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Mixed Palm Oil Waste Utilization through Integrated Mushroom and Biogas Production

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Authors' contributions

This work was carried out in collaboration between all authors. Author SGT collected the study material carried out the mushroom cultivation and anaerobic digestion experiments. Author APM designed the anaerobic digestion experiments, organized the compositional and data analysis and participated in drafting the manuscript. Author IAN managed the analysis of the study and participated in writing the first draft of the manuscript. Author BM edited the first draft of the manuscript. Authors AMM and AKK managed the literature searches read and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Study: The study was to integrate mushroom and biogas production using mixed palm oil to provide both food and energy source to palm oil producing communities as well as reducing environmental pollution.
Design of the Study: Mixed palm oil waste was divided into two portions. One portion was used for mushroom cultivation and afterwards the spent mushroom substrate and the untreated portion

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were used for biogas production.
Methodology: Structural sugars analysis was performed using double acid hydrolysis technique. Total crude protein was determined through kjeldal acid digestion method. Lipids were extracted using a mixture of chloroform and methanol and quantified gravimetrically.
The mushroom strain (*Coprinus scinereus*) was cultivated on the mixed palm oil waste. Afterwards, the spent mushroom substrate and the untreated palm oil waste were subjected to anaerobic digestion in automatic methane potential test system.
Place and Duration of Study: The study was completed in 2 years from 2014-2015. Mushroom cultivation was carried out at the University of Dar-e salaam, Tanzania, whereas feedstock characterization and anaerobic digestion were performed at Lund University, Sweden.
Results: Compositional analysis disclosed that the feedstock contains (% w/w) 0.1 proteins, 3.3 carbohydrates, 22.5 lipids, and 73 lignin. Mushroom yield was 0.64 g /g of substrate at a biological efficiency of 71.4 g/100 g of substrate and productivity of 21.5±0.5%. Consequently total carbohydrates and lipids were decreased by 70% and 76% while the relative content of lignin and protein increased by 23% and 50%, respectively. Particle size reduction (<4 mm) resulted to increased methane yield by 66%. The untreated and biologically treated mixed palm oil wastes yielded 517 and 287 of CH_4 L/Kg VS added which corresponded to 80% and 64.5% of theoretical methane yield, respectively.
Conclusion: Combined mushroom and biogas production offer superior benefits in the utilization of the palm oil waste.

Keywords: Coprinu scinereus; anaerobic digestion; mixed palm oil wastes; mushroom cultivation; spent mushroom substrate.

1. INTRODUCTION

In Tanzania, oil palm cultivation is popular in the western region particularly in Kigoma District, where local farmers have cultivated this crop for the production of palm oil since1920s [1]. The palm oil industry generates large volume of wastes rich in both lipids and lignocelluloses from the oil extraction process [2]. For every ton of fresh fruit bunch processed into crude palm oil, wet empty fruit bunches (22-23%), wet palm press fiber (12-13.5%) and wet endocarp palm kernel shell (5-5.5%) [3] are generated. All these are categorised as solid wastes. The liquid waste commonly known as palm oil mill effluent accounts for 60% of the fresh fruit bunches [3] and comprises of high organic content mainly oil and fatty acids [4]. Other wastes generated include oil sludge (oil sediment), oil palm trunks (once in 25-30 years), oil palm fronds (a by-product of the cultivation of oil palm tree collected during pruning, replanting and harvesting) and palm kernel press cake waste [5]. The current palm oil production system is generally seen as unsustainable because of the detrimental effects on biodiversity such as loss of virgin forests and greenhouse gas emissions associated with current waste disposal methods [6]. So far the palm oil industry in Africa has not used its vast waste streams for bioconversion to value added products such as mushroom and biofuels (e.g. biogas, biodiesel etc).

In Tanzania annual generation of fresh oil palm post harvest wastes has been estimated at 10^5 tons while palm oil processing wastes has been estimated at 1.3×10^5 tons and $1.5 \times 10^5 m^3$ of wastewater [7]. These wastes in Tanzania are mainly used to make household products (e.g. brooms) and building material. Only a small part of the waste is used as fuel feedstock (firewood) in palm oil operations whereas over 90% are not utilised but rather dumped into the environment leading to pollution problems and waste of bioresource [7]. The application of integrated bioconversion approaches to turn these bioresource into value added products such as food, feed, bioenergy (e.g. biogas) and bio-fertiliser would make the palm oil industry more attractive and sustainable [5]. Bioconversion of palm oil wastes into value added products can also save as a strategy for mitigating environmental pollution in Tanzania.

The use of palm oil waste for bioenergy production has been reported in some Asian countries (e.g. Malaysia and Indonesia) and demonstrated to be potential substrate for biogas production [4,8].

However, research on anaerobic digestion (AD) is dominated by palm oil mill effluent as a feedstock with limited studies on solid fractions of palm oil wastes [9]. Mixing different fractions of palm oil waste may enrich and make a more

suited substrate due to different types of nutrients contributed by each component. The biodegradability of different waste streams differs depending on their composition. For example, there is a direct correlation between the absolute biodegradability and the ratio of lignin and cellulose content in different waste streams [10]. High proportions of lignin and cellulose content have a strong negative synergistic effect on biomass digestibility [11]. Also degradation of lipids leads to accumulation of long chain fatty acids (LCFA) which are inhibitory to AD [12]. To alleviate these limitations, physical and biological pre-treatments are usually applied prior to AD [13]. Different strategies have been employed to improve methane yield from lignocellulosic wastes including palm oil wastes such as hydrothermal and alkali pretreatment [14,15]. However, integrated mushroom and biogas production from mixed palm oil waste treating the former as a pretreatment is reported for the first time in this study.

Therefore, the mixed palm oil waste was used as substrate for mushroom cultivation, also considered as biological pretreatment, prior to biogas production from the spent mushroom substrate (SMS). Afterwards, both the untreated and biologically treated mixed palm oil wastes were evaluated for methane production through anaerobic digestion.

2. MATERIALS AND METHODS

2.1 Substrate Collection and Preparation

The palm oil wastes were collected from Kigoma, Tanzania. Four fractions namely, palm oil mill effluent, empty fruit bunch, palm press fiber and palm kernel press cake were obtained after harvesting and processing of fresh fruit bunches for palm oil production. These fractions were manually mixed at a ratio of 8:5:4:3 and afterwards divided into two parts of which one part was used for mushroom (*Coprinu scinereus*) cultivation also considered as a biological pre-treatment. The ratio is based on the contribution of each component to total solids according to composition analysis done in a previous study [7]. Both the untreated portion and biologically treated portion referred to as spent mushroom substrate (SMS) were ground in a laboratory grinder and sieved to three particle sizes i.e. <4 mm (L_1), 4-10 mm (L_2) and ≥11 mm (L_3).

2.2 Mushroom Cultivation

Part of the mixed palm oil wastes mentioned in section 2.1 was used as substrate for cultivation of a species of edible mushroom, *Coprinu scinereus*. A pure isolate of *Coprinu scinereus* (Schaeff) S. Gray s. lat [16] was obtained from Strain bank at the University of Dar es Salaam, Tanzania. Mushroom cultivation was done according to Mshandete and Cuff [17]. Briefly the strain was first activated on malt extract agar at 28℃ and subsequently grown in sorghum grain in 330 mL bottles. Afterwards the bottles were incubated at 28±2℃ until the grains were fully colonised by mycelia. The spawn (5% w/w) were inoculated into sterilised mixed palm oil wastes in polythene bags (ca.1.5 kg) and incubated in dark room at 28℃ to ensure that the substrate does not pin prematurely (i.e. formation of primordia which are white spikes that protrude out of the substrate) for ca. 2 weeks. Harvesting was done after approximately 3 weeks.

2.3 FTIR Measurements

In order to ascertain structural changes caused by the biological pre-treatments on the mixed palm oil wastes, structural and functional group transformation was investigated according to Moshi et al. [18]. The infrared spectra were recorded with FTIR Nicolet IS5 (Thermo Fischer Scientific, USA). Thus, for spectral acquisition about 0.01 mg of each sample was deposited on the surface of the glass disc of the instrument and measured against a pre-established background. All spectra were recorded from 4000 to 550 cm^{-1}. Prior to each measurement the surface of the glass disc was cleaned with methanol.

2.4 Inoculum Collection and Preparation

The inoculum for the batch AD experiments was collected from a mesophilic anaerobic digester treating municipal sewage sludge (Källby, Sweden). The inoculum was pre-incubated at 37℃ for 4 days to deplete the residual easily biodegradable matter [19]. After pre-incubation, a representative sample of the inoculum was analysed in quadruplicate and was found to have an average pH of 7.9, total solids (TS) of 4.2% and volatile solid (VS) of 2.7%.

2.5 Reactor Setup for Anaerobic Digestion

Automatic Methane Potential Test System (AMPTS II) (Bioprocess Control, Lund, Sweden), was used to evaluate methane potential of the untreated and SMS according to Moshi et al. [19]. Two sets of controls were included and

treated in the same way as the test reactors. One control contained only the inoculum to determine the background methane production from the inoculum, and the other control was cellulose (Avicel PH-101, Sigma-Aldrich, USA) to ascertain the suitability of the inoculum. The inoculum to substrate ratio in all experiments was maintained at 2:1 based on grams VS. AD was performed in the AMPTS bioreactors (each 500 mL volume) with working volume of 300 mL. Reactors were purged with O_2 free nitrogen for about 2 minutes and incubated for AD at 37±0.5℃ in a water bath. Stirrers were set to operate intermittently, 30s on and 120 s off at 46 rpm during the whole experiment. Methane volumes were recorded as normalised millilitres.

2.6 Analytical Methods

TS, VS, extractives, structural carbohydrates and lignin were determined according to Sluiter et al. [20,21]. The monomeric sugars released during acid hydrolysis were determined by HPLC (JASCO Corporation, Tokyo, Japan) equipped with a Bio-Rad Aminex HPX-87P column and a refractive index detector (RID). The column temperature was maintained at 85℃. The mobile phase was HPLC-grade water at a flow rate of 0.6 ml/min. All samples were filtered through a 0.45 µm prior to HPLC analysis.

Total lipids were determined according to a protocol described by Folch et al. [22] with some modifications. Briefly, 0.5 g of the mixed palm oil wastes, particle size < 4 mm was suspended in 10 mL of a mixture of chloroform /methanol (2:1). The whole mixture was swirled manually by hand for 20 minutes. The homogenate was centrifuged at 1700 g (Beckman Spinchron Benchtop Centrifuge) for 30 minutes and subsequently filtered through Whatman™ 1004-150 Grade 4 Qualitative filter paper 15 cm diameter, pore size 25 µm, to recover the liquid phase. Subsequently the solvent was washed with 2 mL of 0.9% NaCl (w/v), vortexed and centrifuged twice at 1700 g, and afterwards the upper phase was carefully siphoned off and the lower phase was evaporated to dryness using rotary evaporator and the amount of lipids were determined gravimetrically.

Total protein was determined using bicinchoninic acid (BCA) procedure according to Smith et al. [23]. Samples were extracted using liquid nitrogen and dissolved in Tris-HCl buffer, pH 7.0. After dilution and addition of the BCA reagent, samples were incubated at 37℃ for 25 min. After that; absorbance was read with Elisa microplate reader at λ_{550} nm, (Biochrom Ltd, UK). Total protein was deduced using a pre-established bovine serum albumin calibration curve. Dr. Lange test kits LCK 114 or LCK 914 was used for chemical oxygen demand (COD) analysis and the measurements were performed in a Lasa 100 spectrometer (Dr. Bruno Lange GmbH, Germany).

The degree of COD solubilisation was calculated according to the following formula:

$$\text{COD solubilisation}\,(\%) = \frac{\text{Soluble COD measured after pre-treatment}}{\text{Total COD measured before pre-treatment}} \times 100$$

2.7 Statistical Analysis

All the experiments were performed in triplicates and the data were expressed as mean values ± SD. Analysis of variance (one-way ANOVA) at 95% confidence interval was performed on methane yield data to determine the effect of different degrees of size reduction. The statistical package was installed directly in excel through the Add-In function of Microsoft word 2007.

3. RESULTS AND DISCUSSION

3.1 Substrate Characterisation

Compositional characteristics of the untreated and biologically treated mixed palm oil wastes are presented in Table 1. The composition data include moisture content, TS, VS, total COD (tCOD), soluble COD (sCOD), structural carbohydrates, total lignin, extractives, total lipids and total protein. The values for lipids are slightly higher while those for structural sugar and protein are lower than those reported by Wu et al. [24] for palm oil mill effluent alone. The concentration of total structural carbohydrates (cellulose and hemicelluloses) and lipids decreased by 70% and 76% while lignin and protein increased in relative terms by 23% and 50%, respectively after mushroom cultivation (*Coprinu scinereus*). This could be attributed to utilisation of the readily degradable components such as carbohydrates, lipids and proteins as energy source for the growth of the mushroom [25]. The increase in protein could be attributed to microbial cells in the spent mushroom substrate. The fact that lignin increased in relative terms, implies that *Coprinu scinereus*

does not have ligninolytic enzymes for lignin degradation as reported in other white-rot fungi Zhang et al. [26,27]. The amount of mushroom harvested 0.64 kg /kg of substrate implies that only 64% of total mixed palm oil wastes were converted to mushroom. In terms of organic matter as measured by COD (Table 1) only 18% was converted to mushroom. Thus, in relative terms, 82% of the total organic matter remains in the SMS and this create a problem not only of their disposal but also a significant loss of bioresource. The higher amount of COD in the SMS is due to the fact that while the fungi use organic matter for growth, the microbial cells also add to it.

One of the major environmental hitches in the mushroom producing countries remains the treatment and disposal of the spent mushroom substrates (SMS) [28]. About 5 kg of SMS is produced for each kilogram of mushrooms [29]. Total mushrooms production in 16 leading countries in 2007 was 3,414,392 metric tons which means 17,071,960 metric tons of SMS. In many countries SMS as an agricultural waste has been disposed of without due consideration to the environment [29]. The best option of utilizing this bioresource is production of biogas through anaerobic digestion which also results into an excellent biofertiliser [30,31] and hence closing the production cycle.

Therefore, the integrated approach of mushroom-biogas production is justifiable. The remaining digestate (AD residue) can therefore be used as biofertiliser to enhance vegetable production and thus a zero waste. The tCOD for untreated and biologically pre-treated fraction was in agreement with VS values of 86% and 78%, respectively (Table 1). Decrease in degree of solubilisation by 10% was observed after biological pre-treatment with *C. cinereus*, which indicated decrease of soluble organic components during biological pre-treatment.

3.2 Mushroom Cultivation Biological Efficiency and Productivity

The average yield of the harvested mushroom from triplicate experimental units (1.5 kg bags) was 0.96±0.02 kg (wet weight). The time for first appearance of mushrooms was 10-11 days. The fruiting bodies were harvested while young, firm and fleshly before the caps turned into inky mass. When the mushroom caps turn to inky mass are considered over natured and not suitable for food [16]. Biological efficiency, defined as the percentage conversion of substrate into fruit bodies on a dry weight basis [32], was found to be an average on mixed palm oil waste (71.4 g/100 g substrate). Productivity which was determined from the relationship between fresh weight of mushroom and fresh weight of substrate [33] was 21.3±0.5 (%). The biological efficiency obtained for *C. cinereus* is slightly higher than that reported for the strain on composite sisal decortication waste supplemented with gypsum [17]. The results indicated that the mixed palm oil waste could be recommended as potential substrate for cultivation of bisidiomycetous mushroom, *C. cinereus* which is reported for the first time in Tanzania. The palm oil waste is abundantly found especially in the western part of Tanzania (Kigoma region) where many small scale processors of oil palm are located.

Since this research had a dual purpose, mushroom production and biogas from mixed palm oil waste in addition to mitigation of environmental pollution, composition analysis of the spent mushroom substrate (SMS) was performed. The analysis revealed that it contained up to 91% and 82% of TS and COD of the original mixed palm oil waste, respectively (Table 1). This implies that the SMS is a potential substrate for biogas production and that integrated mushroom and biogas production could accrue double benefit to the communities dealing with oil palm production and processing.

3.3 Effect of Biological Pre-treatment on Structural Composition of Mixed Palm Oil Waste Fractions

The effects on structural transformation following biological pre-treatments are indicated by FTIR spectra Fig. 1 (A and B). The changes were evidenced by disappearance of bands or decrease in intensity of the bands, for functional groups and appearance of new bands (e.g. bands 291593 was 291981 in the treated Fig. 1B). The band in the region of 2915.93 and 2849.51 cm^{-1} in untreated material was observed to have higher intensity than in the biologically treated, and these were assigned to the asymmetric stretch vibration of CH_2 group in cellulose [34]. As compared to spectra of the untreated material, the lignin band at 1585.8 cm^{-1} (aromatic ring of lignin) was enhanced in the biologically pre-treated sample at 1634.05 cm^{-1} and 1539.5 cm^{-1} and this could be due to removal of cellulose and hemicellulose [34]. This observation was correlated with the

compositional analysis (Table 1) wherein a relative increase of lignin content was noted in the biologically treated samples. The disappearance of peaks at 1471.33 cm^{-1}, 1429.43 cm^{-1} and 1417 cm^{-1} (C-H bending of alkane) in pre-treated sample could signify the loss of CH_2 vibration. These changes possibly suggest the loss of carbohydrate and fatty acids after biological pre-treatment. This was also exemplified by relative decrease in peak intensity of FTIR spectra of the biologically treated fraction see Fig. 1B.

3.4 Effect of Size Reduction and Biological Pre-treatment on Methane Yield

The specific VS-based methane yields of the untreated and biologically treated mixed palm oil wastes at different particle sizes are shown in Figs. 2A & C. The highest methane yield achieved in the present study was 517±10.2, 353.40±2.0 and 339.78±4.42 L/KgVS added for the untreated sample with particle size of < 4 mm (L1), ≥ 4.0-11.0 mm (L2) and ≥ 11.0 mm respectively. These corresponded to 80%, 50% and 48% compared to theoretical yield Fig 2B. The theoretical methane yield was estimated as detailed in section 3.4. For SMS methane yield was 287±22.7, 197.20±3.5 and 193.10±19.5 L/kg VS added which corresponded to 64.5% 44.3% and 43.3% compared to theoretical yield. For all particle sizes, the untreated fractions yielded more methane than the biologically treated fractions. This could be explained by the fact that there was loss of lipids and carbohydrate during vegetative growth of *C. Cinereus* and that only 18% and 24% of the total lipids and carbohydrate were available for methane production, respectively (Table 1). However lignin and protein increased in relative terms by 18 and 50% respectively. This implies that the fungi *C. cinereus* does not produce lignin degrading enzymes. It also implies that the microbial cells contributed to the resultant protein increase. Relatively low methane yield of biologically pre-treated substrate has been reported in other studies [35]. However, more than 50% of methane was produced from SMS compared to the untreated mixed palm oil wastes. It is therefore advantageous to use the integrated approach than production of either biogas or mushroom alone from the mixed palm oil wastes. The mushroom can be processed into a variety of functional food products that will be consumed by a sizable proportion of the public to have a wide health benefit which include lowering of the body weight and reduced obesity [36] increased vitamin D levels [37], improved immune system function (because of presence of α and β glucans) [38-40]. The biogas manure can be used as an organic fertiliser to improve production of leafy vegetable commonly consumed by rural, peri and urban populations. Particle size reduction significant improved methane yield ($P \leq 0.05$) with up to 66% and 48% for the untreated and biologically treated mixed palm oil waste (L1) (Figs. 2 B & D). Only a small increase (4 & 3%) between L2 and L3 for the untreated and biologically treated samples respectively, was observed. Varying degrees of increase in methane yield with decrease in particle size of different substrates has been reported in literature [41,42]. Smaller particles

Table 1. Substrate characterization

Parameters measured	Untreated mixed palm oil waste	Biologically mixed palm oil waste
Moisture content (%)	5.9±0.1	12.2±0.3
Total solids (% TS)	94.1±0.1	87.8±0.3
Volatile solids (% VS)	85.89±6	78.41±0.5
Total COD (g/L)	1154±7	948±7
COD solubilisation (%)	34±1	24±0.7
dCOD (g/L)	392±12	224±8
Glucose (%)	1.5±0.1	0.6±0.0
Xylose (%)	1.1±0.0	0.4±0.2
Galactose (%)	0.11±0.1	0±0.0
Arabinose (%)	0.2±0.0	0±0.0
Mannose (%)	0.4±0.0	0±0.0
Total lignin (%)	73±0.1	90±0.2
Extractives (%)	24±0.4	9±0.6
Total recovery (%) (TR)	103.3±0.3	101±0.6
Total protein (%)	0.08±0.0	0.12±0.0
Total lipids (% DWB)	22.5±1	5.5±2

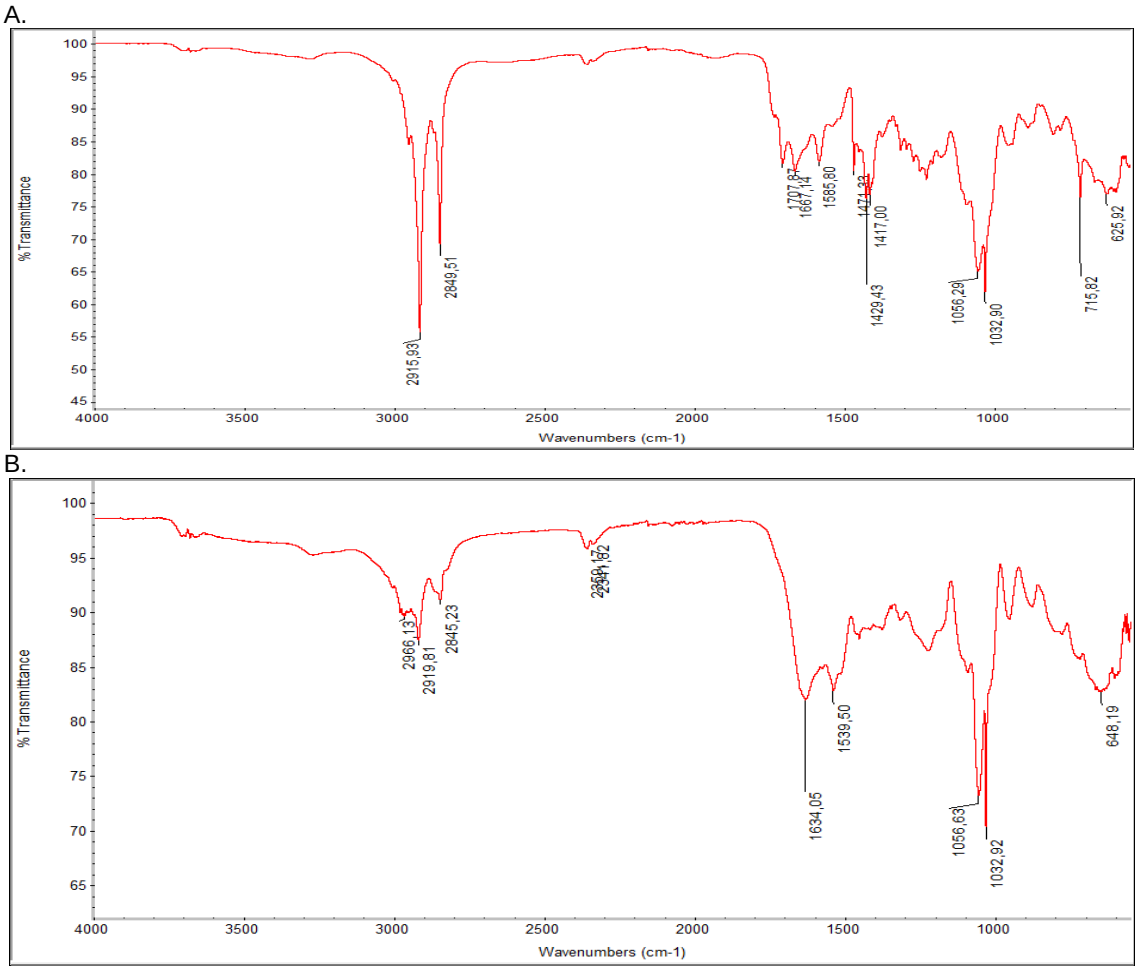

Fig. 1. FTIR: Structural change in palm oil waste due to treatment with *Coprinus cinereus*. A. Untreated palm oil mix waste, B. Biologically treated mixed palm oil waste (*Coprinus cinereus, ca.* 2 weeks, 28℃, and moist condition)

size increases anaerobic biodegradability through increased surface area and hence enhanced microbial and enzymes activity, and consequently increased yield and rate of methane production.

3.5 Estimation of Theoretical Methane Yield of Mixed Palm Oil Wastes

The theoretical methane potential of the mixed palm oil wastes was estimated based on the contribution to VS of the different components. The composition analysis of mixed palm oil wastes included pre-and post-AD lignin determination. Theoretical methane yield was calculated based on the equation:

Total biomethane potential= 415W + 1014 X + 496Y + 727Z according to Triolo [43], where the total potential was given as L CH_4 /Kg VS added considering the proportions of carbohydrate, lipid, protein and lignin per Kg of VS represented as W, X, Y and Z, respectively. Theoretical methane potential of lignin was calculated using the equation founded by Symons, Buswell [44] as follows, lignin $C_{10}H_{13}O_3 + 5.25H_2O = 5.875CH_4 + 4.125CO_2$. From the composition analysis data, total theoretical methane yield of the mixed palm oil wastes, assuming complete degradation of lignin was estimated at 906L/Kg VS added. However, when post AD lignin determination was done and the fraction of lignin degraded in AD considered in the calculation, the total theoretical CH_4 yield of the mixed palm oil waste was 703 L/ Kg VS added. This value was used for calculation of methane yield as percentage of theoretical for all experiments. Post AD lignin determination disclosed that the lignin

degradation during AD was 49%. This contributed significantly to the methane yield. The mechanism of lignin degradation during anaerobic digestion needs further investigation. Perhaps it is related to the composition of the microbial consortia. If this is so then an inoculum could be formulated to include lignin degraders and could be a milestone in AD of lignocellulosic biomass.

There was no inhibition observed during AD of the mixed palm oil waste. It is possible that different fractions of the palm oil wastes resulted to balanced mixture with lipid below that which might have caused inhibition. Higher loading rate may lead to inhibition due to high concentration of long chain fatty acids [45,46]. It has been reported that concentration of lipids in the range of 31 to 47% caused severe inhibition

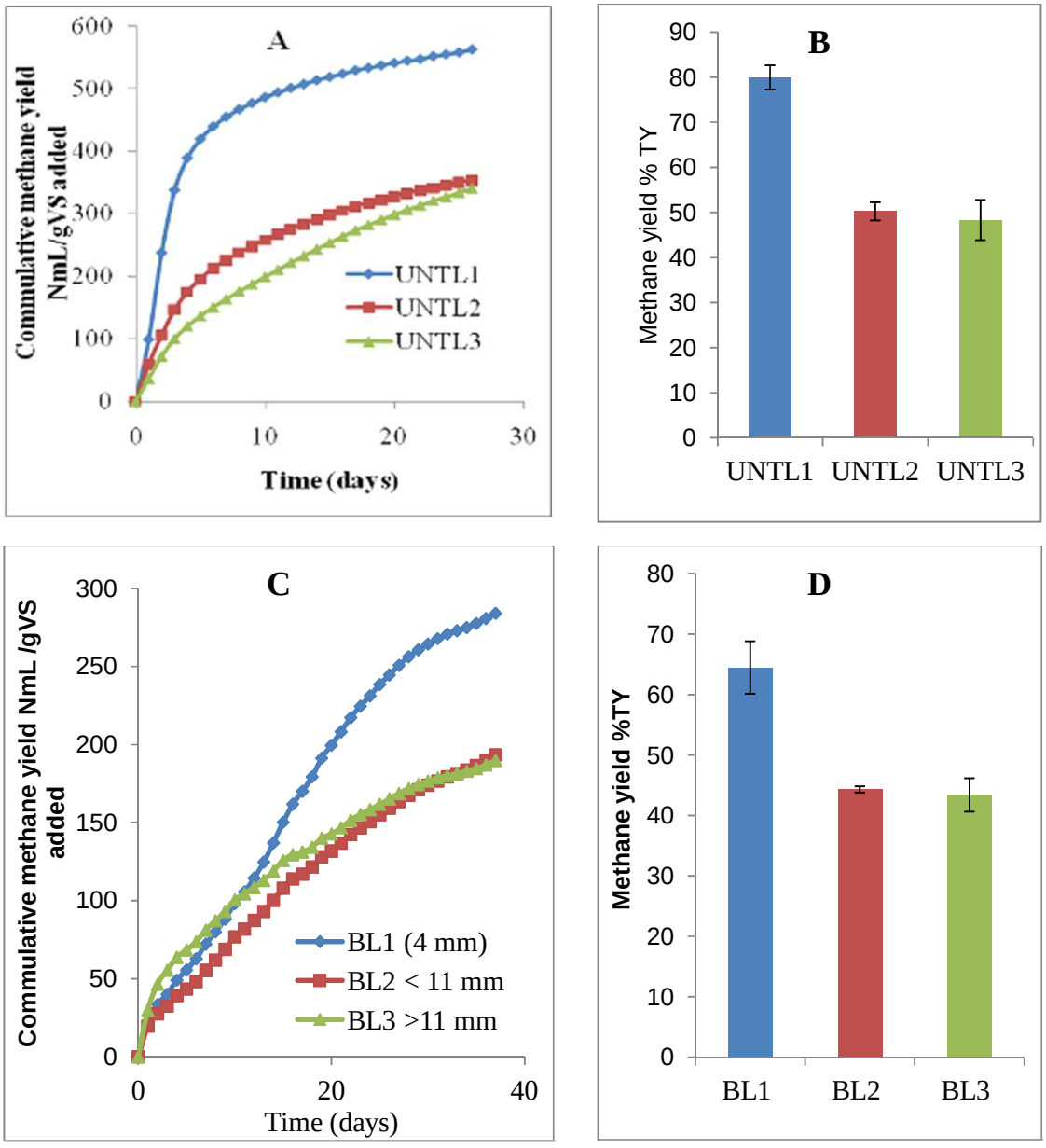

Fig. 2. Cumulative methane yield and methane yield percentage of theoretical yield. A. cumulative methane yield for untreated palm oil mix, B. Cumulative methane for biologically treated palm oil mix, C. Methane yield %TY for untreated palm oil mix, D. Methane yield %TY for biologically treated palm oil mix

Table 2. Biogas production using palm oil waste s different approaches

Substrate	Pretreatment	CH_4 yield mL/gVS added	Reference
Palm oil mill effluent (POME)	Thermophilic AD (45-50℃)	502	[47]
Empty fruit bunch (EFB)	Thermophilic AD (45-50℃)	20 2	[47]
POME+EFB	Thermophilic AD (45-50℃)	276-340	[47]
Oil palm empty fruit bunch (OPEFB)	*N*-methylmorpholine-*N*-oxide at 120℃, 3 h	408	[48]
Oil palm empty fruit bunches (OPEFB)	NaOH (8%), 60 min	240	[49]
Empty fruit bunches)	size reduction <5 mm, 37℃	370	[50]
Mixed palm oil waste (4 different fractions)	size reduction, < 4 mm, 37±2℃	517±10.2	This study
SMS of mixed palm oil waste	size reduction, < 4 mm, biologically treated with *C. cinereus*, 37±2℃	287±22.7	This study

during AD [46]. Mixing of different fractions of the palm oil waste resulted into final lipid content of 22% and 5.5% for the untreated and biologically treated palm oil waste respectively (Table 1), which is well below the amount that could have caused inhibition. The consequence was high methane yield up to 80% and 64.5% of theoretical yield for the untreated and biologically treated mixed palm oil wastes, respectively. Various approaches of biogas production from single and different degrees of mixing of the oil palm fractions are compared in Table 2.

From the analysis done in Table 2 it is evident that mixing different fractions of palm oil waste results into higher methane yield per gram of degradable solids added. This could be explained by a more balanced nutrients supplied by the different fractions of the palm oil waste.

Integrated mushroom–biogas production from the mixed palm oil waste is an outstanding approach since each gram of degradable organic matter ca. 0.8 g of human food (mushroom) is produced. This concomitantly enriches the waste such that more than 50% (Table 2) of methane is obtained from SMS compared to the unused waste. This approach offers the rural community both food and energy, also ensuring clean environment.

Pre and post mushroom cultivation analysis indicated that 70% and 76% of carbohydrates and lipids respectively, were utilized during mushroom growth, whereas protein and lignin increased relatively by 50% and 23% (Table 1). Since lignin degradation (ca, 49%) was observed in the post AD analysis and since lignin ranges second after lipids in methane yield, further studies on lignin degradation in anaerobic reactors is proposed, in a view to optimise the AD process for lignin degradation to maximise methane production from the mixed palm oil waste.

4. CONCLUSION

The mixed palm oil wastes proved to be a promising potential substrate for methane production. Integrated mushroom and methane production from mixed palm oil wastes is a promising strategy of bioconversion of this biomass type to value added products. It produces both food and energy source. It is co-friendly since biogas manure can be used as biofertiliser and hence ending up with zero waste. Significant methane yield was obtained from the spent mushroom substrate 64.5% of theoretical value compared to 80% of the untreated, under the operational conditions applied in this study. Partial degradation of lignin during AD was evident in this study. Therefore, our further studies are focused on optimization of the AD step for better methane yields and improved process economics. Particularly, investigation and optimization of the lignin degradation mechanism is inevitable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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