# Valorization of palm oil wastes into oyster mushrooms (Pleurotus HK-37) and biogas production

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## Abstract

Continued growth of oil palm cultivation for palm oil production has led to higher post-processing wastes that pose environmental management challenges. The goal of this study was to investigate the co-production of oyster mushroom *Pleurotus* HK-37 and biogas as a means to add value to palm oil waste fractions and thus reduce their impact on the environment. A total of 9 blends of solid, semi-solid and liquid palm oil waste fractions were subjected to mushroom production and the resulting spent mushroom substrate (SMS) was used for biogas production. There was a significant difference in mushroom yield (*t-test*, p = 0.00013237) and biological efficiency (*t-test*, p = 0.00044955), with the highest values obtained from substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%). Biogas production was also significantly different among both fresh and pretreated (SMS) waste fractions. The highest biogas volume, methane content and methane yield were observed from SMS waste fractions no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%). Overall, pretreatment of palm oil processing waste with oyster mushrooms increased biogas production and methane yield per kg volatile solids by 102.78% compared to untreated waste. This study has demonstrated that mushroom and biogas production are viable options for the management of palm oil processing waste fractions and thus promoting a circular economy. Further studies should optimize their production and conduct techno-economic

analysis to ascertain economic value.

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## Introduction

### *The palm oil industry worldwide*

The African oil palm (*Elaeis guineensis*) is an economically important crop native to West Africa. It is used primarily to produce palm oil, which is a source of foodstuffs and industrial raw materials. Primarily, red palm oil that is produced from palm oil fruit mesocarp fiber is mainly used for consumption, while the white palm kernel oil is used in the oleochemical industry for making soaps, detergents and toiletry products (Henderson and Osborne, 2000; Poku, 2002; Reinhardt et al., 2007). Oil palm has come to occupy a cultural role among communities where it is grown and where farmers tap its sap to produce palm wine (Obire and Putheti, 2010). Oil palms are established primarily in tropical regions of the world, especially Africa and Asia. Malaysia and Nigeria are the world's largest producers of oil palm, accounting for 85% of the palm oil produced worldwide (Obire and Putheti, 2010; Reinhardt et al., 2007; World Rainforest Movement, 2008). In Africa, oil palm cultivation is mainly distributed parallel to the Atlantic Coast in West and Central Africa, deltas of rivers Niger and Congo and the islands of Madagascar (Henderson and Osborne, 2000; Poku, 2002). Oil palm was introduced to Tanzania and its islands of Pemba by the early slave trade (Gerritsma and Wessel, 1997). Currently, oil palm production is carried out primarily by smallholder farmers living in the western part of the country in the Kigoma region, Southern highlands in the Mbeya region (mostly Kyela district), Pwani region and some parts of the Tanga region (FAO, 2023a, 2023b; TIC, n.d.; UNIDO, 2019).

Tanzania has suitable weather and land suitable for oil palm cultivation and thus could potentially become a leading global producer of palm oil (FAO, 2010; TIC, n.d.). Although the data is scant, by 2004, only 4,500 ha of land had been used (Sulle and Nelson, 2009). In recent years, there has been renewed attention to palm oil production for consumption to plug the gap in edible oil in Tanzania. Currently, the country imports about 500,000 metric tonnes of palm oil per annum (UNIDO, 2019), while FAO (2023) estimates that 98% of palm oil, the most consumed edible oil in Tanzania, is imported.

Other sources estimate that 55.5% of the total edible oil demand in Tanzania is imported, costing the country about 600 billion TZS annually, about 255 million USD (The Citizen, 2021). The government has therefore committed resources to increase palm oil production to meet demand in the country (Mu, 2019; The Citizen, 2021), allocating an equivalent of USD 4.3 million for the purpose (FAO, 2019) and collaborating with other stakeholders such as FAO to fast track the process (FAO, 2023b).

The growth and expansion of the palm oil industry will go hand in hand with an increase in the waste generated by the extraction and processing of the oil. Processing palm oil generates biomass waste products from two processes: one, harvesting and extracting crude oil, and two, processing the crude oil and nuts into a refined product. From the first part of the palm oil production process, the main wastes generated are the solid fiber type wastes in the form of empty fruit bunches (EFB), fruit mesocarp fiber (FMF), also known as palm mesocarp fiber (PMF), palm kernel shells (PKS) and palm press cake (PPC) (Er et al., 2011; Najafpour et al., 2006). Palm oil mill effluent (POME) and sediments (SD) are common liquid and semi-solid wastes, respectively, generated during the refining process. POME is the most polluting organic residue generated from palm oil mills. It has high biological oxygen demand (BOD) due to high organic matter content, mainly oils and fatty acids (Mumtaz et al., 2008). It is estimated that for every ton of fresh fruit bunches processed, 0.5 to 0.75 tonnes of POME are produced (Busu et al., 2010). Like other agro-processing waste products, the by-products of palm oil extraction and processing have limited utility and commercial value. A small proportion of the solid waste fractions are used in agriculture for mulching and soil conservation purposes (Obire and Putheti, 2010; Suhaimi and Ong, 2001). PKS and PMF are also used to generate steam and electricity for palm oil mills in countries like Thailand and Malaysia, while EFB are either incinerated or applied in agricultural fields (Chiew, 2009). The palm press cake is mostly used as animal feed (Basiron, 2007), while POME is often disposed of in an open pond system or discharged into aquatic bodies as a cheap disposal option (Alawi, 2009).

In Tanzania, these wastes have little use in agriculture and a large part of the wastes is discarded. Communities in Tanzania use palm fronds to cover roofs, make temporary fences, and manufacture household items such as brooms. A considerable chunk of the palm oil extraction and processing wastes are left unattended or burned, causing environmental hazards. The gaseous emissions from incineration and steam boilers produce emissions that compound environmental problems. The management of palm oil extraction and processing waste is further complicated by the fibrous lignocellulosic nature of solid waste fractions such as EFB and PMF (Gutiérrez et al., 2009; Ong et al., 2021) and high BOD of liquid wastes (Singh et al., 2020).

The complex lignocellulose nature of the solid wastes from palm oil processing creates environmental problems when they are discarded due to their persistence in the environment. However, these properties are suitable for the production of biological products, such as mushrooms, through the degradation of solid lignocellulose waste and biogas from the organic matter in liquid wastes. Production of valuable biological products such as mushrooms and biogas has been proposed and explored as a viable value-addition pathway for agro-processing wastes. White rot fungi that produce mushrooms, such as oyster mushrooms found in the genus *Pleurotus*, grow and utilize lignocellulose materials for bioconversion by using their lignocellulolytic enzymes that degrade cellulose and lignin into nutrient sources for the fungi (Pathmashini et al., 2010). This ability has allowed them to not only produce valuable products but also act as a biological

pre-treatment of complex wastes for other processes. Mushroom cultivation is an economically important biotechnological industry that can be used for the valorization of agro-industrial residues through solid-state fermentation. It is a low-input activity that is also applicable where land is the limiting factor, and agricultural residues are abundantly available. Further, oyster mushrooms are healthy foods with essential amino acids, essential fatty acids, valuable vitamins, minerals and low-energy carbohydrates that are increasingly gaining importance as a power food (Tabi et al., 2008).

Several studies have reported the use of palm oil processing wastes alone or in combination with other agro-processing wastes in the production of several types of mushrooms, such as *Pleurotus* spp. (Tabi et al., 2008) straw mushroom *Volvariella volvacea* (Triyono et al., 2019), oyster mushroom *Pleurotus ostreatus* (Marlina et al., 2015). Other studies investigated the utilization of palm oil processing waste for biogas production (Sodri and Septriana, 2022; Suksong et al., 2020). In recent years, studies have attempted combined mushroom and biogas production to obtain maximum value from such wastes (Leong et al., 2022; Mmanywa and Mshandete, 2017; Purnomo et al., 2018; Temu et al., 2016). However, there are no studies in Tanzania that determined the feasibility of utilizing palm oil processing waste fractions for oyster mushroom production and biogas. Therefore, this study determined the suitability of nine palm oil waste fractions for the co-production of mushrooms and biogas as a value-added alternative to waste management.

## Material and Methods

### Sample collection

The palm oil processing wastes were collected from a local palm oil processing plant at Bagamoyo, Pwani, Tanzania, and transported to the University of Dar es Salaam Department of Molecular Biology and Biotechnology (UDSM – DMBB) laboratories. We collected 5094 g palm mesocarp fiber (PMF), 5094 g empty fruit bunches (EFB), 675 g palm press cake (PPC), 675 g palm kernel (PK), 135 g palm oil mill effluent (POME) and 135 g sediments. POME and sediments were stored in the refrigerator immediately after arriving at the laboratory until they were used. Other substrates were sun-dried in a screen house, before chopping large-sized wastes like the EFB to reduce the particle size.

### Substrate Characterization

Determination of total solids (TS) and volatile solids (VS) of each palm oil waste fraction and inoculum (for biogas production) was done according to standard methods (APHA, 1998). TS and VS were also determined for waste fractions pre-treated by mushroom cultivation (spent mushroom substrates, SMS). TS and VS were calculated by using the formula:

$$TS = \frac{\text{Weight of substrate at } 105^\circ\text{C}}{\text{Weight of sample}} * 100$$

$$VS = \frac{\text{Weight of substrate at } 105^\circ\text{C} - \text{Weight of substrate at } 550^\circ\text{C}}{\text{Weight of substrate at } 105^\circ\text{C}} * 100$$

## Mushroom production

### *Spawn preparation*

The culture for oyster mushroom *Pleurotus* HK-37 was obtained from the DMBB culture collection. We adopted the method of Mshandete and Cuff (2008) for spawn preparation by using sorghum grains. 1000 g of sorghum grains was washed thoroughly under tap water, drained and par-boiled in 2 L water until the grains were semi-soft. Excess water was decanted over a sieve; the grains were allowed to cool to ambient temperature. To prevent sticking, 5% (w/w) $CaSO_4$ was added, while 10% (w/w) $CaCO_3$ was added to adjust pH. The grains were then filled in 500 mL wide-mouth glass bottles to three-quarters of the total capacity. The bottles were covered with cotton wool, then loosely covered by a piece of aluminum foil before being sterilized at 121 ºC, 1 atmosphere for 15 min. The bottles were allowed to cool to ambient temperature, and then they were aseptically inoculated with three 1-$cm^2$ pieces of $2^{nd}$ generation *Pleurotus* HK-37 mycelia. The mycelia-inoculated grains were then incubated in a dark room at room temperature until the mycelium fully colonized the grains for about 14 days.

### *Substrate preparation*

Water was added to the sun-dried palm oil processing waste fractions to make them moist but not dripping. The palm squeeze test was performed to ensure the right amount of moisture remained in the substrates. No water dripping between palm fingers confirmed that moisture in the substrate was at the correct level to sustain mushroom cultivation. The palm oil processing waste fractions were then blended in respective percentages according to Table 1 to make 500 g wet weight of substrate formulation. POME and sediments were used as supplements in varying percentages, as shown in Table 1. The waste formulations were packed into transparent polypropylene bags, which were then tied loosely by a sisal rope. The bags were steam sterilized in an autoclave at 121 ºC, 1 atmosphere for 1 h and then allowed to cool.

**Table 1.** Blends of palm oil processing waste fractions used for mushroom cultivation

| Blend no. | Palm oil waste fraction blends | Description |
|---|---|---|
| 1 | 98%PMF + 1%POME +1%SD | PMF-based waste fractions |
| 2 | 98%EFB + 1%POME + 1%SD | EFB-based waste fractions |
| 3 | 49%PMF + 49%EFB + 1%POME + 1%SD | 1:1 PMF and EFB |
| 4 | 44%PMF + 44%EFB + 5%PPC + 5%PKS + 1%POME + 1%SD | 1:1 PMF and EFB + PPC and PKS at 5% each |
| 5 | 39%PMF + 39%EFB + 10%PPC + 10%PKS + 1%POME + 1%SD | 1:1 PMF and EFB + PPC + PPC and PKS at 10% each |
| 6 | 44%PMF + 44%EFB + 10%PPC +1% POME + 1%SD | 1:1 PMF and EFB + 10% PPC |
| 7 | 39%PMF + 39%EFB + 20%PPC + 1%POME + 1%SD | 1:1 PMF and EFB + 20% PPC |
| 8 | 44%PMF + 44%EFB + 10%PKS + 1%POME + 1%SD | 1:1 PMF and EFB + 10% PKS |
| 9 | 39%PMF +39% EFB + 20%PKS + 1%POME + 1%SD | 1:1 EFB and PMF + 20%PKS |

Key:

PMF – Palm mesocarp fiber

POME – Palm oil mill effluent

SD - Sediments

EFB – Empty fruit bunches

PKS – Palm kernel shells

PPC – Palm press cake

*Spawning*

Spawning was done by inoculating the sterilized substrate bags of substrate formulations at a rate of 5% w/w, with three replicates per treatment. After inoculation, the bags were closed and placed horizontally on clean wooden shelves in the spawn running room. The shelves were wiped with 70% alcohol to reduce microbial contaminants on the surface. The bags were covered with a black plastic sheet to create darkness and limit fresh air. The room had a clean concrete floor, and the windows and the doorframe were covered with wire gauze to block insects and rodents. The spawning room was kept humid by pouring tap water on the floor and spraying mist on the air every day. The relative humidity range during spawning was 75 - 85%. No artificial lighting was applied to enhance darkness until the substrates were fully colonized by mycelia, an average of 30 days.

*Fructification and fruit body development*

After full mycelium colonization of the substrate bags, fruit body formation was triggered by changing the environmental variables, namely moisture, air exchange, temperature and light in the cropping room. The black nylon cover was removed to allow ventilation and more light to reach the bags. Holes were made through the bags using a 6-inch-long sterilized iron nail to increase air exchange. The bags were subjected to a cold, dry shock by placing them in a -20 $^{o}$C deep freezer for 30 min and later to a cold-wet shock by soaking in a bucket of cold water overnight. These were done to initiate pinhead formation while at the same time lowering carbon dioxide concentration in the bags. The bags were also sprayed with cold tap water twice a day using a hand sprayer to keep them moisturized. The floor of the room was kept wet, and air was misted every day to maintain high humidity.

All replicates of substrate formulations 3, 5, 6 and 7 and 1 replicate of substrate formulations 1, 2, 4 and 8 were re-spawned due to contamination and water lodging. Mycelia colonization failed completely in substrate formulation no 7.

*Harvesting and determination of biological Efficiency (B.E.), mushroom yield (M.Y.) and mushroom size*

Harvesting of *Pleurotus HK-37* fruit bodies was done as recommended by (Mshandete and Cuff (2008). Fruiting bodies of only one flush were harvested before the cap margins started to roll back, approximately 3 to 4 days after pinhead formation. Harvesting was done by gently twisting the mushroom bunches at the base to dislodge them from the substrate.

During harvesting, fresh mushroom bodies from each bag were counted and weighed. Further, three aspects of mushroom crop yield and productivity were evaluated according to Royse et al. (2004).

i. Mean mushroom size was determined as follows:

$$\text{Mean mushroom size} = \frac{\text{Total weight of fresh mushrooms harvested}}{\text{Total number of mushrooms harvested}}$$

ii. Biological efficiency (B.E.) was determined as the ratio of fresh mushrooms harvested (g) per (kg) dry substrate weight, including the supplement weight and expressed as a percentage

iii. Mushroom yield (M.Y.) was determined as the weight of fresh mushrooms harvested (g) per(kg) moist substrate weight, including the supplement weight

## Biogas production

The spent mushroom substrates were used for biogas production in batch anaerobic bioreactors. The working volume was 300 mL, and untreated palm oil processing waste fraction blends were used as controls, according to Table 2. The organic loading rate (OLR) was calculated by using the formula:

$$OLR = \frac{TS * VS(\text{inoculum})}{TS \times VS(\text{substrate})} * 300$$

**Table 2.** Organic loading rate (OLR) for control and SMS substrates for biogas production

| Substrate formulation | Weight of inoculum in control formulations (g fresh weight) | Weight of inoculum in SMS (g fresh weight) |
|---|---|---|
| 1 | 3.6 | 3.93 |
| 2 | 4.45 | 8.2 |
| 3 | 3.29 | 3.6 |
| 4 | 3.05 | 4.03 |
| 5 | 3.7 | 4.6 |
| 6 | 3.38 | 3.4 |
| 7 | 3.67 | 9.58 |
| 8 | 2.76 | 8.21 |
| 9 | 3.02 | 3.25 |

### *Process performance monitoring*

Biogas volume, methane content and methane yield were used to monitor the performance of the anaerobic digestion process. The concentrated alkaline absorption method was used to estimate methane content according to Ergüder et al. (2001) and Mshandete et al. (2005).

Biogas volume was measured using a graduated 100 mL gas-tight plastic syringe with a sample lock. The content of each bioreactor was mixed by gently swirling for 1 min prior to biogas volume measurement. To draw gas from the biogas collection bag, a needle fitted on the graduated syringe was used to pierce through the airtight n-butyl stopper in the gas sampling septum. The gas volume readings were taken on the graduated mark corresponding to the end of the plunger,

which is a point that resists the soft, gentle pulling of the piston. The lock was opened, and the plunger was pushed to withdraw the gas from the bag into the syringe. The process was repeated until the bag was empty. The gas measurements were done at ambient temperature. Total methane production was determined by the cumulative sum of daily gas collection for the entire duration of the study.

Methane yield was determined as the volume of methane produced per unit weight of fresh weight, total solids and volatile solids in the substrate. Biogas was collected for 40 days at an interval of 5 days.

## Results

### Substrate characterization

Generally, the solid waste fractions were rich in biodegradable substances in terms of volatile solids (VS) in the range of 85.67-99.32% for untreated wastes and 87.3 – 90.10% for treated wastes (Table 3). The highest content of biodegradable substances in terms of VS was observed with substrate formulation no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%) for untreated waste fractions (99.32%) and substrate formulation (1:1 PMF:EFB supplemented with 20% PPC, 1% POME and 1% SD) for treated waste fractions (90.10%).

**Table 3.** TS and VS of untreated and pre-treated waste fractions

| Substrate formulation | Untreated waste fractions | | Pre-treated waste fractions | |
|---|---|---|---|---|
| | % TS | % VS of TS | % TS | % VS of TS |
| 1 | 72.47 | 90.01 | 67.60 | 88.46 |
| 2 | 57.34 | 91.76 | 67.75 | 90.06 |
| 3 | 80.90 | 88.45 | 73.80 | 88.70 |
| 4 | 77.71 | 99.32 | 65.03 | 89.88 |
| 5 | 72.31 | 87.66 | 57.64 | 88.60 |
| 6 | 81.70 | 85.67 | 77.57 | 90.02 |
| 7 | 70.65 | 90.82 | 27.25 | 90.10 |
| 8 | 68.54 | 86.50 | 32.81 | 87.3 |
| 9 | 87.20 | 89.31 | 81.22 | 89.12 |

### Mushroom cultivation

The different palm oil waste formulations produced significantly different yields of mushrooms (*t-test*, p = 0.00013237), with the highest yield from substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%). Other substrates with high yields were formulations no. 8, 4, 2 and 5. These formulations were made up of either EFB (no. 2) or 1:1 PMF and EFB waste fractions with various supplementations of POME, sediments, palm press cake and palm kernel shells. Lowest mushroom yields were observed with substrate formulations no. 1 (PMF-

based formulation) and 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD), while all replicates of substrate formulation no. 7 (1:1 PMF:EFB supplemented with 20% PPC, 1% POME and 1% SD) were contaminated and did not produce mushrooms (Figure 1).

Comparison of biological efficiency in different treatments also revealed significant differences (*t-test*, p = 0.00044955). Again, substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%) showed the highest biological efficiency at 75.15%. The rest of the formulations had efficiencies from 53.7% and lower, the lowest levels being for substrate formulations no. 1 (PMF-based formulation) and no. 8 (1:1 PMF:EFB supplemented by 10% PKS, 1% POME and 1% SD) (Figure 1). The mushroom fruiting bodies are shown in Figure 2.

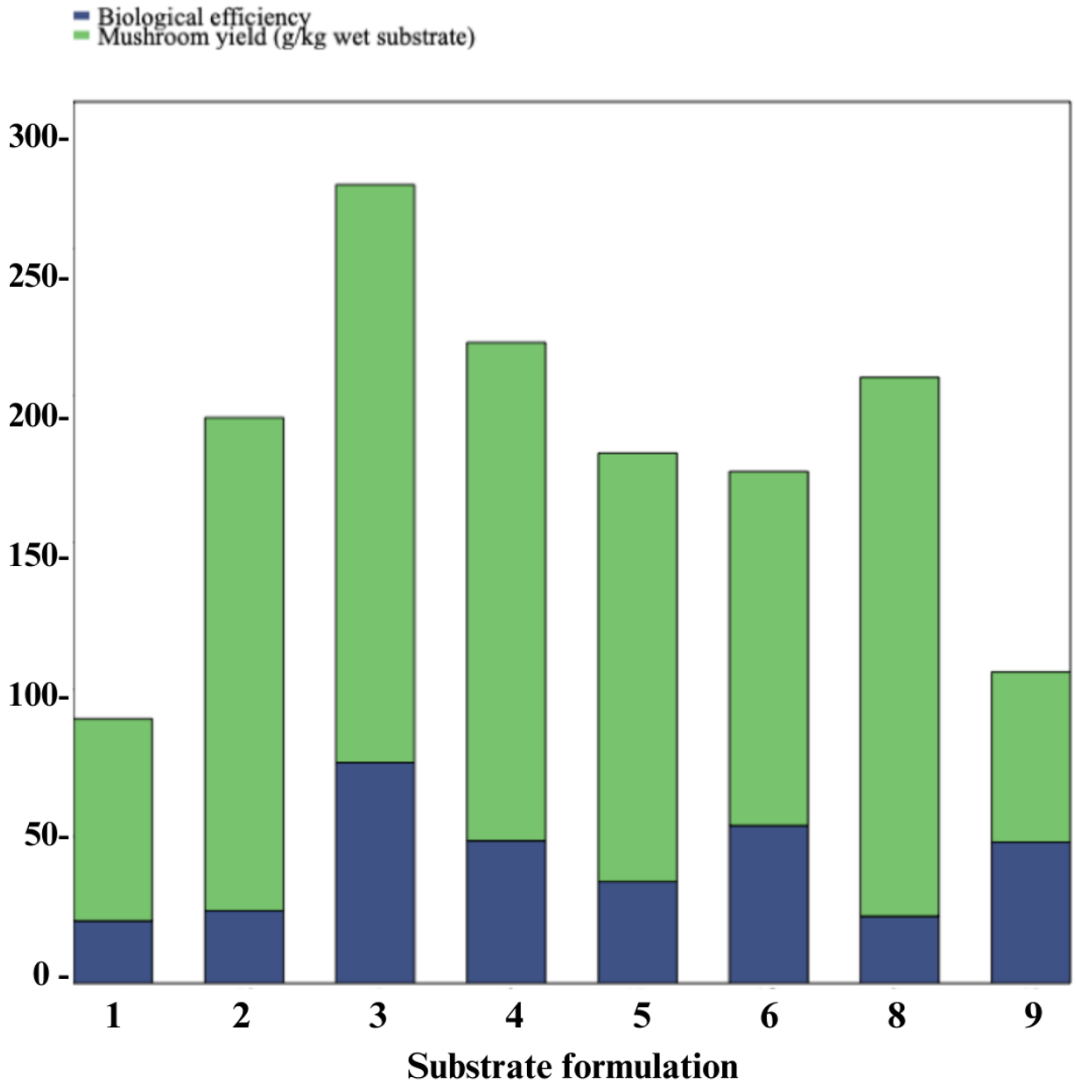


**Figure 1.** A bar chart of mushroom yield and biological efficiency from blends of palm oil processing waste fractions

**Figure 2.** First flush of the oyster mushrooms *Pleurotus HK-37* growing on different palm oil processing waste fractions fully colonized by the mycelia of the mushroom.

In addition, we measured other aspects of mushroom productivity of biological and commercial/consumer interest from the different substrate formulations. We found a significant difference in cap diameter (*t-test*, p = 4.932E-05), stipe length (*t-test*, p = 4.932E-05), mushroom size (*t-test*, p = 0.0015554) and average number of mushrooms in one flush (*t-test*, p = 0.00018289). It was interesting to note that the mushrooms from substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%) that showed the highest yield and biological efficiency also had relatively larger caps, longest stipes, average mushroom size and relatively high number of mushrooms. The largest mushrooms in terms of size and cap diameter were those from substrate formulation no. 6 (1:1 PMF and EFB formulation supplemented with 10% PPC, 1% POME and 1% SD), while substrate formulation no. 2 (98% EFB supplemented with 1% POME and SD) produced the largest number of mushrooms (Table 2).

| Substrate formulation no | Average cap diameter (cm)(cm) | Average stipe length (cm) | Mushroom size | No. of mushrooms |
|---|---|---|---|---|
| 1 | 6.4 | 3.00 | 3.12 | 11.00 |
| 2 | 6.7 | 4.40 | 3.36 | 25.00 |
| 3 | 7.9 | 6.80 | 5.18 | 19.00 |
| 4 | 6.2 | 4.00 | 8.48 | 10.00 |
| 5 | 5.8 | 3.00 | 4.06 | 18.00 |
| 6 | 9.5 | 5.20 | 10.30 | 6.00 |
| 8 | 6.1 | 6.20 | 5.47 | 18.00 |
| 9 | 5.4 | 3.40 | 1.45 | 20.00 |

**Table 2.** Mushroom yield/flush, average cap diameter and average stipe length from blends of palm oil production wastes

## Biogas production

Total biogas volume and percentage methane content in both fresh and pre-treated substrates (spent mushroom substrate (SMS)) are shown in Table 3. For fresh substrate, the highest volume of biogas (1155 ml) was observed from substrate formulation (1:1 PMF:EFB supplemented with 20% PPC, 1% POME and 1% SD), although its methane content was not the highest. Lowest biogas volume (600 ml) was observed from substrate formulation no. 5 (1:1 PMF and EFB (39% total weight) supplemented with PPC and PKS at 10%, POME and SD at 1%) and lowest methane content (74 %) from substrate formulations 8 (1:1 PMF:EFB supplemented by 10% PKS, 1% POME and 1% SD) and 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD).

For pretreated SMS, the highest biogas volume (1568 ml) was observed with formulation no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%), which also had the highest percentage of methane content (88%). The lowest biogas volume (571.5 ml) was observed from substrate formulation no. 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD), which also had the lowest methane content (75%).

**Table 3.** Total biogas volume, methane content and total methane volume of untreated waste fractions compared to spent mushroom substrates

| Substrate formulation no. | Total biogas volume (ml) | | Methane content % | |
|---|---|---|---|---|
| | Untreated waste fractions | Spent mushroom substrates | Untreated waste fractions | Spent mushroom substrates |
| 1 | 745 | 1085 | 85 | 84 |
| 2 | 1010 | 1408.5 | 76 | 82 |
| 3 | 1049 | 1070 | 84 | 81 |
| 4 | 865 | 1568 | 79 | 88 |
| 5 | 600 | 1124 | 79 | 77 |
| 6 | 805 | 1240 | 83 | 73 |
| 7 | 1155 | 1194 | 76 | 84 |
| 8 | 920 | 775.5 | 74 | 77 |
| 9 | 970 | 571.5 | 74 | 75 |

When comparing methane volume from fresh palm oil waste fractions and spent mushroom substrates, the volume was consistently higher in all substrate formulations except for substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%), where the difference was not remarkable, formulation no. 8 (1:1 PMF:EFB supplemented by 10% PKS, 1% POME and 1% SD), and formulation no. 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD). The largest differences in biogas volume were observed from substrate formulations no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%) and 5 (1:1 PMF and EFB (39% total weight) supplemented with PPC and PKS at 10%, POME and SD at 1%).

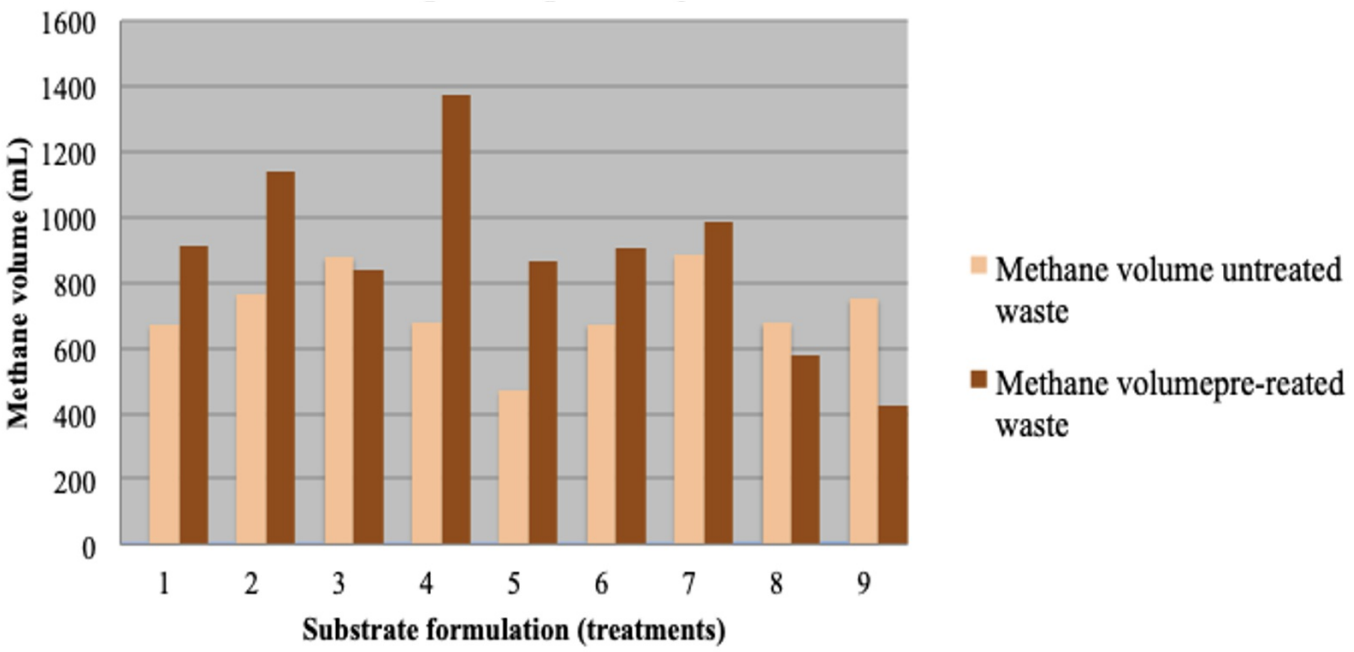


**Figure 3.** Volume of methane produced by fresh and pre-treated (spent mushroom substrates) palm oil waste fractions

Table 4 shows methane yield from fresh palm oil substrate formulations. Methane yield per kg fresh substrate added, per

kg TS added, and per kg VS added was lowest from substrate formulation no. 5 (1:1 PMF and EFB (39% total weight) supplemented with PPC and PKS at 10%, POME and SD at 1%). On the other hand, the highest methane yield per kg fresh substrate added was obtained from substrate formulations no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%) while yields per kg TS and VS were highest from substrate formulation no. 8 (1:1 PMF:EFB supplemented by 10% PKS, 1% POME and 1% SD) (Table 4).

**Table 4.** Methane yield per kg fresh substrate added, kg TS added, and kg VS added of untreated palm oil waste fractions

| Substrate formulation no. | Methane yield ($m^3CH_4$/kg fresh substrate added) | Methane yield ($m^3CH_4$/kg TS added) | Methane yield ($m^3CH_4$/kg VS added) |
|---|---|---|---|
| 1 | 0.186 | 0.258 | 0.287 |
| 2 | 0.172 | 0.301 | 0.328 |
| 3 | 0.267 | 0.33 | 0.373 |
| 4 | 0.222 | 0.286 | 0.288 |
| 5 | 0.127 | 0.176 | 0.201 |
| 6 | 0.199 | 0.244 | 0.285 |
| 7 | 0.240 | 0.341 | 0.375 |
| 8 | 0.245 | 0.379 | 0.428 |
| 9 | 0.252 | 0.285 | 0.319 |

The trend for methane yields from SMS was slightly different. The lowest yields per kg SMS added were lowest from substrate formulation no. 8 (1:1 PMF:EFB supplemented by 10% PKS, 1% POME and 1% SD). Yields per kg TS and VS SMS added were lowest from substrate formulation no. 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD). The highest yields per kg, TS and VS SMS added were highest from substrate formulation no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%) (Table 5).

**Table 5.** Methane yield per kg fresh substrate added, kg TS added, and kg VS added of pre-treated palm oil waste fractions.

| Substrate formulation no. | Methane yield $m^3CH_4$/kg fresh substrate added | Methane yield $m^3CH_4$/kg TS added | Methane yield $m^3CH_4$/kg VS added |
|---|---|---|---|
| 1 | 0.231 | 0.340 | 0.384 |
| 2 | 0.138 | 0.433 | 0.481 |
| 3 | 0.232 | 0.315 | 0.353 |
| 4 | 0.341 | 0.525 | 0.584 |
| 5 | 0.187 | 0.325 | 0.367 |
| 6 | 0.266 | 0.342 | 0.380 |
| 7 | 0.102 | 0.365 | 0.415 |
| 8 | 0.072 | 0.221 | 0.254 |
| 9 | 0.131 | 0.161 | 0.181 |

### Effect of pre-treatment on methane yield of palm oil waste fractions

Percentage increment describes the effect of biological treatment of wastes on methane yield in comparison with methane produced from untreated waste. The effects on methane yield ($m^3CH_4$/kg VS of palm oil wastes added) potential (increase or decrease) at different fractions compared to control (untreated) are presented in Figure 4. An increase in methane yield potential as a result of mushroom cultivation of between 10.67– 102.78 % was observed from all substrate formulations except formulations no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%), 8 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD) and 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD). The highest methane yield increase was observed in substrate formulation no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%).

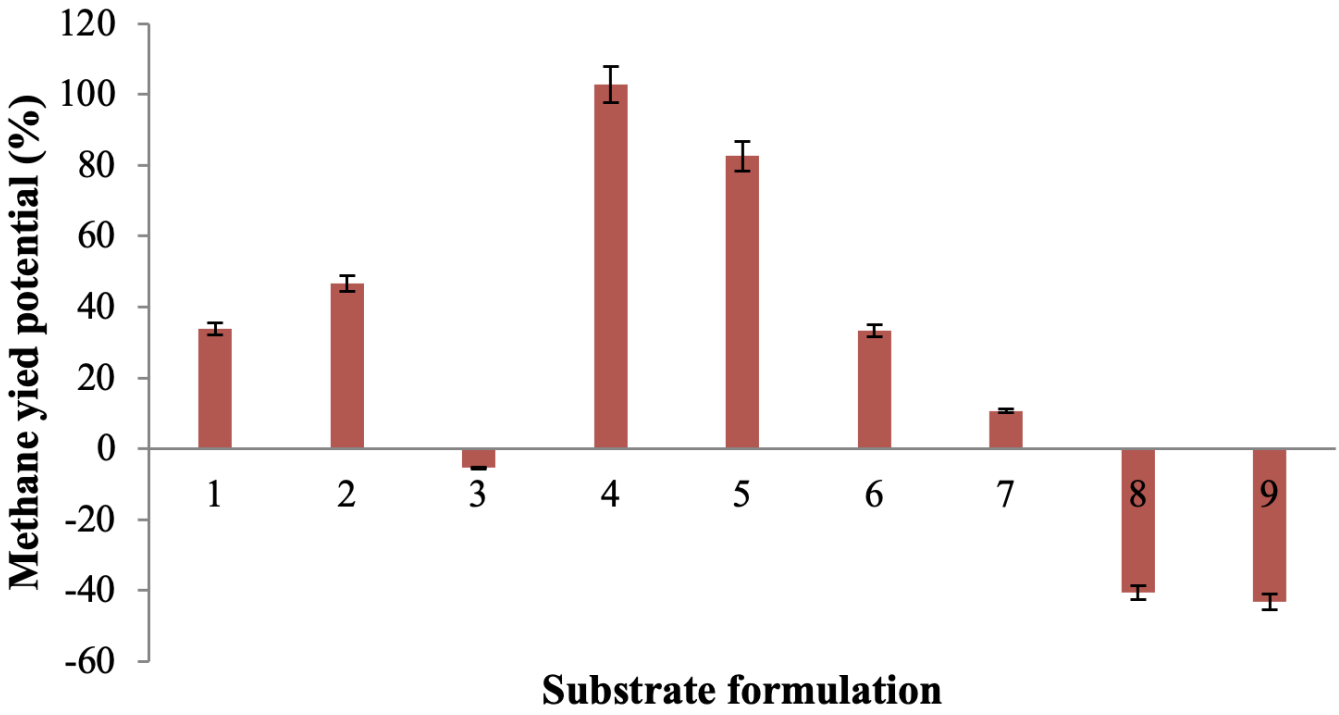


**Figure 4.** Effect of pre-treatment on methane yield of treated (SMS) palm oil waste fractions in comparison with untreated wastes. Error bars indicate the standard error of the mean of the replicates.

## Discussion

### Substrate characterization

The suitability of biomass as feedstock for processing to bioproducts depends mainly on its chemical composition and availability of nutrients measured by the amount of total solids (TS) and volatile solids (VS). VS values are particularly relevant as their levels indicate available organic matter for digestion and thus inform substrate preparation and optimization. In this study, both pre-treated waste blends and spent mushroom substrate (SMS) had a high organic matter

content of up to 99.32% VS for untreated waste fractions and 90.10% VS for treated waste fractions. TS values of the waste fractions and blends of palm oil processing wastes decreased after digestion by mushroom cultivation, indicating that some nutrients were used in the process. Kelly Orhorhoro et al. (2017) investigated the impact of TS and VS of a variety of substrates on biogas production and found the 91.1% VS to be optimum. Other studies utilizing agro-processing wastes for bio-products production made similar observations in core-sisal boles and the leaf stubs (Mshandete et al., 2013), SMS, yard trimmings and wheat straw (Lin et al., 2014) and cattail weed. (Mshandete, 2011)

## Mushroom production

The highest mushroom yield (196.68 g fresh mushroom/kg moist substrate) was observed from substrate no. 3, a blend of 1:1 palm mesocarp fiber (PMF) and empty fruit bunches (EFB) supplemented by palm oil mill effluent (POME) and sediments (SD) at 1% each. This indicates that the formulation may have been easier to colonize by *Pleurotus* mushrooms and hydrolyze to release nutrients for growth, hence more mushroom production. The same substrate formulation had the highest biological efficiency of 75.13%, indicating better substrate conversion into mushrooms. Further, cap diameter and average stipe length correlated with mushroom yield; the higher the mushroom yield, the larger the average cap diameter and the longer the average stipe length, indicating the quality of substrates and their utilization.

Mushroom yield increased progressively from substrate formulation no. 1 (98% PMF supplemented with 1% POME and SD) to substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%). The mushroom yield then decreased steadily from substrate formulations no. 4 to 9 except for substrate formulation no. 8. The reasons for lower productivity from some blends may be the high water holding capacity for PMF-based blends, such as substrate formulation no. 1 (98% PMF supplemented with 1% POME and SD) or the tough fibrous nature of EFB-based blends, such as substrate formulation no. 2 (98% EFB supplemented with 1% POME and SD). High water holding capacity is a property that is unfavorable for mushroom cultivation and may allow colonization of other unwanted microorganisms. In fact, substrate formulation no. 1 was contaminated by other green molds, possibly *Trichoderma* spp, during the study. The fibrous nature of EFB may have made it harder for the fungal enzymes to hydrolyze it to release nutrients during mycelium colonization. The nature of the substrate blends gets more complex from substrate formulations no. 4 to 9 to include more palm kernel shells supplementation, possibly adding cause resistance to hydrolysis and hence low mushroom yield. Mmanywa and Mshandete (2017) utilized similar palm oil waste blends to cultivate *Coprinus cinereus,* where the highest yields were from an EFB-based formulation supplemented with 1% POME and SD. However, the biological efficiency obtained was much lower, up to 35% across all blends. On the other hand, Temu et al. (2016) cultivating *C. cinereus* on a blend of palm oil processing wastes obtained similar yields and biological efficiency. Comparable yields of *Pleurotus* HK-37 were obtained from sisal waste fractions supplemented with cow dung manure, with the highest biological efficiency of 62.87% (Raymond et al., 2013). Mshandete (2011) reported high biological efficiency of up to 100.57% while investigating the cultivation of *Pleurotus* HK-37 on different botanical fractions of cattail weeds. Other studies utilizing palm oil wastes for *Pleurotus* mushroom production found lower yield and biological efficiency (Aubrey et al., 2022), lower yield but higher biological efficiency (Sudirman et al., 2011) and higher yield and biological efficiency with cocoa supplements (Silva et al., 2020).

## Enhancement of methane yield from palm oil waste fractions

Findings show that oyster mushroom production increased the efficiency of biogas production from the majority of the palm oil processing waste blends (7 out of 9) in batch anaerobic reactors. Likewise, methane content was higher in the majority of spent mushroom substrate blends (5 out of 9) compared to untreated palm oil waste blends. However, the most important parameter in biogas production is methane yield per unit volume of volatile solids (VS) contained in the substrate. While biogas volume and methane content are also important parameters, methane yield per unit volume of VS is directly related to the amount of energy that can be extracted from the biogas produced. The methane yield is also an indication of the biodegradability of the substrate, as substrates with low VS/TS, such as lignin, are not easily degraded during biogas production (Sawyerr et al., 2019). In this study, substrate formulation no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%) showed the highest methane yield (0.584 $m^3CH_4$/kg VS), which was twice as much, compared to 0.288 $m^3CH_4$/kg VS obtained from its untreated control. This corresponded to a 102.78% increase in methane yield/kg VS added. Substrate formulations no. 1 (98% PMF supplemented with 1% POME and SD), 2 (98% EFB supplemented with 1% POME and SD), and 5 (1:1 PMF and EFB (39% total weight each supplemented with PPC and PKS at 10%, POME and SD at 1%) also showed a significant increase of methane yield of 33.80%, 46.65% and 82.59%, respectively. The increase in methane yield may be due to improved susceptibility of the substrates to microbial hydrolysis caused by the degradation of lignin by the oyster mushrooms.

Mehta et al. (1990) observed a similar tendency in improved biogas productivity with spent wheat straw, where there was eight times more biogas production from wheat straw treated by cultivation of *Pleurotus florida* mushrooms compared to untreated straw. The spent straw had less cellulose than the original material and an apparent increase in nitrogen content after supporting fungal growth, and this could be the reason for increased biogas production. The tendency of biogas yield to increase with fungal pretreatment of biomass has been reported by other investigators. Bisaria et al. (1983) obtained an increase in biogas yield of 54% from rice straw pre-treated with *Pleurotus sajor-caju,* and Müller and Trösch (1986) observed a doubling in gas yield from wheat straw SMS compared to untreated wheat straw. Utilizing palm oil waste blends similar to the ones in this study, Mmanywa and Mshandete (2017) investigated biogas yield potential increase from palm oil processing wastes SMS pretreated with *C. cinereus* of up to 44.11% (1.4 times), with the highest methane yields per kg VS observed from an EFB-based and PMF and EFB-based SMS compared to untreated waste fractions.

The methane yield per kg VS added for substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%) that produced the highest mushroom yield, and biological efficiency was slightly higher in untreated waste blends compared to SMS. This substrate blend showed a negative methane yield potential per kg VS of -5.36%. This may be due to the utilization of the nutrients during mushroom production and, thus, a lesser amount being left for biogas production. The same may apply to substrate formulation no. 8 (1:1 PMF:EFB supplemented by 10% PKS, 1% POME and 1% SD) that had a negative yield potential between pre-treated waste blends and SMS of -40.65%. However, taking into account the higher yield of mushroom production and modest methane yield, these

substrate formulations performed well on both accounts. A study investigating residual Shorea wood biomass for co-production of oyster mushroom *P. ostreatus* and biogas observed a decrease in lignin content from 31.5% to 23.7% and a decrease in holocellulose from 62.6% to 52.7% (Amirta et al., 2016). Similarly, Temu et al. (2016) observed a decrease in total carbohydrates and lipids from palm oil waste SMS.

The decrease in methane yield potential per kg VS was also observed from substrate formulation no. 9 (1:1 PMF:EFB supplemented with 20% PKS, 1% POME and 1% SD). This substrate formulation seemed resistant to hydrolysis during mushroom production, probably contributed by the higher palm kernel shells supplement; thus, fewer nutrients were exposed for the methanogenic bacteria for biogas production. Substrate formulation no. 7 (1:1 PMF:EFB supplemented with 20% PPC, 1% POME and 1% SD) gave a very low percentage increase of methane yield of 10.67%. This substrate formulation was contaminated during mushroom production and did not produce mushrooms. Therefore, much of the lignin was probably left in the substrate, making it difficult for hydrolysis during biogas production. Similarly, Mmanywa and Mshandete (2017) observed a decrease in methane yield per kg VS from some of the palm oil waste SMS of between -5.26% to -54.76%.

In general, Substrate formulations no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%) is the most suitable for co-production of oyster mushrooms *Pleurotus* HK-37 and biogas as observed by the high mushroom yield (169.58g fresh mushrooms/kg wet substrate) and highest percentage increase of methane yield per VS added of 102.78%. Although it is possible to produce biogas from individual components of palm oil processing wastes (Baharuddin et al., 2010; Singkhala et al., 2021; Suksong et al., 2020), pretreatment ensures maximum utilization of the biomass for both food and energy production but also exposes the nutrients in complex substrates and thus improves the efficiency of the process.

## Conclusion

The goal of this study was to investigate the potential of various waste fractions resulting from palm oil processing for the production of food and biofuel. Substrate formulation no. 3 (1:1 PMF and EFB (each 49% total weight) supplemented with POME and SD at 1%) showed the highest mushroom yield, biological efficiency and mushroom size, while spent mushroom substrate formulation no. 4 (1:1 PMF and EFB (each 44% total weight) supplemented with PPC and PKS at 5% and POME and SD at 1%) showed the highest biogas volume, methane content and yield. These findings indicate that mushroom production is a viable biological pretreatment method for complex lignocellulose waste materials such as palm oil processing wastes. In general, a combination of empty fruit batches and palm mesocarp fiber shows great promise as a substrate for oyster mushroom production and biogas. Based on this research, we recommend integrating mushroom cultivation and biogas production into palm oil production processes to help manage resulting waste products and promote a circular economy. Other substrate formulations investigated in this study may also be used depending on needs and availability. Further studies could optimize oyster mushroom and biogas production by adding other supplements like sources of Nitrogen, or investigate the waste blends on other types of mushrooms.

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## Author Contribution

- Experimental design: A. M. M.
- Sample collection and data generation: A. B. D.
- Data analysis: A. B. D. and A. M. M.
- Manuscript writing: A. B. D. and A. M. M.

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