COMPOSTING AND VERMICOMPOSTING OF SPENT MUSHROOM SUBSTRATE FOR ORGANIC FERTILISER AND CULTIVATION OF *Schizophyllum commune*

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[COMPOSTING AND VERMICOMPOSTING OF SPENT MUSHROOM SUBSTRATE FOR ORGANIC FERTILISER AND CULTIVATION OF *Schizophyllum commune*]

ABSTRACT

An increase in spent mushroom substrate (SMS) disposal as a result of continuous mushroom cultivation will give negative impacts towards the environment. The problem has attracted the attention of many researchers to explore the potential of SMS that still contains high beneficial nutrients after mushrooms cultivation. Reutilizing SMS not only help to reduce the environmental problems but will also benefit farmers economically. *Schizophyllum commune* is popularly consumed in Malaysia due to its delicious taste and medicinal benefits. The cultivation of *S. commune* has increased due to the increase in demand and the short turnover in its cultivation of one month. As a result, large amounts of SMS needs to be disposed. Hence, in this study, the potential of recycling spent *S. commune* substrate via composting and vermicomposting was investigated. SMS of *S. commune* was mixed with goat manure (GM) using three different formulations, 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. The mixtures undertake composting and vermicomposting for 90 days. Samples for analysis of carbon:nitrogen (C/N) ratio and nutrient content (NPK) was taken on day 30, day 60 and day 90 of composting/vermicomposting. The compost and vermicompost were then tested as a fruiting substrate for *S. commune* cultivation by using different substrate formulations. This study revealed that the compost and vermicompost produced after 90 days of decomposition has a low C/N ratio. Among all the compost and vermicompost, compost formulation C (50 SMS:50 GM), compost formulation B (60 SMS:40 GM) and vermicompost formulation C (50 SMS:50 GM) are suitable to be used as organic fertiliser with C/N ratios of CC (23:34), CB (22.66) and VC (21.7). They also have high nitrogen, phosphorus and potassium (NPK) content, CC (N=1.61%, P=0.73%, K=0.81%), CB

(N=1.54%, P=0.74%, K=0.63%) and VC (N=1.51%, P=0.81%, K=0.97%). Whereas, for *S. commune* cultivation, compost CC supplemented with 80% sawdust and 10% rice bran resulted in potentially high mushroom yield of, 80.25 g/bag with biological efficiency, 20.06%. The study also revealed that SMS of *S. commune* had C/N ratio, 66.92 which is almost similar to the C/N ratio of *S. commune* standard formulation, 61.69. Last but not least, the mushroom bags using compost and vermicompost in *S. commune* substrate formulation have no contamination throughout the cultivation period.

Keywords: Carbon:nitrogen ratio; compost; fruiting substrate; mushroom yield; biological efficiency.

[PROSES KOMPOS DAN VERMIKOMPOS BAKI SUBSTRAT CENDAWAN UNTUK BAJA ORGANIK DAN PENANAMAN *Schizophyllum commune*]

ABSTRAK

Peningkatan pembuangan baki substrat cendawan (SMS) akibat daripada penanaman cendawan secara berterusan akan memberi kesan yang negatif terhadap alam sekitar. Hal ini telah menarik perhatian ramai penyelidik untuk meneroka potensi SMS yang masih mengandungi nutrien yang tinggi selepas penanaman cendawan. Penggunaan semula SMS bukan sahaja membantu mengurangkan masalah alam sekitar bahkan turut memberi faedah kepada para petani dalam aspek ekonomi. *Schizophyllum commune* atau lebih dikenali sebagai cendawan kukur dimakan secara meluas di Malaysia kerana rasanya yang sedap dan manfaat perubatannya. Penanaman cendawan kukur semakin meningkat berikutan peningkatan permintaan dan perolehan pendek dalam penanaman sebulan. Akibatnya, sejumlah besar SMS memerlukan pelupusan. Oleh itu, dalam kajian ini, potensi kitar semula SMS cendawan kukur melalui proses penguraian secara kompos dan vermikompos dikaji. SMS cendawan kukur dicampur dengan tahi kambing (GM) menggunakan tiga formulasi yang berbeza iaitu 80 SMS:20 GM, 60 SMS:40 GM dan 50 SMS:50 GM. Campuran tersebut digunakan dalam proses penghasilan kompos dan vermikompos selama 90 hari. Sampel untuk analisis nisbah karbon:nitrogen (C/N) dan kandungan nutrien (NPK) diambil pada hari 30, 60 dan 90 proses penguraian. Kompos dan vermikompos kemudiannya diuji sebagai substrat penanaman cendawan kukur dengan menggunakan formulasi substrat yang berbeza. Hasil kajian mendapati bahawa kompos dan vermikompos yang dihasilkan selepas 90 hari proses penguraian mempunyai nisbah C/N yang rendah. Antara semua kompos dan vermikompos yang dihasilkan, kompos formulasi C (50 SMS:50 GM), kompos formulasi B (60 SMS:40 GM) dan vermikompos formulasi C (50 SMS:50 GM) sesuai digunakan sebagai baja organik dengan nisbah C/N bagi CC (23.34), CB (22.66) dan VC (21.7). Kompos dan

vermikompos tersebut juga mempunyai kandungan nitrogen, fosforus dan kalium (NPK) yang tinggi iaitu CC (N=1.61%, P=0.73%, K=0.81%), CB (N=1.54%, P=0.74%, K=0.63%) dan VC (N=1.51%, P=0.81%, K=0.97%). Manakala, untuk penanaman cendawan kukur pula, kompos CC dicampur dengan 80% habuk kayu dan 10% dedak padi berpotensi memberikan hasil cendawan yang tinggi, 80.25 g/beg dengan kecekapan biologi, 20.06%. Kajian ini juga mendedahkan bahawa SMS cendawan kukur mempunyai nisbah C/N, 66.92 yang hampir sama dengan nisbah C/N substrat komersial cendawan kukur iaitu 61.69. Akhir sekali, beg cendawan kukur yang menggunakan kompos dan vermikompos dalam formulasi substrat tidak dicemari sepanjang tempoh penanaman.

Kata kunci: Nisbah karbon:nitrogen; kompos; substrat penanaman; hasil cendawan; kecekapan biologi.

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Nolisa binti Madrim

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LIST OF SYMBOLS

C	:	Carbon
CO_2	:	Carbon dioxide
cm	:	Centimeter
°C	:	Degree centigrade
g	:	Gram
>	:	Greater than
K	:	Potassium
kg	:	Kilogram
m	:	Meter
N	:	Nitrogen
%	:	Percentage
P	:	Phosphorus
±	:	Plus-minus

LIST OF ABBREVIATIONS

BE	:	Biological efficiency
C/N ratio	:	Carbon-to-nitrogen ratio
CA	:	Compost formulation A (80 SMS:20 GM)
CB	:	Compost formulation B (60 SMS:40 GM)
CC	:	Compost formulation C (50 SMS:50 GM)
CNPK	:	Carbon, nitrogen, phosphorus, potassium
GM	:	Goat manure
MGR	:	Mycelium growth rate
MY	:	Mushroom yield
NPK	:	Nitrogen, phosphorus, potassium
PPBGL	:	Biotechnology Research Centre Glami Lemi University of Malaya
RB	:	Rice bran
SD	:	Sawdust
SMC	:	Spent mushroom compost
SMS	:	Spent mushroom substrate
VA	:	Vermicompost formulation A (80 SMS:20 GM)
VB	:	Vermicompost formulation B (60 SMS:40 GM)
VC	:	vermicompost formulation C (50 SMS:50 GM)

CHAPTER 1: INTRODUCTION

This chapter consist of research background, problem statement, research question, research objectives, scope of work and contribution of study.

1.1 Research Background

Mushroom cultivation is a biotechnological approach to produce food and has become the biggest solid state fermentation industry in the world (Soccol & Vandenberghe, 2003). Many types of mushroom have been cultivated commercially mainly for food and medicinal purpose such as *Agaricus* spp., *Lentinula* spp. and *Pleurotus* spp. (Sánchez, 2010; Herawati *et al*., 2016). Mushroom is widely eaten for health and its delicious taste (Azizi *et al*., 2011). Mushroom contains nutrients like protein, vitamins, lipids, mineral elements and bioactive compounds that can prevent and treat some diseases (Adejoye *et al*., 2007; Sánchez, 2010; Azizi *et al*., 2011). Furthermore, mushroom has high fiber, low fat and low calories that makes it beneficial for human diet (Preecha *et al*., 2015). Moreover, some mushroom produce polysaccharide that has medicinal properties including antioxidant, anticancer, antimicrobial and inducing immune substance for example Lentinan in *Lentinula edodes* and Schizophyllan in *Schizophyllum commune* (Preecha *et al*., 2015).

Schizophyllum commune is a cosmopolitan mushroom having widespread distribution (Imtiaj *et al*., 2008; Arboleda Valencia *et al*., 2011). It is an edible mushroom and known for its medicinal properties such as immunomodulating and anticancer (Figlas *et al*., 2014). This mushroom is also known as split gill which belongs to the division Basidiomycota and class Agaricomycetes. It is consumed in some countries like Nigeria, Malaysia, Indonesia, Thailand, Vietnam, Taiwan and Southern China (Adejoye *et al*., 2007; Imtiaj *et al*., 2008; Herawati *et al*., 2016). Nowadays, the demand for *S. commune* increases due to its ability to produce extracellular polysaccharide known as

schizophyllan that possess various pharmacological and biological activities (Ivanova *et al*., 2014). This exopolysaccharide is a beta-glucan that has multipurpose in various industrial area such as food engineering, biodegradable plastics, agronomy, pharmaceuticals, fuels and others (Joshi *et al*., 2013). Beta-glucan helps boost immune system in human body hence many people consume it as a supplement because the body does not produce beta-glucan naturally (Carbonero *et al*., 2012). In addition, beta-glucan is also used as thickening agents, stabilizer and additive in food product such as salad dressings, frozen desserts, sour cream, and cheese spreads (Teoh *et al*., 2011).

Composting is one of the simplest and oldest techniques in biodegradable of organic wastes decomposition to produce stabilized and sanitized compost which can be used as an amendment for soil health and plant growth (Zhang & Sun, 2014). It usually runs under aerobic condition where oxygen supply is needed. The composting technique has been applied numerously for so long to generate useful end products such as fertilisers, substrates for mushroom cultivation and bio-gas (methane) (Sarkar *et al*., 2016). Composting process has thermophilic phase to kill weeds and destroy the pathogens. Hence, the end product or compost is safe to be applied into the soil and agriculture land. Other than material sanitization, composting is able to break down a large quantity of organic wastes simultaneously in a pile. In addition, various organic wastes can be combined together to produce high quality compost and run under industrial scale (Zhang & Sun, 2014).

When the composting process is added with earthworms, it is called vermicomposting. Vermicomposting is well known for bioremediation to remove heavy metals and produces high quality finer organic fertiliser without any concern of heavy metals content (Azizi *et al*., 2011, 2013, 2014, 2015). It is also enriched with beneficial bacterial and fungal species (Huang *et al*., 2013). Other benefit of vermicomposting is, the final product has better appearance, higher nutrients content and microbial activity making

vermicompost gaining acceptance in the market. The earthworms perform physical and biochemical degradation simultaneously in vermicomposting where the earthworms modify the physical properties through mixing and grinding the organic matters directly. At the same time, the microflora in their intestine decompose the organic wastes and transform them into more stable product (Ndegwa & Thompson, 2000; Loh *et al*., 2005; Fornes *et al*., 2012).

1.2 Problem Statement

The increased imposition of mushroom especially *Schizophyllum commune* throughout the world has led to the large scale of mushroom cultivation hence produce a lot of mushroom waste such as spent mushroom substrate (SMS). SMS which is discarded after harvesting is an organic residue that looks like soil and rich in nutrients. In a mushroom farm in Biotechnology Research Centre Glami Lemi University Malaya (PPBGL) in Jelebu, Negeri Sembilan, ~600 g of *S. commune* SMS generated per bag and over 2,000 bags of the SMS were disposed every week. New farms producing *S. commune* are being developed due to its increasing popularity and demand. Meanwhile, mushroom farmers producing oyster mushrooms in Malaysia discard more than 4,000 tonnes of SMS each month where it usually disposed either via burning or landfilling (Azizi *et al*., 2011, 2013, 2014). The burning process of SMS will produce greenhouse gases which caused greenhouse effect and global warming while SMS landfilling will occupy the land that can be used for other purpose such as farming and housing (Zhang & Sun, 2014).

SMS usually has high C/N ratio, for example, C/N ratio of spent *Pleurotus pulmonarius* substrate is 68 while spent *Hypsizigus marmoreus* substrate is 44.8 (González Matute *et al*., 2011; Wang *et al*., 2015). Landfilling of SMS with high C/N ratio will disturb soil fertility and its microbes because high carbon content in the SMS forced the microbes to use the soil nitrogen. As a result, nitrogen immobilization that

harmful to the microbes occurs (USDA, 2011). SMS also contaminates the soil and ground water around the disposal area due to its nitrogen and phosphorus content. Besides, nitrate and phosphorus can cause eutrophication if leaked into the water source (Kapu *et al*., 2012).

SMS discarded produces a disturbing foul smell due to large piles of SMS tends to undergo an anaerobic decomposition. Moreover, the disposal area may lose their economic value as well as slowing the development progress around it. Hence, SMS reutilization should be enhanced to prevent harmful effect to the environment and economy. However, effective strategy and consistency are needed to recycle SMS so that it becomes useful and safe because one of the major problems in SMS management nowadays is lack of the sustainable efficient management (Jamaludin *et al*., 2012; Phan & Sabaratnam, 2012; Azizi *et al*., 2011, 2013).

SMS can be reutilized in many field including agriculture land and waste management. The approach taken is to address pollution problems and minimize its effect towards the environment. Among all the reutilization, SMS numerously applied in organic fertiliser production because it contains abundant of nutrients and organic matters including nitrogen, phosphorus and potassium (NPK) that add a great value to the soil and crop production (Ntougias *et al*., 2004; Zhang & Sun, 2014). Composting and vermicomposting are two of the widely used process to produce organic fertiliser (Fornes *et al*., 2012). Both processes are the most effective eco-biotechnological approach in waste management because they are low cost and transform the organic wastes into valuable product (Lim *et al*., 2016).

SMS also has potential to be used as fruiting substrate for mushrooms because SMS is still high in nutrients content such as polysaccharide, lignocellulose, protein and NPK which beneficial in the mushroom cultivation (Lou *et al*., 2015). Reused SMS in the cultivation will reduce the cost of mushroom production where mushroom farmers

usually buy their substrate ingredients from manufactures. In addition, reutilizing SMS helps them to manage their mushroom waste hence increase their profit and prevent the problem of the waste.

Other than mushroom cultivation, SMS potential has also been tested for other fungi cultivation such as mold. SMS that is rich in cellulose and hemicellulose was reused as a substrate for *Trichoderma* spp. and *Aspergillus niger* to produce industrially important hydrolytic enzymes namely cellulase, xylanase, amylase and ß-glucosidase (Grujić *et al*., 2015). Therefore, this research is crucial to propose a new solution to overcome the problems of SMS disposal by reutilizing it in the form organic fertiliser and fruiting substrate for mushroom (*S. commune*) in solid state fermentation.

1.3 Research Question

Can spent *S. commune* substrate be composted and reutilized as a mushroom fruiting substrate or organic fertiliser?

1.4 Research Objectives

The specific objectives of this research were:

- To determine the ratio of formulation of SMS for composting and vermicomposting.
- To optimize *S. commune* fruiting substrate formulations using composted SMS.
- To analyse C/N ratio and NPK content of compost as potential organic fertiliser.

1.5 Scope of Work

The research is focusing on using spent mushroom substrate (SMS) rather than spent mushroom compost (SMC) where SMS is the mushroom cultivation residue discarded just after mushrooms harvested. Spent *S. commune* substrate disposed from a mushroom farm in Biotechnology Research Centre Glami Lemi University of Malaya (PPBGL) was selected for study.

We narrow the scope of the study where we emphasize on the quality of the final product of compost and vemicompost produced from SMS of *S. commune* as the main feedstock in this work and mixed with goat manure (GM) that is also available in PPBGL. We only use three mixture formulation in composting and vermicomposting viz 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. The parameters that will be optimized during decomposition are temperature, pH and moisture. We limit the composting and vermicomposting process for only 90 days.

The compost and vermicompost were then tested as fruiting substrate for mushroom cultivation. In this case, we explore its potential as *S. commune* fruiting substrate by using several formulations including supplementation of nitrogen source in the form of rice bran (RB) and carbon source in the form of sawdust (SD). Lastly, we analyze carbon, nitrogen, phosphorus and potassium (CNPK) of the optimized formulation after *S. commune* cultivation to figure out whether its SMS is acceptable as an organic fertiliser so that it can be discard directly or reutilized in agriculture land without any treatment.

1.6 Contribution of Study

This study has opened a new chapter in spent mushroom substrate (SMS) disposal solution. The finding of this project will contribute to the new knowledge of composting and vermicomposting of *S. commune* SMS that will reduce the problem of waste disposal by the mushroom industry as well as produce the useful end products. Furthermore, the

low cost materials was used in composting and vermicomposting, making it affordable to be applied.

The method of recycling *S. commune* SMS via composting and vermicomposting to reutilized as a substrate for mushroom cultivation will contribute to the various application of the compost and vermicompost. Hence, the mushroom growers can easily carry out the procedure to recycle their mushroom waste that can generate extra income.

This project will contribute to the new knowledge and add the database of the research about *S. commune* SMS, composting and vermicomposting process, *Lumbricus rubellus* application in vermicomposting and *S. commune* cultivation using compost or vermicompost as a fruiting substrate.

CHAPTER 2: LITERATURE REVIEW

This chapter discuss the previous studies which are related to the research. The literature review mostly from the current studies.

2.1 Overview of SMS

SMS, SMC, mushroom soil and recycled mushroom compost are terms that refer to the mushroom waste that remains after mushroom cultivation (Mamiro & Royse, 2008). SMS and SMC are used interchangeably to refer to the same co-product of mushroom cultivation which, still contains agro-residues and the mushroom mycelium (Phan & Sabaratnam, 2012). Accurately, SMC is SMS derived compost (Lou *et al*., 2015). SMS becomes the major by-product of the mushroom industry throughout the world where they are discarded regularly (Watabe *et al*., 2004).

Phan and Sabaratnam (2012) stated that the mushroom production worldwide is more than 25 million tons where China as the largest mushroom producer generates high amount of SMS that is over 13 million tons considering their edible mushroom yield every year is more than 22.6 million tons (Gao *et al*., 2015; Lou *et al*., 2015). US produces approximately 240,000 tonnes of *Agaricus* spp. mushroom in 2009 equivalent to more than one million tonnes of SMS production annually (Kapu *et al*., 2012). In Europe, over two million tons of SMS are generated (Ntougias *et al*., 2004).

Meanwhile, estimated production of SMS in Northern Ireland is more than 100,000 tonnes every year (Sharma *et al*., 1999). On the other hand, 150,000 metric tons of SMS were discarded in Iran every year (Tajbakhsh *et al*., 2008). In Malaysia, an average farm generates approximately 438 tonnes of SMS annually (Phan & Sabaratnam, 2012). Other SMS production shown in Table 2.1. In the future, the amount of SMS discarded will increase proportionally to the progress of mushroom cultivation that depends much on the population needs and industry success (Jamaludin *et al*., 2012).

Table 2.1: SMS production in average farm, Ireland and The Netherlands (Cited from Phan and Sabaratnam, 2012).

Country /Place	SMS Production	Time	References
Average farm worldwide	24 tons	Every month	Sing *et al*. (2011)
Ireland	254,000 tons	Per Year	Barry *et al*. (2012)
The Netherlands	> 800,000 ton	Per Year	Oei & Albert (2012)

All the research on SMS until now agreed that SMS is environmentally pollutant (Mamiro & Royse, 2008; Grujić *et al*., 2015). Therefore, the large number and exponent increase of SMS disposal need an efficient management to prevent its adverse or negative impact not just towards the environment but also the economic (Grujić *et al*., 2015). In US alone, it was estimated that the cost of SMS disposal amounted to US$ 7 million annually (Kapu *et al*., 2012).

All researchers that investigate SMS or SMC in their research also unanimously state it has useful nutrient content (Grujić *et al*., 2015; Lou *et al*., 2015). However, the nutrient content depends on the matter used in fresh mushroom substrate production, nutrients uptake during cultivation and duration of SMS left after being disposed and becomes SMC. SMS is still rich in nutrients and organic matters such as carbon (C), hydrogen (H), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Sodium (Na), protein, polysaccharide, lignin, cellulose and hemicellulose (Jordan *et al*., 2008; Lou *et al*., 2015).

To date, SMS potential has been explored in organic fertiliser production (Azizi *et al*., 2014; Zhang & Sun, 2014), fruiting substrate for fungi (Grujić *et al*., 2015; Wang *et al*., 2015), enzyme recovery (Phan & Sabaratnam, 2012), energy feedstock (Kapu *et al*., 2012; Phan & Sabaratnam, 2012), bioremediation (Phan & Sabaratnam, 2012; Azizi *et al*., 2011, 2013, 2015; García-Delgado *et al*., 2015), animal feed (Kim *et al*., 2011; Phan & Sabaratnam, 2012; Fazaeli *et al*., 2014) and casing material (Sharma *et al*., 1999).

2.2 SMS in Organic Fertiliser Production

SMS has desirable characteristics in organic fertiliser production including high NPK contents, high moisture content, a low bulk density, an absence of plant pathogens and high residual enzymes (Zhang & Sun, 2014). The organic fertiliser adds value to the soil by improving soil health and increase crop production making it more demanded compared to chemical fertiliser, which causes serious NO_3^--N pollution towards the surface and groundwater of the soil (Zhang & Sun, 2014; Lou *et al*., 2015). Besides, risk assessment of SMC shows that its heavy metal content meets the safe levels and it could hold 65% nitrogen, hence regarded as a better fertiliser than chemical fertilisers (Lou *et al*., 2015).

A study conducted on SMS as bio-fertiliser that can be applied directly to the soil and crop without waiting for it to become SMC revealed that SMS of *P. ostreatus* degraded wheat straw, sawdust and date palm fibers with C/N ratio range between 22.03 and 35.36 has potential to become natural fertiliser and soil amender in agriculture and horticulture fields (Owaid *et al*., 2017). Another study by Roy *et al*. (2015) reported SMS of oyster mushroom and button mushroom improved the growth and health of *Capsicum annuum* L., where the SMS had the ability to mobilize the soil phosphate into the root and leaf. Moreover, the chlorophyll content in the plant as well as protein and carotenoid content in the fruits were increased (Roy *et al*., 2015).

Lopes *et al*. (2015) had demonstrated the positive effect in fruiting when tomato seedlings were produced with SMC of *A. subrufescens*. They concluded the seedlings led to a higher production of tomato in comparison to the previous report production levels in organic cultivation systems with green, organic and other types of fertilization. However, the SMC was unstable and could underwent changes if it was stored wet for a longer time hence affect the plant growth due to the presence of heavy metals, poor physical properties and excessive salts, that resulted in high electrical conductivity (Lopes

et al., 2015). In addition, the nutrient content in SMC of different mushroom species varied depending on the materials used in the mushroom substrate production, therefore regarded as an inconsistent compost (Jordan *et al*., 2008).

A study to determine the optimum mixture for composting by Kulcu *et al*. (2008) showed the mixture ratio using SMC that suited the composting was 25% carnation waste, 25% chicken manure, 25% cattle manure and 25% SMC with C/N ratio 20.57 or 50% carnation waste, 25% chicken manure and 25% SMC with C/N ratio 22.52 or 50% carnation waste, 25% cattle manure and 25% SMC with C/N ratio 24.39 or 50% carnation waste and 50% SMC with C/N ratio 23.61.

Zhang and Sun (2014) reported co-composted green waste with 35% SMC and 20% biochar produced the highest quality compost product in 24 days compared to traditional composting which required 90-270 days to complete. In addition, the quality of the compost was improved in terms of composting temperature, particle-size distribution, free air space, cation exchange capacity, nitrogen transformation, organic matter degradation, humification, element contents, abundance of aerobic heterotrophs, dehydrogenase activity and toxicity to germinating seeds (Zhang & Sun, 2014).

Jamaludin *et al*. (2012) used five different ratios of sawdust based SMS mixed with GM in composting and vermicomposting. The ratios were 20 SMS:80 GM, 40 SMS:60 GM, 50 SMS:50 GM, 60 SMS:40 GM and 80 SMS:20 GM. They reported the higher usage of GM generated the lower C/N ratio in compost and vermicompost. Furthermore, the longer duration of biodegradation produced the higher quality organic fertiliser. At week 20 of decomposition, the vermicompost derived from 60% of GM and 40% of SMS had C/N ratio of 9.74 which was much lower than the vermicompost that using 40% of GM and 60% of SMS where its C/N ratio was 20.59. (Jamaludin *et al*., 2012).

Act as a control, the compost with the lowest C/N ratio obtained in Jamaludin *et al*. (2012) study was 7.74 at week 20 of composting using formulation 20 SMS:80 GM. The NPK content of the compost was N (2.23%), P (1.77%) and K (0.88%). The C/N ratio and NPK content of the compost at week 20 was better than the compost produced at week 10 that using the same formulation, 20 SMS:80 GM where the C/N ratio was 13.13 while the NPK content was N (1.86%), P (1.51%) and K (0.62%) (Jamaludin *et al*., 2012).

Izyan *et al*. (2009) in their study about the potential of SMC in vermicomposting found out that the highest percentage of nutrient elements was calculated in vermicompost using 80% of cow dung (CD) mixed with 20% of spent *P. sajor-caju* compost. Meanwhile, a study by Azizi *et al*. (2014) indicated the increased of N:P:K ratio from 1.10:0.39:1.15 to 1.16:0.41:1.22 when using SMC of *P. sajor-caju* amended with CD at ratio 2 SMC:1 CD. Further study was carried out where the mixture of the SMC with CD or goat manure (GM) contaminated with landfill leachate produced vermicompost that had low C/N ratio, ranges between 20.65 to 22.93 and was acceptable as soil stabilizer and conditioner. Besides, the vermicompost showed high percentage removal of heavy metals up to 99.81% (Azizi *et al*., 2015).

2.3 Composting Technique

Composting is a process of biological decomposition of organic matters and stabilization of wastes by microorganisms to produce stable product under aerobic condition (Zhang & Sun, 2014). Composting can be manipulated by controlling the composition and environment of a compost pile, including the particle size, initial C/N ratio, mixture ratio, free air space, moisture content, aeration, temperature and pH (Kulcu & Yaldiz, 2007; Kulcu *et al*., 2008). Other than SMS, biodegradable solid wastes like sawdust, fruit and vegetable peelings, fallen leaves, agricultural residues, weeds, egg

shells, coffee grounds, dung and sewage can be used in organic fertiliser production (Sarkar *et al*., 2016).

Traditional composting usually starts with short activation phase followed by thermophilic phase and then mesophilic phase which also known as maturation phase until it achieves stability (Lim *et al*., 2016). Traditional composting took longer time to reach maturity and stabilization which is 90-270 days (Zhang & Sun, 2014). No specific pH required in composting but extreme pH needs to be avoided (Lim *et al*., 2016). The range of moisture content for composting is 55% to 75% where the optimum moisture during thermophilic phase is 60% (Lim *et al*., 2016; Sarkar *et al*., 2016). Meanwhile, the range of initial C/N ratio in composting is between 20 and 50 (Lim *et al*., 2016). The mixtures with high lignocellulose contents, such as sawdust, which in most cases is less biodegradable than other additives, can decrease nitrogen loss in the composting process (Bai & Wang, 2011).

Composting system choices are broad for example, it can be run either using traditional composting which consists of both mesophilic and thermophilic or choosing only one phase such as thermophilic composting. Thermophilic composting also known as hot composting undergoes decomposition process at high temperature range, 45°C to 70°C while mesophilic composting also known as moderate-temperature phase performs biodegradation process at lower temperature range, 20°C to 45°C (Fornes *et al*., 2012).

Zhang and Sun (2014) used two-stage co-composting to decompose green waste with SMC and biochar where they found out green waste mixed with 35% SMC and 20% biochar generated the best quality and maturity compost. The formulation also had longer thermophilic stage, high particle-size distribution and increased nitrification, microbial numbers, enzyme activities and nutrient contents during composting. Co-composting is a simultaneous composting where two or more organic wastes composted together. The

technique was a better choice because it produced high quality compost in shorter time rather than composting the materials separately (Zhang & Sun, 2014).

The method in composting also varied for example, it can be run in open area, reactors, compost pockets, wooden box bin, cinder block bin, garbage can composter, wire mesh bin and so on. Kulcu *et al.* (2008) used reactors in composting to determine the optimum ratio of SMC, chicken manure, cattle manure and carnation waste. Sarkar *et al*. (2016) compared three methods of composting namely heaping or piling, pits and earthen pots where they figured out that heaping or piling was the most efficient method because it offered better aeration, compost sanitization via thermophilic condition and free from foul smell.

On the other hand, Mengistu *et al*. (2017) compared four different methods of composting known as windrow composting, vermicomposting, pit composting and combined windrow and vermicomposting. They found out windrow composting and vermicomposting were better than pit composting because the methods speeded up the decomposition process of organic wastes as well as reduced time duration of stable compost production. Other than that, they also recommended the combined windrow and vermicomposting method in human excreta biodegradation because the method eliminated the pathogens from compost (Mengistu *et al*., 2017).

2.3.1 Composting of SMS to Produce Mushroom Fruiting Substrate

Typically, composting technique uses in organic fertiliser production. However, the technique also can be used in production of fruiting substrate for mushroom cultivation. In fact, *A. bisporus* cultivated on composted wheat straw and horse manure. *Agaricus blazei Murrill* also usually cultivated using the same composting method employed for *A. bisporus*. (González Matute *et al*., 2011; Vos *et al*., 2017).

Following their previous study that indicated 50/50 mixture of non-composted substrate (NCS)/SMC was the most suitable substrate formulation for *A. bisporus* growth (Mamiro *et al*., 2007), Mamiro and Royse (2008) did a further research about the influence of spawn type and strain in *A. bisporus* cultivation using the same formulation of SMC mixed with the NCS. The highest yield (12.8 kg/m^2) and biological efficiency (70.9%) were obtained from the substrate mixture of 50/50 NCS/SMC and spawn type NCS. To date, no report found using SMS/SMC composted with other materials and used in mushroom cultivation.

2.4 Vermicomposting Technique

Vermicomposting defined as an aerobic process undergoes bio-oxidation and stabilization at mesophilic condition to decompose organic wastes using earthworms to fragments, mix and promotes microbial activity with the cooperation of the microorganisms in the earthworm guts and in their feedstock (Gunadi *et al*., 2002). Like composting, vermicomposting is also affected by temperature, pH, moisture content, initial C/N ratio and nature of the organic wastes (Lim *et al*., 2016).

The temperature range for vermicomposting must be around mesophilic condition, 25-40°C, pH between pH 5 and 8 or neutral with high moisture content (40 to 90%) (Fornes *et al*., 2012; Lim *et al*., 2016). The optimal pH for microbial activity was between pH 6.5 and 8.0 while pH range 5.5 to 8.5 was suitable for the earthworms in vermicomposting (Soobhany *et al*., 2015). The pH, temperature and moisture content of vermicomposting reported in Azizi *et al*. (2011, 2013, 2014, 2015) were 7 ± 1, 27 ± 1°C and 70 ± 10% respectively where they maintained via manual turning and watering regularly. Jamaludin *et al*. (2012) kept the humidity of their vermicomposting process in the range of 60% to 70%.

C/N ratio and feed mixture type requirement of the different species of earthworm in vermicomposting are differ. The C/N ratio required by the earthworm and its microflora in vermicomposting was less than 30 (Lim *et al*., 2016). A study by Ndegwa and Thompson (2000) found out the optimal C/N ratio for *E. fetida* was 25. Aira *et al*. (2006) had studied about the effect of C/N ratio towards population structure of *E. fetida* in vermicomposting system using pig manures and they found out the mature earthworms highly calculated (60%) at low C/N ratio while high C/N ratio making the population dominated by juvenile and hatchling earthworms (70%).

Aira *et al*. (2006) result indicated the C/N ratio and food quality influenced the earthworm size, growth and reproduction rates where carbon was the limiting factor. This finding was very important because high growth and reproduction were needed in vermicomposting to accelerate the decomposition and maturity of organic wastes (Aira *et al*., 2006). Vermicomposting process also needs pre-composting phase before earthworms' inoculation to prepare the feedstock for the earthworms and Azizi *et al*. (2011, 2013, 2014, 2015) as well as Jamaludin *et al*. (2012) carried out the pre-composting process for 21 days or three weeks.

Azizi *et al*. (2011) stated that vermicomposting was a realistic and practical technique to solve the SMS disposal problem. Vermicomposting study by Tajbakhsh *et al*. (2008) showed significant reduction in C/N ratio, total organic carbon, total potassium (K), pH, electrical conductivity and enhance of total Kajeldahl nitrogen, total phosphorus (P), micro and macronutrients. Huang *et al.* (2014) found out vermicomposting caused the total carbon and nitrogen content decreased quickly compared to the control (without earthworm) hence regarded as better technique than composting.

In vermicomposting, the earthworms play a great role in vermicompost production. They undergo direct physical and indirect biochemical process (Loh *et al*., 2005). Direct degradation carried out by the earthworms themselves where they improve substrate

aeration by mixing and grinding the feed materials hence reduce the particle size of organic matters as well as increase the surface area of the matters. Meantime, indirect process carried out by the microbes inside the earthworms' intestines where the physical characteristics offered via direct earthworm activity enable them to easily break down the organic matters (Ndegwa & Thompson, 2000).

The presence of symbiotic relationship between gut microbes and earthworm aid with existing microbes in feedstock enhance the biodegradation acceleration therefore the vermicompost can be produced in a short time (Jamaludin & Mahmood, 2010). Moreover, longer period of vermicomposting process increased the vermicompost quality via the decreased of heavy metals content and C/N ratio as well as enriched the macronutrient elements (Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012). Besides, the number of earthworms usually increase in vermicomposting depends on the characteristics of the feed materials and other related parameters such as temperature, pH and moisture (Ndegwa & Thompson, 2000; Aira *et al*., 2006; Fornes *et al*., 2012).

According to Singh *et al*. (2011), the best earthworms for vermicomposting must had high reproductive rate, rapid growth and maturation rate of hatchlings. They also must be possessed high tolerance of environmental stress and high rates of organic matter consumption, digestion and assimilation (Lim *et al*., 2016). There are three groups of earthworms namely epigeic, endogeic and anecic (Lim *et al*., 2016). The study in vermicomposting mostly use epigeic earthworms such as *E. fetida*, *E. andrei*, *E. eugeniae* and *P. excavates* because they have high reproductive rates and live in organic horizons that feed mainly on decaying organic matters (Lim *et al*., 2016). There are also study using anecic earthworms such as *L. rubellus* (Izyan *et al*., 2009; Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012; Azizi *et al*., 2014).

In comparison to regular compost, vermicompost has better C/N ratio and contains stronger modified lignin (Fornes *et al*., 2012). Besides, vermicompost has lower heavy

metals content rather than the compost due to the presence of the earthworms in vermicomposting process where they accumulate the heavy metals in their bodies (Lim *et al*., 2016). However, vermicomposting process usually will not sanitize the vermicompost unless the pre-composting before the vermicomposting is set to run under thermophilic condition (Fornes *et al*., 2012; Lim *et al*., 2016).

Many studies on the combination of composting and vermicomposting ie either composting followed by vermicomposting or vice versa suggested that composting followed by vermicomposting was better (Fornes *et al*., 2012). Recent reports had suggested combining high temperature composting and vermicomposting to obtain high quality product in terms of stability and maturity because hot composting enabled sanitization and elimination of toxic compounds while vermicomposting rapidly reduced particle size and increased nutrients availability (Nair & Okamitsu, 2010).

2.4.1 Vermicomposting of SMS

SMS also used as feed materials for earthworm in vermicomposting. Izyan *et al*. (2009) revealed the potential of vermicomposting process of spent *P. sajor-caju* compost from sawdust combined with cow dung (CD) using *L. rubellus* to produce organic fertiliser where 40:60 of CD:SMC showed the highest percentage of growth and reproduction of the earthworms. Furthermore, vermicompost procured from 80:20 of CD:SMC showed the highest NPK content; N (1.90%), P (0.57%), and K (2.74%) (Izyan *et al*., 2009).

Jamaludin *et al*. (2012) mixed sawdust based SMS with goat manure (GM) in vermicomposting process using *L. rubellus*. They used five different treatments, 20 SMS:80 GM, 40 SMS:60 GM, 50 SMS:50 GM, 60 SMS:40 GM and 80 SMS:20 GM with the same control for each treatment that underwent composting. The analysis for C/N ratio and NPK content was carried out at week 0, week 10 and week 20 of composting and vermicomposting. The best vermicompost they got in their study was vermicompost

generated by using 20 SMS:80 GM where its C/N ratio was 6.39 at week 20 with NPK content of N (2.02%), P (1.93%) and K (0.92%) (Jamaludin *et al*., 2012).

Tajbakhsh *et al.* (2008) used SMC amended with other agricultural wastes, which were potato, fruits and vegetables, pomegranate, stump and tomato underwent vermicomposting for 90 days using *E. foetida* and *E. andrei* to produce vermicompost that was rich in total nitrogen, phosphorus, and other nutrients element beneficial for plants' growth. Their vermicompost also showed good physical properties, low conductivity, low C/N ratios, optimal stability, and maturity therefore, the vermicompost can be used as soil conditioners, healthy organic fertilisers, and good substitutes in potting media (Tajbakhsh *et al*., 2008).

Azizi *et al*. (2014) compared commercial livestock excreta, cow dung (CD) and goat manure (GM) in vermicomposting of SMC of *P. sajor-caju* using *L. rubellus* to produce the nutrient enriched bioproduct. They reported 1:1 of GM:SMC showed the highest multiplication and growth of earthworms in number and biomass. They concluded GM was better than CD in vermiculture and good vermicompost production with SMC as bulking agent. The further study by Azizi *et al*. (2015) reported vermicomposted SMC of *P. sajor-caju* mixed with other organic matters such as CD, GM, sewage sludge and landfill leachate successfully bioremediated heavy metals including Cd, Cr, Pb, Zn and Cu.

2.5 Properties/Characteristics of Organic Fertilisers

Organic fertiliser is a stable final product of biological decomposition process which is humus-rich and complex mixture that improve the soil properties (Watteau & Villemin, 2011). Among all the biodegradation of organic wastes, composting and vermicomposting are two of the best-known technique in stabilization process to produce organic fertiliser known as compost and vermicompost (Fornes *et al*., 2012).

Lim *et al*. (2016) stated the vermicompost under electron microscope was smaller and more scattered than the initial wastes making its texture was finer. Meanwhile, Azizi *et al*. (2011) was reported vermicompost produced from high percentage of SMC combined with sewage sludge had fine texture, dark colour and odourless compare to their initial appearance.

On the other hand, Sarkar *et al*. (2016) reported the matured compost appeared dark without any foul smell. The texture of compost was coarser than vermicompost (Lim *et al*., 2016). At the same time, the volume of compost and vermicompost were reduced after the decomposition process because of the particles downsize during the breakdown process of organic matters by the microbes in composting and joint action of microbes and earthworm in vermicomposting (Fornes *et al*., 2012).

C/N ratio is the ratio of carbon to nitrogen content in the fertilisers that is generally used to characterize them. Specifically, C/N ratio is an essential indicator for the assessment of compost/vermicompost stability and maturity (Jamaludin & Mahmood, 2010; Azizi *et al*., 2015; Soobhany *et al*., 2015). C/N ratio less than 20 was regarded as an acceptable maturity for organic fertilisers while C/N ratio lower than 15 was the indicative of better fertilisers (Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012). Soobhany *et al*. (2015) stated C/N ratio between 15 and 25 revealed a satisfactory degree of the stabilization and maturity of organic wastes.

According to USDA (2011), C/N ratio 24 ruled the soil because the ratio was the perfect balance diet for the soil microbes where they needed C/N ratio near 24:1 to stay alive. C/N ratio much higher than 24 will cause nitrogen immobilization and soil microbes' elimination while C/N ratio less than 24 will trigger the nitrogen mobilization and mineralization (USDA, 2011). Meanwhile, Sánchez-Monedero *et al*. (2010) stated the continuous declined of temperature to ambient air temperature was the indicator of the organic wastes maturity and stability.

2.6 SMS for Mushroom Fruiting Substrate

SMS contains polysaccharide, lignocellulose, protein as well as other nutrients such as NPK, making it beneficial for mushroom cultivation (Grujić *et al*., 2015; Lou *et al*., 2015). SMS had been used as mushroom fruiting substrate in several previous researches (Table 2.2).

Table 2.2: SMS used in mushroom cultivation (Cited from Wang *et al*., 2015).

Mushroom Cultivated	SMS Used	References
Auricularia spp. *Pleurotus* spp.	*Agaricus bisporus*	Sharma & Jandaik (1994)
Lentinula spp		Kilpatrick *et al*. (2000)
Volvariella spp.	*Agaricus bisporus*	Poppe (2000)
Pleurotus spp.	*Lentinula edodes*	Royse (1993), Rinker (2002)
Pleurotus spp.	*Vovariella*	Quimio (1988), Chang & Miles (1989)
Coprinus comatus	*Flammulina* or *Ganoderma*	Rinker (2002)
Lentinula decastes	*Pholiota nameko*	Akamatsu (1998)
Pleurotus spp.	*Lentinula edodes*	Royse (1993), Rinker (2002)
Pleurotus spp.	*Vovariella*	Quimio (1988), Chang & Miles (1989)
Coprinus comatus	*Flammulina* or *Ganoderma*	Rinker (2002)
Lentinula decastes	*Pholiota nameko*	Akamatsu (1998)

A study by Wang *et al*. (2015) concluded SMS of *Hypsizigus marmoreus* was acceptable as an alternative to cottonseed hulls in *P. ostreatus* cultivation where 88% SMS mixed with 10% cottonseed hulls, 1% gypsum and 1% lime showed fastest mycelial growth (5.99 mm/day), followed by 75% SMS added with 20% cottonseed hulls supplemented with 3% wheat bran, 1% gypsum and 1% lime (mycelial growth, 5.56 mm/day), hence decreased costs and reduced disposal problem (Wang *et al*., 2015).

González Matute *et al*. (2011) cultivated *A. blazei* on SMS of *P. pulmonarius*. Their result showed the higher yield obtained when using the SMS supplemented with 20%

vermicompost or 20% of brewery residue or 50/50 combination of the SMS and sunflower seed hulls. In addition, the formulation had a low rate of contamination (González Matute *et al*., 2011).

2.7 Schizophyllum commune

Schizophyllum commune (Figure 2.1) is a mushroom forming white-rot fungus which is cosmopolitan in diversity that can be found in every continent except Antarctica that does not has any wood as a substrate for it to grow (Imtiaj *et al*., 2008; Klaus *et al*., 2011; Figlas *et al*., 2014; Singh *et al*., 2017). It is also known as split gill, one of the seasonal fungi that grows numerously in rainy season, making its availability was limited by the nature (Adejoye *et al*., 2007; Zahida *et al*., 2015).

Figure 2.1: *Schizophyllum commune*

Kingdom : Fungi
Division : Eumycota
Subdivision: Basidiomycotina
Class : Hymenomycetes
Subclass : Holobasidiomycetidae
Order : Agaricales
Family : Schizophyllaceae
Genus : *Schizophyllum*
Species : *S. commune*

Schizophyllum commune is more popular and cultivated commercially in Asia especially East Asia and Southeast Asia where the people here consume it as a healthy food that able to treat several diseases (Imtiaj *et al*., 2008; Figlas *et al*., 2014). Most research of *S. commune* are regarding its ability to produce schizophyllan (Shu & Hsu, 2011; Joshi *et al*., 2013; Singh *et al*., 2017). Schizophyllan is a renewable polymer and a complex chemical structure that is soluble in water (Joshi *et al*., 2013).

So far, the research had proved schizophyllan possessed immunomodulatory, anti-inflammatory, antidiabetic, antimicrobial, antineoplastic, antiviral, antifungal, antitumor, anticancer, antioxidative, anti-neurasthenia and hepatoprotective activities which offered high potential in pharmaceutical industry (Klaus *et al*., 2011; Teoh *et al*., 2011; Joshi *et al*., 2013; Figlas *et al*., 2014; Yao *et al*., 2016; Du *et al*., 2017). It is also best known to have strongest anticancer activity among mushroom-derived substances and had passed its clinical trials, hence several Japanese pharmaceutical companies produce it commercially as anticancer agent (Joshi *et al*., 2013; Ivanova *et al*., 2014).

Besides schyzophyllan production, *S. commune* also widely studied for its ability in enzymes extraction and purification. Irshad and Asgher (2011) and also Asgher *et al*. (2016) reported *S. commune* had high potential in commercial scale production of ligninolytic enzymes such as manganese peroxidase, lignin peroxidase and laccase. Other enzymes that can be isolated from *S. commune* are cellulases, cellobiohydrolases, β-glucosidases and xylanases (Metreveli *et al*., 2017).

Its ability to produce a mixture of lignocellulolytic enzymes necessary to break down complex lignocellulosic biomass triggers growing interest among the researcher due to its possible application in various industries including chemicals, food, fuel, pulp and paper, textile, laundry, animal feed and agriculture (Singh *et al*., 2017). *Schizophyllum commune* also has potential in Chitin-Glucan complex production which possesses immunostimulant properties to boosts the immune system in humans and other animals (Smirnou *et al*., 2011).

2.7.1 Substrate Used in *Schizophyllum commune* Cultivation

Mushrooms are heterotrophs which undergo biochemical decomposition to get their energy source and growth materials from the substrate where they grow. Most cultivated mushrooms are naturally saprophytic mushrooms where they live on dead organic matters

for example, dead trees, stumps, old roots, grass, straw, compost, etc (Chang & Miles, 2004). Each mushroom grows very well in different substrates according to their nutritional requirements. For example, rice straw, wheat straw and horse manure are the substrates used for *A. bisporus* cultivation while wood, sawdust and cotton seed hulls are suitable for *Pleurotus* spp. and *Lentinula* spp. cultivation (Sánchez, 2010).

Schizophyllum commune grow naturally on trees and rotting wood that causes wood decay drastically (Adejoye *et al*., 2007; Imtiaj *et al*., 2008; Arboleda Valencia *et al*., 2011). In large scale of *S. commune* cultivation, sawdust is used as a main substrate supplemented with rice bran (Preecha *et al*., 2015). There was a research to figure out the feasibility of using sunflower seed hulls as a main substrate to cultivate *S. commune* and the result showed the sunflower seed hulls, a cheap by-product of edible oil industry had potential to replace sawdust as a main substrate with biological efficiency, 40.7% and productivity, 1.1% day^{-1} (Figlas *et al*., 2014).

Other substrates that had been tested in *S. commune* cultivation were rice straw and banana stalks to induce higher yield of ligninolytic enzymes namely manganese peroxidase, lignin peroxidase and laccase in solid state fermentation (Irshad & Asgher, 2011; Asgher *et al*., 2016). Meanwhile, Arboleda Valencia *et al*. (2011) used 1% of lignocellulosic fibers from banana stem, sugarcane bagasse, and bamboo to recover lignocellulolytic enzymes such as xylanase, mannanase, polygalacturonase, CMCase, FPase, and avicelase in liquid culture medium. They figured out the sugarcane bagasse and banana stem induced higher hollocellulase activity compared to bamboo (Arboleda Valencia *et al*., 2011).

Fruiting substrate formulation and substrate supplementation play a crucial role in maximizing the mushroom yield. The substrate formulations enhanced *Auricularia polytricha* growth were sawdust mixed with oil palm frond (90:10) added with 15% spent grain and sawdust mixed with empty fruit bunch (50:50) added with 10% spent grain

(Abdul Razak *et al*., 2013). Besides spent grain, wheat bran, rice bran and maize powder are some of the supplementations used in mushroom cultivation (Moonmoon *et al.*, 2011).

Preecha *et al.* (2015) reported the highest yield of *S. commune* cultivation obtained by using formulation 100% sawdust supplemented with 10% rice bran, 75% sawdust added with 25% waste material (reusing cultivated spawn) supplemented with 15% rice bran and 50/50 of sawdust/waste material mixed with 15% rice bran where their yields were 82.85, 81.35 and 80.04 g/bag respectively. Figlas *et al*. (2014) stated sunflower seed hulls had potential to replace sawdust as a main substrate for *S. commune*. Moreover, supplemented it with wheat bran even improved the mushroom yield where the biological efficiency was 48.3% and productivity was 1.6% day^{-1} (Figlas *et al*., 2014). To date, no report on reutilizing of *S. commune* SMS/SMC as a fruiting substrate in its cultivation is found.

2.7.2 Parameters of *Schizophyllum commune* Cultivation

Mushroom life cycle is greatly influenced by various environmental factors including abiotic and biotic factors. Some of the factors can be measured and known as parameter such as temperature, pH and moisture content. The suitable condition is very important for mushroom mycelial growth and fruiting body formation. Majority temperature range for mushroom mycelial growth is 5°C to 33°C while fruiting formation temperature range is 13°C to 24°C where their optimal temperature is around 27°C and 18-21°C respectively (Chang & Miles, 2004).

The yield of the mushroom cultivation maximizes through the substrate formulation aid with suitable parameters. *Agaricus bisporus* pH range for mycelial growth is 3.5 to 9.0 where the optimal pH is 6.8 to 7.0 while temperature range for its mycelial growth is 3-32°C and the optimal temperature is 22-25°C (Chang & Miles, 2004). According to Akinyele and Adetuyi (2005), *Volvariella volvacea* optimum temperature and pH range

are 30°C and 5.5-8.5 respectively. Meanwhile, the humidity enhances *A. polytricha* growth is 85% moisture content (Abdul Razak *et al*., 2013).

In sub-merged culture condition, *S. commune* could grow at a temperature range of 10-40°C and pH range of 3.5-6.5 where its optimal temperature and pH were 25°C and 5.5 respectively to yield the maximum biomass production (Zahida *et al*., 2015). The same finding obtained by Adejoye *et al*. (2007) where *S. commune* collected in Nigeria grew optimally at pH 5.5 and temperature at 25°C. The highest vegetative growth they obtained was 101.05 mg/30 cm while the best mycelial extension was 102.97 mm. The minimal growth of *S. commune* mycelial recorded at 40 °C where it inhibited at low temperature (0 and 10°C) and at high temperature (45 and 50°C) (Adejoye *et al*., 2007).

Ten strains of *S. commune* collected from different geographical origins of Korea, China and Thailand showed the best mycelial growth and density at 30-35°C and the most favorable pH was 5 where the optimal growth measured at pH 5 to 6. The lowest mycelial growth was obtained using pH 9 and temperature at 15°C (Imtiaj *et al*., 2008). To conclude, for mycelial growth, *S. commune* adapted well in high temperature (10-40°C) and low pH (3.5 – 6.5) (Zahida *et al*., 2015).

In other research, the maximum activities of ligninolytic enzymes, manganese peroxidase, lignin peroxidase and laccase synthesized of *S. commune* reported achieved its maximum at pH 4.5, temperature 35°C, moisture content 60% and C/N ratio 20:1 when banana stalks used as a substrate in solid state fermentation (Irshad & Asgher, 2011). The same study conducted by using another substrate (rice straw) indicated the same enzymes maximum recovery were obtained at pH 5.0, temperature 35°C and C/N ratio 20:1. (Asgher *et al*., 2016).

CHAPTER 3: MATERIALS AND METHODS

This chapter will discuss about all materials and methods used along the research. Each method explained in the subtitles below.

3.1 Experimental Design Overview

Figure 3.1 showed the main frame of the whole experimental design of the research. All of the field research was carried out in Biotechnology Research Centre Glami Lemi University Malaya (PPBGL) in Jelebu, Negeri Sembilan.

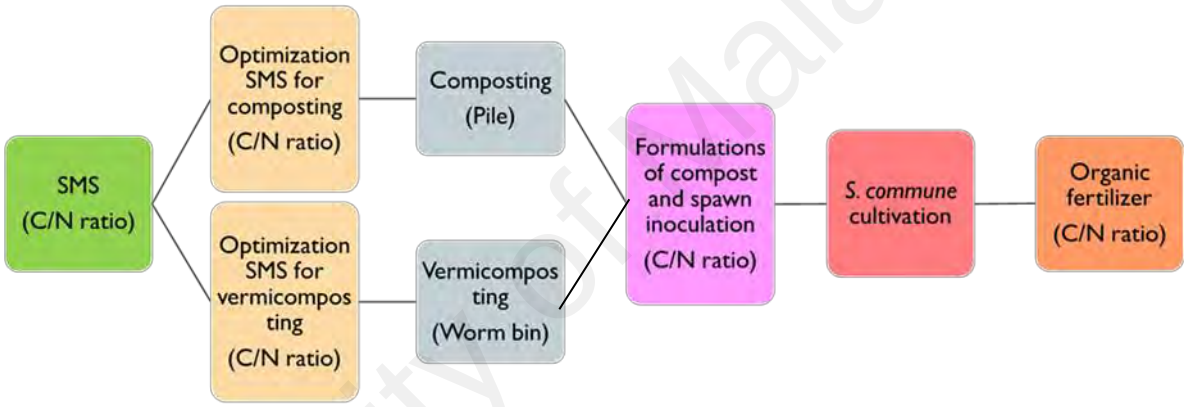


Figure 3.1: Main frame of experimental design of the research.

Spent mushroom substrate (SMS) of *S. commune* was firstly analyzed for its C/N ratio and then mixed with goat manure (GM) at ratios 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. The mixture underwent composting and vermicomposting process for various time intervals during 30, 60 and 90 days which carbon, nitrogen, phosphorus and potassium (CNPK) content were measured. Compost and vermicompost final product were then analysed for the chemical characteristics/suitability as organic fertiliser and use in mushroom cultivation. Lastly, *S. commune* SMS after its cultivation was analyzed to determine either it can be reused as organic fertiliser or not.

3.2 *Schizophyllum commune* SMS

Spent *S. commune* substrate was collected from a mushroom farm in PPBGL. The farm produced more than a tonne of *S. commune* per week. Approximately for every 1 kg of mushroom yield, 10 kg of SMS would be produced. SMS of *S. commune* major contents were sawdust, rice bran and white mycelia. The SMS was still in polyvinyl bags (~600 g each) when discarded at the disposal area near the mushroom farm (Figure 3.2).

In this project, the bags with milky white colour without any visible mold were chosen to minimize the contamination during mushroom cultivation. Their plastic was removed before the SMS was crushed and accumulated in a large bin (Figure 3.3). SMS as a main organic matter of fertiliser production in this work was mixed with GM.

Figure 3.2: Piled thrown bags of *S. commune* SMS lead to the contamination of soil.

Figure 3.3: SMS of *S. commune* crashed using machine.

3.3 Optimization of Composting Preparation

Composting process was carried out by mixing the SMS of *S. commune* with GM at three different formulations. The formulations were 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. The formulations were selected based on the study by Jamaludin *et al*. (2012). Other than that, the formulations were chosen to maximize *S. commune* SMS usage and added with GM which, is needed in composting and vermicomposting to balance the high C/N ratio of the SMS. Besides using different formulations, the composting and vermicomposting parameters such as temperature, pH and moisture content were measured regularly to ensure the parameters were in optimized condition along the process. The parameters were maintained via regular turning and watering of the compost/vermicompost.

3.3.1 Goat Manure Preparation

GM was procured from the goat farm also in PPBGL, located just few meters in front of the mushroom farm. The fresh and wet GM was accumulated and put into several jutes. They were left for several days until the manure became moderately moist range around 60±10%. The moist GM then transferred to the composting area for conducting the experiment.

The GM was crushed by using hand grinder to decrease its size before it was mixed with *S. commune* SMS. Even though the GM contains pathogens that cause the contamination during mushroom cultivation, however the composting process in this project has thermophilic phase to destroy the pathogens.

3.3.2 Formulations of Composting

The formulations used in composting were 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. There was no additional SMS or GM added into the compost throughout the process. The decomposition process was lasted for 90 days or three months. Table 3.1 shows the summarization of the formulation used in the composting. The total weight in the table 3.1 was not fixed because the composting process in this study emphasize the dimension of the pile to achieve the thermophilic composting.

Table 3.1: The weight of SMS, GM and dimension for different mixture ratio in composting preparation.

Formulation / Code	Weight of SMS	Weight of GM	Total Weight	Dimension
80 SMS:20 GM / CA	128 kg	32 kg	160 kg	1m x 1m x 1m
60 SMS:40 GM / CB	120 kg	80 kg	200 kg	1m x 1m x 1m
50 SMS:50 GM / CC	115 kg	115 kg	230 kg	1m x 1m x 1m

3.3.3 Preparation of Composting

Composting process took place in the open area near the mushroom farm. The area was cleared and cleaned before it was used. There is no flooring system used in this work where the pile was placed directly in the ground to prevent the stagnant water as well as reduce the cost of this project. The composting site was divided into three large square area with measure dimension, 1m x 1m x 1m. Bricks and zinc roof supported with woods and ropes were used as divider of the area. Each area was labelled as 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM respectively.

Pile system was chosen following the suggestion by Sarkar, Pal and Chanda (2016) to used heaping or piling that able to trap heat in composting and reach the thermophilic condition. In the system, SMS and GM were placed layer by layer approximately 2 to 3 cm of each layer into each site according to the formulation label set. SMS and GM filled the whole dimension area to form a large pile. The large pile was able to achieve hot composting process for a long time.

All the piles were watered enough until they reached around 60±10% of moisture content. Initial temperature and pH of the piles were measured by using thermometer and pH meter. The piles were then covered with large canvas loosely as roofing system for the piles to reduce water evaporation and allow oxygen to enter and penetrated the piles. The temperature was measured at a depth of about one foot from the surface of the piles daily starting from day 0 of composting.

Meanwhile, pH was measured on weekly basis before turning and watering of the piles. Piles were turned using shovel to allow oxygen circulation. Turning the piles maintained aerobic condition and produced homogenous compost while watering the piles was crucial to preserve their moisture content. Figure 3.4 shows the composting system of this research.

Figure 3.4: Composting system of the research.

3.4 Optimization of Vermicomposting Preparation

Like composting, vermicomposting process also was optimized by using the same formulations, 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. During the decomposition process, the temperature, pH and moisture content were measured regularly to make sure they were at the optimized condition. The temperature, pH and moisture content were optimized via regular turning and watering of the vermicompost.

3.4.1 Goat Manure and Earthworm Preparation

GM was obtained from the goat farm in PPBGL. The fresh and wet GM in the jutes was left for several days until the manure became moderately moist. After that, it was transferred to the vermicomposting area located just few meters away from the goat farm. The GM was soaked in a large bin to soften it. The soft manure was crushed to reduce its size. The earthworm used in vermicomposting was *L. rubellus* (Red earthworm). The red earthworm was obtained from the stock cultures in the Earthworm Reservoir at Institute of Biological Sciences, University of Malaya.

3.4.2 Formulations of Vermicomposting

The formulations used in vermicomposting were 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. No additional SMS or GM was added into the vermicompost along the process. The biodegradation process was carried out for 90 days or three months. Table 3.2 summarizes the formulations used. The worm used in this work is *L. rubellus*. It was chosen because its potential was not explored much as compared to the other worms like *E. fetida*. Furthermore, the findings of this work will add the database about *L. rubellus* hence help the researcher in the future who want to investigate more about it.

Table 3.2: The weight of SMS, GM and red earthworms (*Lumbricus rubellus*) for different mixture ratio and replicates in vermicomposting preparation.

Formulation /Code	Replicate /Bin	Weight of SMS	Weight of GM	Weight of 60 Worms	Average Weight of Worms
80 SMS:80 GM / VA	VA1	4 kg	1 kg	8.60 g	8.38±0.44 g
	VA2	4 kg	1 kg	8.86 g	
	VA3	4 kg	1 kg	8.15 g	
	VA4	4 kg	1 kg	7.89 g	
60 SMS:40 GM / VB	VB1	3 kg	2 kg	9.42 g	9.15±0.26 g
	VB2	3 kg	2 kg	8.80 g	
	VB3	3kg	2 kg	9.17 g	
	VB4	3 kg	2 kg	9.21 g	
50 SMS:50 GM / VC	VC1	2.5 kg	2.5 kg	9.52 g	9.08±0.59 g
	VC2	2.5 kg	2.5 kg	8.38 g	
	VC3	2.5 kg	2.5 kg	9.61 g	
	VC4	2.5 kg	2.5 kg	8.81 g	

3.4.3 Preparation of Vermicomposting

The vermicomposting area was cleared and cleaned before it was used. Vermicomposting process was carried out by using worm bin with measure dimension, 45 cm x 34 cm x 27 cm. GM was soaked in a bin to soften it so that it can be crushed

easily. SMS and GM were put into the bin according to the three ratios formulation, 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM.

Each formulation in every bin was replicated into four replicates. All the bins were labelled with suitable labels by using white masking tape. The GM in the bin was crushed and mixed homogenously with SMS. The mixture was watered until it achieved ±70% of moisture content. After that, the bin was covered with lid with holes to allow air circulation. The holes were made by cutting the middle of the lid to form a large hole. The hole then covered with a net to prevent the worm going out from the bin.

All the bin was placed on vermicomposting racks in the compost site to undergo pre-composting process. The pre-composting was the short composting process before the earthworms' inoculation to prepare the feedstock for the earthworms. The duration of the pre-composting was normally around three weeks so that the large organic matters can be reduced before the inoculation hence the worm can easily absorb and digest the organic matters.

After 21 days of pre-composting, temperature and pH in all bin were measured. Then, 60 adults of *L. rubellus* were introduced into each bin. The day to introduce the earthworms was referred to as day 0 of vermicomposting. Temperature and pH were measured every week before turning and watering process. Manual turning was done by hand to allow the oxygen circulation. Meanwhile, watering the vermicompost was done every week to ensure the moisture content was maintained around 70±10%. The photo of vermicomposting was shown in Figure 3.5.

Figure 3.5: Vermicomposting system of the research.

3.5 Moisture Content, C/N Ratio and NPK Analysis

C/N ratio and NPK analysis of compost and vermicompost was done on day 30, day 60 and day 90 of composting and vermicomposting by sampling 100 g of sample. Each sample was placed in a petri dish and weighed to get their initial wet weight by deducting their initial weight with the weight of the empty petri dish.

After that, the samples were dried in an oven set at 70°C for 24 hours. Then, the dried samples were weighed again to obtain their final weight after the drying process. After that, each sample was placed into the different zip lock plastic and labelled using the masking tape. The samples were sent either to Forest Research Institute Malaysia (FRIM) or University of Putra Malaysia (UPM) for CNPK analysis. The formulation below was used to measure the moisture content.

Moisture Content (%) - (Jeznabadi *et al*., 2016)

$$= \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

3.6 Compost and Vermicompost for *Schizophyllum commune* Cultivation

The final product of composting and vermicomposting were then used in *S. commune* cultivation to investigate its potential as *S. commune* fruiting substrate.

3.6.1 Mushroom Bag Preparation

All the materials needed for the research of *S. commune* cultivation including sawdust, rice bran, wood vinegar and polyethylene bags were available in the mushroom farm in PPBGL. Besides, the inoculation room, incubation room and mushroom cultivation house in the mushroom farm were already adjusted in optimal condition. *Schizophyllum commune* spawn supplied by Mycology Lab of University of Malaya. Figure 3.6 shows the *S. commune* cultivation flow in this study.

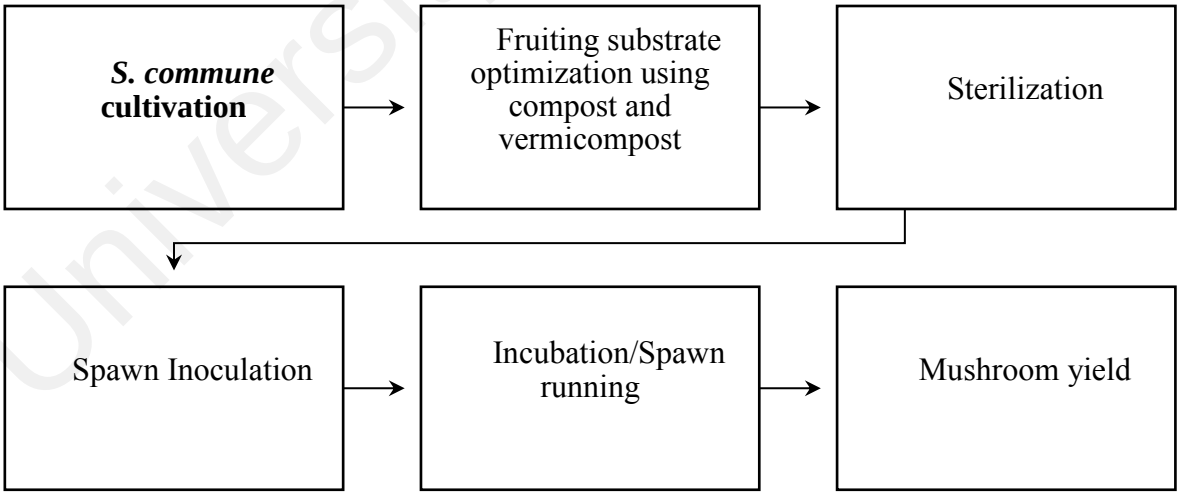


Figure 3.6: *Schizophyllum commune* cultivation flow.

3.6.2 Fruiting Substrate Optimization Using Compost and Vermicompost

Compost and vermicompost were used as fruiting substrate for *S. commune* cultivation in this research. 1000 g from each compost CA (80 SMS:20 GM), CB (60 SMS:40 GM), CC (50 SMS:50 GM) and vermicompost VA (80 SMS:20 GM), VB (60 SMS:40 GM), VC (50 SMS:50 GM) was put into the different polyethylene bag. Each mushroom bag was replicated into five bags. All the bags were compacted by tapping the base of the bags on the flat surface. Then, the bags were closed using plastic necks and caps.

All the mushroom bags were sterilized in multipurpose laboratory autoclave machine in PPBGL. The autoclave was set at 121°C for 90 minutes. After that, the bags were left to cool down until they reached the room temperature. The lid of mushroom bag was opened and the bag was inoculated with *S. commune* spawn in sterilized condition. The bag was closed back by using another mushroom lid that had hole. The lid was autoclaved prior to the spawn inoculation. The function of the hole on the lid was to provide air for growth of the spawn. The hole was covered with micropore tape and the tape was sprayed with alcohol 70% to prevent contamination. After that, all the bags were placed in incubation room in the mushroom farm to allow the growth of *S commune* mycelium.

The mycelium run and density were observed and weekly measured until the mycelium had colonized the substrate. Then, the bag was transferred into the mushroom cultivation house. The bag was sprayed with Clorox 10% before the bag was slit using sterilized scalpel to prevent contamination. The slit bag was watered regularly by spraying misting water around the bags twice a day until *S. commune* was harvested. To optimize the fruiting substrate using compost and vermicompost, five formulations were tested along the research. The list of the formulations were;

1. Unsterilized compost
2. Sterilized compost and vermicompost

3. Sterilized Compost and vermicompost with moisture adjusted to 60% and pH 6
4. Effect of nitrogen source (Add rice bran)
5. Supplementation of sawdust

3.6.2.1 Unsterilized Compost

The main reason pile system was chosen for composting process in this research was to get the sanitation compost that free from pathogens. The compost without autoclave might had potential in *S. commune* cultivation where its fruiting substrate usually need autoclaving or steaming process to remove all the microbes in the substrate. In this case, only compost could be tested because our vermicompost did not achieved sanitation standard.

The compost was placed into a bin to cool it down. Then, 200 g compost was placed on the base of the mushroom bag. The spawn was inoculated on the compost in the bag in the form of large ring. Again, 200 g was placed after the inoculation and it was inoculated again. The process was repeated four times until 1000 g compost was filled into the mushroom bag. The process was carried out in sterilized condition. The unsterilized compost was tested using compost on day 30, day 60 and day 90 of composting.

3.6.2.2 Sterilized Compost and Vermicompost

The compost and vermicompost on day 30, day 60 and day 90 of composting and vermicomposting were used directly as fruiting substrate without any modification. The compost CA (80 SMS:20 GM), CB (60 SMS:40 GM), CC (50 SMS:50 GM) and vermicompost VA (80 SMS:20 GM), VB (60 SMS:40 GM), VC (50 SMS:50 GM) were placed into a bin respectively. 1000 g from each compost or vermicompost was put into the mushroom bag and autoclaved at 121°C. After that, the sterilized compost and

vermicompost were inoculated with *S. commune* spawn under sterilized condition before it placed into the incubation room.

3.6.2.3 Sterilized Compost and Vermicompost with Moisture Adjusted to 60% and pH 6

Both compost and vermicompost were dried under the sun to reduce their humidity. Then, their moisture content was adjusted to 60%. Because their pH was increased after the sun drying, wood vinegar was added until the pH was between pH 5 and 6. Then, the compost and vermicompost were put into the mushroom bag previously described, autoclaved and inoculated with *S. commune* spawn.

3.6.2.4 Effect of Nitrogen Source (Add Rice Bran)

5%, 10% and 15% rice bran (RB) were added into compost CA, CB, CC and vermicompost VA, VB, VC. After their moisture content and pH were adjusted to 60% and 6 respectively, the compost and vermicompost mixed with RB were placed into the mushroom bag and followed the usual procedure of the mushroom bag preparation and cultivation.

3.6.2.5 Supplementation of Sawdust

Compost CA, CB and CC mixed with 10% RB were supplemented with 0%, 20%, 50% and 80 % sawdust (SD). The vermicompost was not used as fruiting substrate in this method because of the findings discussed in the next chapter. Compare to the previous trial using RB, wood vinegar was not added into all substrates supplemented with SD including 0% SD supplementation. It was because SD had low pH and could reduce the pH of the fruiting substrate. So, after the moisture content was adjusted at optimum

parameter, the substrate was mixed homogenously and 1000 g was put into the mushroom bag. As usual, the bag was autoclaved and inoculated with the spawn.

3.6.2.6 Determination of Mycelial Growth Rate, Yield and Biological Efficiency

The mushroom bags were incubated in incubation room in the mushroom farm. The spawn running was observed every day. When mycelium reached the shoulder of the mushroom bag, it was marked as starting point to measure the length of the mycelium run. After 3 days, it was marked again and the length was measured. The same method was repeated until the bag was fully colonized by the mycelium. Three readings of the length were measured per bag to determine the average reading. Mycelial growth rate (MGR) was determined by plotting length of the mycelial growth (cm) against incubation time (days) and calculating the gradient of the curve (Wang *et al*., 2015). Other than mycelial growth rate, the density of mycelium also was observed. The bags with full mycelial growth were then transferred into the mushroom cultivation house.

The bags in the mushroom house were slit to allow the primordia to mature and produced sporophores of *S. commune*. The slit bags were watered every day by spraying them regularly to maintain the optimum humidity for *S. commune* cultivation. The mature fruits were harvested and weighed. The mature fruit of *S. commune* was light to gold in colour and bloom. The yield of *S. commune* was determined by weighing the sporophore every bag. The average weight from replicates bag that produced sporophores was calculated.

Mushroom yield (MY) and biological efficiency (BE) of the *S. commune* cultivation was calculated by using the formulas as follow;

Mushroom yield (g/bag) - (Wang *et al*., 2015)

$$= \frac{\text{Total weight of sporophores/fruiting bodies}}{\text{Bag}}$$

BE (%) - (Moonmoon *et al.*, 2011)

$$= \frac{\text{Weight of fresh fruiting bodies}}{\text{Weight of dry substrate}} \times 100\%$$

3.6.2.7 Analysis of C/N ratio and NPK Assessment of Spent Substrate After Cultivation

A 100 g of sample from the optimized substrate was taken after *S. commune* cultivation. The sample was weighed before its been dried in the oven in the Multipurpose Laboratory of PPBGL. The dried sample was weighed again to calculate the moisture content of the optimized substrate before placed into the zip lock plastic bag. The sample was sent to FRIM for CNPK analysis. Only one sample was sent for the analysis of C/N ratio and NPK assessment of spent substrate after the cultivation.

3.7 Assessment of Compost and Vermicompost as Organic Fertiliser

After 30, 60 and 90 days of composting, the colour and texture of compost were observed. At the same time, 100 g of sample was taken from each compost formulation and sent to FRIM to analyze its C/N ratio and NPK content. Meanwhile, in vermicomposting, the colour, texture and number of *L. rubellus* in vermicompost in each worm bin were observed after 30, 60 and 90 days of vermicomposting. Like compost, 100 g sample of vermicompost also was taken from each vermicompost formulation and sent to FRIM for CNPK analysis.

CHAPTER 4: RESULT AND DISCUSSION

All the result in this study were presented using the picture, graph and table followed by the discussion of the findings.

4.1 Physical and Chemical Characteristics of SMS during Composting

In this study, composting of spent *S. commune* substrate mixed with goat manure (GM) were run for 90 days without any additional organic wastes added. At the end of the duration, compost was odorless and dark in colour compared to the initial appearance. The difference can be seen in Figure 4.1. Sarkar *et al*. (2016) reported the matured compost obtained in their study was dark in colour without any foul smell.

Initial Final

Figure 4.1: Initial and final appearance of compost after 90 days of composting.

The final volume of the compost was reduced. It was due to the decomposition process of the microbes in the composting pile that decreased the particle size of organic wastes. Fornes *et al*. (2012) reported the downsize of particle size as composting process was caused by the fragmentation and decomposition of larger particles.

4.1.1 Temperature

The temperature of composting for three different mixture ratios, CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM) along 90 days of process was shown in Figure 4.2.

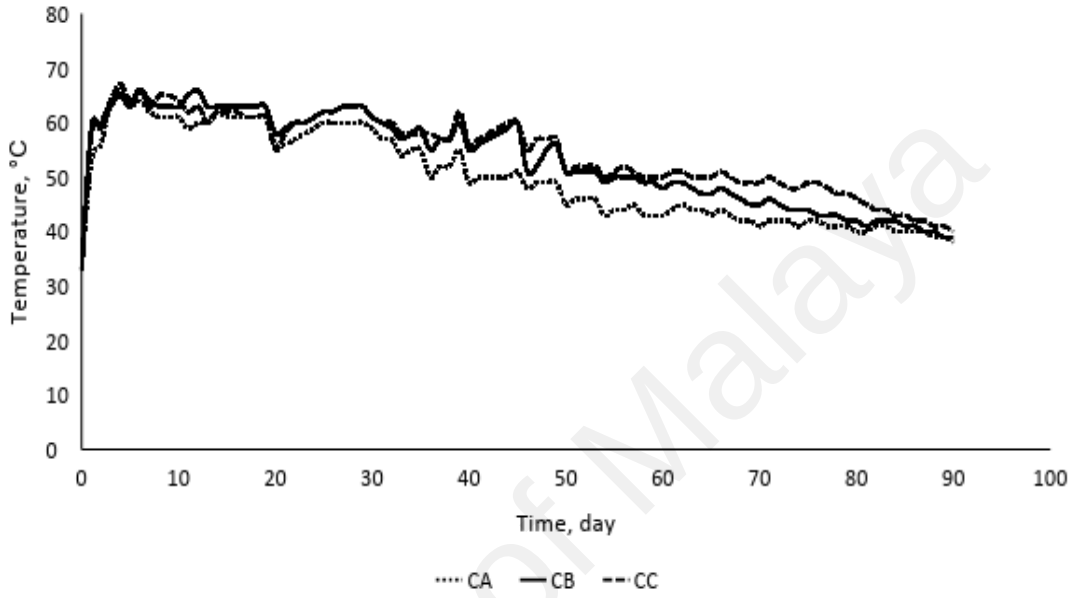


Figure 4.2: Temperature of composting in CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM).

Based on Figure 4.2, the highest temperature recorded was CA (66°C), CB (66°C) and CC (67°C) on day 4 except for CB on day 6 and 12, while the lowest temperature was CA (38°C), CB (39°C) and CC (40°C) measured on day 90. After 90 days of composting, the temperature had not reach the ambient temperature level measured at 32-34 °C which means the compost had not fully achieved its maturity and stability. It might due to the high organic wastes used to build the composting pile hence need a longer time to completely degrade into mature compost where Zhang and Sun (2014) stated composting usually need 90 to 270 days to achieve stability and maturity.

The temperature increased to the thermophilic range for effective composting (50-65°C) on day 1 for all compost formulation. Zhang and Sun (2014) reported the same

finding in co-composting of green waste mixed with 35% or 55% of SMC and 20% or 30% of biochar. In addition, CA, CB and CC were able to retain the best temperature range for thermophiles in hot composting (50-65°C) for more than 30 days. The temperature range (50-65°C) was the most optimal temperature for thermophiles for them to produce high quality compost and effectively kill the pathogens (Bai & Wang, 2011). Besides, water retention of SMS may contribute to the longer duration of hot composting (Zhang & Sun, 2014).

The temperature rose rapidly until day 4 then decreased slightly before it increased again several times and finally reduced slowly until the end of the biodegradation. Initially, the large compost pile was undertaken self-heating and induced the temperature inside the pile until it achieved thermophilic phase. The condition allowed thermophilic microorganisms which increase in population at relatively high temperature carried out decomposition at maximum level thus sanitized the compost by destroying the pathogens (Sarkar *et al*., 2016).

The microbes in the pile used oxygen and broke down the organic matters (Kulcu *et al*., 2008). As a result, heat, carbon dioxide and water released (Kulcu *et al*., 2008). The heat released will heat up the compost pile and the high temperature was sustained by the large pile that was able to trap the heat for a longer time (Sarkar *et al*., 2016). The temperature increased repeatedly because of the turning process. Turning the compost allowed continuous oxygen supply hence accelerated the microbe activity (Sarkar *et al*., 2016). In the end of the process, the temperature reduced slowly because the number of organic matters left to breakdown declined and the composting process was in mesophilic phase also known as maturation phase until it achieves stability (Lim *et al*., 2016).

The compost in this project achieved sanitation standard where hot composting that last longer than three days meet sanitation requirement and free from pathogens (Zhang

& Sun, 2014). Specifically, temperature higher than 55°C are needed to destroy the pathogens in the organic wastes (Lim *et al*., 2016). According to the EPA guidelines for organic wastes sanitization, the temperature of 55°C need to be maintain for 5 consecutive days or at least 15 days (Lim *et al*., 2016). All of our composting materials, CA, CB and CC reach the sanitization temperature level (55°C) on day 2 and maintain the level for more than 30 consecutive days.

The result suggested compost CC with formulation 50 SMS:50 GM showed the better condition for thermophiles where the optimum high temperature (50-65°C) for accelerated degradation and higher quality compost production was last longer which was 66 days followed by compost CB (52 days) then compost CA (38 days).

4.1.2 pH

The pH of composting for three different mixture ratios, CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM) is shown in Figure 4.3.

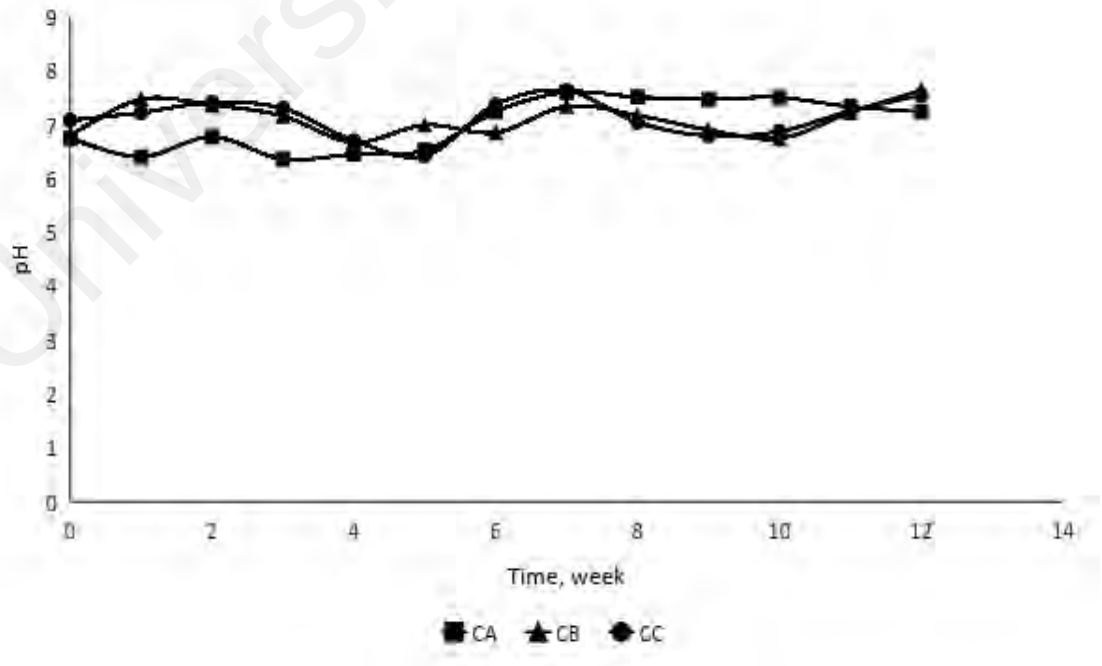


Figure 4.3: pH of composting in CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM).

The result in Figure 4.3 shows that the pH remained neutral along the process where the lowest pH was 6.36 at week 3 recorded in CA and the highest pH was 7.64 at week 12 belong to CB. The initial pH of CA (6.74), CB (6.8) and CC (7.08) was affected mostly by the organic matters used in the feedstock where it clearly indicated the pH of feed materials (SMS and GM) in this study was in neutral condition (Soobhany *et al*., 2015).

The pH changed from time to time throughout the process could be due to initial decarboxylation, ammonification, mineralization, nitrification and formation or breakdown of organic acids which released from the organic matters (Fornes *et al*., 2012). Moreover, ammonification that dominated the process at thermophilic condition also influenced the pH change (Zhang & Sun, 2014).

However, there was no specific range for the best pH in composting process but the optimal pH for microbial activity was between pH 6.5 to 8.0 (Lim *et al*., 2016; Soobhany *et al*., 2015). The temperature measured along the process was optimal for the microbe activities. The final pH of CA (7.24), CB (7.64) and CC (7.54) values were closed to neutral, indicating that the end products could be useful for remediating acid soils (Huang *et al*., 2014).

4.1.3 Moisture

The moisture content of composting process for three different mixture ratios, CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM) is shown in Figure 4.4.

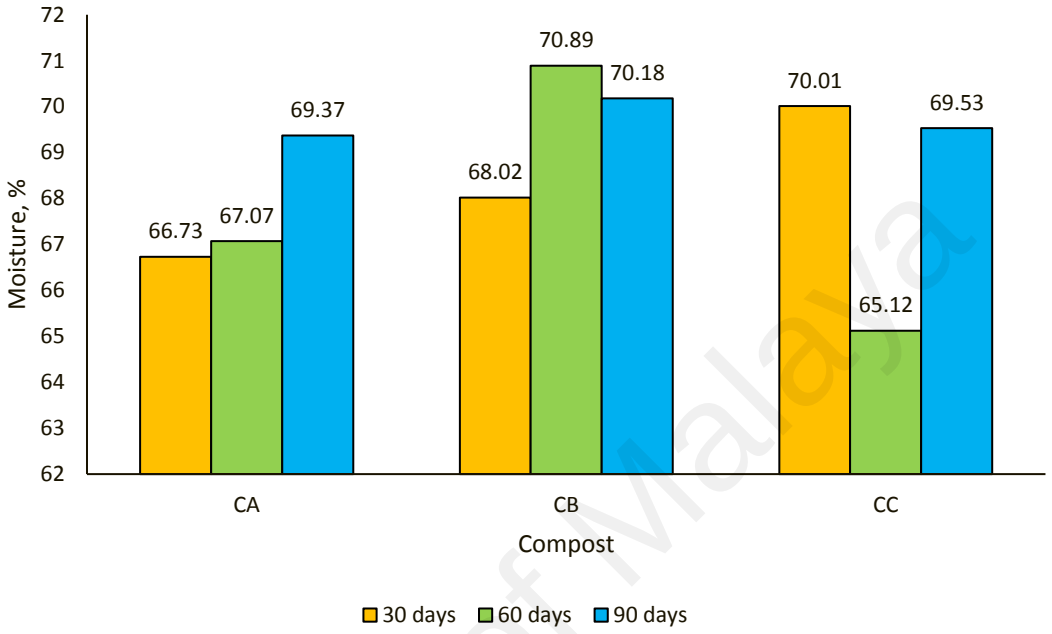


Figure 4.4: Moisture content of composting in CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM).

From Figure 4.4, the moisture content of composting in this study was between 65.12% and 70.89%. The favorable moisture content of composting was between 55% and 75% (Lim *et al*., 2016). Therefore, the humidity in the composting process was still in the range of the favorable moisture content.

Sarkar *et al*. (2016) reported the thermophilic condition could be observed when moisture content was around 60%. Comparatively, the moisture of thermophilic composting in this project which occur mostly at the beginning of the process was close to the optimum value of the thermophilic humidity requirement, making the thermophilic phase in this composting process was last longer for all the compost formulation.

Controlling the moisture during composting process was very important. Low moisture content inhibits microbial activity while high moisture content than the optimum moisture range would slow decomposition and disturb aeration which might cause anaerobic degradation (Sarkar *et al*., 2016). Besides, excessive humidity caused foul smell and nutrient leaching (Sarkar *et al*., 2016).

4.1.4 CNPK Content

The CNPK content of compost CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM) at day 30, day 60 and day 90 of composting was shown in Table 4.1.

Table 4.1: The macronutrients elements of compost CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM) at day 30, 60 and 90 of composting.

Macro-nutrient Elements	**CA**			**CB**			**CC**		
	30	**60**	**90**	**30**	**60**	**90**	**30**	**60**	**90**
C (%)	35.07	34.77	35.36	36.17	34.51	34.89	33.52	38.43	37.58
N (%)	0.79	0.78	1.04	0.85	1.04	1.54	1.06	1.02	1.61
P (%)	-	-	0.69	-	-	0.74	-	-	0.73
K (%)	-	-	0.58	-	-	0.63	-	-	0.81
C/N ratio	44.39	44.58	34	42.55	33.18	22.66	31.62	37.68	23.34

The result in Table 4.1 shows C/N ratio of all compost in this work decreased after 90 days of biodegradation. The lowest C/N ratio in CA, CB and CC was 34, 22.66 and 23.34 respectively, calculated on day 90 of composting. Meanwhile, the highest C/N ratio of compost CA, CB and CC was 44.58, 42.55 and 37.68 respectively, recorded on either day 30 or day 60 of composting.

Jamaludin *et al*. (2012) reported the C/N ratio and N:P:K content of compost with formulation 50:50 of SMS:GM in their study were 32.14 and 1.01:0.47:0.35 respectively

at week 10 (day 70) while at week 20 (day 140) of composting, the C/N ratio was 17.99 and N:P:K content was 1.55:0.95:0.80, also for 50/50 SMS/GM. In comparison to their result, the C/N ratio and N:P:K content obtained at day 90 of composting for the same mixture ratio in this research were 23.34 and 1.61:0.73:0.81 respectively.

The finding in this work and in Jamaludin *et al*. (2012) indicated the longer duration of composting would improve the compost quality in terms of C/N ratio and NPK content. However, NPK content of compost at day 90 in this project was slightly better than the compost produced at week 20 (day 140) in Jamaludin *et al*. (2012) due to the different composting technique used where this study using larger pile system that had longer thermophilic phase. Meanwhile, Jamaludin *et al*. (2012) using plastic containers where the compost was one of the control for their vermicomposting study.

The main factors in C/N ratio change were combination of organic matters used in the composting and the duration of biodegradation process (Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012). Carbon content for all compost formulation was between 33.52% and 38.43%. Total carbon content changed through decomposition process by microbes where they consumed carbon as their main source of energy. Some of the carbon lost via microbial respiration process in form of CO_2 (Ndegwa & Thompson, 2000; Jamaludin *et al*., 2012).

The highest nitrogen content in compost CA, CB and CC also recorded at day 90 of composting. Specifically, compost CC had the highest total N content, 1.61% followed by compost CB with total N content of 1.54% then compost CA, 1.04%. The increase of GM usage in the composting formulation resulted in elevated of total N content in compost where compost CC was formulated by using 50:50 SMS:GM while compost CB and CA mixed with 40% and 20% GM respectively.

The same finding was reported by Jamaludin *et al*. (2012) where the higher usage of GM in their study improved the quality of compost in terms of C/N ratio and NPK content. GM that was naturally had high NPK content enhanced the total N content in the compost (Loh *et al*., 2005; Jamaludin *et al*., 2012). The total N content also influenced by other factors such as ammonification, nitrification, mineralization, immobilization, denitrification and nutrient leaching (Fornes *et al*., 2012).

Ammonification and nitrification by microbes transformed organic nitrogen in organic wastes into ammonia then converted into nitrite and nitrate. Excess nitrogen from decomposition process was released into the compost hence increased their total N content. This process is also known as mineralization (USDA, 2011). Furthermore, SMS as the main organic matter in this research was less biodegradable due to its high lignocellulose content and the characteristic helped reduce the nitrogen loss during the process (Bai & Wang, 2011).

Other potential factors that might contribute to the total N content in the compost were nitrogen fixation process by free living bacteria and loss of water during evaporation (Jamaludin *et al*., 2012). The total P and K content were analyzed at day 90 only to indicated the NPK content of the final products. For total P content, compost CB had the highest value, 0.74% while the lowest total P content obtained in compost CA, 0.69%. In terms of total K content, compost CC had the highest percentage, 0.81% while the lowest percentage calculated in compost CA, 0.58%.

Total P and K content also influenced by the biological microbial activity. The microbes in feedstock used the nutrients for their body synthesis and released remaining minerals in mineralized form (Loh *et al*., 2005). Other than that, endogenic and exogenic enzymes in microbes contributed to the total K content in compost (Azizi *et al*., 2014). Meantime, total P content affected by mineralization and mobilization of phosphorus as

a result of phosphate activity in microbes (Jamaludin & Mahmood, 2010; Azizi *et al*., 2014).

4.2 Physical and Chemical Characteristics of SMS during Vermicomposting

In this study, like composting, vermicomposting of spent *S. commune* substrate also mixed with GM and run for 90 days without added of any additional organic wastes along the process. At the end of the process, vermicompost were odorless and dark in colour. In contrast, vermicompost texture was finer and its colour was darker than compost. Figure 4.5 showed the initial and final appearance of vermicomposting.

Initial Final

Figure 4.5: Initial and final appearance of vermicompost after 90 days of vermicomposting.

Lim *et al*. (2016) stated that the compost texture was coarser while vermicompost was finer instead. The vermicompost under electron microscope was smaller and more scattered than the initial wastes making its texture was finer (Lim *et al*., 2016). Meanwhile, Azizi *et al*. (2011) reported vermicompost produced from high percentage of SMC combined with sewage sludge had fine texture, dark colour and odourless compared to their initial appearance. Like composting, the final volume of vermicompost in this work also declined due to the decrease of the particle size during the breakdown process

of organic matters by joint action of microbes and earthworm activity in vermicomposting (Fornes *et al*., 2012).

After 90 days of vermicomposting, the population of *L. rubellus* in VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM) was increased. Moreover, the earthworm population in VB and VC was obviously higher compared to VA. The highest *L. rubellus* population could be observed in VC. The result was similar to Azizi *et al*. (2014) where the mixture ratio 1:1 of SMC:GM showed the highest *L. rubellus* number.

The multiplication and growth of earthworm population were influenced by their food quality (Aira *et al*., 2006). The increase of the earthworm population indicated the mixture formulation in VB and VC was suitable for *L. rubellus* growth. In addition, the high population of earthworm might due to the high nitrogen content in GM. According to Shahack-Gross (2011), the most suitable dung used in vermicomposting was herbivore dung because it rich in macroscopic and microscopic organic matters that favored the earthworms diet as well as enable the microflora inside their intestine decomposed the organic matter into more stable nutrient (Azizi *et al*., 2014).

4.2.1 Temperature

The temperature of vermicomposting for VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM) is shown in Figure 4.6.

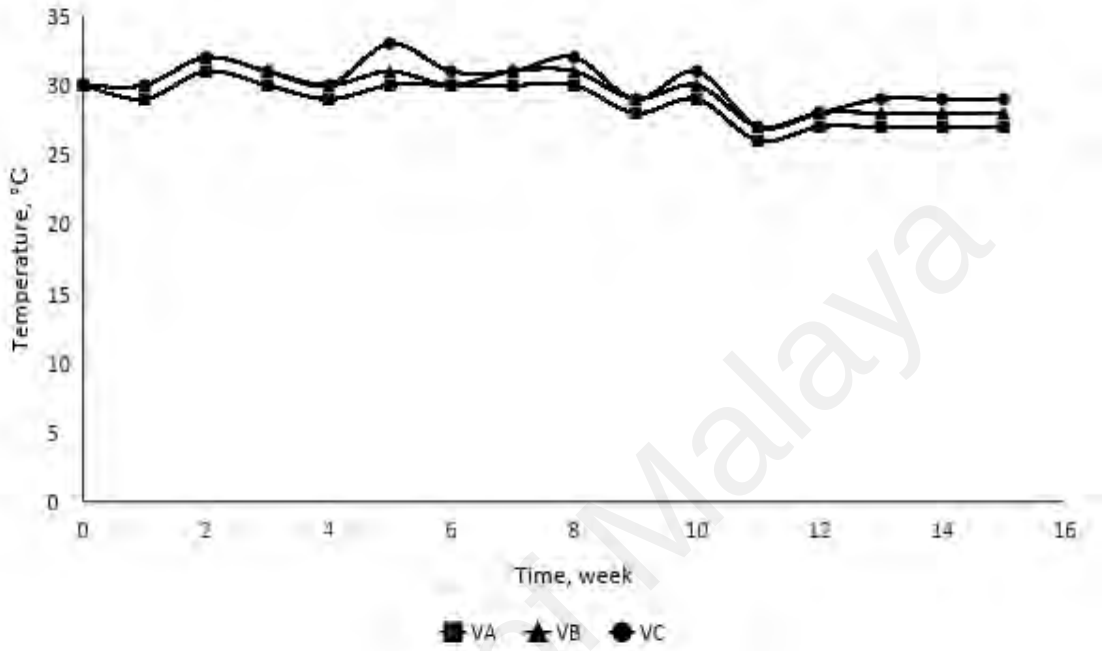


Figure 4.6: Temperature of vermicomposting in VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM).

From Figure 4.6, the temperature remained in mesophilic condition throughout the process where the lowest temperature in VA, VB and VC was 26°C, 27°C and 27°C respectively measured on week 11. Meanwhile, the highest temperature in VA, VB and VC was 31°C, 32°C and 33°C respectively recorded on week 2 except for VC on week 5.

Azizi *et al*. (2011, 2013, 2014, 2015) maintained the temperature at 27±1°C for all the vermicomposting process using *L. rubellus* in their studies. Mesophilic condition range between 25°C and 40°C was crucial in vermicomposting for the earthworms to preserve their large population where the low temperature enable them to grow, multiply and decompose organic matters (Fornes *et al*., 2012). Overall, the temperature measured

along of the vermicomposting process in this project was still at the optimal temperature range for the microbes and earthworms' activities.

4.2.2 pH

The pH of vermicomposting for VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM) is shown in Figure 4.7.

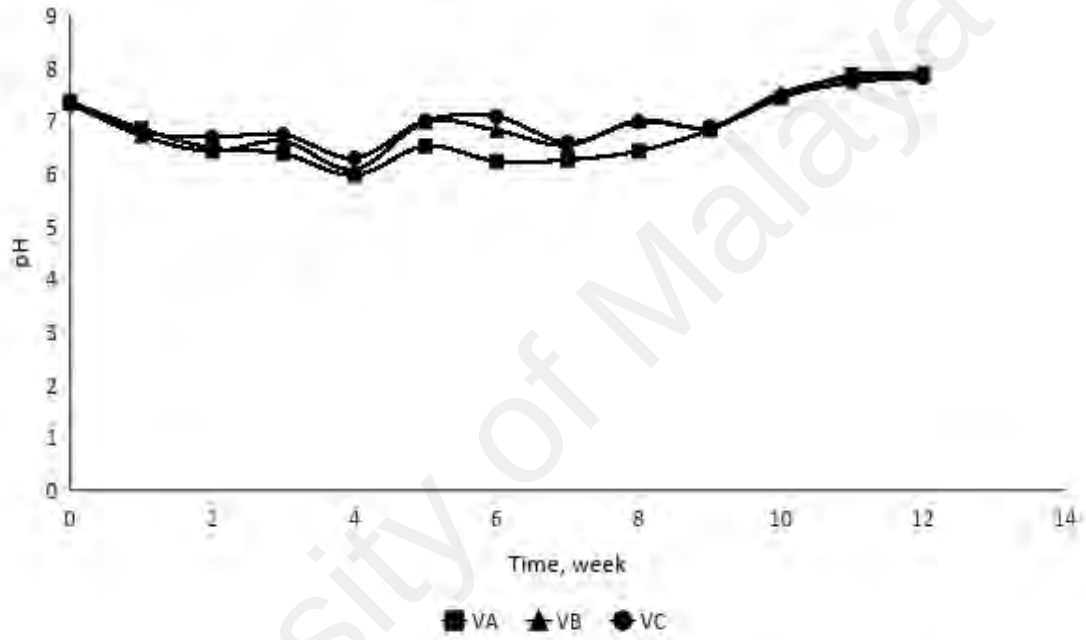


Figure 4.7: pH of vermicomposting in VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM).

Based on Figure 4.7, at the beginning, the pH was in neutral condition, VA (7.36), VB (7.33) and VC (7.31) then it decreased and became slightly acidic at week 4. The lowest pH obtained in VA, VB and VC was 5.97, 6.07 and 6.29 respectively. After week 4, the pH enhanced and became basic or alkaline where the highest pH in VA, VB and VC was 7.88, 7.67 and 7.81 respectively at week 12.

Like composting, the pH depended more on the release of organic acids from the organic matters during the biodegradation process of the microbes and earthworms (Fornes *et al*., 2012). Hence, pH could be varying according to the types and amount of

the organic matters used in the process. According to Soobhany *et al*. (2015), the pH reduction could be the outcome from the production and accumulation of organic acids during the decomposition of polysaccharides in the active decomposition phase.

The increase in pH after week 4 was due to the inoculation of *L. rubellus* where the same result showed by Soobhany *et al*. (2015) that reported the pH increased after *E. eugeniae* introduced into their feed materials. Moreover, SMS contained large number of nitrifying bacteria which dominated mesophilic condition hence contribute to the pH change (Zhang & Sun, 2014).

The best range of pH for vermicomposting was around neutral to maintained the large population of earthworm (Fornes *et al*., 2012). Azizi *et al*. (2011, 2013, 2014, 2015) sustained the pH at 7±1 during vermicomposting using *L. rubellus* where SMC of *P. Sajor-caju* as a bulking agent. Lim *et al*. (2016) reported the pH range for vermicomposting was between pH 5 to 8. To conclude, the pH throughout the vermicomposting process in this study was at optimal range.

4.2.3 Moisture

The moisture content of vermicomposting for VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM) is shown in Figure 4.8.

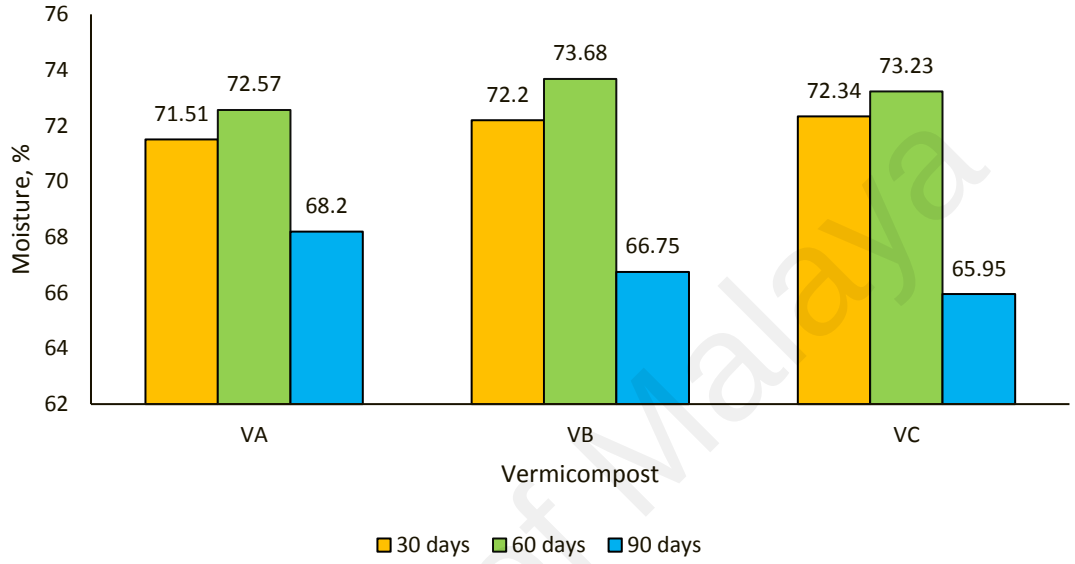


Figure 4.8: Moisture content of vermicomposting in VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM).

The result in Figure 4.8 showed vermicomposting humidity was between 65.95% and 73.68%. Vermicomposting need to be sustained at high humidity (70-90%) to preserve the large population of the earthworms so that the process could be carried out rapidly (Fornes *et al*., 2012). Azizi *et al*. (2011, 2013, 2014, 2015) maintained the moisture content at 70±10% in their all study regarding of vermicomposting. Meanwhile, the humidity range used in a study by Jamaludin *et al*. (2012) was between 60% and 70%.

Like composting, controlling the moisture content along vermicomposting process also was crucial. Low humidity would inhibit the earthworm and microbial activity while excessive humidity would cause anaerobic decomposition, foul smell and nutrient leaching (Sarkar *et al*., 2016). Overall, the moisture content of vermicomposting in this

project was at optimum parameter (Azizi *et al*., 2011, 2013, 2014, 2015; Fornes *et al*., 2012; Jamaludin *et al*., 2012).

4.2.4 CNPK Content

The CNPK content of vermicompost VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM) at day 30, day 60 and day 90 of vermicomposting was shown in Table 4.2.

Table 4.2: The macronutrients elements of vermicompost VA (80 SMS:20 GM), VB (60 SMS:40 GM) and VC (50 SMS:50 GM) at day 30, 60 and 90 of vermicomposting.

Macro-nutrient Elements	VA			VB			VC		
	30	60	90	30	60	90	30	60	90
C (%)	37.80	36.36	37.12	39.17	37.98	35.91	31.95	36.66	32.76
N (%)	0.50	0.95	0.96	1.10	1.09	1.23	1.20	1.31	1.51
P (%)	-	-	0.56	-	-	0.70	-	-	0.81
K (%)	-	-	0.85	-	-	0.92	-	-	0.97
C/N ratio	75.6	38.27	38.67	35.61	34.84	29.2	26.63	27.98	21.7

The result in Table 4.2 shows C/N ratio of vermicompost was decreased where the lowest C/N ratio in VA, VB and VC was 38.27, 29.2 and 21.7 respectively, calculated on day 90 of vermicomposting except for VA on day 60. Meanwhile, the highest C/N ratio of vermicompost VA, VB and VC was 75.6, 35.61 and 27.98 respectively, recorded on day 30 of vermicomposting except for VC on day 60.

Jamaludin *et al*. (2012) reported the C/N ratio and N:P:K content of vermicompost at week 10 (day 70) were 23.26 and 1.31:0.80:0.40 respectively when using mixture ratio of 50:50 SMS:GM while at week 20 (day 140), the C/N ratio and N:P:K content were 13.62 and 1.55:1.17:0.68 respectively. For the same method (vermicomposting) using

same earthworm (*L. rubellus*) and same formulation (50:50 SMS:GM), this project found out the vermicompost had C/N ratio of 21.7 and N:P:K content was 1.51:0.81:0.97.

The findings had proven the longer duration of vermicomposting would decreased the C/N ratio of vermicompost. Furthermore, the NPK content also increased except for the total K content. To be reminded here, Jamaludin *et al*. (2012) using sawdust based SMS in their study while this research had chosen *S. commune* SMS where the standard formulation of *S. commune* consisted mainly of sawdust supplemented with rice bran and $CaCO_3$.

Azizi *et al*. (2014) reported C/N ratio and N:P:K content of *P. sajor-caju* SMC:GM at ratio 1:1 were 24.34 and 1.21:0.49:1.28 respectively at day 70 of vermicomposting using *L. rubellus* while the vermicompost in this work that used the same mixture ratio had C/N ratio of 21.7 and N:P:K content was 1.51:0.81:0.97 at day 90 of vermicomposting. It was again approved that longer period of vermicomposting enhanced vermicompost quality.

Vermicomposting starting materials and the duration of biodegradation process influenced the C/N ratio where the longer decomposition reduced the C/N ratio number (Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012). Jamaludin *et al*. (2012) concluded the higher usage of GM in their vermicomposting formulation generated the lower C/N ratio in vermicompost product because GM was naturally high in NPK content (Loh *et al*., 2005; Jamaludin *et al*., 2012).

The earthworms and microbes needed carbon and nitrogen to sustained their life and population where the carbon requirement for their growth was greater than the nitrogen (Ndegwa & Thompson, 2000). The ideal proportion for initial C/N ratio in vermicomposting was 30:1 so that the earthworms and microbes were able to decompose the organic matters and used the nutrients required for their growth as well as left some

excess nitrogen in vermicompost (Lim *et al*., 2016). Vermicompost VA and VB showed the C/N ratio over than 30 at day 30 and 60 of vermicomposting which indicated the feed materials was not at right proportion for earthworms and microbes although the initial C/N ratio was not analyzed.

Carbon content for all vermicompost formulation was between 31.95% and 39.17%. The changed of total C content throughout the decomposition process could be attributed to the carbon loss via CO_2 production during respiration process of earthworms and microbes (Jamaludin *et al*., 2012; Soobhany *et al*., 2015). Carbon also had function as a main energy for the earthworms and microbes (Ndegwa & Thompson, 2000; Soobhany *et al*., 2015).

The highest nitrogen content in vermicompost VA, VB and VC was 0.96%, 1.23% and 1.51% respectively, recorded at day 90 of vermicomposting. The earthworms in vermicomposting contributed to the change of total N content as a result of its symbiotic decomposition with the microbes in their intestine (Azizi *et al*., 2014). Furthermore, total N content increased through addition of nitrogen by the earthworms in form of mucus, enzymes and nitrogenous excrements (Soobhany *et al*., 2015). The total N content also influenced by other factors such as ammonification, nitrification, mineralization, immobilization, denitrification and nutrient leaching (Fornes *et al*., 2012).

Like compost, the total P and K content were analyzed at day 90 of vermicomposting only to indicate the NPK content of the final products. For total P content, vermicompost VC had the highest value, 0.81% while the lowest total P content obtained in vermicompost VA, 0.56%. In terms of total K content, vermicompost VC has the highest percentage, 0.97% while the lowest total K content was calculated in vermicompost VA, 0.85%. Total P and K content influenced by the biological microbial activity where the

microbes either in feedstock or earthworm gut used the nutrients for their body synthesis and release remaining minerals in mineralized form (Loh *et al*., 2005).

4.3 Potential of Compost and Vermicompost as Organic Fertiliser

C/N ratio is an indicator for the composted and vermicomposted materials to be regarded as a good organic fertiliser or otherwise (Jamaludin & Mahmood, 2010; Azizi *et al*., 2015; Soobhany *et al*., 2015). From the literature review, C/N ratio between 15 and 25 revealed a satisfactory degree of the stabilization and maturity of organic wastes hence accepted as an organic fertiliser (Soobhany *et al*., 2015). Therefore, Table 4.3 showed the compost CA, CB and vermicompost CC which had C/N ratio within the optimum range and could be used as an organic fertiliser after 90 days of composting and vermicomposting.

Table 4.3: The macronutrients elements of compost CB (60 SMS:40 GM), CC (50 SMS:50 GM) and vermicompost VC (50 SMS:50 GM) at day 90 of composting/vermicomposting.

Macronutrient Elements	**Compost/Vermicompost**		
	CB	**CC**	**VC**
C (%)	34.89	37.58	32.76
N (%)	1.54	1.61	1.51
P (%)	0.74	0.73	0.81
K (%)	0.63	0.81	0.97
C/N ratio	22.66	23.34	21.7

Based on Table 4.3, compost CC had the highest nitrogen content, 1.61% while vermicompost VC had the highest P (0.81%) and K (0.97%) content. Therefore, compost CC and vermicompost VC with same formulation (50 SMS:50 GM) were the best compost and vermicompost obtained in this project.

USDA (2011) stated a feed material with C/N ratio of 25 was an almost perfect balanced diet for soil microorganisms that required exactly C/N ratio of 24:1 for its energy and body maintenance. If feed material with C/N ratio of 25 was added to the soil, the soil microbes would consume it quickly without leaving any excess carbon or nitrogen (USDA, 2011).

Hence, compost CB, CC and vermicompost VC that had C/N ratio lower than 25 would cause temporary mineralization if applied to the soil because the soil microbes would consume the compost and left the excess nitrogen in the soil. As a result, soil fertility enhanced and crops could use the nitrogen for their growth (USDA, 2011).

Composting system in this research dominated by thermophilic composting or hot composting. Oxidation process by the thermophiles at high temperature was faster than in mesophilic condition, making it beneficial for fertiliser quality enhancement (Sarkar *et al*., 2016). Besides, the hot composting destroyed and killed the pathogens and weed seeds making the final sanitation compost as safe to be applied into the soil and crops (Sarkar *et al*., 2016).

In vermicomposting, the earthworms which mixing and grinding the organic matters reduced their particle size as well as increased the surface area of the matters resulted the rapid decomposition of microbes in the feedstock and earthworms' intestine (Huang *et al*., 2014). Moreover, rapid growth of the earthworms increased the decomposition thus enhanced the carbon loss (Jamaludin & Mahmood, 2010). Besides, the vermicomposting effectively removed the heavy metals content (Azizi *et al*., 2011, 2013, 2014, 2015).

All the good characteristics of composting and vermicomposting in this study enable the production of high quality compost and vermicompost in 90 days. To conclude, this

research had proven that SMS of *S. commune* can be used to produce a good organic fertiliser with high NPK content.

4.4 Compost and Vermicompost as Substrate for *Schizophyllum commune* Cultivation

In this study, the end product of composting and vermicomposting was used as fruiting substrate for *S. commune* to determine whether they can also be utilized for mushroom cultivation besides as organic fertiliser. Table 4.4 shows the C/N ratio and NPK content of *S. commune* standard formulation, *S. commune* SMS, compost (CA, CB, CC) and vermicompost (VA, VB, VC) at day 90 of composting/vermicomposting. The *S. commune* standard formulation was prepared by using sawdust as the main substrate and supplemented with rice bran and $CaCO_3$.

Table 4.4: C/N ratio and NPK content of *S. commune* standard formulation, *S. commune* SMS, compost (CA, CB, CC) and vermicompost (VA, VB, VC) at day 90 of composting/vermicomposting.

Substrate	Macronutrients element (%)				C/N ratio
	C	N	P	K	
Standard Formulation	40.1	0.65	0.12	0.53	61.69
SMS	40.82	0.61	0.15	0.47	66.92
CA	35.36	1.04	0.69	0.58	34.00
CB	34.89	1.54	0.74	0.63	22.66
CC	37.58	1.61	0.73	0.81	23.34
VA	37.12	0.96	0.56	0.85	38.67
VB	35.91	1.23	0.70	0.92	29.2
VC	32.76	1.51	0.81	0.97	21.7

From Table 4.4, C/N ratio of standard formulation substrate is 61.69. The standard formulation of *S. commune* cultivation in Biotechnology Research Centre Glami Lemi University Malaya (PPBGL) used sawdust as the main fruiting substrate and supplemented with rice bran and $CaCO_3$. The C/N ratio of the standard formulation was lower than its SMS, 66.92.

The C/N ratio was affected by their total C and N content where SMS had higher total C content than the standard formulation and vice versa for the total N content. Other than that, total P content was increased after cultivation and vice versa for the total K content. The difference of CNPK content in the standard formulation and its SMS was due to the nutrients uptake and nutrients release during mycelial growth and fruiting formation of *S. commune* (Chang & Miles, 2004).

Like other species, mushrooms also need carbon and nitrogen for their body maintenance and life activities. Carbon source plays an important role in the structural and energy requirements of the mushroom cells to perform their activities. Meanwhile, the nitrogen is crucial in synthesis of proteins, purines, pyrimidines, chitin and other important elements for mushroom life (Chang & Miles, 2004).

Phosphorus and potassium are other macro elements and minerals which also required in mushroom life cycle. Phosphorus is present in adenosine triphosphate (ATP), nucleic acids and phospholipid. Meanwhile, potassium is the most abundant metallic element found in fungi. Potassium is also a cofactor in some enzyme systems as well as involved in carbohydrate metabolism and maintenance of ionic balance (Chang & Miles, 2004).

The CNPK content in the standard formulation was not far different from its SMS. The finding was useful for the future study where SMS of *S. commune* might have potential to reutilized as a fruiting substrate for *S. commune* cultivation by supplementing it with

other materials to cover back the nutrient loss without went through the composting or vermicomposting process. This finding can be used by other researchers as a reference when *S. commune* SMS was reused as a fruiting substrate for other mushrooms.

Table 4.4 also showed all compost and vermicompost that used as fruiting substrate for *S. commune* cultivation in this project had lower C/N ratio compared to the standard formulation due to the decomposition process. However, they had higher NPK content than the standard formulation. The potential of compost and vermicompost in *S. commune* cultivation was revealed after optimization was carried out as follows.

4.4.1 Unsterilized Compost as Substrate

The main reason why unsterilized compost was tested in this study because the thermophilic composting sanitized the compost and it might have potential to be used as a fruiting substrate for *S. commune* without autoclaving it.

CA CB CC

Figure 4.9: *Schizophyllum commune* mycelial growth using unsterilized compost CA (80 SMS:20 GM), CB (60 SMS:40 GM) and CC (50 SMS:50 GM).

Figure 4.9 shows *S. commune* being cultivated using unsterilized composts. Mycelium only grew surrounding the spawn and did not colonized the substrate and eventually, the

mycelium died after 30 days of incubation. There was no fruiting recorded during *S. commune* cultivation trial using unsterilized compost obtained from day 30, day 60 and day 90 of composting.

Unsterilized compost did not support growth of *S. commune*. This was due to some competitor microbes that might still be present in the substrate which inhibited *S. commune* mycelial growth although the compost achieved sanitation standard which need at least consecutively 3 days of high temperature (Sarkar *et al*., 2016). Initially, the substrate allowed the mycelial grew near the spawn but then the mycelial died because the competitor microbes might quickly gain dominance and prevented the *S. commune* mycelium from developing and colonized the substrate (Chang & Miles, 2004).

Vos *et al*. (2017) stated the unsterilized compost used in commercial *A. bisporus* cultivation was still rich in microbes even though they recorded 80°C as the highest temperature. The compost allowed the mushroom grew because the phase II in the composting process removed unwanted ammonia, reduced number of potentially harmful microbes and suppressed the mushroom competitors. The phase II had pasteurization stage where the steam was supplied to destroy spores of contaminating microorganism (Vos *et al*., 2017). This indicates that compost for *S. commune* cultivation needs additional heat treatment by pasteurization to a higher temperature than during composting recorded at 67°C. Therefore, further optimization as fruiting substrate was done using sterilized compost.

4.4.2 Sterilized Compost and Vermicompost as Substrate

The next trial was tested with sterilized compost and vermicompost without any modification. The autoclave used in sterilization of compost and vermicompost was set at 121°C for 90 minutes.

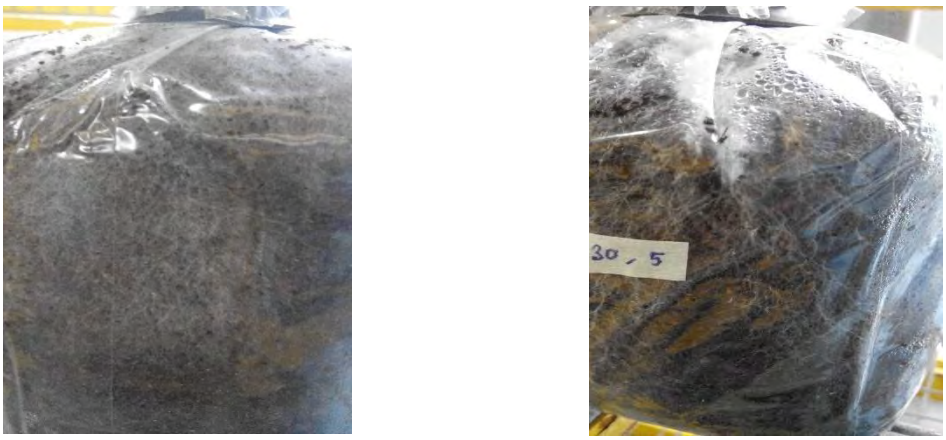
CC VC

Figure 4.10: *Schizophyllum commune* mycelial growth using sterilized compost CC (50 SMS:50 GM) and vermicompost VC (50 SMS:50 GM).

Figure 4.10 which also represent the result of other mushroom bags using CA, CB, VA and VB showed the mycelial growth was not uniform and the mycelial only grew on the certain area in the substrate. Fruiting bodies did not develop and mature even after mycelium colonization. This might be due to the high moisture content of the substrate that reduces aeration and produced sparse mycelial growth. Optimal moisture content to cultivate *S. commune* was 60% but moisture content of compost and vermicompost in this work was over 65% (Figlas *et al*., 2014).

4.4.3 Effect of Moisture and pH Adjustments of Sterilized Compost and Vermicompost

Referring to the previous result, the moisture content of compost and vermicompost was reduced via sun drying. Since the pH was increased after the drying process, wood vinegar was added to reduce the pH until the substrate reached the optimal pH for *S. commune* cultivation, between pH 5 and 6.



CC VC

Figure 4.11: *Schizophyllum commune* mycelial growth using adjusted sterilized compost CC (50 SMS:50 GM) and vermicompost VC (50 SMS:50 GM).

Figure 4.11 showed *S. commune* mycelial growth using adjusted sterilized compost CC and vermicompost VC. The figure also represents the result for the mushroom bags using CA, CB, VA and VB. Based on the figure, modification of moisture and pH of compost and vermicompost allowed uniform mycelial growth however, still thin to support fructification. This indicates the lack of nutrient content in the substrate to support formation of fruiting bodies in mushroom.

This is displayed in Table 4.4 whereby, CNPK content of compost and vermicompost obviously differ from the *S. commune* standard formulation substrate due to the decomposition process that resulted in decreasing of C/N ratio in compost and vermicompost (Tajbakhsh *et al*., 2008; Huang *et al*., 2014; Zhang & Sun, 2014). Thus, further cultivation trial was done by supplementation with nitrogen and carbon sources into the compost and vermicompost.

4.4.4 Effect of Supplementation of Rice Bran as Nitrogen Source

Rice bran (RB) as a main nitrogen source of *S. commune* cultivation was added because a high concentration of nitrogen encouraged mycelial growth (Chang & Miles, 2004). Three different percentages, 5%, 10% and 15% of RB were supplemented into the compost and vermicompost. Overall, RB supplementation enhanced the mycelial density. Besides, the mycelial grew evenly and fully colonizing the substrate well.

4.4.4.1 Mycelial Growth

Table 4.5 shows the mycelial growth rate (MGR) of *S. commune* using compost and vermicompost as fruiting substrate supplemented with 0%, 5%, 10% and 15% RB.

Table 4.5: Mycelial growth rate of *S. commune* using compost and vermicompost supplemented with rice bran.

Substrate (5 replicates)	Mycelial growth rate (cm/day)			
	0% RB	5% RB	10% RB	15% RB
CA	0.87 ± 0.06	0.88 ± 0.02	0.52 ± 0.01	0.77 ± 0.10
CB	0.57 ± 0.18	0.74 ± 0.06	0.56 ± 0.02	0.30 ± 0.50
CC	0.67 ± 0.08	0.77 ± 0.01	0.57 ± 0.05	0.47 ± 0.09
Mean	0.70 ± 0.15	0.80 ± 0.07	0.55 ± 0.03	0.51 ± 0.24
VA	0.90 ± 0.28	0.92 ± 0.11	0.48 ± 0.13	0.74 ± 0.22
VB	0.52 ± 0.02	0.80 ± 0.03	0.73 ± 0.22	0.76 ± 0.09
VC	0.81 ± 0.34	0.89 ± 0.03	0.61 ± 0.05	0.42 ± 0.01
Mean	0.74 ± 0.20	0.87 ± 0.06	0.61 ± 0.13	0.64 ± 0.19

Based on Table 4.5, the highest MGR was presented by all compost and vermicompost supplemented with 5% RB where their average MGR was 0.80±0.07 cm/day and 0.87±0.06 cm/day. Meanwhile, the lower average of MGR could be seen when using 15% RB in compost (0.51±0.24 cm/day) and 10% RB in vermicompost (0.61±0.13 cm/day).

From the result in Table 4.5, we concluded the MGR was different when using different levels of supplementation. The same finding was obtained by Wang *et al*. (2015) where the increase supplementation of SMS and wheat bran in cotton seed hulls showed the different MGR of *P. ostreatus*.

According to Moonmoon *et al*. (2011), the MGR of *L. edodes* was influenced by the bioavailability of nitrogen. The mycelial grew better when there was nitrogen available in the substrate (Moonmoon *et al*., 2011). However, the MGR result alone was not enough to indicated the good mycelial growth of *S. commune*. It must be supported with the result of *S. commune* mycelial density.

4.4.4.2 Mycelial Density

Mycelial density of *S. commune* using compost and vermicompost as fruiting substrate supplemented with RB was sustained until the mycelial colonized the substrate. The incubation time taken was a month or four weeks.

Figure 4.12: Mycelial density of *S. commune* using vermicompost VC (50 SMS:50 GM) supplemented with rice bran.

Figure 4.12 shows the picture of the mycelial density for vermicompost VC supplemented with 0%, 5%, 10% and 15% RB. The pictures represent the density of all

compost and vermicompost supplemented with the same percentage of RB. The density using substrate of compost and vermicompost without RB was very thin and could hardly be seen.

As RB supplementation increased, the mycelial density also increased. The density was similar and denser when using compost and vermicompost supplemented with 10% and 15% RB. There were also some primordia formed in the substrate supplemented with 10% and 15% RB during the incubation time. The incubation time was around a month.

From the result, we concluded RB as a source of nitrogen was very important to produce the dense mycelium of *S. commune*. Besides, the dense mycelium could be sustained along the incubation time as long as parameters such as temperature, pH and moisture content were set at optimum level. There was no mushroom bag contamination occurred until the end of the incubation.

The mycelial density was affected by the nitrogen source available in the substrate (Moonmoon *et al*., 2011). In addition, substrate materials, substrate formulation, supplementation levels and optimum parameters including temperature, pH and moisture content also affected the mycelial growth and density (Jonathan & Fasidi, 2001).

Imtiaj *et al*. (2008) reported the mycelial density of *S. commune* adapted to high temperature and low pH. The dense mycelial could be seen when temperature was set between 30°C and 35°C using pH range between pH 5 and 6 (Imtiaj *et al*., 2008). In this study, the pH was set around 6 therefore the dense mycelial could be produced when RB supplementation was 10% and over.

4.4.4.3 Mushroom Yield and Biological Efficiency

Schizophyllum commune was able to produce fruiting bodies when compost and vermicompost supplemented with RB was used as substrate. The increase in mycelial density enabled the production of fruiting bodies. However, the mushroom bags could only be harvested once. According to Sánchez (2010), the failure of second flush in mushroom fruiting formation occurred because of inadequate substrate nutrition.

CC 5% RB CC 10% RB CC 15% RB

Figure 4.13: *Schizophyllum commune* yield using compost CC (50 SMS:50 GM) supplemented with rice bran.

Figure 4.13 shows the picture of *S. commune* fruiting bodies grow on compost CC (50 SMS:50 GM) supplemented with 5%, 10% and 15% RB. From Figure 4.13, the compost supplemented with RB allowed the formation of *S. commune* fruiting bodies. Meantime, Table 4.6 showed mushroom yield (MY) and biological efficiency (BE) of *S. commune* cultivation using compost and vermicompost supplemented with 5%, 10% and 15% RB. 0% RB supplementation was not included in Table 4.6 because there was no fruiting formation in compost and vermicompost supplemented with 0% RB.

Table 4.6: *Schizophyllum commune* yield using compost and vermicompost supplemented with rice bran.

Substrate (5 replicates)	Substrate Dry Weight (g)	5% RB		10% RB		15% RB	
		MY (g/bag)	BE (%)	MY (g/bag)	BE (%)	MY (g/bag)	BE (%)
CA	400	3.01 ±0.12	0.75 ±0.05	7.84 ±0.10	1.96 ±0.06	7.72 ±0.23	1.93 ±0.16
CB	400	3.32 ±0.37	0.83 ±0.29	9.89 ±0.27	2.47 ±0.10	9.93 ±0.52	2.48 ±0.32
CC	400	3.44 ±0.35	0.86 ±0.11	10.27 ±0.60	2.57 ±0.23	10.19 ±0.15	2.55 ±0.03
VA	400	2.79 ±0.86	0.70 ±0.17	5.06 ±0.16	1.27 ±0.02	5.10 ±0.14	1.28 ±0.06
VB	400	3.05 ±0.22	0.76 ±0.15	6.61 ±0.31	1.65 ±0.07	6.59 ±0.11	1.65 ±0.05
VC	400	3.20 ±0.07	0.80 ±0.08	6.79 ±0.40	1.70 ±0.09	6.77 ±0.12	1.69 ±0.08

From Table 4.6, high MY and BE were acquired when using compost CC supplemented with 10% RB (10.27±0.60 g/bag, 2.57±0.23%) and vermicompost VC supplemented with 10% RB (6.79±0.40 g/bag, 1.70±0.09%). Meanwhile, the lowest MY and BE were obtained in the substrate consisted of compost CA mixed with 5% RB (3.01±0.12 g/bag, 0.75±0.05%) and vermicompost VA supplemented with 5% RB (2.79±0.86 g/bag, 0.70±0.17%). In comparison between compost and vermicompost, the compost supplemented with RB had better yield and BE than the vermicompost.

From the result in Table 4.6, we could conclude compost and vermicompost derived from *S. commune* SMS mixed with GM were proven could be used as fruiting substrate for *S. commune* cultivation by supplementing the substrate with RB where RB supplementation improved the mycelial growth and density.

Figlas *et al*. (2014) also had proven the yield and BE of *S. commune* using sunflower seed hull (SSH) as substrate with supplementation were better than without any supplementation. Figlas et al. (2014) reported the yield and BE of *S. commune* cultivated in SSH supplemented with 7.5% wheat bran were 193.1 g/bag and 48.3% respectively compared to SSH without supplementation where the yield and BE were 162.1 g/bag and 40.7% respectively.

According to Jeznabadi *et al*. (2016), the barley straw substrate supplemented with RB produced the highest mushroom fresh weight of *P. eryngii*, 83.49 gkg^{-1} substrate. However, RB supplementation in compost and vermicompost in this project produced low yield and BE of *S. commune*. Perhaps it was due to the lack of the carbon source. Therefore, we conducted the next trial using sawdust supplementation. Sawdust was the main substrate in *S. commune* commercial cultivation.

4.4.5 Effect of Supplementation of Sawdust

The last trial of *S. commune* cultivation in this research was sawdust (SD) supplementation. In this trial, the vermicompost was not used as fruiting substrate. Following the previous result, all compost supplemented with 10% RB was chosen because the substrates showed the better yield and BE than others as well as dense mycelium. Besides, the substrates had no contamination until the end of the incubation. Furthermore, it had low cost of production compared to compost supplemented with 15% RB which also showed better *S. commune* yield.

4.4.5.1 Mycelial Growth

Table 4.7 shows the MGR of *S. commune* cultivation using compost CA, CB and CC plus 10% RB supplemented with 0%, 20%, 50% and 80% SD.

Table 4.7: Mycelial growth rate of *S. commune* using compost plus 10% rice bran supplemented with sawdust.

Substrate (5 replicates)	**Mycelium growth rate (cm/day)**			
	0% SD	**20% SD**	**50% SD**	**80% SD**
CA+10% RB	0.57 ± 0.71	0.32 ± 0.82	0.37 ± 0.28	0.82 ± 0.09
CB+10% RB	0.51 ± 0.44	0.42 ± 0.17	0.52 ± 0.29	0.93 ± 0.73
CC+10% RB	0.53 ± 0.56	0.50 ± 0.06	0.54 ± 0.65	0.90 ± 0.31
Mean	0.54 ± 0.03	0.41 ± 0.09	0.48 ± 0.09	0.88 ± 0.06

From Table 4.7, initially, the MGR in the substrate CA, CB and CC decreased until 20% of SD supplementation then increased and achieved the highest average MGR, 0.88±0.06 cm/day when using 80% SD supplementation. According to Jonathan and Fasidi (2001), the mycelial grew very well when the good substrates were supplemented with the required nutrients and cultivated at optimal parameters including temperature, pH and moisture content.

However, the MGR of the standard formulation of *S. commune* cultivation in PPBGL is 1.57±0.07 cm/day that is higher than the highest MGR recorded in this trial, 0.93±0.73 cm/day. The standard formulation of *S. commune* cultivation used as comparison is using sawdust as the main fruiting substrate and supplemented with rice bran and $CaCO_3$.

4.4.5.2 Mycelial Density

Mycelial density of *S. commune* that grew on compost CC+10% RB supplemented with SD was shown in the pictures in Figure 4.14. The pictures represent the mycelial density of all compost supplemented with the same percentage of SD. The incubation time taken was a month or four weeks.

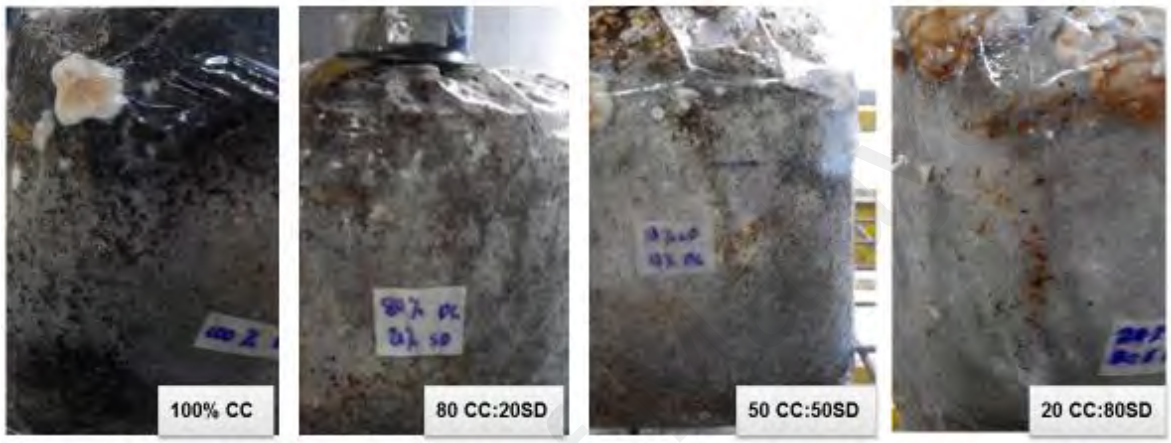


Figure 4.14: Mycelial density of *S. commune* using compost CC (50 SMS:50 GM) plus 10% rice bran supplemented with sawdust.

Based on Figure 4.14, the mycelial density of *S. commune* was very thin when using compost CC+10% RB without SD supplementation. It was because the pH was not set at optimum pH for *S. commune* mycelial growth where wood vinegar was not added into the substrate. The initial pH of compost CC+10% RB without SD supplementation was around pH 7.

The mycelial became denser when SD supplementation was increased. It was because the SD used had low pH, range between pH 4 and 5 hence reduced the pH of the substrate. Imtiaj *et al*. (2008) reported the optimum pH range for mycelial density of *S. commune* was between pH 5 and 6. To conclude, supplementation of SD was suitable to reduce the pH of *S. commune* fruiting substrate where the pH measured after SD supplementation was around pH 6.

4.4.5.3 Mushroom Yield and Biological Efficiency

The pictures in Figure 4.15 showed the fruiting bodies of *S. commune* using compost as substrate supplemented with SD and RB.

20 SD: 80 DC 50 SD:50 DC 80 SD:20 DC

Figure 4.15: *Schizophyllum commune* yield using compost CC (50 SMS:50 GM) plus 10% rice bran supplemented with sawdust.

Based on Figure 4.15, the increase in sawdust supplementation caused increase of *S. commune* fruiting production. It was due to the high SD percentage added into the substrate hence enhanced carbon content needed by *S. commune* for fruiting formation. No wood vinegar was added because the pH in this formulation was around pH 6 that was suitable for *S. commune* growth (Imtiaj *et al*., 2008).

Table 4.8: *Schizophyllum commune* yield using compost plus 10% rice bran supplemented with sawdust.

Substrate (5 Replicates)	Substrate Dry Weight (g)	CA		CB		CC	
		MY (g/bag)	BE (%)	MY (g/bag)	BE (%)	MY (g/bag)	BE (%)
100% DC	400	7.77 ±0.19	1.94 ±0.06	9.88 ±0.66	2.47 ±0.20	10.09 ±0.42	2.52 ±0.17
20 SD: 80 DC	400	18.72 ±0.90	4.68 ±0.42	20.45 ±0.17	5.11 ±0.08	20.91 ±0.09	5.23 ±0.02
50 SD:50 DC	400	22.37 ±0.51	5.59 ±0.12	38.85 ±0.38	9.71 ±0.19	39.02 ±0.88	9.76 ±0.26
80 SD:20 DC	400	42.37 ±0.11	10.59 ±0.04	78.85 ±0.71	19.71 ±0.25	80.25 ±0.33	20.06 ±0.10

Schizophyllum commune yield of the final trial was shown in Table 4.8. All of the mushroom bags only could be harvested once because of the contamination during cultivation. The highest yield was harvested from the mushroom bag consisted compost CC+10% RB supplemented with 80% SD, 80.25±0.33 g/bag followed by compost CB+10% RB supplemented with 80% SD, 78.85±0.71 g/bag and compost CA+10% RB added with 80% SD, 42.37±0.11 g/bag. It was indicated that the compost+10% RB supplemented with 80% SD produced the highest yield compared to the 50% and 20% of SD supplementation.

Like mushroom yield, BE also increased when SD supplementation increased. The highest BE was 20.06±0.10% recorded when using CC+10% RB supplemented with 80% SD while the lowest BE was 1.94±0.06%, using substrate CA+10% RB without SD supplementation. Overall, compost CC showed the best yield and BE when supplemented with SD and RB followed by compost CB and compost CA. By using the finding, we compared the yield and BE of *S. commune* cultivation when compost CC used had different duration of composting. The substrate supplemented with 10% RB and 80% SD.

80 SD:20 CC (day 30)

80 SD:20 CC (day 60)

80 SD:20 CC (day 90)

Figure 4.16: *Schizophyllum commune* yield using compost CC (50 SMS:50 GM) plus 10% rice bran supplemented with 80% sawdust (compost CC produced at day 30, day 60 and day 90 of composting).

The pictures in Figure 4.16 indicated *S. commune* grew very well in the substrate consisted of compost, RB and SD although the compost used had different composting duration. Furthermore, the substrate formulated with compost had zero contamination throughout the incubation period hence reduced the loss of mushroom bags as well as increased the yield during cultivation. It possibly happened due to the compost production which undergo thermophilic composting that able to destroy and kill pathogens (Sarkar *et al*., 2016).

The *S. commune* yield and BE using the fruiting substrate consisted of compost CC that had different composting duration supplemented with RB and SD were presented in Table 4.9.

Table 4.9: *Schizophyllum commune* yield using compost CC (50 SMS:50 GM) plus 10% rice bran supplemented with sawdust (compost CC produced at day 30, day 60 and day 90 of composting).

Substrate (5 replicates)	Substrate Dry Weight (g)	30 days		60 days		90 days	
		MY (g/bag)	BE (%)	MY (g/bag)	BE (%)	MY (g/bag)	BE (%)
100% CC	400	7.72 ±0.06	1.93 ±0.04	9.45 ±0.16	2.36 ±0.09	10.09 ±0.42	2.52 ±0.17
20 SD: 80 CC	400	17.94 ±0.13	4.49 ±0.07	19.91 ±0.44	4.98 ±0.12	20.91 ±0.09	5.23 ±0.02
50 SD:50 CC	400	28.55 ±0.30	7.14 ±0.18	35.41 ±0.41	8.85 ±0.23	39.02 ±0.88	9.76 ±0.26
80 SD:20 CC	400	44.83 ±0.09	11.21 ±0.05	60.82 ±0.32	15.21 ±0.12	80.25 ±0.33	20.06 ±0.10

From Table 4.9, we could observed that increased in time duration of composting would increase the *S. commune* yield and BE. Probably, it was due to the high NPK content in compost CC at day 90 of composting compared to the compost produced at day 30 and day 60. It also might because of the different stabilization and maturity level of the compost where the compost after 90 days of composting was more stable and mature (Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012; Azizi *et al*., 2015; Soobhany *et al*., 2015).

The best yield (80.25±0.33 g/bag) and BE (20.06±0.10%) were showed by the compost CC at day 90 of composting which supplemented with 10% RB and 80% SD while the lowest yield (7.72 g/bag) and BE (1.93%) were belonged to the substrate consisted of 100% compost CC at day 30 of composting. SD supplementation was proven to improve the MY and BE of *S. commune* cultivation. In addition, increase of SD supplementation enhanced MGR and mycelial density.

According to Moonmoon *et al*. (2011), SD supplemented with 25% wheat bran produced the highest number of fruiting bodies, biological yield (153.3/500 g packet) and BE (76.6%) of *L. edodes*. They also reported the growth and yield of *L. edodes* on SD are significantly influenced by different levels of wheat bran (WB), RB, maize powder (MP) and their combination supplements. The highest MGR of *L. edodes* (0.4 cm/day) was recorded using SD supplemented with 20% RB or 15-25% of combination of WB, RB and MP (Moonmoon *et al*., 2011).

Meanwhile, the study by Preecha *et al*. (2015) revealed that the formula of 100% SD supplemented with 10% RB produced the highest yield, 82.85 g/bag followed by SD mixed with 25% of waste material (reusing cultivated spawn) supplemented with 15% RB, 81.35 g/bag and 50/50 of SD/waste material (reusing cultivated spawn) adding with 15 % RB, 80.04 g/bag.

4.5 Potential of Compost and Vermicompost as Substrate for *Schizophyllum commune* Cultivation

Commercial cultivation of *S. commune* utilizes sawdust as a main substrate indicated that it required high carbon content to generate high mushroom yield (Preecha *et al*., 2015). It was supported by the analysis of CNPK content of the standard formulation of *S. commune* substrate that used to produce *S. commune* commercially in Biotechnology Research Centre Glami Lemi University Malaya (PPBGL) in Jelebu, Negeri Sembilan where the substrate had high C/N ratio, 61.69.

Irshad and Asgher (2011), Asgher *et al*. (2016) and Metreveli *et al*. (2017) had proven *S. commune* produced a wide range of enzymes to degrade the lignocellulosic substrates during vegetative growth. The enzymes were peroxidases and laccases for lignin degradation and various types of cellulases and xylanases for cellulose and hemicellulose

degradation. The enzymes' activities during the vegetative growth dictate a connection to the regulation of the fruiting body development in *S. commune* (Chang & Miles, 2004).

In comparison to the standard formulation of *S. commune* cultivation, the compost and vermicompost in this project had lower C/N ratio, ranges between 21.7 to 38.67. The compost and vermicompost could not cultivated *S. commune* alone. It must be supplemented with RB to improve the mycelial density. Furthermore, SD supplementation was needed to increase the yield and BE of *S. commune.*

The highest yield and BE that we obtained in this research were 80.25±0.33 g/bag and 20.06±0.10% respectively, using compost CC at day 90 of composting as substrate supplemented with 10% RB and 80% SD. However, the highest BE recorded (20.06±0.10%) in this project was still very low compared to the BE of the standard formulation of *S. commune* cultivation in PPBGL (89.25±0.05%) that used SD as the main substrate supplemented with RB and $CaCO_3$.

Furthermore, the highest yield and BE reported in this study used 80% SD which was too high to be considered as supplementation. Therefore, compost and vermicompost were suitable to be used as supplementation rather than as a main substrate for *S. commune* cultivation.

4.6 C/N ratio and NPK Analysis of Recycled Substrate After *Schizophyllum commune* Cultivation

Table 4.10 showed the CNPK analysis of substrate compost CC supplemented with 10% RB after *S. commune* cultivation. The formulation had the best yield among the optimized substrate using RB supplementation. We did not analyze the optimized substrate supplemented with RB and SD because the best yield was shown by using the substrate added with 80% SD that was high in carbon content.

Table 4.10: CNPK analysis of SMS after *S. commune* cultivation using substrate compost CC (50 SMS:50 GM) supplemented with 10% rice bran.

SMS	C (%)	N (%)	P (%)	K (%)	C/N ratio
CC + 10% RB	23.67	2.08	0.81	0.87	11.38

The result in Table 4.10 showed the SMS had lower C/N ratio, 11.38 and high NPK content (N=2.08%, P=0.81%, K=0.87%) especially for total N content due to the RB supplementation where RB was the main nitrogen source in *S. commune* standard formulation. C/N ratio less than 15 was regarded as a good fertiliser (Jamaludin & Mahmood, 2010; Jamaludin *et al*., 2012). USDA (2011) stated the organic fertiliser with low C/N ratio would provide more nitrogen for the plant growth.

Overall, the spent *S. commune* substrate degraded compost CC supplemented with 10% RB can be disposed directly or used in agriculture without giving any harmful effect to the soil. It is because its C/N ratio is still in the safe range that can be digested by the soil microbes as well as left some available nitrogen that can be absorbed by the plant for their growth (USDA, 2011).

CHAPTER 5: CONCLUSION AND FUTURE WORK

Constant increase of spent mushroom substrate (SMS) disposal due to the growth of mushroom industry whose demand was influenced by human population gave harmful effects to environment if not recycled properly. Hence, this project was conducted by composting and vermicomposting the spent *S. commune* substrate with goat manure for fertiliser production and *S. commune* cultivation to achieve the three objectives of this study.

The main objective is to determine the ratio formulation of SMS for composting and vermicomposting. The formulations of SMS for composting and vermicomposting were 80 SMS:20 GM, 60 SMS:40 GM and 50 SMS:50 GM. From the formulation, C/N ratio and NPK content of compost as potential organic fertiliser were analysed to achieved one of the objectives of this work.

Compost CC and vermicompost VC with mixture ratio of 1:1 of SMS:GM showed the best quality of organic fertiliser. Compost CC had low C/N ratio, 23.34 and high NPK content where N was 1.61%, P equaled to 0.73% and K was 0.81%. Meanwhile, vermicompost VC with C/N ratio 21.7 also had high NPK content of N, 1.51%, P, 0.81% and K, 0.97%. Compost CB with formulation 3:2 of SMS:GM also acceptable as an organic fertiliser with C/N ratio 22.66 and N, P, K content 1.54%, 0.74% and 0.63% respectively.

We also proved that compost and vermicompost of SMS mixed with GM could cultivate *S. commune* by supplementing them with rice bran (RB) and sawdust (SD) where the highest yield was 80.25 g/bag with biological efficiency, 20.06% acquired from the substrate consisted of compost CC supplemented with 10% RB and 80% SD. This finding fulfills another objective of this study.

The study also revealed that SMS of *S. commune* had high potential to reutilized in its cultivation again considering its nutrients content that was almost similar to the standard formulation. Furthermore, the mushroom bags using compost and vermicompost had no contamination throughout the incubation period.

The compost in this research was obtained from thermophilic composting process that sanitized the compost by destroying and killing the pathogens and weed seeds which detrimental to the crops. However, further study is needed to evaluate the compost and vermicompost potential using various crops. As a conclusion, the compost and vermicompost in this research are not only has potential for crops but also suitable for *S. commune* cultivation.

The future research can improve the organic fertiliser production using SMS of *S. commune* by mixing it with other organic matters such as cow dung, green waste and so on using the same method of composting and vermicomposting or the other methods that is more convenient. This kind of research will enrich the recent knowledge and database regarding organic fertiliser production. We also suggest to include phytotoxicity evaluations for the organic fertiliser.

We also recommend the future studies to improve and explore more about the potential of spent *S. commune* substrate in its cultivation or other mushroom by improving the method to produce the high yield and biological efficiency (BE) of the cultivation considering the lower BE obtained in this work compared to the standard formulation. The improvement will help to give the alternative method to reduce the cost of the *S. commune* cultivation that still used sawdust as the main substrate.

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