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Valorization of spent mushroom substrate (SMS) along with rice straw as feedstock for efficient bio gasification

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ABSTRACT

Wastes generated after mushroom harvesting are known as spent mushroom substrate (SMS) which causes contamination of the environment. Anaerobic digestion is an economical and viable technology used for the management of several types of agricultural wastes, thus generating syngas that has many thermal applications. The current manuscript reports the potential of SMS for anaerobic digestion in five different combinations with rice straw, cattle dung, and bio-digested slurry. The kinetics of the biogasification process occurring in digesters was analyzed by modified Gompertz model. Results showed that maximum biogas (3,12,664 ml) was produced in digester C containing SMS, rice straw, cattle dung, and bio-digested slurry in the ratio of 8:2:1:1 as compared to control (48,968 ml) consisting of only SMS and water in the ratio of 8:2. Evaluation of feedstock for proximate and chemical composition recorded a notable reduction in total solids (TS), volatile solids (VS), cellulose, hemicellulose, lignin content as compared to control. On the other hand, ash content showed a significant increase after anaerobic digestion. Modified Gompertz equation revealed that maximum biogas production potential (P) of 6,150.50 mLg^{-1} VS with a biogas production rate (R_m) of 134 $mLg^{-1}d^{-1}$ and lag phase (λ) of 12.80 days was observed in digester C as compared to the control digester A (P= 12.24 mLg^{-1} VS; R_m = 8.56 $mLg^{-1}d^{-1}$; λ=8.80 days). The study demonstrated that the co-digesting SMS with rice straw can be successfully implicated for producing biogas as an energy source rather making dumped at the village site for causing pollution.

Key words: *Biogasification, Biomass, Anaerobic digestion, Gompertz equation, Spent mushroom substrate (SMS).*

Introduction

Mushrooms are biological resources with high nutritional content and several biotechnological applications. They are enriched in high-value proteins, carbohydrates, minerals, phenols, and vitamins which could be a replacement for proteins of animal origin (Ramos *et al*., 2019). Unsaturated fatty acids existing in mushrooms are very much helpful in cardiovascular health. Additionally, mushrooms contained a higher fraction of dietary fiber which has immune system boosting and anticarcinogenic activity (Deng, 2013). Mushrooms are also endowed with anti-tumor, anti-diabetic, and anti-oxidant properties (Nowacka-Jechalke *et al.,* 2018).

The mushroom industry has been extending significantly owing to the increment in the world's population. Mushrooms have a marvelous nutritional profile with a distinctive flavor and are enriched in bioactives in preventing several ailments and afflictions. Certain mushrooms sp. such as Oyster mushroom (*Pleurotus* sp.), shiitake (*Lentinula edodes*), button mushroom (*Agaricus bisporus*), and others are consumed worldwide for nutritional benefits (Valverde *et al*., 2015). This has led to increased cultivation and consumption of mushrooms globally with market value expected to escalate by 20.84 million by 2026 (Atallah *et al*., 2021). Cultivation of mushrooms has biotechnological significance also as agro-industrial residues such as corncob, wooden chips, manure, sawdust, etc. are recycled and utilized (Koji´c *et al.,* 2021).

Cotton waste, paddy straw, sawdust, and oil palm empty fruit bunch, and other agricultural wastes are used as the growth media for mushroom cultivation (Oseni *et al.,* 2012). Despite the expeditiously proliferating mushroom industry, a generous amount of SMS is generated after harvesting the mushroom crop (Atallah *et al*., 2021). It has been estimated that for per kg of mushroom crop produced 5 kg of SMS is generated (Williams *et al*., 2001). Improper disposal of these waste products results in air, water, and soil pollution and might cause detrimental effects on human and animal health conditions. Therefore, the exploration of alternative strategies for the efficient management of SMS is the need of the hour.

Anaerobic digestion (AD) is a traditional and efficient technique for waste treatment wherein organic compounds are converted to clean biofuel such as biogas along with the generation of biogas slurry which has application as organic fertilizer (Gao *et al*., 2021). The process of AD can be carried out either in the liquid phase (total solids (TS) <15%) or in the solid phase (TS >15%). Solid-phase anaerobic digestion offers many benefits such as higher productivity, lesser mobile parts, and energy costs as compared to liquid-phase anaerobic digestion(Li *et al*., 2011). The biogas production process is a spontaneous operation that occurs in anaerobic conditions producing a gaseous mixture known as biogas which is composed of 40-70% methane (CH_4), 20-30% carbon dioxide (CO_2), 100-3000 ppm hydrogen sulfide (H_2S) and water, trace gases, and other impurities (Ramaraj *et al.,* 2016). The generated syngas can be employed for heat and electricity production, and biofuels for vehicles.

In agricultural countries like India, several tons of crop residues are generated every year. Rice straw contributes significantly (about 34%) to the total residues generated (Bhattacharyya *et al.,* 2015) which is managed by burning. Crop residue burning has serious effects on the environment by releasing polluting substances. However, rice straw can be treated by anaerobic digestion thereby producing clean fuel like biogas

Anaerobic digestion involves a complex interaction of microbiological, biochemical, and physical-chemical processes generating biogas and biofertilizer as major end products. The process is separated into four phases namely fermentative and hydrolytic (Phase 1), acidogenic (Phase 2), acetogenic (Phase 3), and methanogenic (Phase 4). Each phase is catalyzed by four different trophic groups of microbes (Kwietniewska and Tys, 2014). Optimum conditions are necessary to maintain a balance between the microbial population involved in the process. The factors affecting the system are temperature, moisture, TS, H^+ or OH^- ions, retention time, organic loading rate, C/N ratio, and mixing (Aslanzadeh, 2014).

The present study was conducted in the Biogas laboratory, Department of Renewable Energy Engineering, Punjab Agricultural University, Ludhiana, Punjab. The major goal of the study was to evaluate the anaerobic digestion potential of the spent mushroom substrate (SMS). To meet this end, SMS was mixed with various combining ratios of rice straw, bio-digested slurry, and cattle dung in different biodigesters and its biogasification potential was studied. Furthermore, the kinetics of the biogasification process undergoing digesters was studied by a modified Gompertz model. Ultimately, the proximate and chemical composition of feedstock were analyzed.

Materials and Methods

Raw materials

SMS was procured from a local mushroom farmer in the Sangrur district of Punjab. The waste was dried at 50°C to remove extra moisture and chopped into small pieces to enhance substrate surface area for faster decomposition by the microbial population. The rice straw was procured from research fields of

PAU, Ludhiana. These were chopped into small pieces of 5-10 cm in size to increase the surface area for easy and rapid degradation by bacteria during anaerobic digestion. Cattle dung and bio-digested slurry were collected from the dairy farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Cattle dung acts as a substrate and inducer along with providing necessary bacteria for anaerobic digestion.

Solid-state anaerobic digestion

Experimentation was carried out in 2-L solid-state anaerobic digestion reactors**.** Digesters were closed with a rubber stopper and provided air-tight connections with Araldite as an adhesive. The digester stopper has two outlets. One for connection with a collecting container and another with a liquid reservoir. Reactors were operated in batch mode under mesophilic conditions. Biogas production was noted every 24 hours for 30 days by the monophasic water displacement method. The temperature was recorded using a thermometer. After completion of the anaerobic digestion, feedstock was taken for composition analysis.A total of five digesters (in triplicates) were established each containing various mixing ratios of SMS, bio-digested slurry, and cattle dung was fed to each digester, and biogas production was noted after every 24 hours. The composition of each digester labeled as A to E is given in Table 1.

Analytical methods

Proximate components (Total solids (TS), volatile solids (VS), ash) and chemical components i.e. cellulose, hemicellulose, lignin, and silica of the feedstock fed to different digesters were calculated by standard protocols of AOAC (2002). Reduction in total volatile solids (VSR%) after anaerobic digestion was measured by using the following relation:

$$VSR(\%) = \frac{(VS_{bd} - VS_{ad})}{VS_{bd}} \times 100$$

VS_{bd} and VS_{ad} denote volatile solids before and after anaerobic digestion.

Kinetics of the biogasification process

Calculation of cumulative biogas production (mL biogas g^{-1} VS) was done and then fitted with the modified Gompertz equation. Various parameters were calculated using MS Solver of Excel 2010. The Gompertz equation (Prajapati *et al.,* 2015) is given below:

$$M = P \times \exp\left[-\exp\left\{\left(\frac{R_{m \times e}}{P}\right) \times (\lambda - t) + 1\right\}\right]$$

Where M is the cumulative biogas yield (mL biogas g^{-1} VS added), P is the enhancement in ultimate biogas yield (mLg^{-1} VS), R_m is the maximum rate of biogas production (mL$g^{-1}d^{-1}$) and ë is the lag phase (days) and e = 2.718.

Statistical analysis

All experiments were performed in triplicates. Values are present in Mean ± Standard deviation. Tukey's HSD Multiple Range test using SPSS Statistics 22 software was used to compare means.

Results and Discussion

Composition of feedstock

The proximate and chemical composition of feedstock before and after anaerobic digestion is represented in Table 2. Determination of feedstock characteristics before the anaerobic digestion process enables the selection of better process parameters during the anaerobic digestion process (Abdelgadir *et al.*, 2014). TS observed in SMS were 93.74% and they enhanced to 99.64% when rice straw, bio-digested slurry, and cattle dung were mixed. Similarly, VS increased from 82.34% to 76.73%. Higher TS and VS content indicates that SMS is an organic matter-enriched substrate (Luo *et al.*, 2018). After anaerobic digestion, a significant decrease in total solid and volatile solid content of feedstock was observed as

Table 1. Composition of different digesters

Digester Label	Feedstock content
A	800 g SMS+200 ml H_2O
B	800 g SMS+ 200g bio-digested slurry
C	800 g SMS+ 100g rice straw+ 50 g cattle dung + 50 g bio-digested slurry
D	800 g SMS + 100g rice straw+200 g cattle dung
E	800 g SMS + 50 g cattle dung+ 150 g bio-digested slurry

Table 2. Proximate and chemical composition of the feedstock

Digester label	Proximate composition (%)						Chemical composition (%)					
	Total solids (TS)		Volatile solids (VS)		*Ash		Cellulose		Hemicellulose		Lignin	
	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion
A	93.74	91.49	82.34	82.17	17.66	17.83	32.00	30.00	24.00	22.00	18.00	18.00
	2.40^{e}		0.21^{e}		0.96^{e}		6.25^{e}		8.33^{e}		0.00^{e}	
B	96.71	83.40	81.73	74.41	18.27	25.59	36.00	28.00	22.00	18.00	20.00	16.00
	13.76^{c}		8.96^{c}		40.07^{c}		22.22^{c}		18.18^{c}		20.00^{c}	
C	99.64	78.07	76.73	61.43	23.27	38.57	38.00	26.00	28.40	18.00	28.00	20.00
	21.65^{a}		19.94^{a}		65.75^{a}		31.58^{a}		36.62^{a}		28.57^{a}	
D	97.09	81.99	82.63	74.12	17.37	25.88	36.00	26.00	26.00	20.00	22.00	16.00
	15.55^{b}		10.30^{b}		48.99^{b}		27.78^{b}		23.08^{b}		27.27^{b}	
E	99.13	91.73	77.13	74.51	22.87	25.49	36.00	30.00	22.00	20.00	26.00	24.00
	7.46^{d}		3.40^{d}		11.45^{d}		16.67^{d}		9.09^{d}		7.69^{d}	

Values superscripted by different alphabets in columns are percent reductions in various proximate and chemical parameters after anaerobic+digestion and are calculated by comparing means with Tukey's test using IBM SPSS Statistics 22; *Ash content showed a percent increase after anaerobic digestion.

compared to the present initially. In digester C, the TS decreased from 99.64 to 78.07 % i.e. 21.65% reduction and VS decreased from 76.73 to 61.43% i.e. 19.94% reduction, which showed their consumption by microbial population involved in the gasification process. TS and VS are the two very crucial factors for determining the final the organic loading rate. One of the most important attributes of the substrate consumed by the microbial population for anaerobic digestion is total volatile solids which are finally converted into methane (Morosini *et al*., 2016). In methane production, initially, hydrolysis of large polymers to small molecules occurs which are then converted to alcohols and volatile fatty acids (VFA) by the enzymatic action of fermentative bacteria. Then acetogenins convert acetate to carbon dioxide and hydrogen and ultimately methane is produced from acetate (Tsapekos *et al*., 2017). Ash content which represents the number of minerals present in biomass for normal metabolism of the plant increased significantly after anaerobic digestion (Siegal-Willott *et al*., 2010). Biomass conversion to biogas contributed to the increased ash content. Additionally, a significant decrease was also observed in cellulose, hemicellulose, and lignin content. Cellulose reduced from 38 to 26% (31.58%), hemicellulose from 28.40 to 18% (36.62%), and from 28 to 20% (28.57%), respectively in digester C which was the maximum reduction among all the digesters. This was followed by digester D whereby cellulose, hemicellulose, and lignin content decreased from 36 to 26% (27.78%), 26 to 20% (23.08%), and 22 to 16% (27.27%), respectively. The minimum reduction was observed in control digester A. The possible reason for the decrease in the cellulose, hemicellulose, and lignin content after anaerobic digestion is that the hydrolase enzyme released during the hydrolysis step by the facultative and obligate anaerobic bacteria community degrades the larger polymeric substances like carbohydrates, fats, cellulose, and proteins into monomeric units. But the degradation of lignocellulosic biomass and lignin occurs slowly and incompletely (Boontian, 2014). The degradability of lignocellulosic feedstock enormously depends on enzymatic activity and hydrolysis of lignocellulosic residues by microbial population and is the rate-limiting step in the anaerobic digestion (Yu and Schanbacher, 2010). Li *et al.* (2014) reported that as SMS has higher cellulose, but lower hemicellulose and lignin it has a higher degradation rate as compared to other lignocellulosic substrates such as yard trimmings and wheat straw which has higher hemicellulose and lignin content contributing to their recalcitrancy (Meng and Ragauskas, 2014).

Solid-state anaerobic digestion

Fig. 1. represents the cumulative biogas yield from different digesters after solid-state anaerobic digestion of different combining ratios of SMS, rice straw, cattle dung, and bio-digested slurry. Solid-state anaerobic digestion presents several advantageous features over conventional liquid anaerobic digestion e.g. more methane production, lesser input requirements, and easy digestate handling (Zhu *et al.,*2015). Zhu *et al.* (2015) demonstrated that solid-state anaerobic digestion of corn stover with SMS enhanced cumulative methane yield by 40% (98.5 L/kg VS) and decreased lag phase from 11 to 4 days. During the cultivation period, most lignin and hemicellulose portion of the mushroom is pre-degraded enhancing its fermentation efficiency, thus, making it an efficient substrate for the biogasification process (Leong *et al*., 2022). Its mycelia are also disrupted thereby providing enhanced surface area for the anaerobic digestion process, thus making it more prone to enzymatic and microbial action (Nuchdang *et al*., 2015).

It is evident from Fig. 1 that the maximum cumulative biogas production in digester C containing SMS, rice straw, cattle dung, and bio-digested slurry in a ratio of 8:2:1:1 was 312.66 liters in 30 days. Biogas production increased upto the last day, i.e. 30th day of anaerobic digestion. This was followed by digester D containing SMS, rice straw, and cattle dung in 8:1:1. Digester D also followed a similar trend as digester C in the biogas production where it increased upto the 30th day of the digestion process. Hence, both digesters C and D showed higher biogas production as compared to the control digester A containing only SMS and water in an 8:2 ratio producing only 48.97 liters of biogas in the period of 30 days. Higher biogas production in digesters C and D among all other combinations is because both involve co-digestion of rice straw with SMS. Many researchers have reported the co-digestion of mushroom waste with feed stocks such as food waste, palm oil mill effluent (POME), and sugar mill wastewater (Wang *et al*., 2022; Mamimin *et al*., 2021, Kumar *et al*., 2021). Co-digestion of two feedstock lengthens the time required for fermentation while maintaining the stability of the system resulting in increased biogas yield (Leong *et al*., 2022).

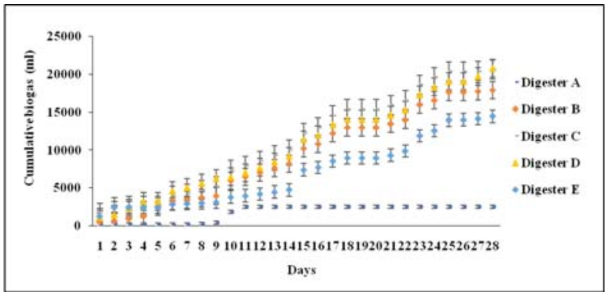

Fig. 1. Cumulative biogas production in solid-state anaerobic digestion

In the control digester A, the increase in biogas production was slow but it continued up to the 11th day of anaerobic digestion and it was significantly lesser than other digesters. After that, biogas production became constant. The reason for the lesser production of biogas in the control digester A might be the insufficient number of methanogens required for biogas production. SMS is a highly degradable substrate and during the process of solid-state anaerobic digestion, large amounts of volatile fatty acids were produced. If the system has insufficient dilution or lower buffering capacity, it will cause the overall pH to drop which will cause the inhibition of the activity of methanogens, and decreased consumption of volatile fatty acids, thus further intensifying their accumulation and decreased consumption rate. A previous study reported that the anaerobic digestion process is greatly affected by the highly degradable nature of SMS leading to lesser methane generation. Hence, to the maintenance of the stability of the process, SMS was mixed with dairy manure (Luo *et al*., 2018). However, this problem can be alleviated in the liquid phase because VFAs could be diluted easily in liquid systems (Lin *et al.,* 2014) which is difficult to achieve in solid systems. Production of biogas in digesters B and E increased with time and it continued to increase for the remaining experimental days and total biogas produced was 258.98 and 195.15 liters, respectively. Variable methane production values are obtained in different sets of digesters containing various combining ratios of SMS, rice straw, cattledung, and biodigested slurry. This is because methane yield produced in anaerobic digestion of lignocellulosic material is enormously influenced by feedstock to inoculum ratio (F/I) with a higher F/I ratio resulting in complete stoppage of the system. Córdoba *et al.* (2016) observed that by reducing the SMS concentration from 80% to 40%, methane production enhanced.

Cumulative biogas data obtained in this study corroborates well with the environmental temperature. In the present experiment during the entire study period temperature ranged from 32 °C to a maximum of 36°C, i.e. temperature was at mesophilic range. Significantly higher biogas was produced in digester C followed by digesters D, E, B, and A. Maximum biogas production is observed at the mesophilic temperature range as compared to psychrophilic and thermophilic temperature ranges. The most significant factor influencing the rate of microbial metabolism during the anaerobic digestion process is temperature (Tian *et al*., 2018). At psychrophilic or low temperatures, methanogenic bacteria use fewer amounts of volatile acids which reduces their maximum specific growth, and biogas generation also decreases (Dhadse *et al*., 2012). On the other hand, thermophilic temperature causes affect gas solubility shifting the chemical equilibrium from ammonium to ammonia generation and finally inhibiting the whole process (Garcia and Angenent, 2009). In a previous study Arikan *et al.* (2015) observed biogas yield from anaerobic digestion of dairy manure in field scale digesters at operating temperatures 22 and 28 °C was 70% and 87%, respectively of that obtained at 35 °C.

Kinetics of the biogasification process

Table 3 and Fig. 2 showed the results obtained after fitting of modified Gompterz equation. Digester C has the maximum biogas production potential (P) of 6,150 mLg^{-1} VS with a biogas pro-duction rate (R_m) of 134 $mLg^{-1}d^{-1}$ with a lag phase (λ) of 12.80 days which has SMS, rice straw, cattle dung and bio-digested slurry in ratio 8:2:1:1. This was higher than the control digester A (P) = 12.24mLg^{-1} VS, (R_m) = 8.56 $mLg^{-1}d^{-1}$, lag phase (λ) = 8.80 days) containing only SMS and water in 8:2. This was followed by digester D (containing SMS, rice straw, and cattle dung in a ratio 8:1:1) with P, R_m and λ of 5,115.67 mLg^{-1} VS, 105.47 $mLg^{-1}d^{-1}$ and 13.80 days, respectively. The initial period required for acclimatization of methane-producing bacteria to substrates present in an anaerobic digester is represented by the lag phase λ i.e. lag phase derived from the Gompterz model (Syaichurrozi *et al.*, 2016). Biogas production in all the digesters started after a certain lag period i.e. 8.80, 3.74, 12.80, 13.02, and 9.73 days in digesters A, B, C, D, and E, respectively. The predicted biogas yield (mLg^{-1} VS) and experimental biogas yield (mLg^{-1} VS) calculated in five digesters were in close agreement with each other. The assumption behind the modified Gompertz equation is that the growth rate of bacteria under varying environments in different digesters is in direct proportion to the amount of methane produced and has a sigmoidal nature (Nopharatana *et al*., 2007). VS present in substrates is converted into biogas during the digestion process and the quantity of volatile substrates converted or removed is expressed as percent VS removal (Syaichurrozi, 2018). The highest percent reduction in VS was in digester C (19.94%) followed by digester D (10.30 %) which was significantly higher than control digester A (0.21%). The maximum percent reduction in the volatile solids content of digesters C and D justifies the higher biogas production yield obtained in both digesters as compared to

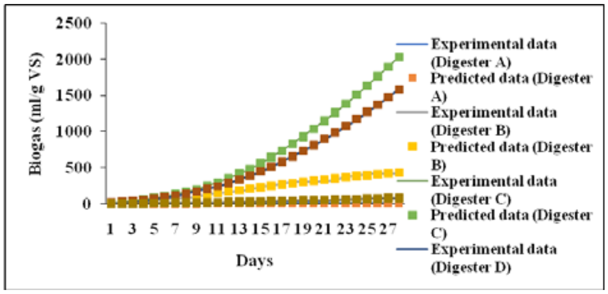

Fig. 2. Variation and fitting of the cumulative biogas data with the modified Gompertz equation for different digesters (A-E) with time

Table 3. Kinetic constants estimated using a modified Gompterz equation

Digester label	Total biogas production (Litres)	P (mLg^{-1}VS)	R_m ($mLg^{-1}d^{-1}$)	ë (d)	VSR (%)
A	48.97^{e}	12.24	8.56	8.80	0.21
B	258.98^{c}	536.44	20.36	3.74	8.96
C	312.66^{a}	6,150.50	134.00	12.80	19.94
D	295.32^{b}	5,115.67	105.47	13.02	10.30
E	195.15^{d}	322.64	4.43	9.73	3.40

the control.

In a previous study, Wang *et al.* (2022) employed the modified Gompertz model and first-order kinetic model for simulating the co-digestion of SMS and food waste under different combining ratios at thermophilic conditions. They observed the favorable and collaborative effect of co-digestion. They further reported that 69% and 15.9% higher methane was produced at a ratio of 3:7, the lag phase of 4.9 days, and k_h of 0.102 d^{-1}as compared to mono-digestion of SMS and food waste, respectively.

Conclusion

The present investigation revealed that SMS has enormous methane production potential. It can be successfully used as feedstock for anaerobic digestion alone or in co-digestion with lignocellulosic wastes like rice straw etc. This serves a dual purpose, first is proper management of SMS which otherwise causes environmental pollution. Second, the generation of biogas, a renewable bioenergy resource. The findings in the present study enable the sustainable biogas production from anaerobic co-digestion of SMS and rice straw as co-feedstock that will redress the incessant use of fossil fuels, thereby reducing the emission of greenhouse gases (GHGs) and hence alleviating the global environmental issue.

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Conflict of Interests

No conflicts of interest.

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