International Journal of Agriculture, Environment and Biotechnology
Citation: *IJAEB*: **17**(04): 731-741, December 2024
DOI: 10.30954/0974-1712.04.2024.12

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

REVIEW PAPER

Spent Mushroom Substrate- Prospects and Challenges of Agro-waste Management into Sustainable Solutions: A Review

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Paper No. 1192 **Received:** 23-08-2024 **Revised:** 11-11-2024 **Accepted:** 29-11-2024

ABSTRACT

India being an agrarian country produces huge amount of agro-wastes which can be sustainably bio converted through mushroom cultivation. It entails minimum investment for a small scale enterprise and ameliorate income of rural households of India to combat poverty. As a by-product of mushroom production, the Spent Mushroom Substrate (SMS) is generated which consists of unused fungal parts, semi mineralized lignocellulosic waste and retrievable mineral nutrients. The genesis and application of SMS comprises an integrated approach of rural bio-entrepreneurship because it can be converted into several aspects supporting circular economy. The semi fermented biomass can be re-utilized as source of compost, bio fertilizer, or as substrate for a fresh or 2nd cycle of mushroom production. Plant pathogens can be minimized by SMS derived bio pesticides. SMS can be used as an amendment of livestock and aquaculture feed thus reducing the cost of procuring commercial foods. Biofuels can be extracted from SMS and various bioremediation processes can be achieved by its Biochar. Industrially important lignocellulosic enzymes are retrieved from SMS and used in various applications minimizing agro-wastes from the field. With such versatile benefits at hand, no critical review was observed addressing the challenges and constraints associated with SMS application like standardization steps, toxicity concerns and commercial feasibility. Thus, this review article focusses to abridge various aspects of SMS utilization with the technical pros and cons to reduce and safeguard environmental consequences.

HIGHLIGHTS

- Genesis of SMS from various lignocellulosic agro-wastes.
- Potential use and application of SMS.
- Challenges and prospect of SMS utilization.

Keywords: SMS, mushroom, agro-waste, prospects, challenges

The increasing demand for food supply has climaxed in huge production of agricultural crops and its waste. Annually, the data from the year 2019-2020, states that 500 MT (million tonnes) of agricultural wastes are produced in India where wheat occupies 98.38 MT, paddy 284.95 MT, sugarcane (377.77 MT), maize (26.26 MT) and cotton (33.09 MT) (IARI, 2012). A sustainable approach is to convert these wide-range of lignocellulosic-wastes like wheat, paddy straw, sugarcane bagasse, cotton seeds and corn cobs into substrates for edible mushroom cultivation (Girotto and Piazza, 2022; Subedit *et al*. 2023). Mushrooms' unique shapes and '*umami*' taste have always fascinated human palatability and curiosity. Not only do these fungi have nutritional and medicinal versatility, but they also serve as an important purpose in ecology, agriculture and pharmacology. Fig. 1 highlights the all-round prospective of SMS.

How to cite this article: Sarkar, S., Bayen, S., Samanta, S. and Pal, D. (2024). Spent Mushroom Substrate- Prospects and Challenges of Agro-waste management into sustainable solutions: A Review. *Int. J. Ag. Env. Biotech.*, **17**(04): 731-741.

Source of Support: None; **Conflict of Interest:** None

Table 1: Utilization of different agro-wastes by the mushroom for genesis of SMS

Substrates	Production (MT)	Mushroom type	References
Paddy straw	284.95	*Pleurotus ostreatus, Volvariella volvacea, Lentinula edodes*	Singh *et al.* 2021; Akter *et al.* 2022; Gao *et al.* 2020; Samantaray and Singh 2024
Wheat straw	98.38	*Pleurotus ostreatus, Cordyceps militaris*	Akter *et al.* 2022; Bilik *et al.* 2024
Sugarcane Bagasse	377.77	*Pleurotus ostreatus, Cordyceps militaris*	Akter *et al.* 2022
Cotton waste	33.09	*Volvariella volvacea*	Saskiawan *et al.* 2023; Pandirwar *et al.* 2023
Maize	26.26	*Pleurotus ostreatus, Ganoderma annularis*	Castorina *et al.* 2023

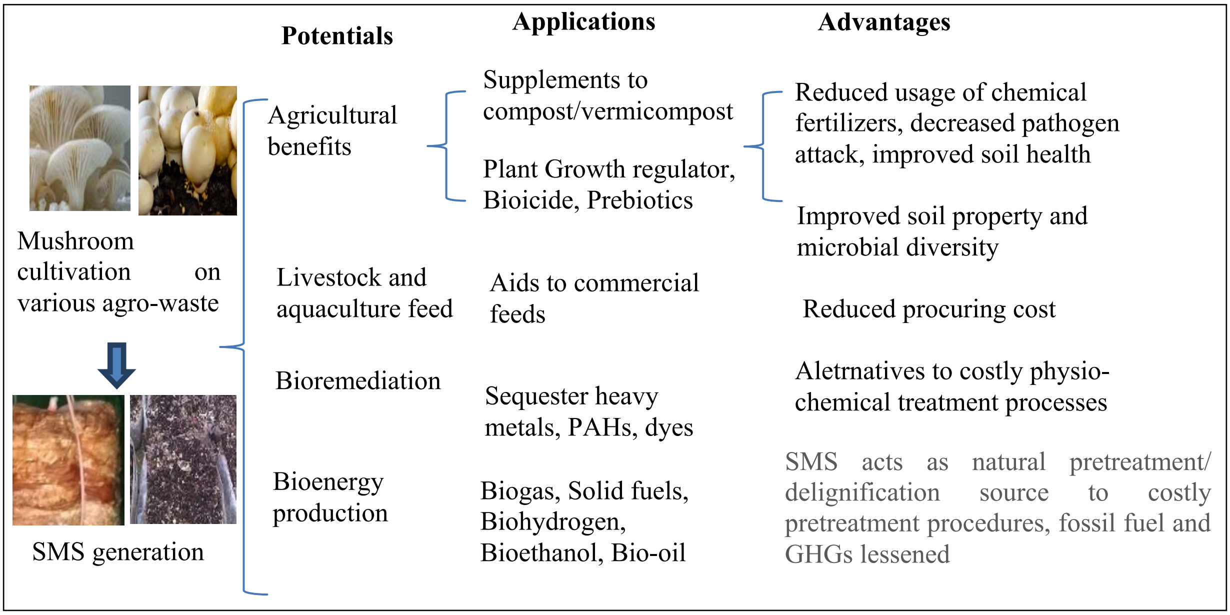

Fig. 1: Broad spectrum uses of SMS

Commonly cultivated edible mushroom species are *Agaricus*, *Pleurotus*, *Volvariella*, *Calocybe* and *Lentinula edodes* which are compatible for growing in varied agro-climatic conditions of Indian subcontinent. The preferred substrates for mushroom cultivation are paddy and wheat straws in India and accounts for 998 million tonnes of agro-wastes generated annually (Raman *et al.* 2021). Table 1 represents the varied quantity of agro-waste produced and utilization capacity of the fungus.

Mushrooms don't just stop being beneficial after their harvest; the "Spent Mushroom Substrate" (SMS) offers an auxiliary journey towards realm of sustainability, environmental awareness and circular economy (Beckers *et al.* 2019; Antunes *et al.* 2020; Wan Mahari *et al.* 2020; Zied *et al.* 2020; Alhawari *et al.* 2021; Shirur *et al.* 2021; Leong *et al.* 2022; Martin *et al.* 2023; Vasilakis *et al.* 2023; Kousar *et al.* 2024). Apparently this trivial by-product has enormous potential in diverse areas like agriculture, biofertilizers, animal feed, biocontrol agents, bioremediation, biosorbents, biomaterials and renewable energy source. The genesis of SMS takes place on diverse agricultural waste and as the cultivated mushroom metabolize the substrate's nutrients, they leave behind 75-80% of retrieval minerals like nitrogen, phosphorus, sulphur, potassium, magnesium and enzymes (Liu *et al.* 2018; Hernandez *et al.* 2021; Umor *et al.* 2021). The substrates are rich in organic biomass with varied proportion of cellulose, hemicellulose and lignin. To maintain sustainable method of waste disposal abiding environmental regulations, valorising SMS into multiple biologics and encouraging integrated SMS-agro systems is the economical and viable solution. In this study, the prospective utilization of

SMS generation

SMS in India with challenges as sustainable solution of agricultural waste management are discussed.

The Potential of SMS

1. Agricultural benefits

The composition of SMS makes it a valuable fertilizer for crops due of its high nutritional content, high cation exchange capacity and low mineralization rate, all of which preserves its value as an organic matter. The bulk and light weight of SMS belie its high water content (45%) but the composted spent substrate acts as a good growing medium for agricultural crops, vegetables and orchards, with proven multiple benefits in enhancing crop yield, quality and in controlling plant diseases (Becker *et al*. 2019; Kumla *et al*. 2020; Širić *et al*. 2022). Recomposting of SMS is very much necessary before it is used as soil amendment as this process enhances nutrient and water holding capacity of SMS, induces microbial growth, and improves physical and chemical texture of soil (Rajavat *et al*. 2019; Velusami *et al*. 2021). Residues of fungicide, insecticide and heavy metals are reduced upon recomposting and can be successfully used as biomanure or biofertilizer after supplementation with deficient minerals (Carrasco *et al*. 2018; Martín *et al*. 2023). This can abate the cost of buying chemical fertilizers and minimizes the risk of pest infestation on agricultural and horticultural crops. Numerous reports on agricultural applications are reported from successful uses of SMS as compost which has equivalent mineral compositions as commercial fertilizers. Ageing of SMS improves its stability and bioavailability, makes it more porous, surges bulk density, air permeability which directly impacts soil health and crop productivity (Othman *et al*. 2020; Velusami *et al*. 2021; Leong *et al*. 2022). Additionally, de-lignified SMS is enriched with various hydrolytic and oxidative enzymes which can be reutilized for second round of mushroom cultivation (Kumla *et al*. 2020). Table 2 highlights the major agricultural inputs of SMS.

Table 2: Current pertinent research highlighting SMS utility in agriculture

SMS	Co-composting materials	Crops benefitted	Effect on soil flora, soil health and microbial diversity	References
SMS	Rice soil (with 20%, 50%, and 80% SMS)	Rice seedling growth	Abundant diversity of *Bacillus*, *Bacteroidetes* and *Ascomycota* group. Increase in superoxide dismutase in seeds	He *et al*. 2024
Enoki mushroom, *A.bisporus*, *Auricularia auricula*	Replaced 25 % chemical fertilizers (Nitrogen)	Farmland crops	Abundance of Proteobacteria, Actinobacteria, Mortierellomycota in nutrients rich soil.	Chen *et al*. 2022
Wheat straw of *Hypsizygus marmoreus*, *Pholiota nameko* and *Hericium erinaceusin*	Wheat bran and gypsum	*P. ostreatus* mushroom	Improved growth performance of the fungus by 66–73%	Lisiecka *et al.* 2021
P. ostreatus	$CaCO_3$ and wheat bran	*P. ostreatus* mushroom	Improved biological efficiency of the fungus by 53–56%	Zakil *et al*. 2022
Liquid organic fertilizer from SMS	Anoxic conditions	Pak-choi	Rhizosphere soil was improved	Huang *et al.* 2022
SMS	—	Lettuce	Minerals found were equal to commercial chemical fertilizers	Hernández *et al*. 2021
SMS	Amendments (peat and vermiculite) mixed with *Bacillus amyloliquefaciens*	(*Hibiscus sabdariffa*)	Improved plant growth and chlorophyll. Enhanced soil enzymes and minerals	Ngan and Riddech, 2021
A. bisporus and *P. ostreatus*	Peat	Baby Leaf Lettuce	Better yield under induced pressure of *Pythium irregulare* (plant pathogen)	Hernández *et al*. 2021
SMS	—	*Spinacia oleracea* (spinach)	Increased produce and quality	Muchena *et al.* 2021
SMS	Biogas residues, pig manure	Tomato seed germination	Better quality of tomato seedlings compared to commercial seeds	Wang *et al*. 2024
SMS Biochar	*P. ostreatus*	Better mushroom yield and quicker fruiting bodies	Increased surface area, retains substrate, moisture and prevents nutrient leaching	Hu *et al*. 2022

2. Biochemical and Medicinal Applications

According to the available literary evidences, SMS possess bioactive components that display antioxidant and antibacterial effects applicable into a range of biomedical settings, from wound healing to nutraceutical products. SMS harbours an active pool of industrially important ligninolytic enzymes like cellulase, laccase, α-amylases, manganese peroxidase, β-glucosidase and xylanases due to the microbial activity on lignocellulosic substrates (Rajavat *et al*. 2019; Martin *et al*. 2023; Sabaratnam *et al*. 2024). The retrieved enzyme concentration is influenced by the stages of mushroom growth, the number of flushes passed by the fungi and the type of substrates used for cultivation (Lisiecka *et al*. 2021). The growing demand for prebiotics has compelled exploration of fungal SMS for production of a prebiotic substrate, xylooligosaccharides (XOS), one of the components of hemicellulose degradation.

3. Animal Feed

The discarded mushroom substrate can be recycled into a highly valued and nutritious animal feed with the appropriate processing and blending techniques. Relevant report shows increased resistance towards pathogenic bacteria and improved innate immunity as the stalk waste contains high amount of vitamins, minerals, polysaccharides like cordycepin and adenosepin. For example, in Catfish (*Clarias gariepinus*), fish growth, digesting ability and survivality showed 16% more improvement than commercial fish feed. Further observations of Qi *et al*. (2020) suggests use of fermented mushroom substrate with help from *Bacillus* sp., as diet supplemented to weaned pigs. Mushroom stalk waste acts as prebiotic in aquaculture feeds and acts as promising alternative source to antibiotics and its toxic effects (Kannan *et al*. 2022). Inclusion of *Cordyceps militaris* SMS in the diet of weaning pigs improved the IgA concentration and growth performance of pig with simultaneous decrease in effect of pathogenic bacteria, blood cholesterol, interleukin-1 and tumor necrosis factor-α. Parallel observations were seen in broiler chickens and Nile tilapia respectively where considerable results were obtained in their growth (Martín *et al*. 2023). The major factors are thought to be components like bacteriocin and enterocin produced from spent substrate, responsible for favouring the growth of beneficial bacteria over pathogenic microbes. Even the enteric emission of methane, a greenhouse gas was reduced in *Holstein steers* when they were fed with SMS-based silage of *Flammulina velutipes* mushroom. The SMS-silage modifies rumen fermentation through the presence of bioactive compounds like phenolic and lignocellulolytic enzymes which increases the digestibility power of the ruminants if supplemented with the agro feed. Rice straw fermented with spent substrate of *Pleurotus sajor-caju* was used as dietary feed for Alpine dairy goats which exhibited improved milking performance (Fan *et al*. 2022).

4. Biofuel production

To meet the burgeoning demand for sustainable energy sources, the carbohydrate remains of partially digested SMS is exploited for biofuel production. Biofuels like Biogas, Biomethane, Bioethanol and Biohydrogen comparable to International Standard of Solid Biofuels can be generated form SMS alone or by mixing with other wastes (Alvesa *et al*. 2021; Kumar *et al*. 2021; Shu *et al*. 2022; Leong *et al*. 2022; Gu *et al*. 2023; Devi *et al*. 2023; Martin *et al*. 2023; Kousar *et al*. 2024; Sabaratnam *et al*. 2024). Co-digestion of SMS in different proportions with livestock manure (Gao *et al*. 2021), corn stover (Du *et al*. 2021), palm oil effluent (Mamimin *et al*. 2021), sugar mill wastewater (Kumar *et al*. 2021), food waste (Wang *et al*. 2022; Zhang *et al*. 2022; Gu *et al*. 2023) generates high value biofuel than untreated agro-waste. Even high grade biofuel was obtained when SMS from *L. edodes*, *Pleurotus ostreatus*, *Agaricus subrufescens* was supplemented with casing material like peat, saw dust and achiote capsule (Chen *et al*. 2021; Gu *et al*. 2023). Even, the anaerobic sludge generated after Biogas formation was used as Biofertilizers for crops (Najafi *et al*. 2019; Naeem *et al*. 2020; Moreira *et al*. 2020; Baharin *et al*. 2022). Low-emission fuel pellets like Bio-Coke was also achieved from SMS for pasteurization of mushroom substrate (Kamal Baharin *et al*. 2020). The sturdiness to remain intact when charred at high temperature reflects the potential of SMS to be used as biofuel-biocoke. The partially digested lignocellulosic matrix offers worthy biogas fermentation efficiency, reduced lignin and hemicellulose content, large surface area for anaerobic digestion (Mamimin *et al*. 2021; Gao *et al*. 2021; Leong *et al*. 2022; Vasilakis *et al*. 2023). Considering the huge amount of SMS generated, mushroom cultivation and biogas production can be integrated and valorization of these agro residues can be an integral solution of waste management.

5. Waste Management

Bioremediation of several recalcitrant environmental pollutants starting from organic xenobiotics to heavy metal can be adsorbed and removed by SMS or biochar converted SMS (Kulshreshtha, 2019; Wu *et al*. 2019; Chang *et al*. 2021; Ghose and Mitra, 2022; Grim *et al*. 2022; Sathiya *et al*. 2022; Schallemberger *et al*. 2023; Martin *et al*. 2023). Biochar has large bioavailable surface area and wide functional groups by which they bind to organic and inorganic pollutants like heavy metals by adsorption, chemical precipitation, complexation and reduction pathways (Wei *et al*. 2020; Chang *et al*. 2021; Menaga *et al*. 2021). Wide research studies regarding the bioremediation potential of SMS are evident from. Similarly, textile dye degradation was also achieved by the lignocellulosic enzymes of SMS like endocellulases, exocellulases, β-glucosidase, laccase, amylase and dextranases recovered from SMS of different mushroom. Emerging pollutants like UV creams which are difficult to remove conventional treatment processes, are successfully bio remediated by SMS of *Pleurotus djamor* in a bioreactor based design (Yang *et al*. 2021). Even various hydrocarbons, antibiotics and pesticides like linuron, diazinon myclobutanil and metsulfuron methyl were degraded and expunged by spent substrate of *Pleurotus* and *Agaricus blazei* (Antón-Herrero *et al*. 2022). Phytoremediation by guinea grass root amended by *Pleurotus ostreatus* SMS to clean up black oil contaminated soil was also achieved successfully. In another phytoremediation endeavour of cleaning lead-zinc mines, *Macleaya cordata* amended with SMS (*Lentinus edodes*) and $CaCO_3$ exhibited enhancement of heavy metals phytoabsorption (Cai *et al*. 2021). Even heavy metals like Cr, Cd, Pb, Cu and Zn from sewage sludge could be mitigated by using spent mushroom compost from *Pleurotus sajor-caju* along with vermicompost. Based on the performance capability of SMS to remediate ecosystem from recalcitrant xenobiotic it can be concluded that the unused agro waste holds huge potential in bioremediation.

Challenges and Future Directions

Mushroom cultivation compounded with Biotechnological processes ensure reutilization of various agro-waste minimizing waste disposal problem. But there are certain obstacles like proper standardization process, toxicity and economic evaluation which must be overcome for its sustainable use.

1. Standardization

SMS utilization in various sectors is in its nascent stage and hence no standardized protocol exists. Agricultural waste like paddy, bagasse, areca and saw dust has high lignin content preferred by *Pleurotus* sp. whereas *Agaricus* sp., prefer high cellulose, hemicellulose supplemented with manure (Grimm *et al*. 2024; Zied *et al*. 2020). Zied *et al*. 2020 designed some SMS maturation standardized steps like phase I and phase II to obtain ideally good compost. Phase I step necessitates homogenous mixing of SMS with added amendments, good aeration and moisture content. Phase II needs ideal pasteurization temperature of 57–60 °C for 8–12 hrs and acclimatizing condition for 3 to 5 days at 45–48 °C. Again depending on the substrate type, storing conditions and nutrient deficiency in SMS, different formulations and incorporation stages has been suggested by different researchers (Zied *et al*. 2020). For example, Koutrotsios *et al*. 2018 emphasized on elemental fingerprinting of SMS prior to its use, as presence of rare earth elements influences the secretion of fungal lignocellulosic enzymes. Pre-treatment processes like acid and alkali treatment is chosen based on the recalcitrant components of SMS like Lignin (Beig *et al*. 2021). Beckers *et al*. 2019, performed a protocol of separating carbohydrate fraction from lignin using thermochemical pre-treatment procedures at 192 °C and organosolvents (10 wt % of ethanol–water mixtures) to attain soluble lignin fractions. In many cases, combinatorial use of acid and alkali are suggested for complete sugar reduction with no generation of inhibitors. Although constraints are observed when certain pre-treatment procedures yielded unsuitable substrates for SMS reutilization. Comparatively alkaline treatment with sodium hydroxide or ammonia improved the pre-treatment conditions (Beckers *et al*. 2019).

For Biofuel production from SMS, the pyrolysis temperature is the deciding factor, otherwise direct combustion at 800–1600 °C, generates low grade fuels with emission of greenhouse gasses (Deng *et al*. 2020; Atallah *et al*. 2021; Leong *et al*. 2022). Additionally, high Alkali Index of SMS leads slagging and fouling during burning of SMS biomass (Jasiunas *et al*. 2017). Several literature sources cited evidences of physical treatment process like thermal, hydrothermal, microwave, and grinding process for increasing degradation efficacy of SMS but the high cost and labour intensive course belies the treatment procedures. The purpose of physical treatment endorses increasing surface-volume area, partially degrade crystalline cellulose fraction and increase accessibility of SMS to anaerobic digestion. Chemical (acid or alkaline hydrolysis), or biological (microbial or enzymatic) treatment are further suggested of recalcitrant SMS which operates at cheap cost (Prasad *et al*. 2020; Meneses *et al*. 2022; Leong *et al*. 2022). Biogas production is another alternative but challenges like high C/N ratio, moisture content in SMS, as well as refractory nature of lignin are the faced obstacles (Moreira *et al*. 2020; Leong *et al*. 2022). During the fermentation process by the fungi, SMS accumulates decent amount of nitrogen in their matrix which increases the C/N ratio and inhibits fermentation (Leong *et al*. 2022). Moreover the initial reaction of fermentation leads to acidogenesis components which generates volatile fatty acids and hampers the efficiency of biogas production (Gao *et al*. 2021).

Keeping in mind the sustainable trend of waste minimization and low emission of Green House Gases (GHGs), SMS offers 2nd generation biofuels due to the partially digested lignocellulosic biomass otherwise raw agri-substrate requires costly physical or chemical treatment for use as biofuel. Though mushroom cultivation decreases the recalcitrance of crystalline cellulose and lignin content and makes the SMS more reachable to anaerobic microbes, bioaugmentation by several other anaerobic species like white-rot fungi needs to be explore and standardized further for biofuel production.

2. Toxicity Concerns

When considering utilizing the high organic component of SMS as fresh substrate, manure or animal feed, it is pertinent that SMS should be free from pathogenic microbes, recalcitrant wastes and toxins. SMS retain large surface area with well-developed microporous structure as well as plenty reactive functional groups like hydroxyl, phenolic or phosphoryl, that serve substantial role in metal-ion binding. While using digestate of pig slurry or chicken manure, close inspection of SMS for occurrence of faecal bacterial pathogens like *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* should be checked (Okechi *et al*. 2023). Even the presence of pathogenic bacteria like *Pseudomonas syringae* and fungal species like *Trichoderma*, present in SMS component should be verified by toxicity test to ascertain formation of harmful intermediates by metabolic action of microbes prior to use. Also weathering of SMS to rainfall and varied temperature is necessary to stabilize it before it can be reutilized to ensure removal of extra salt and other harmful substances. Kucaj *et al*. (2019) suggested phytotoxicity test of soil to ensure presence of various chemical compounds by planting the indicator plant, *Lepidium sativum*. Excessive use of SMS in soil can produce organic component rich SMS leachate leading to water pollution by dissolving the soil organic matter. Instead a sustainable solution like converting the SMS to biochar could reduce the leaching process and hold on nutrient uptake in the soil.

3. Economic Viability

Challenge is to convert SMS into viable option to reduce incurring cost of fresh mushroom growth, chemical fertilizers and livestock feed without hampering the agronomical parameters and ecosystem pollution. If recycled SMS incurs loss to mushroom farm or hampers livestock then implementation becomes unsuitable. Mushroom cultivation biologically pre degrades the lignocellulosic biomass, thus saving the cost of pre-treatment processes for biofuel production (P´erez-Ch´avez *et al*. 2019). According to several authors, approximately 62.3 billion m^3 of biogas is produced globally whereas different experimentations revealed SMS of various substrate sources generated the same fuel as 8.920, 282, 249.7, 11.9 and 314.1 L biogas/kg vS respectively (Richard *et al*. 2020; Leong *et al*. 2022; Kumar *et al*. 2022, Najafi *et al*. 2019). N, P and K are the essential macro elements for agricultural crops and in order to obtain incentive on its commercialization, the cost-effectiveness of SMS needs to be estimated. Dependency on chemical fertilizers could be minimized by employing SMS derived biofertilizer for crop improvement. Thus usage of SMS will reduce the cost of procuring chemical fertilizers and offers an excellent alternative in the circular economy concept.

4. Public Awareness

Dissemination of the knowledge of potential benefits of using SMS as a sustainable solution to agro waste dumping can be achieved profusely by spreading awareness among local people. The usage gap can only be bridged by conducting awareness campaigns, educational programs, workshops and participation in mushroom training programs to instil in them a better understanding of the business profitability. It is essential to promote the advantages of SMS due to its various capabilities like to improve revenue via the integration of holistic utilization and cost reduction in animal or livestock feed. SMS offers an environmentally conscious approach to handle organic waste. Thus, instead of being disposed into landfills or burnt openly, creating greenhouse gases, SMS may be valorised for many purposes to create value added products.

CONCLUSION

Accumulation of agro-wastes and incumbent pollution has triggered the scientific community to harnesses bioeconomy prospects from the discarded waste. An integrated approach of circular economy by channelizing this waste to different value-added products is ongoing. In addition, encouraging mushroom consumption or various value added products as easy protein source can reduce nutritional deficiency and food insecurity of Indian villages. Moreover integrating mushroom production with SMS circular economy can help Indian farmers' dependence on synthetic chemical fertilizers and contribute resilience to their income. Retrieving value added compounds from the SMS can provide sustainable solution to various environmental challenges imposing minimum risk on ecosystem health. Thus SMS re-utilization as an integrated approach with mushroom cultivation should be harnessed more aggressively to derive the benefits of its potential.

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