horticulturae

MDPI

Article

Biotransforming the Spent Substrate of Shiitake Mushroom (*Lentinula edodes* Berk.): A Synergistic Approach to Biogas Production and Tomato (*Solanum lycopersicum* L.) Fertilization

Pankaj Kumar [1,†], Ebrahem M. Eid [2,3,†], Mostafa A. Taher [2,4], Mohamed H. E. El-Morsy [5,6], Hanan E. M. Osman [7,8], Dhafer A. Al-Bakre [9], Bashir Adelodun [10,11], Sami Abou Fayssal [12,13], Madhumita Goala [14], Boro Mioč [15], Valentino Držaić [15], Fidelis O. Ajibade [16], Kyung Sook Choi [11], Vinod Kumar [1,*] and Ivan Širić [15,*]

[1] Agro-Ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar 249404, Uttarakhand, India; rs.pankajkumar@gkv.ac.in
[2] Biology Department, College of Science, King Khalid University, Abha 61321, Saudi Arabia; ebrahem.eid@sci.kfs.edu.eg (E.M.E.); mtaher@kku.edu.sa (M.A.T.)
[3] Botany Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
[4] Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt
[5] Deanship of Scientific Research, Umm Al-Qura University, Makkah 24243, Saudi Arabia; mhmorsy@uqu.edu.sa
[6] Plant Ecology and Range Management Department, Desert Research Center, Cairo 11753, Egypt
[7] Biology Department, Faculty of Science, Umm-Al-Qura University, Makkah 24243, Saudi Arabia; heosman@uqu.edu.sa
[8] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo 11651, Egypt
[9] Biology Department, College of Science, Tabuk University, Tabuk 47512, Saudi Arabia; dalbakre@ut.edu.sa
[10] Department of Agricultural and Biosystems Engineering, University of Ilorin, PMB 1515, Ilorin 240103, Nigeria; adelodun.b@unilorin.edu.ng
[11] Department of Agricultural Civil Engineering, Kyungpook National University, Daegu 41566, Korea; ks.choi@knu.ac.kr
[12] Department of Agronomy, Faculty of Agronomy, University of Forestry, 10 Kliment Ohridski Blvd, 1797 Sofia, Bulgaria; sami.aboufaycal@st.ul.edu.lb
[13] Department of Plant Production, Faculty of Agriculture, Lebanese University, Beirut 1302, Lebanon
[14] Nehru College, Pailapool, Affiliated Assam University, Cachar, Silchar 788098, Assam, India; madhumitagoalap@gmail.com
[15] University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia; bmioc@agr.hr (B.M.); vdrzaic@agr.hr (V.D.)
[16] Department of Civil and Environmental Engineering, Federal University of Technology, PMB 704, Akure 340001, Nigeria; foajibade@futa.edu.ng
* Correspondence: drvksorwal@gkv.ac.in (V.K.); isiric@agr.hr (I.Š.)
† These authors contributed equally to this work.

check for updates

Citation: Kumar, P.; Eid, E.M.; Taher, M.A.; El-Morsy, M.H.E.; Osman, H.E.M.; Al-Bakre, D.A.; Adelodun, B.; Abou Fayssal, S.; Goala, M.; Mioč, B.; et al. Biotransforming the Spent Substrate of Shiitake Mushroom (*Lentinula edodes* Berk.): A Synergistic Approach to Biogas Production and Tomato (*Solanum lycopersicum* L.) Fertilization. *Horticulturae* **2022**, *8*, 479. https://doi.org/10.3390/horticulturae8060479

Academic Editor: Agnieszka Jasińska

Received: 6 May 2022
Accepted: 26 May 2022
Published: 27 May 2022

Abstract: Agro-wastes, such as crop residues, leaf litter, and sawdust, are major contributors to global greenhouse gas emissions, and consequently a major concern for climate change. Nowadays, mushroom cultivation has appeared as an emerging agribusiness that helps in the sustainable management of agro-wastes. However, partial utilization of agro-wastes by mushrooms results in the generation of a significant quantity of spent mushroom substrates (SMS) that have continued to become an environmental problem. In particular, Shiitake (*Lentinula edodes* Berk.) mushrooms can be grown on different types of agro-wastes and also generate a considerable amount of SMS. Therefore, this study investigates the biotransformation of SMS obtained after Shiitake mushroom cultivation into biogas and attendant utilization of slurry digestate (SD) in tomato (*Solanum lycopersicum* L.) crop fertilization. Biogas production experiments were conducted anaerobically using four treatments of SMS, i.e., 0% (control), 25, 50, and 75% inoculated with a proportional amount of cow dung (CD) as inoculum. The results on biogas production revealed that SMS 50% treatment yielded the highest biogas volume (8834 mL or 11.93 mL/g of organic carbon) and methane contents (61%) along with maximum reduction of physicochemical and proximate parameters of slurry. Furthermore, the biogas digestate from 50% treatment further helped to increase the seed germination (93.25%), seedling length (9.2 cm), seedling root length (4.19 cm), plant height (53.10 cm), chlorophyll content (3.38 mg/g), total yield (1.86 kg/plant), flavonoids (5.06 mg/g), phenolics (2.78 mg/g), and tannin (3.40 mg/g) contents of tomato significantly ($p < 0.05$) in the 10% loading rate. The findings of this study suggest sustainable upcycling of SMS inspired by a circular economy approach through synergistic production of bioenergy and secondary fruit crops, which could potentially contribute to minimize the carbon footprints of the mushroom production sector.

Keywords: circular economy; crop fertilization; greenhouse gas emissions; spent mushroom substrates; vegetable production

1. Introduction

In the last decades, increased mushroom production has resulted in tremendous post-cultivation residues where each kg of mushrooms left around 5 kg of spent substrate [1], accounting for an average of 10–50 million metric tonnes annually [2]. These residues are disposed of haphazardly without any pre-treatment [3]. Generally, farmers use a wide range of harmful pesticides, which persist in crop residues and are further used for mushroom production. Therefore, the spent mushroom substrates (SMS) may hold a considerable quantity of nitrates, which pose a risk of surface and groundwater contamination. This alarming situation pushed the European Union (EU) in 1991 to develop a legislation "Nitrate Directive" aiming to protect the vital source of life [4]. This legislation positioned SMS as compost or livestock manure. In this context, a study detected higher inorganic nitrogen liberation (increased by around 8%) within leachate containing SMS compared to the control (no added SMS to the leachate) [5]. This increases the high risk of nitrate leaching in soil and water as a result of SMS discharge onto the environment.

Enclosing around 2% N (dry matter basis), SMS has been suggested for subsequent use as compost and casing soil for mushroom production [6]. However, the relatively depleted holo-cellulose contents have made this use non-profitable economically unless supplemented with good sources of hemicellulose and cellulose. SMS is beneficial in the bioremediation process of contaminated soils in addition to being a neutralizer of the latter's acidity [7]. Different types of SMSs originating from several species' cultivation (e.g., *Agaricus bisporus*, *Flammulina velutipes*, *Grifola frondosa*, *Hipsizigus marmoreus*, *Pleurotus eryngii*, and *Pholiota nameko*) have been previously used as growing media for the transplant production of lettuce. Among them, spent *Agaricus* substrate was the most appropriate for maximal nutrient affordability needed for plant growth [8]. The growth promotion of honeydew melon seedlings was successfully attained when the spent substrate of *Flammulina velutipes* was incorporated into the former's growing medium [9]. SMS was also used as a biofertilizer due to its richness in phosphorus needed for root growth, calcium, and protein needed for plant tissue formation and photosynthesis, respectively [10,11]. Although mushrooms succeeded to act as natural bio-accumulators of potentially toxic elements [12–14], prominent amounts of the latter in SMS are still challenging. This promotes the concept of "circular economy" in the mushroom industry (Figure 1) as part of the sustainable production and consumption agenda of the United Nations on Sustainable Development Goals [15].

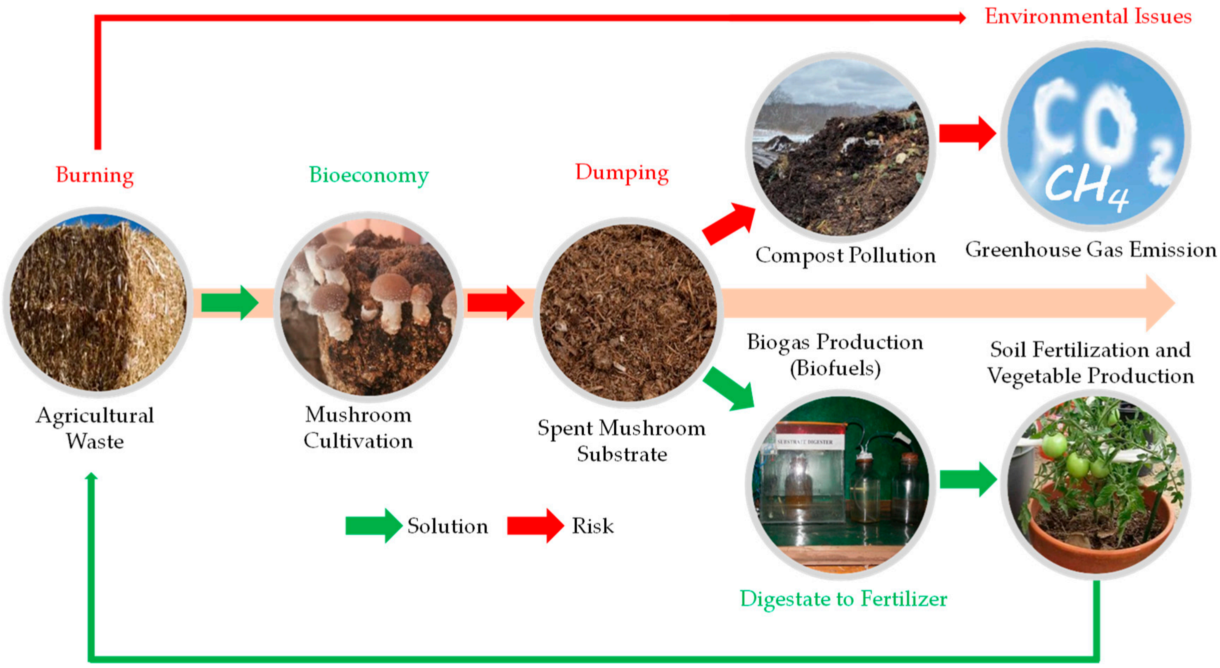


Figure 1. The circular economy concept for sustainable management of agricultural wastes through mushroom cultivation, bioenergy generation, and crop fertilization.

Shiitake mushroom (*Lentinula edodes*) has been widely grown on several types of agro-industrial wastes aiming for a successful bioremediation process [13]. Spent mushroom substrate is rich in holo-celluloses as shiitake mushroom generally consumes only 15%, 56%, and 23% of hemicellulose, cellulose, and lignin contents found in initial substrates, respectively [16]. SMS contains a considerable amount of protein and carbohydrates, which has prompted researchers to suggest its incorporation in biofuels' production and more precisely, biogas production [17]. As biogas production is a costly process, the search for cheap raw materials has become indispensable. Among these materials, wastes rich in lignocellulosic components were well suited as being pre-digested biologically [18], therefore facilitating bacterial decomposition. In this context, SMS used for the solid-state anaerobic digestion (AD) succeeded in the increase of methane production by 1.5 times and solid waste reduction per kilogram of woodchips by around 8 times [19]. An increased cumulative methane yield (by 414%) was detectable from SMS supplemented with chicken manure [20]. Moreover, SMS supplemented with sugar mill wastewater had a considerable biogas yield [21]. Other agricultural wastes were reported to be suitable for biogas production such as flower wastes, which are generated in significant amounts by temples [22]. The modeling of biogas production after AD of *Azolla pinnata* revealed a good value (around 3600 mL) [23]. Additionally, water hyacinth (*Eichhornia crassipes* [Mart.] Solms) supplemented with sugar mill effluent led to a considerable biogas production (around 6800 mL) when the digester was incubated at 40 °C [24].

Hence, the resultant digestate contains significant amounts of nitrogen (N), phosphorus (P), and potassium (K), thus allowing it to play the role of organic mineral fertilizers. When digestate was added to alfalfa, an increase in macro-element contents in crop leaves was detected compared to manure, while no heavy metals were observable [25]. The study on the effect of digestate on perennial grasslands, intercropped triticale, clover grass, and silage maize yields revealed that it could complement mineral fertilizers by reducing the use of the latter by around 66% [26]. The yield of several cereal crops (spring wheat, triticale, and barley) was improved by 20–25% when they were fertilized with digestate compared to synthetic nitrogen fertilizer [27]. A digestate prepared from *Carica papaya* peels via AD and used for maize crop fertilization positively impacted several vegetative indices. An increase in the number of leaves, leaf area, plant height, stem girth, total shoot, root biomass, and length was obtained compared to chemical fertilizer (NPK 15:15:15) [28].

The effect of liquid digestate supplemented with biochar and pelleted digestate on the productivity and quality of tomato (*Solanum lycopersicum*) crop was previously assessed, which showed promising results [29]. However, the current study is the first to assess the biogas production efficiency of Shiitake SMS and the effect of SMS digestate addition on growing soil properties as well as vegetative and productive indices of tomato crops.

2. Materials and Methods

2.1. Experimental Materials

Wheat straw-based SMS was obtained from the Agro-ecology Pollution Research Laboratory of Gurukula Kangri (Deemed to be University), Haridwar, India (29°55′10.4″ N and 78°07′08.0″ E) right after the cultivation of Shiitake (*Lentinula edodes* Berk.) mushroom using the bag-log method in March 2021. The initial substrate used for the mushroom cultivation contained a blend of wheat straw (*Triticum aestivum* L.: 80%), sugarcane bagasse (10%), and wheat bran (9%) with added fertilizer (1% $CaCO_3$). SMS was sun-dried (2 days until a constant weight was achieved) immediately after harvesting the final flush of Shiitake mushroom and converted into a powder form for further analysis. Cow dung (CD) was obtained from the cattle shed of Gurukula Kangri (Deemed to be University), Haridwar, India (29°55′23.1″ N and 78°07′38.0″ E) in sterile self-sealing poly bags (500 g capacity). For the crop cultivation experiments, the soil was obtained from the departmental garden, while certified healthy seeds of Pusa Early Dwarf (PED) variety of tomato (*Solanum lycopersicum* L.) were obtained from Beej Bhawan, Pusa Complex, New Delhi, India.

2.2. Experimental Design for Biogas Production Using SMS

The biogas production experiments were conducted using the water displacement method, as previously described by Kumar et al. [24]. The three glass reactors (substrate digester, gas collection, and water displacement units) were cast-off to produce biogas anaerobically using SMS and CD blends. Biogas was produced using three different treatments of SMS, i.e., 25% (62.5 g SMS + 187.5 g CD), 50% (125.0 SMS + 125.0 g CD), and 75% (187.5 g SMS + 62.5 g CD), and a control treatment using CD (250 g) with no SMS addition (Figure 2). Distilled water was added to adjust the total biogas slurry volume to 750 mL. Finally, the preparative slurry digester was placed inside a thermostat unit for biogas production at 35 °C for 21 days. Three replicates of each treatment were used for biogas production. The volume of produced biogas was measured at an interval of each third day by measuring the volume of displaced water.

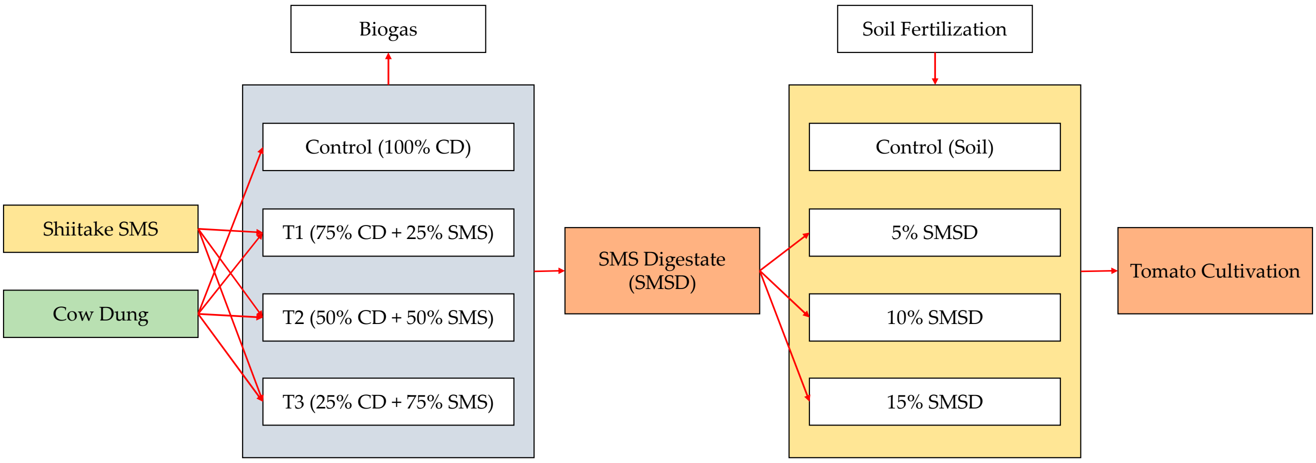


Figure 2. Experimental design for biogas production using SMS and soil fertilization using SMSD for tomato cultivation.

2.3. Experimental Design for Crop Cultivation Using SMS Digestate

The digested slurry was sun-dried to remove water content right after the termination of biogas experiments. Tomato cultivation was performed using one control treatment (garden soil as reference substrate) and three biogas digestate treatments (5, 10, and 15% dw.) (Figure 2). Purposely, a total of 15 kg mixture of soil and biogas digestate was filled in polyvinyl chloride (PVC) bags (20 kg capacity). Before the germination step, the seeds were soaked in carbendazim solution (2% for 24 h) to avoid any indigenous fungal infection. Then, tomato seed was sown in the beaker (250 mL) containing 200 g of soil obtained from a previously prepared base and allowing for seed germination for 15 days. Seven replicates of each treatment were performed to study the effect of SMS digestate on the growth response of tomato seedlings. After that, three seedlings were transferred into the cultivation bags of their respective treatments for further emergence. Bags were placed in a greenhouse to protect them from any animal or pest attack and watered equally using normal water supply, when necessary, until June 2021. During this period, the plants were raised under greenhouse conditions (16 h light/ 8 h dark period, 76% humidity, and 23 °C temperature) and watered using a 2 L hand sprayer. The experiments lasted for 100 days with appropriate harvesting of fruits when they reached a marketable stage in terms of size, weight, and color.

2.4. Chemical, Analytical, and Instrumental Methods

In this study, the SMS, CD, and garden soil were analyzed for selected physicochemical and proximate parameters following standard methodologies [30]. For this, pH and electrical conductivity were measured using a digital meter (1615, ESICO, Parwanoo, India). Total solids (TS), volatile solids (VS), cellulose, hemicellulose, Klason-lignin, and total ash contents were determined according to Kumar et al. [21]. Organic carbon (OC) and total nitrogen (N) were estimated by using Walkley and Black [31] and Kjeldahl's methods [32], respectively. Similarly, the contents of total phosphorus (P) and potassium (K) were measured using a spectrophotometer (60 Cary, Agilent, Santa Clara, CA, USA) and flame-photometer (1382, ESICO, Parwanoo, India) instruments, respectively. Biogas was carefully collected in a 50 mL gas syringe. The composition of produced biogas (CH_4, CO_2, H_2S, and water vapors) was conducted using gas chromatography (GC) equipped with a thermal conductivity detector (TCD) and argon (Ar) as career gas (Nucon-5765, Nucon Engineers Ltd., New Delhi, India). For this, the temperature of the injection column and TCD detector units were 60 and 90 °C with a career gas flow rate of 30 mL/min, respectively. On the other hand, percent seed germination (%), seedling length (cm), seedling root length (cm), plant height (cm), chlorophyll content (mg/g), total yield (kg/plant), flavonoids (mg/g), phenolics (mg/g), and tannins (mg/g) were also estimated. The following Equation (1) was used to calculate the percent germination:

$$\text{Germination (\%)} = (\text{No. of Seed Germinated} \times 100)/\text{No. of Total Seed} \tag{1}$$

A calibrated scale (75 cm) and digital balance (Samson HI-600K, Edapally, India) were used for heigh and weight estimation. Total chlorophyll contents (mg/g) were determined after extraction in 80% acetone solution followed by spectrometric determination as previously reported by Kumar et al. [33], while total phenolics and tannins were estimated by the ethanol extraction method as discussed by Ahmed et al. [34]. Similarly, total phenolics were estimated by using Folin–Ciocalteu phenol reagent as the extraction solution followed by spectroscopic determination at 517 nm. The contents of tannins were determined by digesting 1 g of dried tomato fruit powder in 10 mL of 4% copper acetate solution followed by incineration at 550 °C for 1 h. The contents of tannins were represented as weight differences before and after ashing.

2.5. Data Analysis and Software

For all analyses, the samples were proportionally pooled from three replicates of individual treatment, and chemical analysis was carried out thrice to obtain mean and standard deviation values. The reduction in physicochemical and proximate parameters of biogas slurry was evaluated using the removal efficiency index [24] as provided in Equation (2):

$$\text{Removal efficiency (\%)} = [(V_I - V_F)/V_I] \times 100 \quad (2)$$

where V_I and V_F are the initial and final values of biogas slurry parameters, respectively. Moreover, the data obtained in this study were analyzed using selected statistical tools such as unpaired Student's *t*-test, one-way analysis of variance (ANOVA), principal component analysis (PCA), and Pearson correlation. The various software packages, including Microsoft Excel (Version 2019, Microsoft Corporation, Redmond, WA, USA), OriginPro (Version 2022a, OriginLab Corporation, Northampton, MA, USA), and Statistical Package for Social Sciences (SPSS) (Version 23, IBM, Chicago, IL, USA) were used for data analysis. All tests were performed at a confidence interval of 95%, i.e., $p < 0.05$ to indicate statistical significance.

3. Results and Discussion

3.1. Physicochemical and Proximate Composition of CD and SMS

Table 1 summarizes the physicochemical and proximate characteristics of the Shiitake SMS and CD. The physicochemical and proximate parameters of SMS were significantly ($p < 0.05$) higher compared to the pH, EC, and C:N ratio. As the initial Shiitake substrate was formulated using wheat straw, wheat bran, and sugar cane bagasse, SMS also contained residual cellulose, hemicellulose, and lignin. Due to the limited bioconversion and utilization by mushrooms, a major portion of lignocellulose is left in the SMS [35]. The breaking of a complex β(1–4)-linked chain of glucose molecule bonds is necessary to release several sugars from the lignocellulose wastes. In this, three types of cellulase enzymes ((1) endo-β-1,4-glucanase (EG), (2) cellobiohydrolase (CBH), and (3) β-glucosidase (BGL) play an important role in its hydrolysis. Being acidic, the release of these enzymes onto the substrate by fungal mycelia might be the reason behind the reduced pH in the SMS [36]. In contrast, the CD is relatively more pre-digested by the ruminants (cow) as a process of cellulase activity. However, the main purpose of utilizing CD was the amalgamation of microbial communities into the biogas slurry to facilitate efficient AD. In general, SMS had more digestible contents compared to CD inoculum. Therefore, VS, OC, and N, as main components were observed within the optimum range in SMS, i.e., 57.10, 35.28, and 9.05% suitable for biogas production, respectively. A previous study by Lin et al. [19] found that woodchips obtained after Shiitake pre-treatment had optimum levels of digestible contents (OC: 47.50%, N: 0.34%, TS: 89.20%, and VS: 99.20%) to support solid-state AD. Most recently, Kumar et al. [13] investigated the properties of paddy-straw-based Shiitake SMS and confirmed that it had significant loads of organic and inorganic nutrients. They suggested that the Shiitake mushroom partially consumed organic and inorganic nutrients; therefore, it can be used for secondary purposes such as biogas production.

Table 1. Physicochemical and proximate characteristics (mean ± SD; *n* = 3) of the Shiitake SMS and CD used in this study.

Characteristics	Spent Mushroom Substrate (SMS)	Cow Dung (CD) Inoculum	Unit	Student's *t*-Test ^	
				***t*-Statistics**	***p*-Value**
pH	5.72 ± 0.03	8.02 ± 0.02	—	110.48	<0.01
Electrical conductivity	4.80 ± 0.16	5.92 ± 0.25	dS/m	6.53	<0.01
Total solids	40.02 ± 1.24	7.20 ± 0.08	%	45.74	<0.01
Volatile solids	57.10 ± 2.05	10.64 ± 0.10	%	39.20	<0.01
Cellulose	23.07 ± 0.81	5.12 ± 0.13	%	37.89	<0.01

Table 1. *Cont.*

Characteristics	Spent Mushroom Substrate (SMS)	Cow Dung (CD) Inoculum	Unit	Student's *t*-Test ^	
				t-Statistics	*p*-Value
Hemicellulose	14.21 ± 0.94	3.02 ± 0.05	%	20.58	<0.01
Lignin	17.36 ± 2.07	6.18 ± 0.27	%	9.27	<0.01
Total ash	4.03 ± 0.12	3.86 ± 0.09	%	1.96	<0.01
Organic carbon	35.28 ± 3.40	21.20 ± 2.00	%	6.18	<0.01
Nitrogen	9.05 ± 0.05	4.10 ± 0.24	%	34.97	<0.01
C:N ratio	3.87	5.17	—	—	—
Phosphorus	0.74 ± 0.02	0.51 ± 0.01	%	17.81	<0.01
Potassium	1.30 ± 0.05	0.40 ± 0.03	%	26.73	<0.01

^ Significantly different from each other at $p < 0.05$ based on unpaired Student's *t*-test; —: not applicable.

3.2. Changes in Biogas Slurry before and after AD

The reduction in the physicochemical and proximate parameters of biogas slurry prepared using different proportions of SMS and CD is given in Table 2. After 21 days of AD, a significant ($p < 0.05$) reduction was observed in the values of analyzed parameters as indicated by the one-way ANOVA test. Remarkably, the maximum percentage reduction in pH (25.76%), EC (31.72%), TS (41.13%), VS (65.84%), cellulose (30.97%), hemicellulose (52.41%), lignin (70.86%), total ash (6.72%), OC (74.04%), N (51.18%), P (10.40%), and K (16.47%) was observed in 50% SMS treatment. However, the reduction patterns greatly varied with the SMS to CD ratio and increasing order of parameter removal efficiency in selected treatments were identified as 75% < control < 20% < 50% (Figure 3). It was also observed that as SMS loading was increased from 25 to 75%, the slurry became less digestible due to the decreasing volume of inoculum. The complex structures of cellulose, hemicellulose, and lignin are first converted into simple organic molecules to release the sugar contents, which are later used by methanogens to produce CH_4 [37]. In this, acidogenic microbes play an important role in the breakdown and supply of carbon and nitrogen for acetogenins, and later to methanogens. In fact, the OC reduction rate of anaerobes was much higher than that of N, which might be due to the rapid utilization rate (usually 10–20 times higher) of anaerobes. Generally, C is a major energy source for microbes, while N is essential and not required at high levels as in the case of C. Therefore, methane-producing microbes utilize more C as compared to the N source [24,38]. Slighter reduction in other parameters such as total ash, P, and K are associated with their lesser utilization and also, they do not migrate out of the reactor with biogas, unlike carbon-based molecules. However, the availability of nutrients and optimum quantity of microbial cells (substrate to inoculum ratio) is the main factor that derives the methane production process in an anaerobic digester [39]. Thus, the results proved that a 50:50 ratio of SMS and CD could be more advantageous in terms of maximum removal of physicochemical and proximate parameters of biogas slurry.

Recent studies have shown substantial reductions in biogas slurry parameters after AD [24,38,40]. In a study, Yang et al. [40] investigated the effect of AD on parameters of food waste-based slurry. They reported TS and VS removal rates as 52 and 63% after 14 days, respectively. In another study, Kumar et al. [24] performed biogas production using *Eichhornia crassipes* plant residues supplemented with sugar industry effluent. They recorded a significant drop in biogas slurry properties after the 15th day, i.e., pH (6.4), TS (21%), N (0.82%), OC (14.19%), and VS (16.53%). Similarly, Nong et al. [38] noted the highest reductions in TS (70%), VS (64.76%), and COD (66.55%) of biogas slurry prepared using water primrose (*Ludwigia peploides*) plant residues with CD after 14 days. Thus, these reports also suggested efficient biodegradability of different waste materials in combination with CD.

Table 2. Changes in the characteristics of SMS and CD-based biogas slurry before and after AD.

Characteristics		**Treatments**			
		Control (0%)	**25% SMS**	**50% SMS**	**75% SMS**
pH	Before	8.02 ± 0.02	7.45 ± 0.03	6.87 ± 0.05	6.30 ± 0.02
	After	6.30 ± 0.05 *	5.66 ± 0.07 *	5.10 ± 0.03 *	5.02 ± 0.04 *
Electrical conductivity	Before	5.92 ± 0.25	5.64 ± 0.13	5.36 ± 0.12	5.08 ± 0.09
	After	4.45 ± 0.10 *	4.07 ± 0.04 *	3.66 ± 0.06 *	3.90 ± 0.15 *
Total solids	Before	7.20 ± 0.08	15.41 ± 0.25	23.61 ± 1.08	31.82 ± 2.06
	After	5.01 ± 0.02 *	10.24 ± 0.11 *	13.90 ± 0.72 *	23.10 ± 1.17 *
Volatile solids	Before	10.64 ± 0.10	22.26 ± 0.29	33.87 ± 1.90	45.49 ± 1.50
	After	5.58 ± 0.14 *	10.03 ± 1.20 *	11.57 ± 0.44 *	26.05 ± 2.31 *
Cellulose	Before	5.12 ± 0.13	9.61 ± 0.48	14.10 ± 0.20	18.58 ± 0.86
	After	4.17 ± 0.20 *	7.10 ± 0.62 *	9.73 ± 0.36 *	15.28 ± 0.20 *
Hemicellulose	Before	3.02 ± 0.05	5.82 ± 0.09	8.62 ± 0.12	11.41 ± 0.28
	After	2.01 ± 0.02 *	3.45 ± 0.15 *	4.10 ± 0.45 *	8.23 ± 0.19 *
Lignin	Before	6.18 ± 0.27	8.98 ± 0.30	11.77 ± 0.19	14.57 ± 0.10
	After	3.58 ± 0.09 *	4.03 ± 0.12 *	3.43 ± 0.23 *	9.19 ± 0.23 *
Total ash	Before	3.86 ± 0.09	3.90 ± 0.18	3.95 ± 0.08	3.99 ± 0.05
	After	3.71 ± 0.03 ^{ns}	3.72 ± 0.07 *	3.68 ± 0.05 *	3.87 ± 0.07 ^{ns}
Organic carbon	Before	21.20 ± 2.00	24.72 ± 1.46	28.24 ± 0.91	31.76 ± 1.09
	After	8.84 ± 0.65 *	7.82 ± 0.60 *	7.33 ± 0.70 *	15.26 ± 0.63 *
Nitrogen	Before	4.10 ± 0.24	5.34 ± 0.19	6.58 ± 0.23	7.81 ± 0.40
	After	2.63 ± 0.18 *	3.08 ± 0.07 *	3.21 ± 0.14 *	5.30 ± 0.25 *
Phosphorus	Before	0.51 ± 0.01	0.57 ± 0.02	0.63 ± 0.02	0.68 ± 0.02
	After	0.48 ± 0.02 ^{ns}	0.52 ± 0.01 *	0.56 ± 0.02 *	0.64 ± 0.03 ^{ns}
Potassium	Before	0.40 ± 0.03	0.63 ± 0.02	0.85 ± 0.02	1.08 ± 0.05
	After	0.36 ± 0.01 ^{ns}	0.55 ± 0.02 *	0.71 ± 0.04 *	0.98 ± 0.06 ^{ns}

* and ^{ns}: significantly different and not different from initial values at $p < 0.05$.

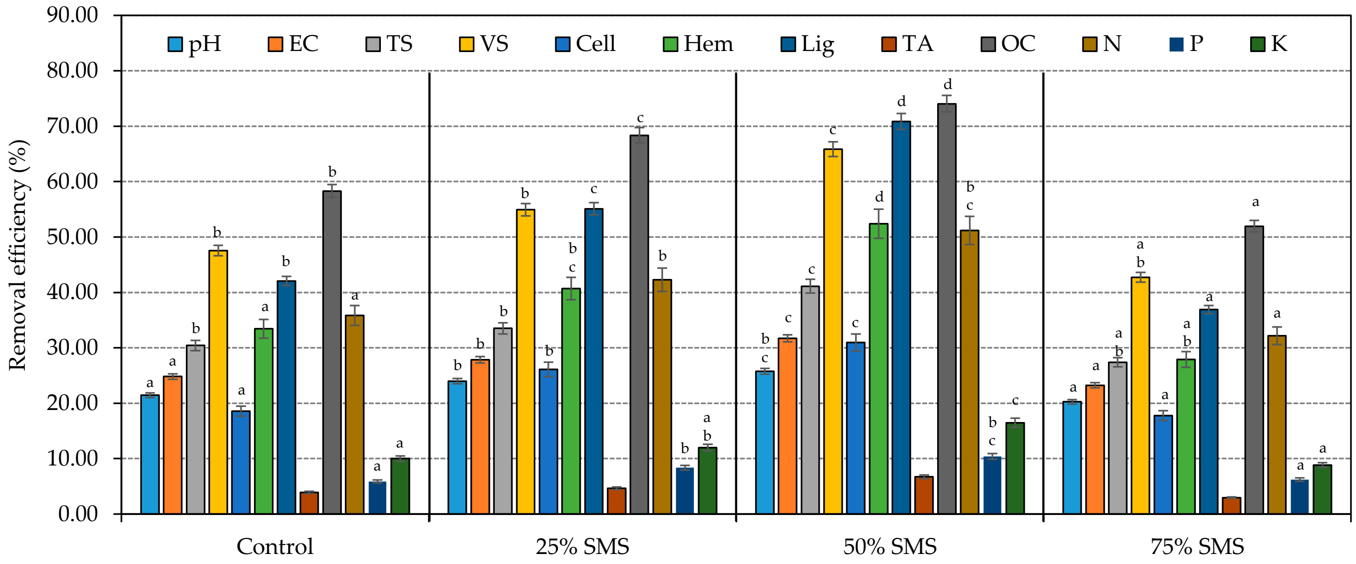


Figure 3. Removal efficiency (%) of physicochemical and proximate properties of biogas slurry prepared using different mixtures of SMS and CD (EC: electrical conductivity; TS: total solids; VS: volatile solids; Cell: cellulose; Hem: hemicellulose; Lig: lignin; TA: total ash; OC: organic carbon; N: nitrogen; P: phosphorus; K: potassium; the same letters (a–d) indicate no significant difference among treatment group values at $p < 0.05$).

3.3. Biogas Production Potential of Shiitake SMS

At the end of the AD experiments, the volume and composition of produced biogas were determined using water displacement and GC-TCD techniques. The final volume of produced biogas was calculated to represent cumulative yield (mL). Results indicated that biogas production started right after the second day conferring to rapid adaptation of microbial communities inside the digester. As shown in Figure 4a, the highest volume of biogas (8834 mL or 11.93 mL/g OC) was achieved using 50% SMS loading followed by 25% (6880 mL or 10.06 mL/g OC). These results are also consistent with the slurry parameters, confirming positive relation between a load of digestible contents and their removal efficiency. Although, the biogas yield increased from the control to 50% treatment and drastically decreased in 75% SMS loading. By using the control and 75% SMS treatments, only 4881 mL (8.37 mL/g OC) and 4024 mL (7.74 mL/g OC) of biogas were produced, which is approximately half of that achieved in 50% SMS loading. The curve showed low biogas yield during the first 3 days following an exponential inclination up to 18 days, which later became stationary. Stabilization in biogas yield after 18 days might be due to exhausting nutrients and the aging of microbial cells inside the digester. CH_4 and CO_2 are the main constituents of biogas produced by methanogens during microbial metabolism as a terminal step [21]. In terms of biogas composition, the maximum contents of CH_4 (61.00%) were also identified in 50% SMS treatment as given in Figure 4b. Nevertheless, the contents of CO_2 and H_2S were relatively lesser (39.00 and 0.06%) compared to other SMS treatments, i.e., control, 25, and 75%. For all mixing ratios, the composition of water vapor contents was similar, i.e., <0.01% of total biogas volume. The lowest CH_4 yield in 75% SMS loading might be related to incomplete digestion, a result of the low inoculum mixing that carries lesser microorganisms to assist AD. A 100% SMS treatment was not performed as a limitation of no inoculum mixing that would eventually not support biogas production. Inoculum size is the major factor that plays an important role in AD. A 25% CD mixing could have reduced the abundance of certain mesophilic microbes in 75% SMS treatment in the present study. The abundance of mesophilic microbe (*Bacillus* sp., *Proteus* sp., *Methanobacterium* sp., etc.) in CD actively contributes to enhancing the methane yield in AD as previously described by Kumar et al. [21].

Studies have shown that SMS could be efficiently upcycled through bioenergy recovery. Recently, Kumar et al. [21] conducted laboratory-scale experiments for electro-assisted AD of SMS supplemented with industrial effluents. After 15 days of digestion, results showed a maximum of 10344 mL (8.73 mL/g OC) biogas was produced having relative CH_4 contents of 63.05%, respectively. Similarly, Najafi and Ardabili [41] also produced biogas from SMS with varying C:N ratios (12.2, 20, 30, and 40). After 14 days of thermophilic digestion (55 °C), the highest recovered biogas volume was 44.1001 mL/g VS confirming the suitability of SMS as bioenergy feedstock. Moreover, Gao et al. [20] studied the effectiveness of SMS loading on biogas production from different livestock manure wastes. They concluded that a 15% SMS loading resulted in maximum biogas yield (111.0 mL/g VS) after 45 days of digestion time. Thus, SMS obtained after the cultivation of Shiitake can be effectively used for high-quality biogas production that helps in reducing its disposal issues.

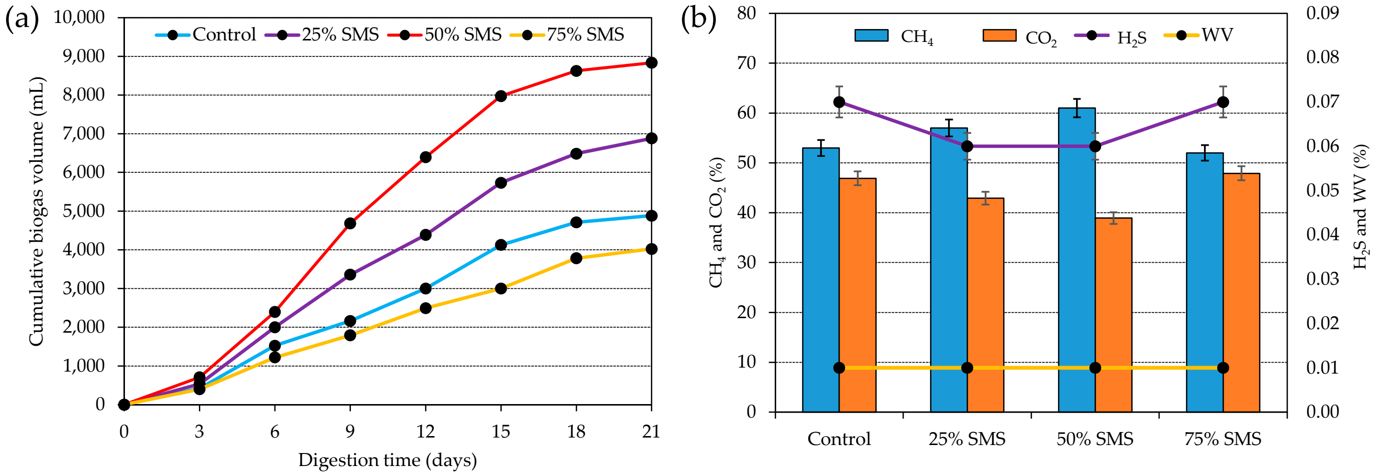


Figure 4. Cumulative volume (mL) (**a**) and composition (**b**) of biogas produced using different treatments of Shiitake SMS and CD (CO$_2$: carbon dioxide; H$_2$S: hydrogen sulfide; WV: water vapor).

3.4. Effect of SMSD on Soil Properties

The digested slurry (SMS digestate: SMSD) obtained after the biogas production experiments was sun-dried and further utilized as a soil supplement at different mixing rates (*w*/*w*). The SMSD obtained from 50% SMS treatment of biogas production experiments was further used for soil fertilization since it yielded the highest biogas quantity and should be recommended for pilot-scale implementation compared to other treatments. Table 3 summarizes the physicochemical and major nutrient properties of control and SMSD amended soil. As indicated from the data, the garden soil used in this study had above neutral (7.30) pH with EC, OC, N, C:N ratio, P, and K values of 1.23 ± 0.08 dS/m, 0.10 ± 0.01%, 12.30, 0.48 ± 0.02%, and 0.17 ± 0.01%, respectively. After SMSD mixing, the soil properties were significantly ($p < 0.05$) increased. In this study, the nutrient proportions increased with an increase in the SMSD from 0 to 15%. The reduction in the soil pH after mixing might be due to the acidic nature of incorporated SMSD as AD already reduced it from 6.87 ± 0.05 to 5.10 ± 0.03 (Table 2). The use of SMSD has always been accepted as an alternative soil nourisher due to its richness in various organic and inorganic constituents that support crop growth and productivity. Studies addressing the effects of SMS mixing have indicated improvements in soil health and beneficial microbial communities [18]; however, reports on using SMSD are almost lacking. A study by Medina and co-workers [42] investigated the interactions of SMS mixing on soil properties. They reported a positive correlation between SMS mixing and available C, N, and P activities of soils. Additionally, Lipiec et al. [43] investigated the impact of SMS addition on available OC in sandy soils. They found that SMS addition was helpful to reclaim the OC of sandy soils, which ranged from 45.8 to 117.8% in various depth treatments. Thus, the results reported by them are in line with those reported in the current study on SMS-based soil fertilization.

Table 3. Effect of spent mushroom substrate digestate (SMSD) mixing on soil nutrient properties.

Characterstics	Control	5% SMSD	10% SMSD	15% SMSD	Unit
pH	7.30 ± 0.03 ^{a}	6.96 ± 0.05 ^{b}	6.61 ± 0.02 ^{c}	6.27 ± 0.06 ^{d}	-
Electrical conductivity	2.17 ± 0.05 ^{a}	2.44 ± 0.02 ^{b}	2.71 ± 0.03 ^{c}	2.97 ± 0.05 ^{d}	dS/m
Organic carbon	1.23 ± 0.08 ^{a}	2.64 ± 0.05 ^{b}	4.05 ± 0.10 ^{c}	5.47 ± 0.09 ^{d}	%
Nitrogen	0.10 ± 0.01 ^{a}	0.43 ± 0.03 ^{b}	0.76 ± 0.06 ^{c}	1.09 ± 0.03 ^{c}	%
C:N ratio	12.30	6.16	5.35	5.03	-
Phosphorus	0.48 ± 0.02 ^{a}	0.51 ± 0.02 ^{a}	0.54 ± 0.03 ^{ab}	0.57 ± 0.02 ^{b}	%
Potassium	0.17 ± 0.01 ^{a}	0.21 ± 0.02 ^{ab}	0.26 ± 0.02 ^{c}	0.30 ± 0.03 ^{c}	%

The same letters (a–d) indicate no significant difference among treatment group values at $p < 0.05$.

3.5. Effect of SMSD on Seedling, Growth, and Yield of Tomato

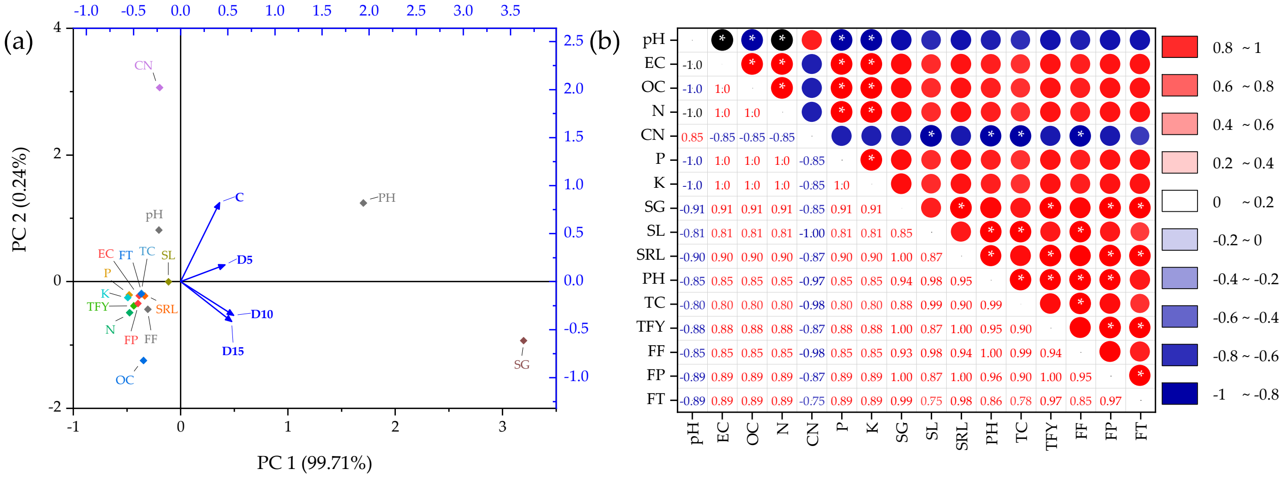
The SMSD used in this study was rich in terms of certain nutrient elements, including C, N, P, and K, which are essential components of healthy soil. Therefore, it was used for tomato fertilization at different mixing rates, i.e., 0, 5, 10, and 15% *w/w* of soil. As shown in Table 4, it was evidenced from the findings that SMSD was helpful to improve the seed germination, growth, biochemical constituents, and productivity of the tomato crop. In general, the maximum significant ($p < 0.05$) seed germination (93.25%), seedling length (9.2 cm), seedling root length (4.19 cm), plant height (53.10 cm), chlorophyll content (3.38 mg/g), total yield (1.86 kg/plant), flavonoids (5.06 mg/g), phenolics (2.78 mg/g), and tannin (3.40 mg/g) contents were recorded in the 10% SMSD mixing soil when compared to control treatment having no digestate addition. However, all the parameters increased from 0 to 10% mixing, which again declined in 15% SMSD treatment. The highest germination and seedling length in 10% treatment might be due to optimum availability of nutrients and supportive physical structure of the soil, which facilitated early maturation and emergence as compared to the control treatment. Overall, the response of tomato crop was observed in a decreasing order of 10% > 15% > 5% > control. Figure 5a shows a PCA loading biplot for interaction between soil properties and tomato response. Based on the PCA results, the data were ordinally transformed into two major components, i.e., PC1 and PC2 having variance coverages of 99.71 (eigenvalue: 1980.38) and 0.24 (eigenvalue: 4.79), respectively. The axial-vector lengths showed that SMSD mixing had a strong influence on tomato response parameters. Similarly, the Pearson correlation matrix in Figure 5b also indicated a significantly ($p < 0.05$) positive relationship between SMSD mixing on soil physicochemical properties except for pH and C:N ratio, which had a negative interaction. Moreover, the seeding, growth, biochemical, and yield parameters of tomatoes also showed a similar positive response with SMSD addition as indicated by correlation coefficient values (>0.75).

Table 4. Effect of spent mushroom substrate digestate (SMSD) mixing on germination, growth, biochemical, and yield response of tomato.

Response	Control	5% SMSD	10% SMSD	15% SMSD
Seed gemination (%)	67.28 ± 2.50 ^{a}	75.80 ± 3.47 ^{b}	93.25 ± 1.78 ^{c}	90.24 ± 2.36 ^{c}
Seedling length (cm)	7.27 ± 0.20 ^{a}	8.99 ± 0.14 ^{b}	9.26 ± 0.26 ^{bc}	9.15 ± 0.11 ^{b}
Seedling root lengh (cm)	3.10 ± 0.05 ^{a}	3.50 ± 0.02 ^{b}	4.19 ± 0.06 ^{c}	4.02 ± 0.10 ^{c}
Plant height (cm)	42.08 ± 1.36 ^{a}	49.20 ± 0.94 ^{b}	53.10 ± 1.02 ^{b}	51.50 ± 1.30 ^{b}
Total chlorophyll content (mg/g fwt.)	2.53 ± 0.03 ^{a}	3.18 ± 0.04 ^{b}	3.38 ± 0.02 ^{c}	3.24 ± 0.05 ^{c}
Total fruit yield (kg/plant)	0.98 ± 0.12 ^{a}	1.30 ± 0.23 ^{b}	1.86 ± 0.15 ^{c}	1.70 ± 0.08 ^{c}
Fruit flavonoids (mg/g)	3.10 ± 0.01 ^{a}	4.43 ± 0.05 ^{b}	5.06 ± 0.09 ^{c}	4.80 ± 0.04 ^{c}
Fruit phenolics (mg/g)	1.76 ± 0.02 ^{a}	2.14 ± 0.10 ^{b}	2.78 ± 0.07 ^{c}	2.62 ± 0.13 ^{c}
Fruit tannins (mg/g)	2.64 ± 0.05 ^{a}	2.75 ± 0.03 ^{a}	3.40 ± 0.05 ^{bc}	3.31 ± 0.02 ^{b}

The same letters ($^{a-c}$) indicate no significant difference among treatment group values at $p < 0.05$.

It has been observed that biofertilizer addition promotes the aeration and nutrient exchange capabilities of soils, which later aids in efficient seed germination [44]. Lesser emergence of seeds in 15% treatment might be due to high N-load, which results in soil acidification leading to a slowdown of plant growth [45]. On the other hand, optimum nutrient proportions also support CO_2 exchange capacity, thereby resulting in high rates of photosynthetic pigment formation (chloroplasts). Certain secondary metabolites such as flavonoids, phenolics (aromatic-benzene compounds), and tannins (family of protective bioactive compounds) are also improved as a result of biofertilizer application [46]. However, a high load of fertilizer may also inhibit the beneficial soil microbial communities, which later results in lesser crop yields. Moreover, crop fertilization of fermented-SMS may also depend on the type of agro-waste used, type of mushroom cultivated, days and duration of cultivation and composting, fermentation condition, percentage of cow-dung/ farmyard manure, microbes involved in anaerobic fermentation, metabolites in (siderophores, organic acid) fermented SMS, type of crop plants selection, type and quality of soil, and environmental condition and water irrigation. Previously, the SMS has been widely accepted for crop fertilization; however, reports on the use of SMS digestate obtained after biogas production are still limited, particularly for tomato crops. As for the results of Meng et al. [47], the individual and mixed applications of composted biogas slurry and SMS obtained after *Auricularia auricula* mushroom cultivation was helpful to improve soil nutrient profiles supporting tomato seedling and yield. Similarly, Collela et al. [48] also conducted pot-scale experiments to study the impact of SMS obtained after *Agaricus bisporus* production. They reported that SMS addition helped to raise tomato production by 20% compared to other tested commercial fertilizers. Therefore, it was evidenced by the results of the current study that Shiitake SMS can be effectively used for attendant biogas production and later for soil supplementation under tomato cultivation.



	pH	EC	OC	N	CN	P	K	SG	SL	SRL	PH	TC	TFY	FF	FP	FT
pH																
EC	-1.0															
OC	-1.0	1.0														
N	-1.0	1.0	1.0													
CN	0.85	-0.85	-0.85	-0.85												
P	-1.0	1.0	1.0	1.0	-0.85											
K	-1.0	1.0	1.0	1.0	-0.85	1.0										
SG	-0.91	0.91	0.91	0.91	-0.85	0.91	0.91									
SL	-0.81	0.81	0.81	0.81	-1.00	0.81	0.81	0.85								
SRL	-0.90	0.90	0.90	0.90	-0.87	0.90	0.90	1.00	0.87							
PH	-0.85	0.85	0.85	0.85	-0.97	0.85	0.85	0.94	0.98	0.95						
TC	-0.80	0.80	0.80	0.80	-0.98	0.80	0.80	0.88	0.99	0.90	0.99					
TFY	-0.88	0.88	0.88	0.88	-0.87	0.88	0.88	1.00	0.87	1.00	0.95	0.90				
FF	-0.85	0.85	0.85	0.85	-0.98	0.85	0.85	0.93	0.98	0.94	1.00	0.99	0.94			
FP	-0.89	0.89	0.89	0.89	-0.87	0.89	0.89	1.00	0.87	1.00	0.96	0.90	1.00	0.95		
FT	-0.89	0.89	0.89	0.89	-0.75	0.89	0.89	0.99	0.75	0.98	0.86	0.78	0.97	0.85	0.97	

Figure 5. PCA biplot (**a**) and Pearson correlation matrix (**b**) for the effects of SMS digestate on the response of tomato (PC: principal component; D: SMSD load as %; EC: electrical conductivity; OC: organic carbon; N: nitrogen; CN: carbon to nitrogen ratio; P: phosphorus; K: potassium; SG: seedling germination; SL: seedling length; SRL: seedling root length; PH: plant height; TC: total chlorophyll; TYF: total fruit yield; FF: fruit flavonoids; FP: fruit phenolics; FT: fruit tannins).

4. Conclusions

We conclude that spent mushroom substrate (SMS) obtained after the cultivation of Shiitake mushroom had significant loads of organic and inorganic constituents supportive of biogas production. The maximum cumulative biogas yield (8834 mL and 61.00% CH_4) was produced using 50:50 SMS to cow dung inoculation with the most reduction in slurry parameters after 21 days of anaerobic digestion (AD). Furthermore, the SMS digestate applied to soil helped to increase the nutrient profile under tomato cultivation as indicated by the principal component and Pearson correlation analyses. The maximum significant ($p < 0.05$) germination, growth, biochemical parameters, and yield of tomato plants were produced using 10% digestate *w/w* soil application. The fermented SMS elevated the nutrient availability and may have accelerated the growth and bio-stimulation effect in crops. Therefore, the findings of this study provide a method for utilizing the agricultural residues through mushroom cultivation, bioenergy production, soil fertilization, and vegetable production as inspired by a circular economy concept. Future studies on compositional analysis of SMS digestate in terms of beneficial microbial communities and their role in soil are highly recommended.

Author Contributions: Conceptualization, P.K. and M.G.; data curation, P.K. and M.G.; formal analysis, P.K.; funding acquisition, E.M.E. and I.Š.; investigation, P.K.; methodology, P.K.; project administration, E.M.E.; software, B.A.; supervision, V.K.; validation, E.M.E., M.A.T., M.H.E.E.-M., H.E.M.O., D.A.A.-B., B.A., S.A.F., M.G., B.M., V.D., F.O.A., K.S.C., V.K. and I.Š.; visualization, P.K.; writing—original draft, P.K. and S.A.F.; writing—review and editing, E.M.E., M.A.T., M.H.E.E.-M., H.E.M.O., D.A.A.-B., B.A., M.G., B.M., V.D., F.O.A., K.S.C., V.K. and I.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by King Khalid University (grant number RGP. 1/182/43), Abha, Saudi Arabia; and Umm-Al-Qura University (grant number 22UQU4320730DSR01), Makkah, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work is a person-to-person collaboration between P.K., E.M.E., and I.Š. The authors are grateful to their host institutes for providing the necessary facilities to conduct this study. M.A.T. extends his appreciation to King Khalid University for funding this work through the Research Group Project under grant number RGP. 1/182/43; H.E.M.O. expresses her gratitude to Umm-Al-Qura University for funding this work under grant number 22UQU4320730DSR01. All individuals included in this section have consented to the acknowledgment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Royse, D.J.; Baars, J.; Tan, Q. Current Overview of Mushroom Production in the World. In *Edible and Medicinal Mushrooms*; John Wiley & Sons, Ltd.: Chichester, UK, 2017; pp. 5–13.
2. Gonani, Z.; Riahi, H.; Sharifi, K. Impact Of Using Leached Spent Mushroom Compost As A Partial Growing Media For Horticultural Plants. *J. Plant Nutr.* **2011**, *34*, 337–344. [CrossRef]
3. Carrasco, J.; Zied, D.C.; Pardo, J.E.; Preston, G.M.; Pardo-Giménez, A. Supplementation in Mushroom Crops and Its Impact on Yield and Quality. *AMB Express* **2018**, *8*, 146. [CrossRef] [PubMed]
4. EEA European Environment Agency. Nitrate Directive. 2022. Available online: https://www.eea.europa.eu/archived/archived-content-water-topic/water-pollution/prevention-strategies/nitrate-directive (accessed on 3 May 2022).
5. Stewart, D.P.C.; Cameron, K.C.; Cornforth, I.S. Inorganic-N Release from Spent Mushroom Compost under Laboratory and Field Conditions. *Soil Biol. Biochem.* **1998**, *30*, 1689–1699. [CrossRef]
6. Gerrits, J.P.G. Samenstelling van Afgewerkte Champignonmest. *Champignoncultuur* **1992**, *36*, 91–93.
7. Ahlawat, O.P.; Gupta, P.; Kumar, S.; Sharma, D.K.; Ahlawat, K. Bioremediation of Fungicides by Spent Mushroom Substrate and Its Associated Microflora. *Indian J. Microbiol.* **2010**, *50*, 390–395. [CrossRef] [PubMed]
8. Kwack, Y.; Song, J.H.; Shinohara, Y.; Maruo, T.; Chun, C. Comparison of Six Spent Mushroom Composts As Growing Media for Transplant Production of Lettuce. *Compos. Sci. Util.* **2012**, *20*, 92–96. [CrossRef]
9. Van Tam, N.; Wang, C.-H. Use of Spent Mushroom Substrate and Manure Compost for Honeydew Melon Seedlings. *J. Plant Growth Regul.* **2015**, *34*, 417–424. [CrossRef]
10. Lou, Z.; Sun, Y.; Zhou, X.; Baig, S.A.; Hu, B.; Xu, X. Composition Variability of Spent Mushroom Substrates during Continuous Cultivation, Composting Process and Their Effects on Mineral Nitrogen Transformation in Soil. *Geoderma* **2017**, *307*, 30–37. [CrossRef]
11. Zhu, H.-J.; Sun, L.-F.; Zhang, Y.-F.; Zhang, X.-L.; Qiao, J.-J. Conversion of Spent Mushroom Substrate to Biofertilizer Using a Stress-Tolerant Phosphate-Solubilizing Pichia Farinose FL7. *Bioresour. Technol.* **2012**, *111*, 410–416. [CrossRef]
12. Abou Fayssal, S.; el Sebaaly, Z.; Alsanad, M.A.; Najjar, R.; Böhme, M.; Yordanova, M.H.; Sassine, Y.N. Combined Effect of Olive Pruning Residues and Spent Coffee Grounds on Pleurotus ostreatus Production, Composition, and Nutritional Value. *PLoS ONE* **2021**, *16*, e0255794. [CrossRef]
13. Kumar, P.; Eid, E.M.; Al-Huqail, A.A.; Širić, I.; Adelodun, B.; Abou Fayssal, S.; Valadez-Blanco, R.; Goala, M.; Ajibade, F.O.; Choi, K.S.; et al. Kinetic Studies on Delignification and Heavy Metals Uptake by Shiitake (Lentinula edodes) Mushroom Cultivated on Agro-Industrial Wastes. *Horticulturae* **2022**, *8*, 316. [CrossRef]
14. Kumar, P.; Kumar, V.; Adelodun, B.; Bedeković, D.; Kos, I.; Širić, I.; Alamri, S.A.M.; Alrumman, S.A.; Eid, E.M.; Abou Fayssal, S.; et al. Sustainable Use of Sewage Sludge as a Casing Material for Button Mushroom (Agaricus bisporus) Cultivation: Experimental and Prediction Modeling Studies for Uptake of Metal Elements. *J. Fungi* **2022**, *8*, 112. [CrossRef] [PubMed]
15. Adelodun, B.; Kareem, K.Y.; Kumar, P.; Kumar, V.; Choi, K.S.; Yadav, K.K.; Yadav, A.; El-Denglawey, A.; Cabral-Pinto, M.; Son, C.T.; et al. Understanding the Impacts of the COVID-19 Pandemic on Sustainable Agri-Food System and Agroecosystem Decarbonization Nexus: A Review. *J. Clean. Prod.* **2021**, *318*, 128451. [CrossRef]

16. Royse, D.J. Recycling of Spent Shiitake Substrate for Production of the Oyster Mushroom, Pleurotus sajorcaju. *Appl. Microbiol. Biotechnol.* **1992**, *38*, 179–182. [CrossRef]
17. Fang, W.; Ye, J.; Zhang, P.; Zhu, X.; Zhou, S. Solid-State Anaerobic Fermentation of Spent Mushroom Compost for Volatile Fatty Acids Production by PH Regulation. *Int. J. Hydrogen Energy* **2017**, *42*, 18295–18300. [CrossRef]
18. Mohd Hanafi, F.H.; Rezania, S.; Mat Taib, S.; Md Din, M.F.; Yamauchi, M.; Sakamoto, M.; Hara, H.; Park, J.; Ebrahimi, S.S. Environmentally Sustainable Applications of Agro-Based Spent Mushroom Substrate (SMS): An Overview. *J. Mater. Cycles Waste Manag.* **2018**, *20*, 1383–1396. [CrossRef]
19. Lin, Y.; Ge, X.; Liu, Z.; Li, Y. Integration of Shiitake Cultivation and Solid-State Anaerobic Digestion for Utilization of Woody Biomass. *Bioresour. Technol.* **2015**, *182*, 128–135. [CrossRef]
20. Gao, X.; Tang, X.; Zhao, K.; Balan, V.; Zhu, Q. Biogas Production from Anaerobic Co-Digestion of Spent Mushroom Substrate with Different Livestock Manure. *Energies* **2021**, *14*, 570. [CrossRef]
21. Kumar, P.; Kumar, V.; Singh, J.; Kumar, P. Electrokinetic Assisted Anaerobic Digestion of Spent Mushroom Substrate Supplemented with Sugar Mill Wastewater for Enhanced Biogas Production. *Renew. Energy* **2021**, *179*, 418–426. [CrossRef]
22. Kumar, V.; Kumari, S.; Kumar, P. Management and Sustainable Energy Production Using Flower Waste Generated from Temples. In *Environmental Degradation: Causes and Remediation Strategies*; Agro Environ Media—Agriculture and Ennvironmental Science Academy: Haridwar, India, 2020; pp. 154–165.
23. Kumar, V.; Kumar, P.; Kumar, P.; Singh, J. Anaerobic Digestion of Azolla pinnata Biomass Grown in Integrated Industrial Effluent for Enhanced Biogas Production and COD Reduction: Optimization and Kinetics Studies. *Environ. Technol. Innov.* **2020**, *17*, 100627. [CrossRef]
24. Kumar, V.; Singh, J.; Nadeem, M.; Kumar, P.; Pathak, V.V. Experimental and Kinetics Studies for Biogas Production Using Water Hyacinth (Eichhornia crassipes [Mart.] Solms) and Sugar Mill Effluent. *Waste Biomass Valorization* **2020**, *11*, 109–119. [CrossRef]
25. Koszel, M.; Lorencowicz, E. Agricultural Use of Biogas Digestate as a Replacement Fertilizers. *Agric. Agric. Sci. Procedia* **2015**, *7*, 119–124. [CrossRef]
26. Ehmann, A.; Thumm, U.; Lewandowski, I. Fertilizing Potential of Separated Biogas Digestates in Annual and Perennial Biomass Production Systems. *Front. Sustain. Food Syst.* **2018**, *2*, 12. [CrossRef]
27. Doyeni, M.O.; Stulpinaite, U.; Baksinskaite, A.; Suproniene, S.; Tilvikiene, V. The Effectiveness of Digestate Use for Fertilization in an Agricultural Cropping System. *Plants* **2021**, *10*, 1734. [CrossRef]
28. Dahunsi, S.O.; Oranusi, S.; Efeovbokhan, V.E.; Adesulu-Dahunsi, A.T.; Ogunwole, J.O. Crop Performance and Soil Fertility Improvement Using Organic Fertilizer Produced from Valorization of Carica papaya Fruit Peel. *Sci. Rep.* **2021**, *11*, 4696. [CrossRef]
29. Ronga, D.; Caradonia, F.; Parisi, M.; Bezzi, G.; Parisi, B.; Allesina, G.; Pedrazzi, S.; Francia, E. Using Digestate and Biochar as Fertilizers to Improve Processing Tomato Production Sustainability. *Agronomy* **2020**, *10*, 138. [CrossRef]
30. Latimer, G.W. *Official Methods of Analysis of AOAC International*, 21st ed.; AOAC International: Rockville, MD, USA, 2019.
31. Walkley, A.; Black, I.A. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
32. Chromý, V.; Vinklárková, B.; Šprongl, L.; Bittová, M. The Kjeldahl Method as a Primary Reference Procedure for Total Protein in Certified Reference Materials Used in Clinical Chemistry. I. A Review of Kjeldahl Methods Adopted by Laboratory Medicine. *Crit. Rev. Anal. Chem.* **2015**, *45*, 106–111. [CrossRef]
33. Kumar, V.; Kumar, P.; Singh, J.; Kumar, P. Potential of Water Fern (Azolla pinnata R.Br.) in Phytoremediation of Integrated Industrial Effluent of SIIDCUL, Haridwar, India: Removal of Physicochemical and Heavy Metal Pollutants. *Int. J. Phytoremediat.* **2020**, *22*, 392–403. [CrossRef]
34. Ahmed, S.; Rahman, F.B.; Ahmed, S.; Mohammad Shohael, A. Insights into the Bioactive Compounds, Antioxidant Potential and TLC Profiling of Different Extracts of Tomato Plants. *Jahangirnagar Univ. J. Biol. Sci.* **2018**, *7*, 65–77. [CrossRef]
35. Kumar Pandey, A.; Rajan, S.; Sarsaiya, S.; Jain, S.K. Mushroom for the National Circular Economy. *Int. J. Sci. Res. Biol. Sci.* **2020**, *7*, 61–69.
36. Mathew, G.M.; Sukumaran, R.K.; Singhania, R.R.; Pandey, A. Progress in research on fungal cellulases for lignocellulose degradation. *J. Sci. Ind. Res.* **2008**, *67*, 898–907. Available online: https://hdl.handle.net/123456789/2417 (accessed on 20 March 2022).
37. Awe, O.W.; Zhao, Y.; Nzihou, A.; Minh, D.P.; Lyczko, N. A Review of Biogas Utilisation, Purification and Upgrading Technologies. *Waste Biomass Valorization* **2017**, *8*, 267–283. [CrossRef]
38. Nong, H.T.T.; Unpaprom, Y.; Whangchai, K.; Ramaraj, R. Sustainable Valorization of Water Primrose with Cow Dung for Enhanced Biogas Production. *Biomass Convers. Biorefin.* **2020**, *12*, 1647–1655. [CrossRef]
39. Li, Y.; Chen, Y.; Wu, J. Enhancement of Methane Production in Anaerobic Digestion Process: A Review. *Appl. Energy* **2019**, *240*, 120–137. [CrossRef]
40. Yang, L.; Huang, Y.; Zhao, M.; Huang, Z.; Miao, H.; Xu, Z.; Ruan, W. Enhancing Biogas Generation Performance from Food Wastes by High-Solids Thermophilic Anaerobic Digestion: Effect of PH Adjustment. *Int. Biodeterior. Biodegrad.* **2015**, *105*, 153–159. [CrossRef]
41. Najafi, B.; Faizollahzadeh Ardabili, S. Application of ANFIS, ANN, and Logistic Methods in Estimating Biogas Production from Spent Mushroom Compost (SMC). *Resour. Conserv. Recycl.* **2018**, *133*, 169–178. [CrossRef]

42. Medina, E.; Paredes, C.; Bustamante, M.A.; Moral, R.; Moreno-Caselles, J. Relationships between Soil Physico-Chemical, Chemical and Biological Properties in a Soil Amended with Spent Mushroom Substrate. *Geoderma* **2012**, *173–174*, 152–161. [CrossRef]
43. Lipiec, J.; Usowicz, B.; Kłopotek, J.; Turski, M.; Frąc, M. Effects of Application of Recycled Chicken Manure and Spent Mushroom Substrate on Organic Matter, Acidity, and Hydraulic Properties of Sandy Soils. *Materials* **2021**, *14*, 4036. [CrossRef]
44. Sánchez, Ó.J.; Ospina, D.A.; Montoya, S. Compost Supplementation with Nutrients and Microorganisms in Composting Process. *Waste Manag.* **2017**, *69*, 136–153. [CrossRef]
45. Raza, S.; Miao, N.; Wang, P.; Ju, X.; Chen, Z.; Zhou, J.; Kuzyakov, Y. Dramatic Loss of Inorganic Carbon by Nitrogen-Induced Soil Acidification in Chinese Croplands. *Glob. Change Biol.* **2020**, *26*, 3738–3751. [CrossRef] [PubMed]
46. Rachel, K.V.; Sirisha, G.V. Effect of Bio-Fertilizers Application on Qualitative, Quantitative Yield of Phytochemicals in Three Divergent Groups of Plants and Their Antioxidant Activities. *Res. J. Life Sci. Bioinform. Pharma. Chem. Sci.* **2016**, *2*, 56–77.
47. Meng, X.; Dai, J.; Zhang, Y.; Wang, X.; Zhu, W.; Yuan, X.; Yuan, H.; Cui, Z. Composted Biogas Residue and Spent Mushroom Substrate as a Growth Medium for Tomato and Pepper Seedlings. *J. Environ. Manag.* **2018**, *216*, 62–69. [CrossRef] [PubMed]
48. Collela, C.F.; Costa, L.M.A.S.; Moraes, T.S.J.d.; Zied, D.C.; Rinker, D.L.; Dias, E.S. Potential Utilization of Spent Agaricus bisporus Mushroom Substrate for Seedling Production and Organic Fertilizer in Tomato Cultivation. *Cienc. Agrotecnol.* **2019**, *43*, 7119. [CrossRef]