Recovery of nitrogen and phosphorus nutrition from anaerobic digestate by natural superabsorbent fiber-based adsorbent and reusing as an environmentally friendly slow-release fertilizer for horticultural plants

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Abstract:

[Purpose] To help minimize the negative impact of chemical fertilizers on the environment, recycle nitrogen and phosphorus nutrients of anaerobic digestate and reduce loss of nutrients via leaching, an eco-friendly slow-release fertilizer was prepared through recovery of nitrogen and phosphorus nutrition from digestate using superabsorbent fibers extracted from soybean curd residue as an adsorbent. **[Methods]** The preparation method was proposed, and the fiber composite-based adsorbent was characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscope (SEM) techniques. **[Results]** The successful incorporation of N and P into the fiber composite-based adsorbent via adsorption was confirmed by results of these analyses. Also, the swelling capacity as well as water retention capability of the obtained fiber composite-based adsorbent were evaluated. The release behavior of N and P from impregnated fiber composites was examined and was found to be partially in good accordance with the standard of the Committee of European Normalization, showing its good slow-release and water-retention properties. Furthermore, in order to assess the fertilizer quality of the prepared materials, the effects of different fertilizers (commercially available fertilizer and prepared slow-release fertilizer) on tomato plant growth and soil microbial communities were investigated. **[Conclusions]** The obtained results have demonstrated the potential of fiber composite-based slow-release fertilizer system for recycling N and P nutrition from digestate, improving the effectiveness of fertilizer as well as protecting the environment.

Keywords: resource recovery; bio-fertilizer; water absorbency; encapsulation; soil microbial communities; pyrosequencing

1. Introduction

Water and fertilizer play crucial roles in intensive agricultural production [1-3]. Over the past several decades, large amounts of commercially available chemical fertilizers (e.g. nitrogen and phosphorous fertilizers) have been applied around the world to enhance the crop yields to meet the increasing demand for foods [4,5]. However, suboptimal or over-fertilization not only causes huge economic and resource losses due to the leaching and volatilization of nutrients, but also causes severe ecological, environmental and health issues (e.g. sharp decline in biodiversity, water eutrophication and water resource pollution) [6-8]. Currently, water deficiency is also a global problem [9].

Considering these issues, one of the most promising solutions is considered to be synthesis of controlled-release fertilizers (CRFs) or slow-release fertilizers (SRFs) using superabsorbent polymers or composites as carriers [10]. The CRFs or SRFs products were developed to release nutrients gradually for plant uptake during the plant growth. Hence, the CRFs or SRFs hold great potential in improving nutrient use efficiency and mitigating the related environmental pollution issues [6,7]. Hitherto, many superabsorbent materials applied to the CRFs or SRFs had been studied, including chitosan-based polymer [11], carboxymethyl cellulose [12,9], starch-based polymer [5], biochar-based copolymer [7,4], waste paper [13], and wheat straw-based adsorbent [14]. More recently, Chen et al. [7] published a good review to summarize the natural materials used in environmentally friendly fertilizers and demonstrated that various natural materials (e.g. chitosan, starch, cellulose, lignin, biochar, and agricultural residues) have been successfully employed as carriers or coatings to regulate nutrients release and improve fertilizer use efficiency in varying degrees. Nevertheless, most of the previous studies were based on lab-scale experiments; the ecological and economic limitations for scaling up of the technologies for production of CRFs or SRFs remain to be explored. In addition, most of the CRFs or SRFs release nutrients fast in the early stages of crop growth (e.g. around 65-100% release within 20-40 days [15,4,12], whereas plants still need to uptake nutrients during the medium and late stages of growth [4]. Thus, novel CRFs or SRFs with advantages in the longer term slow release of nutrients and higher ecological and economic feasibility could be developed by using renewable and biodegradable carrier materials (e.g. superabsorbent fibers from bio-wastes) and cheap nutrient sources (e.g. anaerobic digestate).

Soybean curd residue (SCR) is a relatively inexpensive and abundant soy food processing by-product in the soy product factories [16,17]. SCR is rich in crude fibers composed of cellulose, hemicellulose, and lignin, accounting for about 50% of the dry weight in soybean [18,19]. The fibers in SCR could be manufactured into biodegradable and superabsorbent polymers, acting as a nutrient carrier of SRFs. Compared to traditional superabsorbent carriers synthesized via polymerization, the fiber-based materials have considerable advantages i.e. abundant raw materials, low cost, biodegradability, renewability, and easy operation [20,21,14].

Currently, the large quantity of digestate generated in biogas plants is a critical issue globally. The liquid digestate in anaerobic digesters can be a low-cost nutrient sources, especially for N and P [22,23]. Hence, utilization of biogas digestate as a replacement fertilizer could benefit the digestate

management and resource recovery simultaneously [24]. Compared to the traditional chemical fertilizers, the alternative bio-fertilizer is more environmentally friendly and has a longer-term release period [25]. It can be hypothesized that the nutrient contents of digestate can be integrated into fiber-based superabsorbent materials to generate a more ecological slow-release fertilizer. Hitherto, however, the fiber-based slow release fertilizer using digestate as nutrition resource have not been investigated.

Therefore, the main objective of the present work was to develop an eco-friendly slow-release fertilizer through recycling nitrogen and phosphorus nutrition from anaerobic digestate using the natural superabsorbent fiber-based adsorbent. The physicochemical properties of the fiber composite-based adsorbent were characterized using FT-IR, XRD and SEM techniques. The swelling capacity and water retention capability of the obtained fiber composite-based adsorbent were evaluated. Then, a slow-release fertilizer (e.g. N and P adsorbed onto fiber composite-based adsorbent) was prepared, and its nutrient release behavior was evaluated. Finally, the efficiency of the slow release fertilizer was investigated using the potted tomato as a model plant. This study was motivated by the need for eco-friendly fertilizer production in a sustainable manner. Compared to the traditional chemical fertilizer, fertilization via the developed eco-friendly fertilizer could help reduce the usage of chemical fertilizer, thereby reducing environmental pollution, particularly in the long run. The cleaner production of eco-friendly fertilizer using two wastes could play a vital role in the overall environmental sustainability of modern agriculture and horticulture.

2. Materials and Methods

2.1. Materials

Hydrochloric acid (HCl), sodium hydrate (NaOH), sodium chloride (NaCl) were purchased from Merck Company. Other chemicals used in this work were all of analytical grade and commercially available. Soybean curd residue (SCR) was obtained from the Fortune Food Manufacturing Pte Ltd in Singapore. The SCR was chopped and then dried in an oven at 70 $^{\circ}$C for 48 h. The commercially available fertilizer (Doo Worx Liquid Soil Supplement, composted organic cow manure) was purchased from Equipment Engineering Pte Ltd in Singapore. The tomato seeds were purchased from the Singapore Horti-Flora Company. The soil for tomato experiments was FERTIPLUS universal potting soil purchased from the Far East Flora Pte Ltd in Singapore. The main characteristics of the soil were summarized in Table S1 in supplementary material. The digestate was collected from a 20 L anaerobic

digester treating food waste in the E2S2-CREATE lab in Singapore. The characteristics of SCR and digestate are shown in Table 1.

Table 1. Characteristics of soybean curd residue and anaerobic digestate used in this study.

Characteristics	Units	Soybean curd residue	Anaerobic digestate
Crude fiber	wt%[2]	55.58 ± 0.16	-
Extractives[1]	wt%[2]	44.42 ± 0.13	-
Total solids (TS)	wt%[3]	23.13 ± 0.02	4.90 ± 0.11
Volatile solids (VS)	wt%[3]	22.03 ± 0.01	3.45 ± 0.03
VS/TS	-	0.952	0.704
pH	-	6.91 ± 0.02	8.21 ± 0.10
Total nitrogen	mg/L	-	3325 ± 225
Total phosphorus	mg/L	-	62.25 ± 13.75
NH_3-N	mg/L	-	3301 ± 145
NO_3^--N	mg/L	-	0.75 ± 0.25
C	wt%[2]	44.73 ± 0.18	36.66 ± 0.12
H	wt%[2]	7.36 ± 0.04	5.04 ± 0.03
N	wt%[2]	4.03 ± 0.02	5.90 ± 0.04
S	wt%[2]	ND[4]	2.41 ± 0.02
P	wt%[2]	0.26 ± 0.01	1.85 ± 0.03
K	wt%[2]	1.22 ± 0.02	0.47 ± 0.02

Note: [1]: Extractives represent organic compounds (e.g. carbohydrate, protein and fat) extracted from the sample with the neutral detergent (3% w/v SDS). [2]: On dry basis. [3]: On wet basis.[4]: ND refers to not detected in the samples.

2.2. Experimental design and procedures

2.2.1. Extraction of crude fibers from soybean curd residues

Fig. 1 shows the proposed preparation process of the fiber composite-based slow-release fertilizer, including extraction of crude fibers from soybean curd residues. First, the dried SCR was cut into small

pieces (approximately 3-6 mm in length). The SCR was suspended in distilled water and incubated at 90 ˚C for 6 h to solubilize the water-soluble components (e.g. sugars). Water-soluble fiber components were then precipitated using ethanol. Subsequently, the mixture solution was filtered with the sintered filter funnel. Subsequently, the intermediate product was treated with 3% w/v SDS neutral solution at 100 ˚C for 1 h to remove the proteins and carbohydrates. After filtration, crude fiber was successively washed several times with distilled water and 95% ethanol until neutral. Finally, the neutral fibers were dried using an oven at 60 ˚C for 24 h and the resultant product was weighed and used as the fiber-based superabsorbent materials (FBSM). The overall yield of crude fibers in this study was around 56 wt% on dry basis.

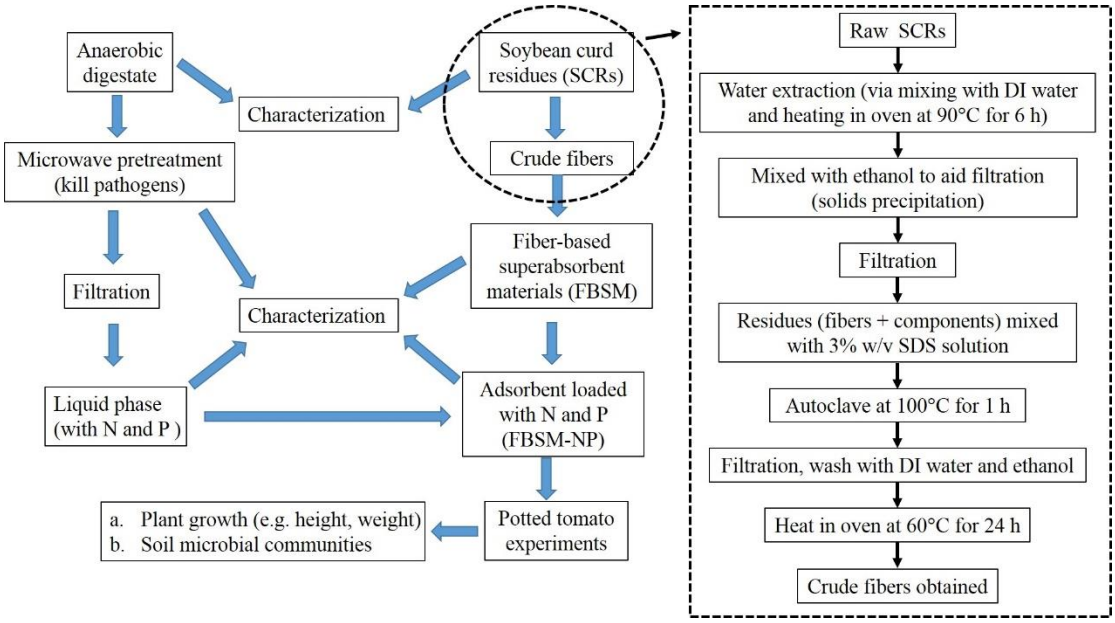

Fig. 1. Flowchart of preparation of fiber-based NP fertilizer (FBSM-NP), aiming at the integral use of anaerobic digestate and soybean curd residues.

2.2.2. Microwave pretreatment of anaerobic digestate for pathogen reduction

The main function of microwave pretreatment (MW) process is to kill the pathogenic microorganisms or pathogens in the anaerobic digestate (Tong et al., 2016). Raw anaerobic digestate was first pretreated by using a 28 L microwave oven (350 mm (w) × 360 mm (l) × 245 mm (h), NN-ST651M, Panasonic Corporation, Japan) with a 320-mm turntable and a power requirement of 230-240V, 50Hz. The microwave oven had a power range of 0-1000 W. The MW pretreatment was operated at 800 W for 20 min with an agitation rate of 45 rpm. After MW pretreatment, the original and pretreated anaerobic

digestate were sampled to analyze the abundance of the antibiotic resistance genes (ARGs). Afterwards, the pretreated digestate was centrifuged at 10,000 rpm for 10 min and then filtrated through 0.45 μm filters to obtain the liquid phase. The concentration of total nitrogen and total phosphate in the liquid phase was determined as 3325 and 62.25 mg/L, respectively. The liquid phase of the digestate was utilized as the nutrition source of N and P in subsequent experiments.

2.2.3. Preparation of fiber-based superabsorbent materials loaded with N and P (FBSM-NP)

As shown in Fig. 1, before loading with N and P, the dried crude fibers were ground into homogeneous granules ranging from 0.135 to 0.170 mm in diameter (90-110 meshes). The loading of N and P onto the fiber-based superabsorbent material was performed by swelling of the dried fiber granules (m_0) in the liquid phase of the digestate for 24 h. The swollen loaded composite were then filtered, dried at 60 ˚C for 48 h in a vacuum oven, and then weighed (m_1) with electronic balance. The loading percentage was calculated through the following equation: Loading percentage (%) = 100 * (m_1-m_0)/m_0. Finally, the fiber-based superabsorbent materials loaded with N and P (FBSM-NP) was further characterized and evaluated as a fertilizer in potted tomato tests.

2.2.4. Measurement of water absorbency of FBSM and FBSM-NP in swelling experiment

Approximately 2 g (a_0) of the dry FBSM or FBSM-NP sample was accurately weighed and immersed into 300 mL distilled water and allowed to soak at 25 ˚C for 24 h till the swelling process was completed. The swollen FBSM or FBSM-NP was filtered by using an 80-mesh sieve to remove all the unabsorbed water and then weighed (a_1). The water absorbency was measured using the next equation: Water absorbency = (a_1 - a_0)/a_0. The pH-sensitivity behavior of FBSM and FBSM-NP was investigated in solutions with different pH values (3, 5, 7, 9 and 12). Diluted 0.1 M solutions of HCl and NaOH were used to adjust the pH values.

2.2.5. Measurement of water-retention capacity of FBSM and FBSM-NP in soil

The water-retention capacity of FBSM and FBSM-NP in soil was measured using the water evaporation ratio of soil as an indicator according to the method [26]. Briefly, a 3 g sample of FBSM or FBSM-NP was well-mixed with 80 g of dry soil and transferred into a plastic beaker. Each beaker was supplemented with identical 100 g of distilled water and was then weighed (W_0). A control beaker

without FBSM or FBSM-NP was performed at the same conditions. All the beakers were stored at room temperature and were weighed every 1-3 days (W_x) over a period of 60 days. Finally, the water evaporation ratio (W%) of different soil samples was measured according to the following equation:

$W\% = 100*(W_0 - W_x)/100$

2.2.6. Slow release behavior of FBSM-NP fertilizer in soil

The slow release behavior of FBSM-NP fertilizer in soil was investigated using the method described by Wu et al. [26]. The slow release property of the fertilizer formulation was then evaluated using a standard of Committee of European Normalization (CEN) [27]. The standard defined that a fertilizer can be described as a slow-releasing one if its nutrients show slow-release properties under a temperature of 25 °C, and accord with the following three criteria: 1) no more than 15% released in 24 hours, 2) no more than 75% released in 28 days, and 3) at least about 75% released at the stated release time.

2.2.7. Potted tomato experiment

The fertilizer quality of FBSM-NP was evaluated in the potted tomato trial (Figure S1 in supplementary material). Initially, 1 kg soil was weighed and transferred into each flowerpot. All the flowerpots were then randomly allocated into four groups corresponding to four different fertilization treatments: control without fertilizer (T0); fertilization with 100 g FBSM-NP (T1); fertilization with 100 g FBSM (T2); fertilization with 100 g commercial fertilizer (T3). After fully mixing the soil and fertilizer, approximately 5 tomato seeds were sowed at the soil depth of 5 mm in each flowerpot according to the instructions of the manufacturer. Each treatment consisted of three flowerpots; each flowerpots cultivated two tomato plants after germination through removing 1 or 2 plants per flowerpot. The tomato growth period in this study was about 100 days. The growth process of the tomato was recorded by taking photos every 7 days. At the end of the tomato experiment, the effects of four treatments were compared in terms of tomato root system and the height of plants. In addition, the soil was sampled from each flowerpot at the end of the experiment and the effects of different treatments on soil microbial communities were investigated using pyrosequencing technology.

2.3. Analytical methods

Fourier transform infrared (FT-IR) absorption spectra of the samples were recorded using a Bruker Equinox 55 FT-IR equipped with an ATR accessory. The wavenumber range was 200-4000 cm^{-1}. The resolution and sample scan time was 4 cm^{-1} and 32 scans, respectively. The FBSM and FBSM-NP samples were dried at 45 $^{\circ}C$ for 2 h and were crushed for analysis. Crystal structure of the sample was investigated using an X-ray diffraction (XRD) meter (Bruker D8 Advance, Germany) using a Cu Kα radiation (λ = 0.154 nm) with an acceleration voltage of 40 kV and a beam current of 30 mA. The scanning range of 2θ was 5-75° and the scanning speed was 0.04° per second. The morphology of selected samples was observed using a field emission scanning electron microscope (SEM, JEOL-6700F, USA). Concentration of total nitrogen, NH_4^+-N, NO_3^--N, and total phosphate of liquid samples were measured using a DR900 multi-parameter portable colorimeter (HACH, USA) via corresponding kits according to the manufacturer's manual. A pH meter (Agilent 3200P, USA) was used to measure pH of samples. Volatile solids (VS), total solids (TS) and elemental composition (C, H, N, S, P and K) were determined using the methods described in Zhang et al. [28]. The crude fiber contents were measured using the method modified from van Soest et al. [29].

2.4. DNA extraction and 16S rRNA gene pyrosequencing of soil microbial communities

Following the manufacturer's protocol, total DNA of microbial communities was extracted in soil and digestate samples (solid phase), respectively, using the PowerSoil DNA extraction kit (MoBio Laboratories, Inc., USA). The quality of the collected DNA was checked by determining its absorbance at 280 nm and 260 nm with a UV-Vis Spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000, USA). Afterwards, the PCR reactions were performed to amplify DNA fragments of the microbial communities. In particular, the primers 1369F and 1492R were used [30]. Then, following the procedures described by Zhang et al. [31,32], IIIumina Hiseq 2000 pyrosequencing technique was utilized to investigate the microbial community structure. To attain the effective sequencing data, the pretreatment procedure was first performed on the raw sequencing data following the instructions by Zhang et al. [28]. Subsequently, the effective sequences were aligned via a sequence aligner (MOTHUR) with the help of a SILVA database. The aligned sequences were then clustered into operational taxonomic units (OTUs) via Usearch software using the average neighboring clustering algorithm at the 97% similarity level. Afterwards, the OTU-based analysis of the sequences was carried out using the MOTHUR software.

2.5. Statistical analysis

Each sample analysis or treatment in this study was carried out in triplicate. The statistical significance among different treatments was determined through analysis of variance (ANOVA) using SAS software (SAS Institute Inc., USA). A threshold value of $p < 0.05$ was used for statistical significance.

3. Results and Discussion

3.1. Characterization of fiber-based superabsorbent materials (FBSM) and FBSM-NP

3.1.1. Elemental analysis

The elemental contents in the FBSM-NP, FBSM and commercial fertilizer are presented in Table 2. As shown in Table 2, the N content in FBSM-NP was 3.65 wt%, which was 1.5-fold and 2.1-fold higher that of FBSM and commercial fertilizer, respectively. The P content in FBSM-NP was 0.14 wt%, which was 7-times higher than that of FBSM, but was only 35% of P content in the commercial fertilizer. Compared to FBSM, the relatively high N and P content in FBSM-NP confirmed the successful incorporation of N and P into FBSM.

Table 2. Elemental contents of the FBSM-NP, FBSM and commercial fertilizer.

Elements (wt%[1])	FBSM-NP	FBSM	Commercial fertilizer
C	49.38 ± 0.14	48.97 ± 0.12	25.61 ± 0.07
H	7.33 ± 0.04	7.14 ± 0.05	2.69 ± 0.03
N	3.65 ± 0.07	2.51 ± 0.03	1.77 ± 0.02
P	0.14 ± 0.02	0.02 ± 0.00	0.40 ± 0.03
K	0.42 ± 0.03	0.11 ± 0.01	0.81 ± 0.04
S	0.67 ± 0.01	< 0.50	0.55 ± 0.02
Na	0.67	0.48	0.20
Ca	0.11	0.04	4.18
Mg	0.04	0.01	0.35
Fe	< 0.01	< 0.01	0.20
B	< 0.01	< 0.01	ND[2]

Al	< 0.01	< 0.01	0.71
Cu	< 0.01	< 0.01	< 0.01
Cr	ND	ND	< 0.01
Mn	ND	ND	0.02
Ni	ND	ND	< 0.01
Zn	ND	ND	0.01

[1] On dry basis. [2]: ND refers to not detected in the samples.

3.1.2. SEM, XRD and FT-IR analysis

The SEM images of the FBSM and FBSM-NP fertilizer formulations are shown in Fig. 2. As can be seen in Fig. 2a and b, both FBSM and FBSM-NP had porous structures, which might be due to the physical cross-linking effect in the fiber structure [33]. Compared to FBSM, FBSM-NP showed some crystals of loaded NP fertilizer (Fig. 2b), which was consistent with the higher NP content results of elemental analysis (see Section 3.1.1). The FBSM-NP composite formed by incorporation of N and P into a porous fiber structure can retain the nutrient and water in the composite and delay dissolution of the incorporated NP fertilizer, which would contribute to the slow release behavior of the FBSM-NP [34].

To investigate the structure and crystallinity of the prepared samples, XRD patterns of FBSM and FBSM-NP were recorded. The XRD patterns of FBSM and FBSM-NP (Fig. 2c) were characterized by two characteristic peaks at $2\theta = 21°$ and 36°, which could be attributed to the fact that the amorphous cellulose material contained several typical peaks at $2\theta = 20.53°$, 38.87° and 80.90° [35]. With loading of NP onto the FBSM, an obvious loss of the diffraction peaks was observed (Fig. 2c), indicating that the FBSM-NP had a lower crystallinity due to distortion of typical fiber crystalline structure by loaded NP fertilizer compounds, compared to FBSM.

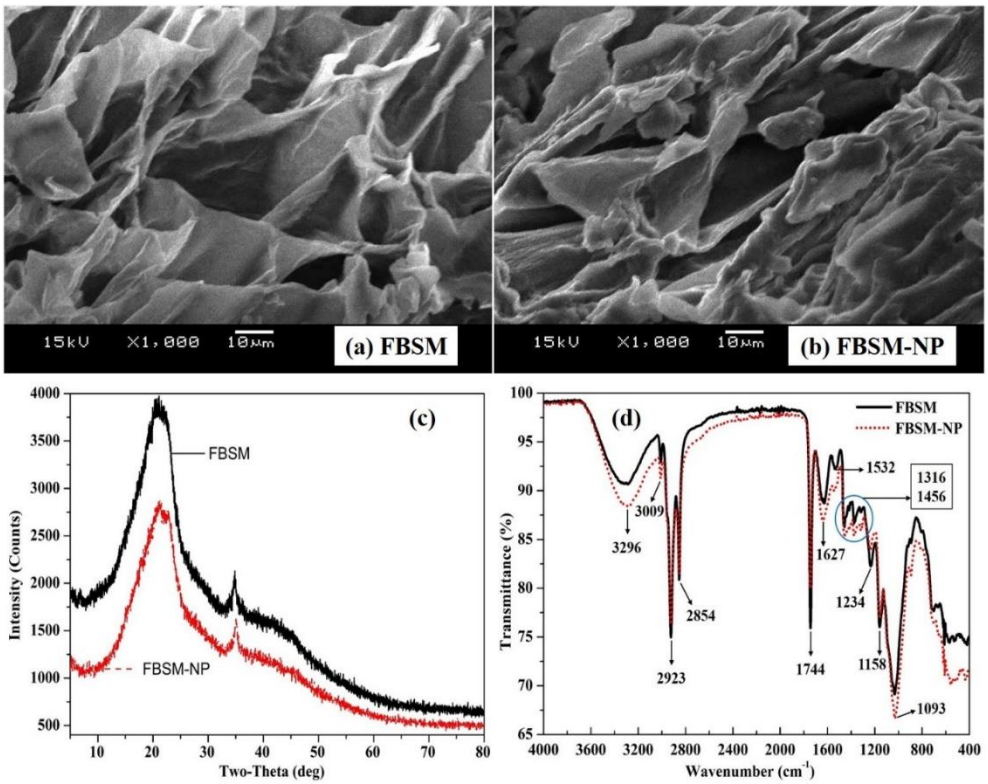

Fig. 2. SEM images (a, b), XRD patterns (c) and FT-IR spectra (d) of FBSM and FBSM-NP.

Fig. 2d shows the FT-IR spectra of FBSM and synthesized FBSM-NP. As shown in FBSM and FBSM-NP spectrum, three peaks appeared at 3296 cm^{-1} and 2923-2854 cm^{-1} were related to the N-H and O-H stretching vibrations, respectively. Furthermore, the peaks found at 1744 cm^{-1} and 1627 cm^{-1} indicated the stretching vibration of the C=O bonds. Also, the peaks appeared at 1456 cm^{-1}, 1158-1234 cm^{-1}, and 1093 cm^{-1} were attributed to the stretching vibrations of the P=O bonds, the C-N bonds and the C-O bonds, respectively. Comparing FT-IR spectra of FBSM with FBSM-NP spectra, an increase of the N-H intensity (3296 cm^{-1}) as well as a decrease of the C=O intensity (1744 cm^{-1}) were observed, which could be attributed to the successful encapsulation of NP-containing compounds within the superabsorbent fiber network (FBSM).

3.2. Effect of microwave pretreatment on pathogen reduction in anaerobic digestate

The feasibility of using microwave pretreatment (MW) to kill the bacterial pathogens in the anaerobic digestate was investigated. As shown in Fig. 3, compared to the original digestate, microwave pretreatment decreased the relative total abundance of bacterial pathogens by 72%. Specifically, a total of 11 bacterial pathogens were identified from sludge samples of original and microwave-pretreated

digestate at the genus level, including *Streptococcus*, *Mycobacterium*, *Escherichia-Shigella*, *Syntrophomonas*, *Clostridium*, *Bacillus*, *Lysinibacillus*, *Acidovorax*, *Klebsiella* and *Proteiniphilum*. This dramatic removal of bacterial pathogens was consistent with the previous results [36,32], showing that microwave pretreatment was a promising approach to eliminate pathogens of the bacterial community. Similarly, it has been reported that microwave pretreatment could efficiently reduce the antibiotic resistant bacteria concentration, and most antibiotic resistance genes concentrations tended to attenuate during the pretreatment [37]. In addition, two bacterial pathogens (e.g. *Mycobacterium* and *Lysinibacillus*) dominated the communities after microwave pretreatment. *Mycobacterium* is a pathogenic bacterial genus which causes pulmonary infection in primates [38]. Bacterial genus *Lysinibacillus* includes some pathogenic species such as *Bacillus cereus* and *Bacillus anthracis*, which could cause food poisoning and human diseases, respectively [39]. Hence, it is imperative to deal with these two pathogenic bacteria using modified microwave pretreatment techniques such as extending microwave pretreatment period and intensity in future studies.

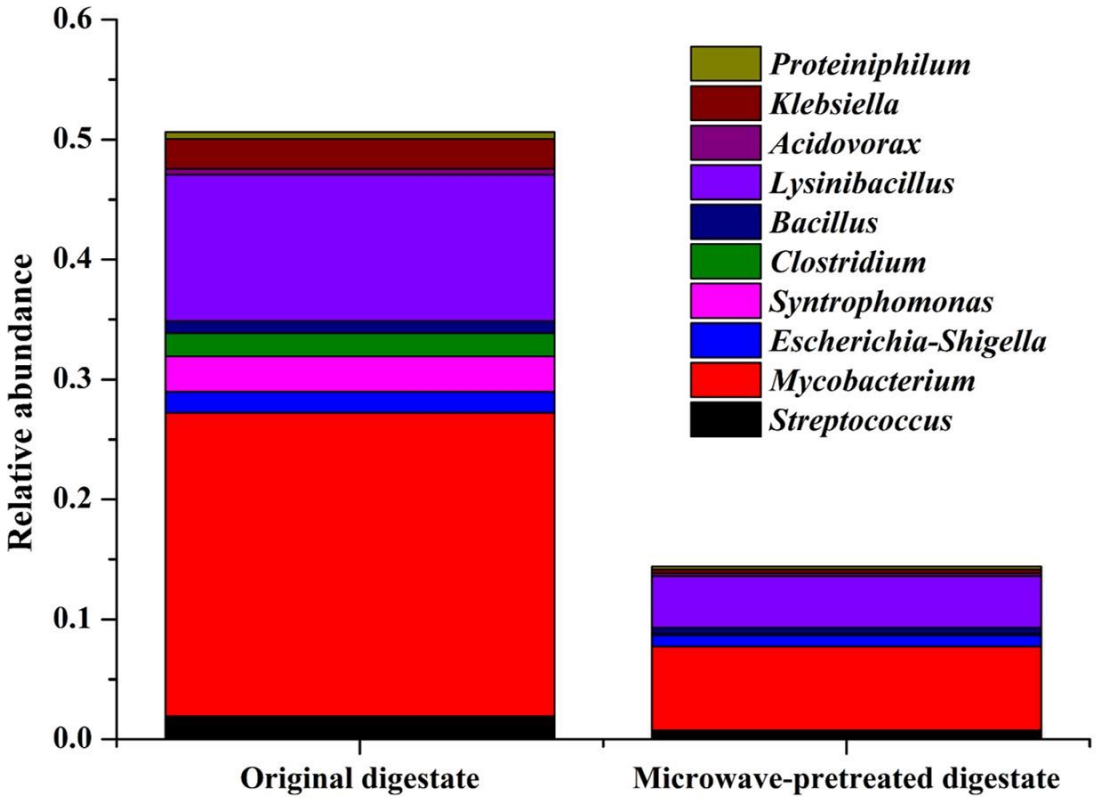

Fig. 3. Relative abundance of bacterial pathogens at the genus level in original and microwave-pretreated anaerobic digestate.

3.3. Water absorbency of FBSM and FBSM-NP in swelling experiment

The water absorbency and its reversibility of FBSM and FBSM-NP were investigated in swelling studies. The samples were put in the distilled water to induce swelling and then dried at a temperature of 60 ˚C, which was repeated 4 times. As shown in Fig. 4a and b, the average water absorbency of FBSM-NP at pH = 7 was 12.6 g/g, which was 25% higher than that of FBSM, indicating that absorbance capabilities of FBSM-NP was better than FBSM. During the 4 sorption-desorption repetitions, water absorbency reversibility of FBSM and FBSM-NP was acceptable. From Fig. 4, the water absorbance capabilities of FBSM and FBSM-NP did not change significantly in environments with different acidities, which demonstrated that the FBSM and the prepared FBSM-NP were insensitive to solution pH. This results indicated that the prepared FBSM-NP could be potentially applied to the different soils with a wide range of pH scope to improve its capacity of water absorbency.

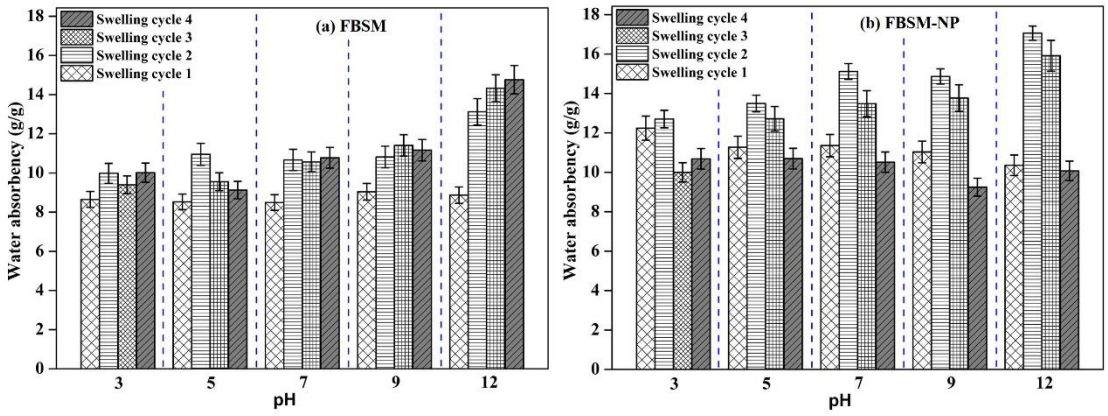

Fig. 4. Swelling repeatability and water absorbency of (a) FBSM and (b) FBSM-NP in solutions with different pH values.

3.4. Water retention behavior of FBSM and FBSM-NP in soil

Water retention capacity of the prepared FBSM-NP is another important parameter due to the fact that it represents the effective utilization of water in the soil environment. Fig. 5 shows the water retention behavior of the oven-dried soil with FBSM, FBSM-NP and without fertilizer formulation (control). As shown in Fig. 5, the time needed for 70 wt% water evaporation was 30 days for the soil without FBSM-NP, while it was 50 days for the soil with FBSM-NP. After 30 days, the water content of the soil with

FBSM-NP was 44 wt%, while that of the soil with FBSM and control was 40 wt% and 31 wt%, respectively. Hence, the FBSM-NP had a good water retention capacity in soil, which could be ascribed to the fact that the high porous fiber structure in FBSM-NP provided a high water absorbency (see Sections 3.1.2 and 3.3). Consequently, the soil with FBSM-NP could potentially save more water for the growth of plants.

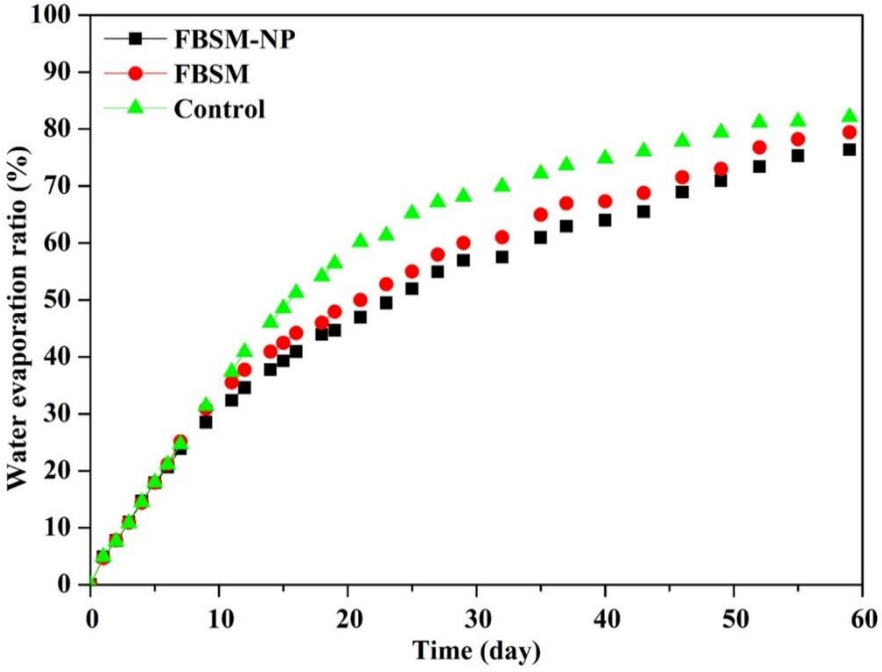

Fig. 5. Water retention behavior of the oven-dried soil with FBSM, FBSM-NP and without fertilizer formulation.

3.5. Slow release behavior of FBSM-NP in soil

The slow-release property was one of the most key characteristics of the FBSM-NP. The release of nitrogen (NH_4^+) and phosphorus ($H_2PO_4^-$) from FBSM-NP was investigated in soil, results of which are shown in Fig. 6. As shown in Fig. 6, the cumulative releases of NH_4^+ and $H_2PO_4^-$ from FBSM-NP reached 17.6 and 5.0 wt%, respectively, in 24 hours, while they were 84.9 and 52.8 wt%, respectively, within 30 days. At day 60, the cumulative releases of NH_4^+ and $H_2PO_4^-$ from FBSM-NP reached 93.2 and 62.8 wt%, respectively. Although the slow-release property of N and P from FBSM-NP did not perfectly meet the European standards of the slow-release fertilizer, the above results demonstrated the potential feasibility of using FBSM as an adsorbent for recycle of N and P from anaerobic digestate

followed by the application of the nutrients-loaded carrier (FBSM-NP) as an economic alternative fertilizer in the current fertilizer market.

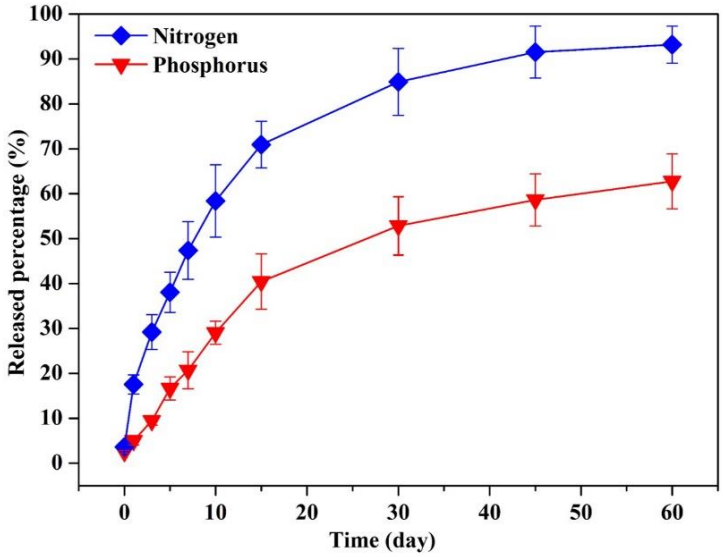

Fig. 6. Slow-release behavior of nitrogen (NH_4^+) and phosphorus ($H_2PO_4^-$) from FBSM-NP in soil.

3.6. Potted tomato experiments

3.6.1. Effect of different fertilizers on tomato plant growth

The average length and weight of tomato plants in different pots are presented in Fig. 7. As shown in Fig. 7 (a), the greatest length (19.4 cm) and the greatest weight (0.53 g) was obtained by the group P1 (with FBSM-NP), followed by group P3 (with commercial organic fertilizer; 17.5 cm and 0.50 g), followed by group P2 (with FBSM; 17.0 cm and 0.42 g), and then followed by group P4 (control; 14.2 cm and 0.34 g). Compared to the control, application of the FBSM-NP or FBSM, or commercial fertilizer improved the plant growth in terms of the plant length and weight. The enhanced plant growth could be attributed to the exogenous N and P nutrients from FBSM-NP or commercial fertilizer, or the improved water retention capability from FBSM. Compared to group P2 (with FBSM), group P1 (with FBSM-NP) had a better facilitating effect on plant growth, indicating that the N and P nutrition in the liquid phase of the treated anaerobic digestate was successfully adsorbed by the FBSM. The fiber-based fertilizer (FBSM-NP) showed a greater performance than commercial fertilizer, which demonstrated that it is technically feasible to use FBSM as an adsorbent for recycle of N and P from anaerobic digestate and use the end product as a bio-fertilizer. Nonetheless, the results of group P1

(FBSM-NP) were only marginally greater with length greater by 11% and weight by 4%. Hence, the effects of larger dosages of the FBSM-NP on plant growth could be investigated in future studies, with the hope of showing a significant high improvement.

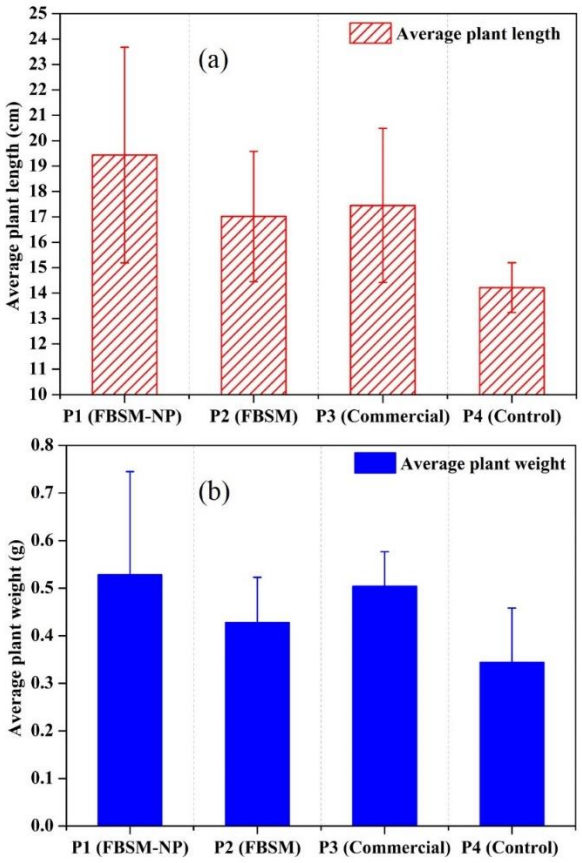

Fig. 7. Average plant length (a) and plant weight of tomato plants in different pots. P1 (FBSM-NP), P2 (Control), P3 (Commercial) and P4 (FBSM).

3.6.2. Effect of different fertilizers on soil microbial communities

In order to investigate the shifts of microbial compositions in soils with different fertilization treatments over a 100-day period, 16S rRNA gene pyrosequencing was performed. 80887 ± 8373 non-chimeric sequence reads on average were derived from 4 samples. A gradual saturation was obtained for the rarefaction curves and rank abundance curves (Fig. S2 in supplementary material), which demonstrated that a sufficient samples for sequencing had been taken and that the extracted sequence reads were efficient to further examine the major soil microbial compositions in all samples. In addition, this point was also validated by the Good's coverage of 0.998-0.999. All the clean reads were clustered into operational taxonomic units (OTUs) with a sequence similarity of ≥ 97%; subsequently,

370 OTUs were acquired from all the samples for analysis.

Alpha diversity analysis on basis of OTUs clustering was conducted to investigate the soil microbial diversity in each sample. Diversity indices derived from 16S rRNA gene sequence data of soil microbial communities are summarized in Table 3. The alpha diversity analysis via ACE, Chao1, Shannon, and Simpson indices revealed that the species richness among the four samples fluctuated with variable fertilization conditions. The different microbial shift due to different fertilizer pressure might explain this phenomenon. Previously, Zeng et al. [40] reported that nitrogen fertilization directly affected soil bacterial diversity and indirectly affected bacterial community composition through soil acidification and plant community change. Simultaneously, it had been reported that more diverse soil microbial communities could induce improving the functioning of the ecosystems [41]. To provide a deep insight into the community shift, classification analysis of microbial communities was performed at the phylum level (Fig. 8).

Table 3. Diversity indices derived from 16S rRNA gene sequence data of soil microbial communities.

Samples	ACE	Chao1	Shannon	Simpson	Goods coverage
P1 (FBSM-NP)	348.103	347.667	7.149	0.988	0.999
P2 (Control)	346.825	347.769	6.916	0.986	0.998
P3 (Commercial)	343.503	342.571	7.200	0.989	0.999
P4 (FBSM)	342.079	341.214	6.973	0.985	0.998

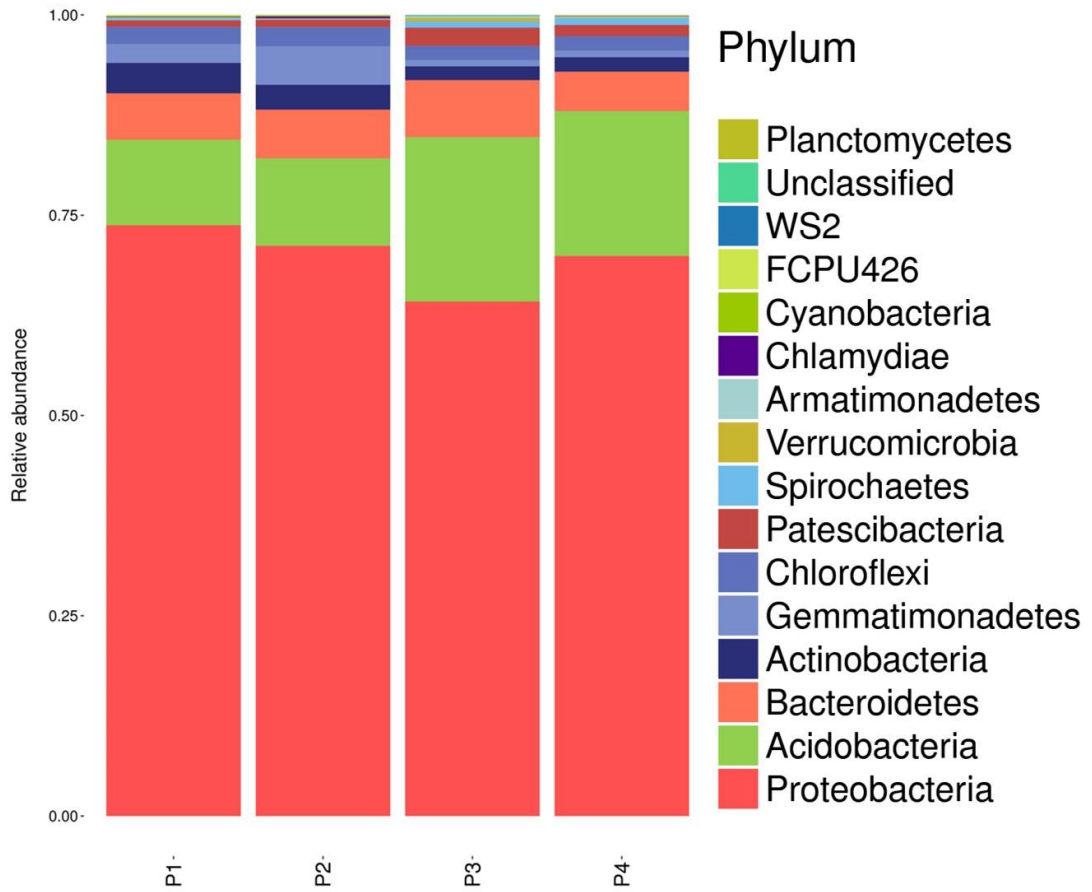

Fig. 8. Species taxonomy of soil microbial communities at the phylum level in group P1 (FBSM-NP), P2 (Control), P3 (Commercial) and P4 (FBSM).

As shown in Fig. 8, top 6 predominant species in the FBSM-NP loaded soil on basis of the relative abundance (> 1%) were *Proteobacteria* (73.8%), *Acidobacteria* (10.7%), *Bacteroidetes* (5.8%), *Actinobacteria* (3.8%), *Gemmatimonadetes* (2.4%) and *Chloroflexi* (2.2%), which covered 98.7% of the total abundance. Although most of the top 6 predominant species were commonly shared by the four soil samples, the relative abundance of these species in different samples varied along with variable fertilization conditions. Among the top 6 dominant species, the overpowering dominance of *Proteobacteria*, *Acidobacteria* and *Bacteroidetes* has been reported widely in agricultural soils with different fertilization conditions [42-45,40]. PCoA analysis (Fig. S3 in supplementary material) revealed that the dominant soil microbes species composition induced by different fertilization conditions were manifestly different among the four samples. Given that bacteria belonging to the same phylum may have significantly different phenotype and genotype [46], the analysis of correlation between soil microbial compositions and the fertilization conditions at the phylum may be insufficient.

Therefore, examination of the soil microbial communities was further performed at the genus level (Fig. 9).

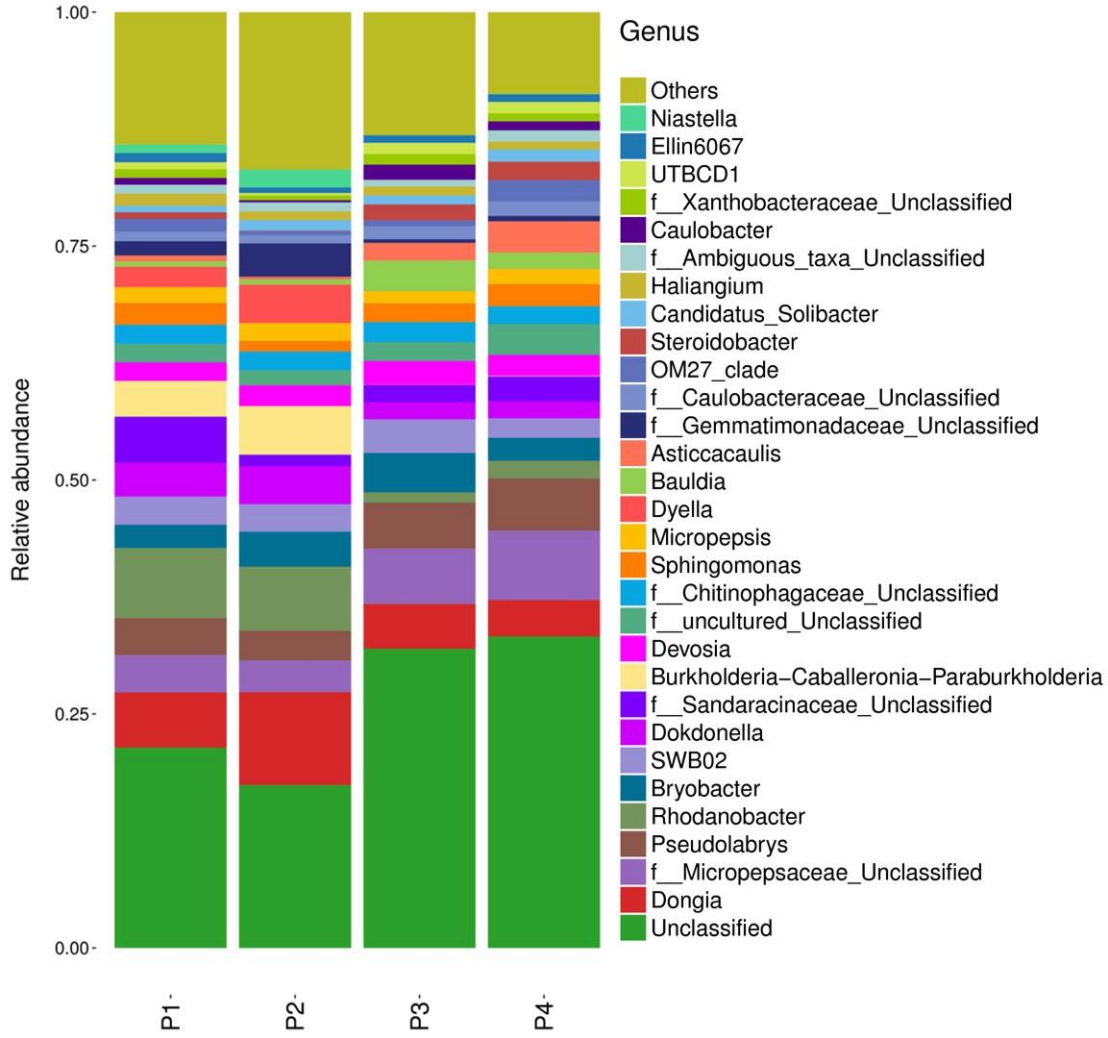

Fig. 9. Species taxonomy of soil microbial communities at the genus level in group P1 (FBSM-NP), P2 (Control), P3 (Commercial) and P4 (FBSM).

From Fig. 9, predominant bacterial genus (with a ≥ 1% relative abundance) included *Micropepsaceae* (5.2 ± 1.6%), *Pseudolabrys* (4.4 ± 0.9%), *Rhodanobacter* (4.3 ± 2.9%), *Bryobacter* (3.2 ± 0.8%), *SWB02* (2.9 ± 0.5%), *Dokdonella* (2.8 ± 1.0%), *Sandaracinaceae* (2.7 ± 1.4%), *Chitinophagaceae* (2.0 ± 0.1%), *Sphingomonas* (2.0 ± 0.5%), and *Micropepsis* (1.6 ± 0.2%). *Micropepsaceae*, *Pseudolabrys* and *Rhodanobacter* play a significant role in fixing, nitrifying, and denitrifying nitrogen among plant biomass, soil ecosystem and atmosphere [47-49]. Among these ten

predominant genera, *Sandaracinaceae* and *Sphingomonas* were found to be selectively enriched in the P1 (with FBSM-NP), showing 4.0-times and 2.0-times higher abundance than that of the control (P2), respectively. *Sandaracinaceae* species (e.g. *Sandaracinus amylolyticus*) were recognized as plant growth-promoting bacteria in soil responsible for degradation of starch, chitin, and cellulose [50,51]. *Sphingomonas* was reported to be functionally important in accelerating plant growth by producing plant growth hormone such as gibberellins and IAA (Indole-3-acetic acid) [52,53]. As a result, the enrichment of *Sandaracinaceae* and *Sphingomonas* could have contributed to the enhanced tomato plant growth in terms of length and weight in P1 (FBSM-NP).

4. Conclusions

A novel natural superabsorbent fiber-based adsorbent was obtained from soybean curd residue and used to recycle the N and P nutrition from anaerobic digestate and reused as a multifunctional fertilizer for slow release of nutrients. The reclaimed fertilizer had a nitrogen content of 3.65 wt% and a phosphorus content of 0.14 wt%, showing an average water absorbency of 12.6 g/g at pH = 7. The SEM, XRD and FT-IR analysis confirmed the porous composite structure of the fiber-based adsorbent and successful incorporation of nitrogen-containing and phosphorus-containing compounds into the adsorbent material. Feasibility of using microwave pretreatment to reduce pathogens in anaerobic digestate was validated. Slow release behavior of the reclaimed fertilizer in soil was determined, results of which demonstrated that the reclaimed fertilizer exhibited preferable slow-release properties: N released 84.9% within 30 days and P released 62.8% within 60 days in neutral soil. In potted tomato tests, the enhanced plant growth in terms of the plant length and weight could be partially attributed to the extra N and P nutrients and the improved water retention capability due to the reclaimed fertilizer. Analysis of soil microbial communities suggested that beneficial species *Sandaracinaceae* and *Sphingomonas* were selectively enriched by 2 to 4 times in the soil with the reclaimed fertilizer, contributed to the enhanced tomato plant growth. In light of the above encouraging results, the developed low-cost and eco-friendly slow-release fertilizer made of two kinds of wastes (soybean curd residue and anaerobic digestate) could have a great potential for application in the agricultural and horticultural environments.

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