46 ± 0.02			
Salt content	mg/kg	527.75 ± 4.92	7846.21 ± 77.49			
Organic matter	%	4.28 ± 0.35	34.51 ± 2.55			
Total nitrogen	g/kg	1.63 ± 0.31	24.16 ± 0.93			
Alkali hydrolyzed nitrogen	mg/kg	197.75 ± 9.40	1937.15 ± 62.34			
Total phosphorus	mg/kg	814.31 ± 94.38	1110.71 ± 163.80			
Available phosphorus	mg/kg	43.13 ± 1.167	726.20 ± 33.38			
Water soluble protein	mg/kg	13.65 ± 0.34	100.94 ± 2.76			
Polysaccharide	mg/kg	58.25 ± 3.42	883.80 ± 34.62			
Total potassium	g/kg	7.24 ± 1.30	7.56 ± 0.68			
Available potassium	mg/kg	101.70 ± 2.22	509.85 ± 5.46			
Cr	mg/kg	41.08 ± 1.67	102.62 ± 2.35	1000	3000	150
Ni	mg/kg	53.08 ± 3.76	140.44 ± 6.66	300	420	40
Cu	mg/kg	28.60 ± 1.91	152.18 ± 3.35	1000	4300	50
Pb	mg/kg	ND	ND	750	840	250
Cd	mg/kg	ND	ND	20	85	0.3
Zn	mg/kg	65.88 ± 3.83	1467.42 ± 54.37	2500	7500	200

SSAD: anaerobic digestate from sewage sludge; ND: below detection line; CEC: Permissible limits set for sewage sludge by the commission of European Communities (CEC) (Khan et al., 2013); USEPA: Permissible limits set for sewage sludge by the United State Environmental Agency (USEPA) (Lu et al., 2007); SEPA: Permissible limits set for soil pH < 6.5 by the State Environmental Protection Administration (SEPA) (Khan et al., 2013).

Table 2
The properties of SSBC.

Properties	Unit	SSBC
pH		8.09 ± 0.02
Surface area	m^2/g	111.80 ± 0.02
Total pore volume	cm^3/g	0.22 ± 0.02
Average mesopore radius	nm	19.62 ± 0.47
C content	%, w	14.05 ± 0.32
N content	%, w	1.39 ± 0.02
S content	%, w	4.50 ± 0.02
C/N	–	10.13 ± 0.55
Available nitrogen	mg/kg	64.51 ± 15.98
Available phosphorus	mg/kg	1505.63 ± 32.63
Available potassium	mg/kg	90.54 ± 2.98

2.3.2. Mixing SSAD and SSBC as green substrate

Green substrate was prepared by adding SSBC into SSAD in different proportion. Four experiment groups were set (10%-SSBC, 20%-SSBC, 30%-SSBC and 40%-SSBC). The proportion of SSAD and SSBC was shown in Table 3. SSAD and SSBC were mixed to prepare the initial substrate. Then quartz sand and perlite are added, accounting for 25% and 60% (see Section 2.2, 60% is the most suitable additive amount) of the total weight of initial substrate, respectively. The moisture content is 30%. After mixing them evenly, the green substrate was put in seedling tray.

2.4. Pot experiment

The experiment took place in a greenhouse with controllable temperature, which was set at 27 °C. The prepared substrate was filled into the seedling tray, then the ryegrass seedlings germinated to 2 cm were transplanted into the seedling tray. Each well of the seedling tray was planted nine seedlings. The inoculated seedling tray was place in the greenhouse with artificial light for 15 h per day and growth period was 20 days. Seedling survival rate and individual biomass were detected.

2.5. GHG and ammonia emission

Gas collection and analysis method was according to existing method (Wang and Wang, 2003). Soil, SSAD and the green substrate (SSAD: SSBC = 6:4, w/w, according to the result of plant growth in different substrate, 6:4 is the most suitable substrate for plant growth) were put in the bottom of a transparent polyethylene cylinder (diameter 20 cm, height 65 cm), respectively. Each sample was evenly laid on the bottom of the cylinder, the thickness of each sample was about 3.0 ± 0.5 cm. There is a circular opening with a diameter of 8 cm in the top center of the cylinder, which is sealed during the experiment. Gas tee was installed on the top of the device. The gas fluxes were collected every day at same time. Before collecting gas fluxes, in order to mix the gas in the device evenly, a 50 mL plastic syringe with a three-way stopcock was used to pump the internal gas 20 times. The gas in the device was collected by the syringe. The collected gas sample was taken to lab and analyzed CO_2, CH_4, and N_2O within 24 h by gas chromatograph (Agilent 7890B). The gas emission fluxes were calculated; the method of GHG emission calculation was according to formula (1) (Hou et al., 2020; Ortiz-Gonzalo et al., 2018). According to the CO_2, N_2O and CH_4 emission fluxes, the cumulative emission (formula (2)) and global warming potential (GWP) (formula (3)) were calculated. The formulas are according to Hou et al. (2020) and Somplak et al. (2019). For collecting NH_3, sulfuric acid was absorbent to absorb NH_3 and replaced it every day. The beaker containing 50 mL sulfuric acid was placed inside the cylinder. The beaker is 5 cm from the top of the device. NH_3 was absorbed by sulfuric acid solution and detected by Nessler's Reagent Colorimetry. The formulas are as follows:

$$F = \frac{dc}{dt} \cdot \frac{M}{V_0} \cdot \frac{P}{P_0} \cdot \frac{T_0}{T} \cdot H \quad (1)$$

where F is gas emission fluxes ($mg \cdot m^2 \cdot h^{-1}$); dc/dt is the rate of gas concentration change during the gas sampling period. M is the gas molar mass ($g \cdot mol^{-1}$), V_0 is the gas molar volumes at standard atmospheric pressure ($L \cdot mol^{-1}$). P_0 and T_0 are the standard atmospheric pressure (kPa) and standard atmospheric temperature (K), respectively. T and P are the actual temperature and pressure, respectively. H is the height of the sampling chamber (m).

$$R = \sum (F_{i+1} + F_i)/2 \times (t_{i+1} - t_i) \times 24 \quad (2)$$

R is the cumulative emission of GHG in three substates ($kg \cdot hm^{-2}$); F_i is the GHG emission flux of the i-th sampling ($mg \cdot m^2 \cdot h^{-1}$), t_i is the i-th sampling time, d.

The global warming potential (GWP) was calculated, the formula is as followed,

$$GWP_{(CH4+N2O)} = R_{CH4} \times 25 + R_{N2O} \times 298 \quad (3)$$

where R_{CO2}, R_{CH4}, and R_{NO2} was the cumulative emission of CO_2, N_2O and CH_4, respectively.

2.6. Analysis of microbial community

The microbial community in Soil, SSAD and green substrate (SSAD: SSBC = 6:4, w:w) were analyzed. Microbial diversity was detected by Shanghai Majorbio Bio-pharm Technology company. Genomic DNA was extracted with Fast DNA SPIN KIT, which is a special kit for the study of soil microorganism extraction, following the manufacturer's instructions. All PCR reactions were carried out in a TransGen AP221-02. And 1% agarose gel was used to check the size of PCR samples. The entire DNA extract was used for Miseq Sequencing and obtained PE reads. PE reads slices according to the overlap relationship, meanwhile performs quality control and filtering on the sequence quality. After distinguishing samples, it conducts OUT cluster analysis and species taxonomy analysis. The data were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

2.7. Data analysis

The date was analyzed using SPSS software. The analysis of variance (ANOVA) and *t*-test were performed to compare differences between the different treatment groups. When $P < 0.05$, the values were considered to be significantly different. Drawing data results were using Origin 2021.

Table 3
The proportion of SSAD and SSBC in pot experiment.

Component	Ingredients	10%-SSBC	20%-SSBC	30%-SSBC	40%-SSBC
Initial substrate (IS)	SSAD (%)	90	80	70	60
	SSBC (%)	10	20	30	40
Additive	Quartz sand (% weight of IS)	25	25	25	25
	Perlite (% weight of IS)	60	60	60	60

3. Results

3.1. The growth of ryegrass in green substrate

Perlite was added into SSAD to improve porosity, and the results were shown in Fig. 1. Ryegrass couldn't survive in SSAD without perlite.

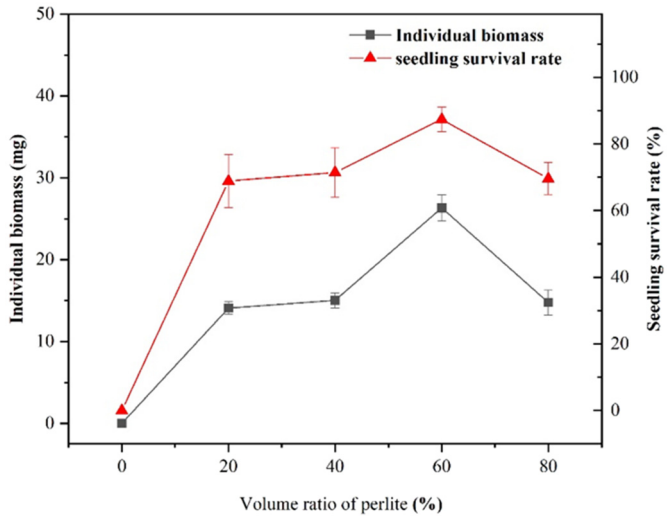

Fig. 1. Ryegrass seedling survival rate and individual biomass in SSAD mixed with different ratio of perlite.

When the addition amount of perlite increased from 0 to 60%, seedling survival rate and individual biomass increased obviously and both reached the maximum value at 60% perlite adding. The maximum value of seedling survival rate and individual biomass were 87.41% and 26.34 mg, respectively, but when the addition amount of perlite was over 60%, the growth of ryegrass was inhibited. Therefore, the most suitable addition amount of perlite was 60%.

The optimum addition dosage of SSBC was also explored and the results are shown in Fig. 2. Five ratios of SSAD and SSBC were explored, which were 10:0, 9:1, 8:2, 7:3 and 6:4 (w/w). The seedling survival rate and individual biomass in soil were 98.7% and 83.78 mg (68.67 in aboveground, 15.11 mg in underground), respectively. Seedling survival rate in SSAD only added perlite was 87.4%, which was 11.4% lower than that in soil. The individual biomass was 30.23 mg (26.34 mg in aboveground, 3.89 mg in underground), which was 63.9% lower than that in soil. When the dosage of SSBC was gradually increased, the seedling survival rate and individual biomass increased obviously. When 40% SSBC was added into SSAD, the seedling survival rate and individual biomass reached the maximum value, which were 100% and 100.19 mg respectively (92.09 mg in aboveground, 8.10 mg in underground). The seedling survival rate and individual biomass in 40%-SSBC treatment group

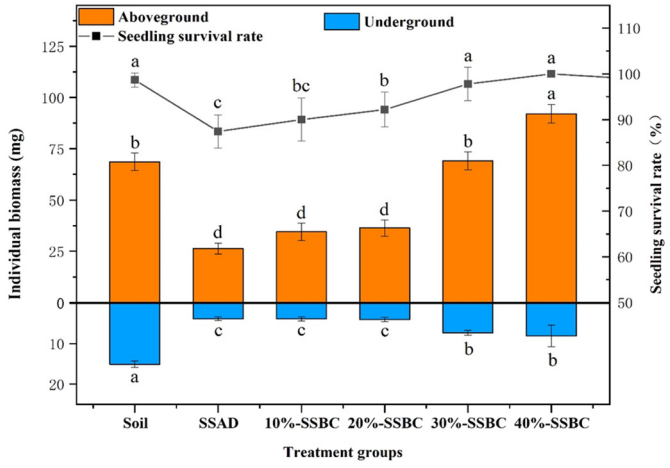

Fig. 2. The seedling survival rate and biomass of ryegrass in different ratio of SSAD and SSBC. (a, b, c, d and bc were the symbol of significant difference.)

were 1.32% and 19.59% higher than those in soil, which proved that ryegrass would grow better in green substrate than in soil. In 50%-SSBC treatment group, seedling survival rate and individual biomass decreased by 1.1% and 32.1%, compared with 40%-SSBC treatment group. Therefore, the most suitable addition amount of SSBC was 40%. The green substrate was prepared by mixing SSAD and SSBC (6:4, w: w), perlite (60% weight of initial substate) and quartz sand (25% weight of initial substrate) as filler (in the following discussion, 40%-SSBC was used to represent the green substrate).

3.2. GHG emissions

3.2.1. The emission of N_2O

The emission flux of N_2O is shown in Fig. 3a. In total, the emission flux of N_2O in SSAD was obviously higher than those in green substrate

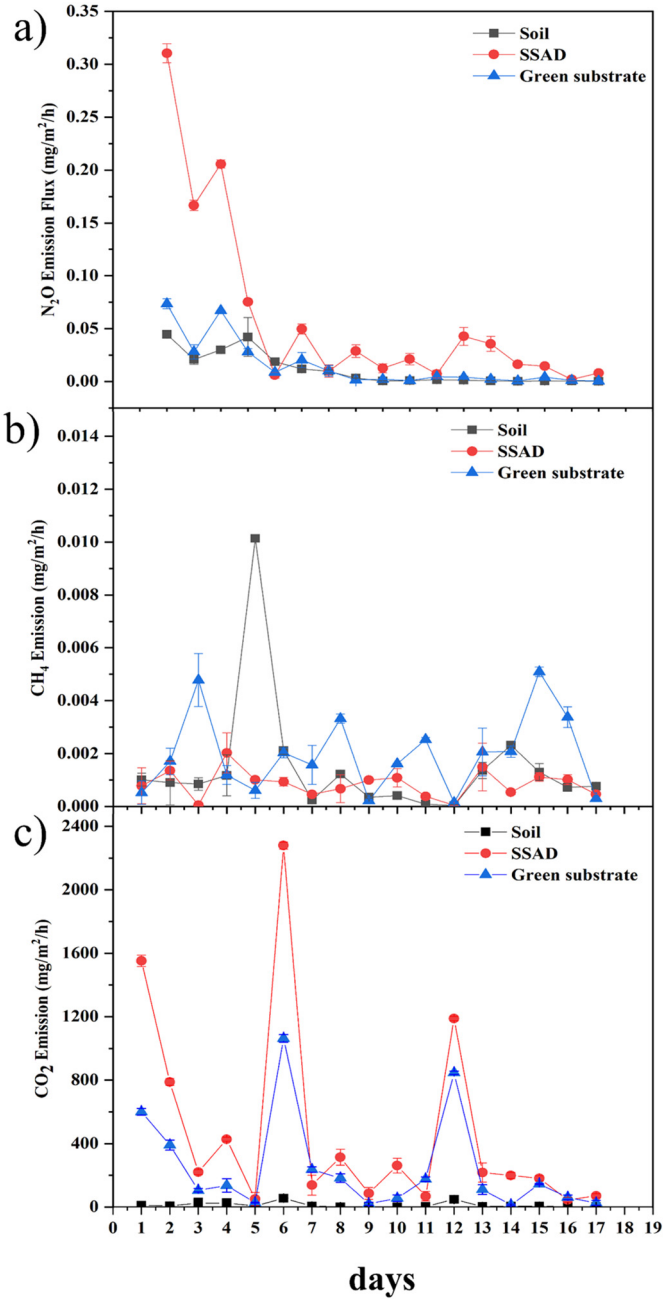

Fig. 3. GHG emission in soil, SSAD and green substrate.

and soil. They all reached a maximum value at first day, which were 0.311, 0.074 and 0.045 $mg \cdot m^{-2} \cdot h^{-1}$ in SSAD, green substrate and soil, respectively (Fig. 3a). The emission flux in SSAD was 4.2 times and 6.9 times higher than those in green substrate and soil at the maximum value level. With the extension of time, the emission flux decreased and kept stable. The emission flux in green substrate and soil were similar after sixth day, which demonstrated that green substrate had the comparative impact on the environment from the emission of N_2O. The cumulative emission of N_2O in soil, SSAD and green substrate were 4.517, 24.229 and 6.198 $kg \cdot m^{-2}$, respectively (Table 4). The cumulative emission of N_2O in green substrate was 37.19% higher than that in soil, but 74.42% lower than that in SSAD.

3.2.2. The emission of CH_4

The emission flux of CH_4 is shown in Fig. 3b. They were all kept in a relatively low level and constantly fluctuating. The emission flux of CH_4 in soil had a maximum value (0.151 $mg \cdot m^{-2} \cdot h^{-1}$) at the fifth day, which was the highest emission flux during the sampling time, but during the other sampling time, the emission in soil was always low and the cumulative emission was 0.710 $kg \cdot m^{-2}$. The emission flux of CH_4 in green substrate had four "peak value" at the third day, i.e., the 8th day, the 11th and the 15th day, which were 0.0048, 0.0032, 0.0025 and 0.0051 $mg \cdot m^{-2} \cdot h^{-1}$. The cumulative emission in green substrate was 0.792 $kg \cdot m^{-2}$, approximated to that in soil. However, the emission flux in SSAD was lower than those in soil and green substrate. The maximum emission flux of CH_4 in SSAD was only 0.0020 $mg \cdot m^{-2}$. The cumulative emission of CH_4 in SSAD was 0.340 $kg \cdot m^{-2}$, which was 57.07% lower than that in green substrate. The addition of SSBC increased the emission of CH_4 in SSAD.

3.2.3. The emission of CO_2

As shown in Fig. 3c, the maximum CO_2 emission flux in three substrates was SSAD > green substrate > soil. Compared with SSAD and green substrate, the emission in soil was stable and the maximum value was 56.198 $mg \cdot m^{-2} \cdot h^{-1}$. The trend of CO_2 emission in SSAD and green substrate was similar; they both reached a higher emission flux at the first day and then declined; two "peak values" appeared at the 6th and 12th day. The emission flux at the first day were 1551.850 $mg \cdot m^{-2} \cdot h^{-1}$ and 600.571 $mg \cdot m^{-2} \cdot h^{-1}$ in SSAD and green substrate. The emission flux of CO_2 in SSAD was 2279.090 $mg \cdot m^{-2} \cdot h^{-1}$ and 1188.180 $mg \cdot m^{-2} \cdot h^{-1}$ at the 6th day and the 12th day, which were 2.14 times and 1.40 times higher than those in green substrate. Therefore, the addition of SSBC decreased the emission of CO_2 in SSAD. As for the cumulative emission of CO_2, they were 5120.39, 193,214.49 and 100,388.49 $kg \cdot m^{-2}$ in soil, SSAD and green substrate, respectively. Since the organic matter in SSAD was much higher than that in soil, and SSBC was a carbon-rich material, the emission of CO_2 in SSAD and green substrate will be higher than that in soil. The addition of SSBC decreased the emission of CO_2 in SSAD by 48.04%.

3.3. NH_3 volatilization

The emission of NH_3 emission in soil, SSAD and green substrate were shown in Fig. 4. NH_3 emission in soil was almost 0; the NH_3 emission amount of SSAD was increasing with the extension of time and the rising of temperature. The NH_3 emission tended to be stable and fluctuated around 5.5 μg/g and the temperature was stabilizing at 27 °C after the 15th day, which demonstrated that temperature might affect the emission of NH_3.When 40% SSBC was added into SSAD, NH_3 emission was obviously reduced. The maximum emission (2.5 μg/g) in green substrate was at first day, then the emission of NH_3 dropped sharply in the first five days and remained stable at about 0.5 μg/g after the 6th day.

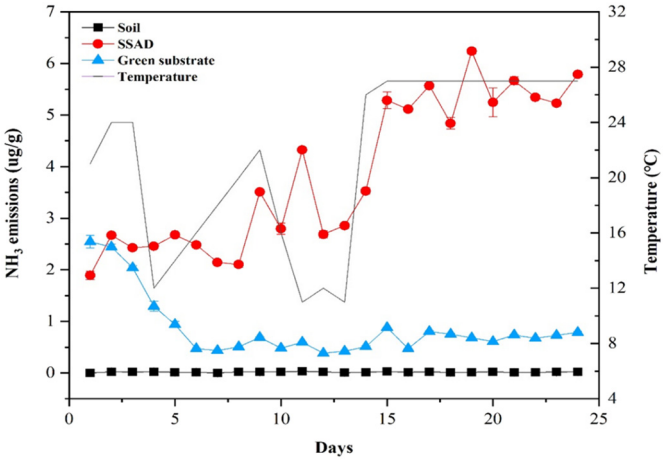

Fig. 4. The NH_3 emission in soil, SSAD and green substrate (SSAD: sewage sludge anaerobic digestate).

3.4. Comparison of microbial community structures

3.4.1. Bacteria community

Bacteria communities of soil, SSAD and green substrate were compared from the phylum and class level, and the results of main groups (>0.01) are shown in Fig. 5a and b. According to Fig. 5a, *Proteobacteria*, *Actinobacteria* and *Firmicutes* were the most abundant phylum in three samples. Among them, *Proteobacteria* was the most abundant phylum group in three substrates, which accounted for 74.52%, 32.59% and 77.29% in SSAD, soil and green substrate, respectively. The abundance of *Proteobacteria* was similar in SSAD and green substrate, but was approximately 2 folds high in soil. *Actinobacteria* accounted for 10.52%, 24.21% and 8.17% in SSAD, soil and green substrate, respectively. The abundance of *Actinobacteria* in green substrate was 22.34% and 66.25% lower than those in SSAD and soil. *Firmicutes* was reported to be dominant in AD system (Nguyen and Khanal, 2018), which was 13.50%, 7.06% and 6.71% in SSAD, soil and green substrate. In total, there were no significant differences in the relative abundance of these three main phylum groups in SSAD and green substrate. Therefore, the effect of SSBC was not obvious at the phylum level.

At the class level (Fig. 5b), the main groups (>0.01) in SSAD and green substrate was similar in total, but different from soil. *Gammaproteobacteria*, *Alphaprotecbacteria*, *Bacilli*, and *Actinobacteria* were the dominant group in three substrates. The relative abundance of *Gammaproteobacteria* in SSAD (50.90%) and green substrate (45.96%) were 42.76% and 37.82% higher than that in soil (8.14%). The abundance of *Alphaprotecbacteria* was 23.61%, 22.48% and 31.31% in SSAD, soil and green substrate, respectively. It was not significantly different in SSAD and soil, but after adding 40% SSBC, the abundance of it was increased by 32.61%. The abundance of *Actinobacteria* in SSAD and green substrate were 13.70% and 16.05% lower than that in soil, but the abundance of *Bacilli* was 7.37% and 0.7% higher than that in soil. However, only considering the SSAD and green substrate, the addition of SSBC decreased the abundance of *Actinobacteria and Bacilli* by 22.34% and 51.71%.

3.4.2. Archaeal community

The relative abundances of archaeal at family level in three substrates are illustrated in Fig. 6. The dominant groups at family level were similar

Table 4
GHG cumulative emission and GWP in different substrate.

Treatments	N_2O cumulative emission ($kg \cdot m^{-2}$)	CH_4 cumulative emission ($kg \cdot m^{-2}$)	$GWP_{(CH4+N2O)}$ ($kg\ CO_2 \cdot hm^{-2}$)
Soil	4.517	0.710	1363.816
SSAD	24.229	0.340	7228.742
Green substrate	6.198	0.792	1842.012

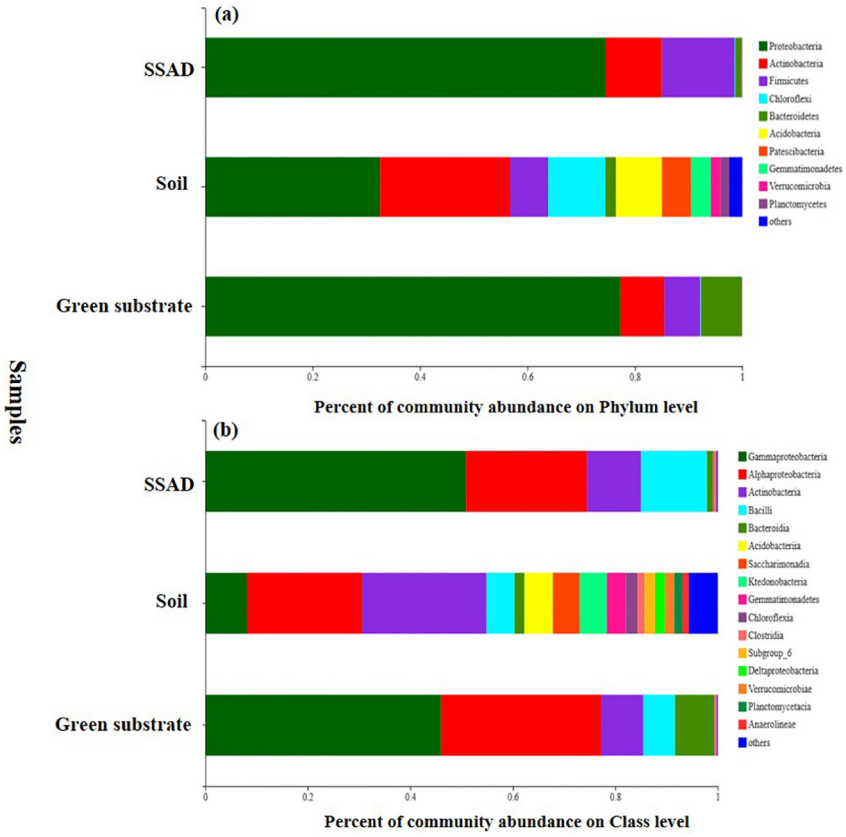

Fig. 5. The relative abundance of bacteria community at the phylum level (a) and class level (b).

in SSAD and green substrate, but significantly different from that in soil. *Nitrososphaeraceae*, which was dominant group at the family level in soil, accounts for 81.61%, but it did not exist in SSAD and green substrate. *Methanobacteriaceae* and *Methanosarcinaceae* were the dominant group at family level in SSAD and green substrate. *Methanobacteriaceae* accounted for 79.39% and 88.61%, respectively. The addition of SSBC increased the abundance of *Methanobacteriaceae* by 11.61% in SSAD. *Methanosarcinaceae* accounted for 17.40% and 5.71% in SSAD and green substrate, which decreased by 67.18% after adding SSBC.

4. Discussion

4.1. The effect of perlite and SSBC on the ryegrass growth

Ryegrass could hardly survive in SSAD, which is attributed to poor permeability. Perlite is an agriculture filler with less nutrient content and high permeability (Broxtermann et al., 2017; Hu et al., 2014). The porosity of SSAD was improved with adding perlite. Perlite is a kind of glassy rock which was formed by volcano eruption, including 1–5% of

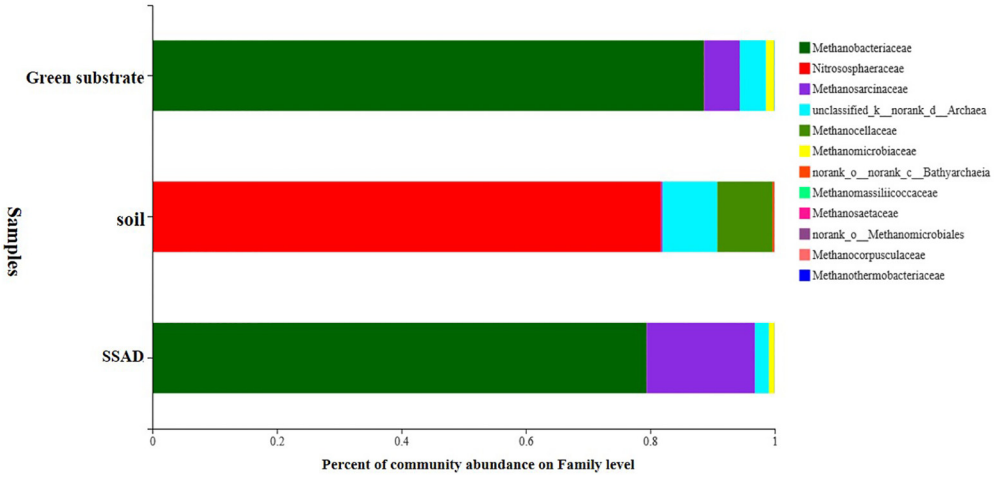

Fig. 6. The relative abundance of Archaeal community at the family level.

combined water (Raji et al., 2019; Silber et al., 2010). The main compound of perlite is silicon oxide (70–75% of weight), but it also contains metal mineral nutrient, such as 12–15% aluminum oxide (Al_2O_3), 3–5% potassium oxide (K_2O), 3–4% sodium oxide (Na_2O), 0.5–2% iron oxide (Fe_2O_3), 0.5–1.5% calcium oxide (CaO) and 0.2–0.7% magnesium oxide (MgO_2) (Agar et al., 2019). Therefore, the addition of perlite also provides metal mineral nutrients for the growth of ryegrass and dilutes the high salt and nutrient contents to avoid inhibiting the growth of ryegrass. According to calculation, when adding 60% of perlite, the value of salt content, organic matter, total nitrogen, phosphorus was 3138.48, 9660.00 and 444.28 mg/kg, respectively, and organic matter was 13.80%. They were still higher than that in soil even adding 80% of perlite, but 80% of perlite was not beneficial to containing water, and 40% of perlite was too less to dilute the high salt concentration. Therefore, the optimum addition of perlite in this study was 60%. When adding 60% (of total weight) perlite, the seedling survival rate and biomass increased to the maximum extent (Fig. 1).

After SSAD was pyrolyzed into biochar under high temperature (600 °C), the nutrient content decreased in some degree (Table 2). Compared with SSAD, available nitrogen and available potassium of SSBC decreased by 96.7% and 82.2%, respectively. Available phosphorus of SSBC increased by 107.3%. Khan et al. (2013) also reported a decrease of nitrogen content and an increase of phosphorus content, when sludge was pyrolyzed into biochar. Generally, available N is closely related to the NH_4^+-N content (Moller and Muller, 2012), which is easy to convert nitrate, resulting in a decrease in the content of available N. Besides, NH_4^+-N can be captured by the SSBC in pores to chemical reactions, which makes higher NH_4^+-N removal (Thant Zin and Kim, 2021; Zhang and Wang, 2016).There are more polyphosphate compounds in SSAD compared to nature soil (Singh and Agrawal, 2007). They might decompose to increase the content of available P. On the other hand, SSBC possessed large pore size and surface area (Table 2), which would help improve the air and water permeability of SSAD to enhance the root breath. The porosity and nutrients content were well adjusted after mixing SSAD and SSBC. When 40% of SSBC was added into SSAD (with 60% of perlite), the seedling survival rate and biomass reached the maximum value (100% and 100.19 mg) and biomass was obviously higher than that in soil (Fig. 2). Furthermore, SSBC would provide shelter for microbes due to large surface area (Zhu et al., 2017). Some microbes (e.g., *Proteobacteria*, *Actinobacteria* and *Firmicutes*) can promote the growth of ryegrass by improving their absorption of nutrients or decomposing some substances in the substrate into easily absorbed components; these microbes had a higher abundance in green substrate (Fig. 5). In a word, the growth of ryegrass in green substrate was improved by these pathways.

4.2. The effect of substrate properties on the gas emission

The emission of GHG and NH_3 is related to two main factors, i.e., substrate properties and microbial community (Xiao et al., 2019). Here the substrate properties were mainly discussed, including TN, oxygen content, organic matter (OM), pH and temperature.

TN and oxygen content affect the emission of N_2O and NH_3. The emission of N_2O is related to nitrification and denitrification process (Brar et al., 2013), especially an active denitrification process. Due to the poor permeability of SSAD, it was easy to form anaerobic environment and facilitated denitrification process, which leading to higher N_2O emission. The higher release of N_2O from river deposits is also due to this reason (Song et al., 2021). Meanwhile, the content of TN in SSAD was 14.8-fold higher than that in soil, indicating more nitrate was denitrified to produce N_2O. For these reasons, SSAD had the highest gas emission. When adding 40% SSBC into SSAD to prepare green substrate, oxygen content in substrate was increased by improving permeability and the content of TN decreased, leading to a decrease of N_2O emission. Khan et al. (2013) reported the same result that the emission of N_2O in paddy soil was significantly reduced by adding SSBC. As for NH_3 volatilization, it is a main pathway of N loss (Wang et al., 2018). NH_3 volatilization increased with the increase of pH and temperature. Since alkaline condition is more favorable for NH_3 volatilization and higher molecule movement with the rising of temperature (Yang et al., 2018).

The emission of CO_2 was mainly affected by OM and soil respiration. OM was decomposed by respiration to produce CO_2 (Hou et al., 2020). The microorganisms that can promote the decomposition of OM had a higher abundance (see Section 4.4) in SSAD, and the OM content was SSAD>green substrate>soil. Therefore, the emission of CO_2 was higher in SSAD than that in green substrate and soil.

CH_4 emission was related to anaerobic decomposition of OM, which was mainly affected by carbon content and microbial activity (Ji et al., 2020). SSBC was a carbon-rich material with 14.05% C content (Table 2), the addition of 40% SSBC increased the C content in green substrate, which might increase the emission of CH_4. It is reported that biochar is an effective additive to improve methane production (Shen et al., 2021; Tu et al., 2019). Meanwhile, due to large surface area of biochar (Table 2), it can enhance direct interspecies electron transfer, which would improve syntrophic action and bacterial growth (Baek et al., 2018). The addition of SSBC might improve electron transfer by reducing acid stress (Sunyoto et al., 2016), which simultaneously enhance methanogenic activity.

What's more, pH value has effect on all gases. The pH of SSAD was mainly controlled by the concentration of NH_4^+, PO_4^{3-}, CO_3^{2-} and HCO_3^- (Hjorth et al., 2010). The pH of SSAD was 8.46 ± 0.02 (Table 1), which was beneficial to the hydrolysis of ammonium carbonate, resulting in the production of CO_2 and NH_3 (Moller and Muller, 2012). The gas emission of green substrate was also higher than that in soil because the pH value of SSBC was 8.09 ± 0.02 (Table 2). Besides, pH in soil and biochar was crucial factor impacting the emission of N_2O and NH_3 (Sha et al., 2019). Biochar can increase pH to stimulate the N_2O reductase enzymatic activity of denitrifying bacteria to suppress the N_2O emission (Liu et al., 2010; Zhu et al., 2017). Furthermore, variation of pH may lead to generation of aluminum and iron hydroxide precipitations in substrate, and the substrate ammonium would be mainly adsorbed by negatively charged organic functional groups, thus reducing NH_3 volatilization (Sha et al., 2019; B. Wang et al., 2015). Temperature also affected the emission of NH_3, especially in SSAD. The trend is similar to that of temperature (Fig. 4). This may be attributed to the higher molecule movement with the rise of temperature (Yang et al., 2018).

4.3. Global warming potential

The cumulative emission and global warming potential (GWP) of GHG are shown in Table 4. Compared to the cumulative emission in SSAD and green substrate, after adding 40% SSBC, the cumulative emission of N_2O and CO_2 in SSAD decreased by 74.41% and 48.04%, the cumulative emission of CH_4 increased by 57.07%. GWP was integrated by GHG (CH_4 and N_2O) emission fluxes. After adding 40% SSBC, $GWP_{(CH4+N20)}$ decreased by 74.52% in SSAD. Similar results were also observed in other studies, so it is considered to be more environmental-friendly after adding SSBC into SSAD. The emission of N_2O and CH_4 in green substrate was not significantly different from soil, and the $GWP_{(CH4+N20)}$ in green substrate was 1.35 times higher than that in soil. It is reported that the $GWP_{(CH4+N20)}$ was 1.18 times higher than nature soil after using nitrogen, phosphorus and potassium fertilizer (Wang et al., 2019). The GWP was not much higher than that in soil, whereas the growth of ryegrass in green substrate was significantly increased. Therefore, the green substrate was suitable for a plant substrate.

4.4. Microbial community changes the emission of gas

The microbial community structure in SSAD and green substrate was different from that in soil (Fig. 5a and b). *Proteobacteria*, *Actinobacteria*

and *Firmicutes* were the main bacterial groups with different abundance (Fig. 5a). They were also the common phyla group in the mesophilic AD system (Ji et al., 2020; Nguyen and Khanal, 2018). They could enhance the production of CO_2 and CH_4 (Fig. 3b and c). Since *Proteobacteria* had a higher abundance in SSAD and green substrate (74.52%–77.29%), which could induce cell lysis and release intracellular substances to enhance the hydrolysis and acidification process (Cheng et al., 2018). Actinobacteria is responsible for the degradation of some complex substrates (e. g. alkyl ethers, tetrahydrofuran (Kim et al., 2008)) by producing hydrolytic enzymes (Cheng et al., 2018; Ruan et al., 2019). *Firmicutes* is responsible for the degradation of complex organic matter and denitrification (Kumar et al., 2010). Anaerobic fermentation in the substrate was enhanced under these effects, and the production of CO_2 and CH_4 would finally be enhanced resulting in the increase of emission. Besides, the abundance of *Actinobacteria*, *Firmicutes*, *Chloroflexi* in SSAD and green substrate was lower than that in soil, which had less inhibition effect on denitrification (Dos Santos et al., 2012; McGrath et al., 1995). The N_2O was an intermediate product in the process of denitrification, thus the emission of N_2O was higher than that in soil (Fig. 3a). After adding 40% of SSBC, the abundance of them decreased (Fig. 5a), therefore the emission in green substrate was lower than that in SSAD.

From the class level, *Gammaproteobacteria* and *Alphaprotecbacteria* belong to phylum *Proteobacteria*, they are the main consumers of glucose and VFAs (Ariesyady et al., 2007). The decomposition of VFA was faster in SSAD and green substrate, which could enhance the production of CH_4 (Fig. 3b). Meanwhile, *Alphaprotecbacteria*, which is related to N cycling, belongs to Proteobacteria phylum (Burges et al., 2020). *Actinobacteria* and *Bacilli* might influence the N cycle (Dos Santos et al., 2012; McGrath et al., 1995). The abundant of them in green substrate increased when 40% SSBC was added, which may result in decreasing in the emission of N_2O and NH_3.

As for archaeal community, *Nitrososphaeraceae* was the most abundant group in soil (Fig. 4). It is a common ammonia-oxidizing archaea, which plays a key role in NH_3 oxidation (Li et al., 2019; Li et al., 2015). Therefore, the emission of NH_3 in soil was much lower than that in SSAD and green substrates. *Methanobacteriaceae* and *Methanosarcinaceae* are two major methanogen families annotated (Y.B. Wang et al., 2015), which are responsible for the production of CH_4. They were the most abundant group in SSAD and green substrate (Fig. 6) resulting that the higher emission of CH_4 (Fig. 3b). After adding 40% SSBC, the abundance of *Methanobacteriaceae* was increased (79.39%–88.61%), which may cause an increase of the CH_4 emission in green substrate (Fig. 3b).

5. Conclusion

The green substrate was prepared by mixing SSAD and SSBC (SSAD: SSBC = 6:4, w/w) as an initial substrate, and then add quartz sand (25% weight of initial substrate) and perlite (60% weight of initial substrate) as filler. The seedling survival rate and individual biomass of ryegrass in green substrate was 1.32% and 19.59% higher than those in soil, which proved that green substrate was suitable for plant growth, it can be as a plant substrate. Except for CH_4, the NH_3 and GHG emission of SSAD were reduced by adding SSBC, reducing the impact of SSAD on the environment during the application. According to the analysis of microbial community, the abundance of nitrogen-fixing bacteria was increased by adding SSBC and methanogens was also enhanced, which caused the different gas emission, but the total emission amount in green substrate compared with soil was not so high. The green substrate can not only provide better fertility than soil, but also increase the usage amount of SS and relieve the pressure of high SS production. Meanwhile, the addition of SSBC made the structure and diversity of microbial community change, the NH_3 and GHG emission were reduced, which made less impact on the environment. Furthermore, the biogas produced in the AD process could be the energy source for the pyrolysis of SSBC; and it will reduce the cost and achieve resource recycling.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was funded by the National Natural Science Foundation of China (Nos. 21806100, 21677093), Natural Science Foundation of Shanghai (No. 18ZR1414100).

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