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Two-stage banana leaves wastes utilization towards mushroom growth and biogas production

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Abstract

Banana leaves wastes (BL) were subjected to fungal treatment using *Pleurotus ostreatus* to produce edible mushrooms and biogas in the anaerobic digestion process. Effects of fungal treatment on mushrooms production, lignin degradation, trace elements compositions and biogas yield during the anaerobic digestion process were evaluated. Treatment with *P.ostreatus* for 36 d resulted in the production of 181 ± 19 g of edible mushrooms per 2 kg of BL with biological efficiency of $37 \pm 4\%$. Lignin concentration in fungal treated BL decreased by 10% indicating an improvement on its digestibility. Important trace elements (Fe, Mn, Mo, Co and Ni) necessary for the improvement of the anaerobic digestion process were also significantly reduced ($P < 0.05$) during the fungal treatment process. The biogas yield for the fungal treated BL was $282 \text{ mL g}^{-1} \text{ VS}^{-1}$ of which this study suggests that could be improved through trace element supplementation during the anaerobic digestion process.

Keywords Banana leaves · Mushrooms · Trace elements · *Pleurotus ostreatus* · Biogas

Introduction

Lignocelluloses materials contain an abundant source of sugars and, therefore, presents an excellent potential for the resources re-use and recovery; however, they comprise lignin, cellulose, and hemicellulose, which are highly recalcitrant and require pre-treatment to enhance their biodegradability (Akpınar and Urek 2017; Kucharska et al. 2018;

Thakur et al. 2013; Vasco-Correa and Shah 2019). In tropical countries such as Tanzania, several tons of unutilized banana by-products, including banana leaves, are generated daily (Padam et al. 2014). Banana leaves wastes are mainly generated at the markets due to their application as wrapping materials for food, clothes, clay pots and cultural applications (Kennedy 2009). Since banana leaves are lignocellulosic, to utilize these wastes for energy recovery such as biogas production would require the pre-treatment to enhance their digestibility.

Different pre-treatment methods such as chemical, physical, physical–chemical and biological are used to enhance the digestibility of the lignocelluloses substrates (Monlau et al. 2013). Pre-treatment of lignocellulosic substrates with chemical, organosolv, ionic liquids or ozonolysis techniques require high operating costs, therefore, are not feasible at large scale applications (Ariunbaatar et al. 2014; Kumar and Sharma 2017). Additionally, handling by-products of chemicals pre-treatment substrates pose an environmental challenge due to formation of inhibitory compounds such as furans, phenolic compounds and carboxylic acids which can inhibit the growth of fermentative microorganisms (Baral and Shah 2014; Behera et al. 2014). Biological pre-treatment, on the other hand, is considered environmentally friendly and does not necessarily

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require chemicals and can be performed at mild conditions (Chaturvedi and Verma 2013; Rodriguez et al. 2017; Sari and Budiyo 2014). Some of the drawbacks of biological pre-treatments include microbes utilize part of carbohydrates during the pre-treatment process, and the process is prolonged (Cesaro and Belgiorno 2014; Mishra et al. 2018). Biological pre-treatment for enhancement of bioethanol and biogas production has mainly focused on pre-treatment by the specific microbial consortium, fungal pre-treatment, partial composting, enzymatic pre-treatment and ensiling (Rouches et al. 2016; Wagner et al. 2018). Among all the available biological pre-treatments, fungal pre-treatment (FP) using a white-rot fungi species such as *Pleurotus ostreatus* (oyster mushroom), *Ceriporiopsis Subvermispora*, *Trametes Versicolor*, *Coriolus Versicolor*, *Phanerochate Chrysosporium*, and *Cyathus Stercoreus* have been considered as most capable of degrading the lignin of the most lignocellulosic substrates and of enhancing their digestibility for their subsequent applications (Abdel-Hamid et al. 2013; Rodríguez-Couto 2017; Thomsen et al. 2016).

Fungal species (e.g., *Pleurotus ostreatus*) have been used to pre-treat and to enhance hydrolysis process for biogas production of different substrates such as wheat straw, shore wood biomass, spent coffee grounds; and sugar bagasse to mention a few (Albornoz et al. 2018; Amirta et al. 2016; Wobiwo et al. 2018; Tuyen et al. 2013). Previous studies indicated that the lignocellulosic substrates such as wheat straw and shore wood could be pre-treated with *P. ostreatus* to improve their digestibility and hence to improve the anaerobic digestion process with a resultant increase of biogas and methane production (Albornoz et al. 2018; Amirta et al. 2016). However, to the best of our knowledge, there is no published report on the influence of fungal treatment on banana leaves wastes using *P. ostreatus* on trace elements, biomass composition and impacts on biogas production. Enhanced biogas production to the pre-treated samples is due to the fungal degradation of lignin which provides accessibility of cellulose for bacteria degradation in the AD process (Pérez-Chávez et al. 2019). However, different results by some studies (Wobiwo et al. 2018; Tuyen et al. 2013) showed that the pre-treatment with *P. ostreatus* on spent coffee grounds and sugarcane bagasse could lead to the loss of carbohydrates and hence the decrease in biogas in the anaerobic digestion process. Therefore, the recovery of the resources such as biogas (after fungal treatment) highly depends on the amount of organic matter left, type of fungal species used, pre-treatment duration, and the number of days for the anaerobic digestion process and the purpose of the pre-treatment. This study aimed to investigate the utilization of banana leaves towards mushroom production from *P. ostreatus* and biogas production. The influence of fungal treatment on banana leaves wastes using *P. ostreatus* on trace

elements, biomass composition and biogas production was evaluated in this study.

Materials and methods

Banana leaves wastes characterization

The banana leaves wastes (BL) used in this study were collected in woven bags from National Milling Corporation (NMC) market, commonly known as Samunge in Arusha city, Tanzania ($3^{\circ} 22' 36.0768''\text{S}$, $36^{\circ} 41' 10.5396''\text{E}$). Banana leaves wastes comprises 23.6% of organic market wastes (Quantified under this study between May and September in the year 2019) as indicated in Fig. 1. Banana leaves are produced from several activities including ripening of bananas and as carrying materials for clay pots. The BL wastes were manually sorted out from the mixed waste streams at the market before the collection. The BL wastes were then brought to the Nelson Mandela African Institutions of Science and Technology (NM-AIST) laboratory where they were chopped into 5–10 cm in length using a bush knife and further air-dried for 7 days before characterization and the fungal treatment work. During characterization, a portion of BL was shredded into small pieces and then grounded with a mortar and a pestle. Total solids (TS), volatile solids (VS), fixed solids (FS) and moisture contents (MC) were measured gravimetrically following standard methods for the examination of water and wastewater samples (APHA 2012). To determine the carbon–nitrogen ratio (C/N), the organic wastes samples were dried in an oven (Binder-Ed 53) at 70°C for 24 h, and the dried samples were crushed and grounded into powders using mortars and pestles before sieving them to obtain fine powders. About 3.3 mg of each sample was analyzed for carbon, hydrogen, and nitrogen using a CHNSO analyzer (Flash 2000 organic elemental analyzer). For pH determination of organic wastes, 10 g of the dried wastes were mixed

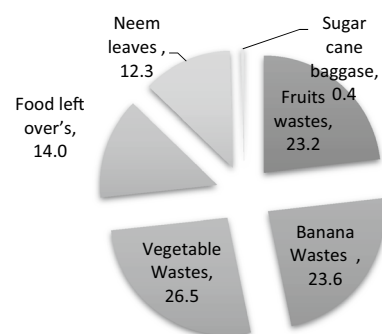


Fig. 1 Organic fraction market wastes compositions at Samunge market ($3^{\circ} 22' 36.0768''\text{S}$, $36^{\circ} 41' 10.5396''\text{E}$)

with 100 ml deionized water and centrifuged for 15 min, then filtered through Whatman filter papers. The pH of the filtered solution was determined using a pH meter (HI 2209 pH/mV Meter). Biomass compositions (lignin, cellulose and hemicelluloses) were determined according to the Chesson (Datta 1981; Maryana et al. 2014). The trace elements in BL were analyzed before and after the fungal treatment at Tanzania Atomic Energy Commission, Arusha, Tanzania, using Energy Dispersive X-ray Fluorescence technique (EDXRF) (Spectro Xepos, serial No. 4R0138, operated by X-lab ProTM software). Some portion of the chopped and dried banana leaves were stored at -20°C to maintain their characteristics before anaerobic digestion process. Statistical analyses for the means differences in trace elements composition of the fungal treated and un-treated samples were conducted using *T* test in Microsoft Excel 2010. Three replicates were used for each sample and results are expressed as mean and standard deviation.

Fungal treatment of banana leaves wastes using *Pleurotus ostreatus*

Fungal treatment of BL was carried out using the Spawn of the fungus, *Pleurotus ostreatus* (PoH) which was procured from the Sokoine University of Agricultural in Tanzania. The spawns were stored at 4°C at the NM-AIST laboratory before use. Fungal treatment was performed using locally available equipment and with the concept of industrial applications in developing countries perspectives. The treatment hut was locally constructed using the tree barks and covered at the top with the coconut leaves. Generally, the treatment involved pasteurization, spawning running (colonization) and formation of the fruiting bodies. Before pasteurization, the BL (5–10 cm) were soaked in water for two hours to soften it and was left to drain until no water was dripping from the substrates. The drained substrates were placed in layers into woven sacks sheets and pasteurized into 0.225 m^3 metal drums with a platform of stones with sieves at the top to the height of 18 cm from the bottom. Firewood was used as the source of fuel, and for one batch of pasteurization for three hours, one bundle (15 kg) of firewood was used. About 20 l of water was poured inside the drum, the substrates were placed on the top of the platform, and the drum was covered with a metal lid. The pasteurization process took about 3 h, and the temperatures inside the drum were measured using an infra-red thermometer (WT900, -50 to 950°C). The temperature inside the pasteurization drum varied from 92 to 124°C . The pasteurized substrates were allowed to cool and drain inside the drum for 1 day. Transparent polyethene sheets were used as packaging materials for the cooled pasteurized substrates. Before packaging, the sheets were cut into small pieces (45 cm length), and the ends sealed using a candle flame. About 2 kg of the loosely

pasteurized substrates (corresponding to 490 g dry weight) were weighed using a portable electronic spring balance and inoculated with *P. ostreatus* strain at the top and both sides inside the polyethene sheets. A total of 10 bags were used in this experiment. The already packed spawn substrates were incubated on wooden shelves which were disinfected with antibacterial sprays (Dettol) in the pre-treatment hut. To ensure enough darkness was available for spawn running, dark clothes were used to cover the spawned bags. In accordance to Tesfaw et al. (2015), light and relative humidity are pre-requisites for pinhead initiation after the completion of the spawn running. To ensure enough light and humidity the dark clothes covering the spawned bags were removed, and tiny holes were made into the bags using a syringe needle and clean water was sprayed once a day to the spawned bags. Temperature and humidity were measured using pocket weather meter (kestrel 3000) and were kept in the average temperature of 22°C and 70–85% humidity. Biological efficiency which is the measure to assess the growth potential of mushrooms was determined as the percentage ratios of the fresh mushrooms harvested to the weight of the dry substrates (Vieira and de Andrade 2016).

Anaerobic digestion process of fungal treated and un-treated banana leaves wastes

Anaerobic digestion process for biogas production of the fungal treated and un-treated banana leaves was carried out in batch reactors. The batch reactor with cow dung only was treated as the control experiment. The other two batch reactors each contained 20 g of the fungal-treated and un-treated banana leaves which were ground and sieved to 2 mm sizes and added with 400 mL of cow dung adjusted to total solids (TS) of 6.1% with tap water. The cow dung was collected in a closed bucket from the Roman Catholic Church cattle hut beside the NM-AIST Campus in Arusha, Tanzania. All experiments were performed in duplicate in a 500 mL Erlenmeyer flask with an effective volume of 350 mL. The batch reactors were sealed with rubber stoppers and were incubated in a water bath for 40 days at 37°C and stirred manually three times a day for about 2 min. The volume of the biogas produced was collected by displacement of water.

Results and discussion

Characteristics of banana leave wastes used in the fungal treatment and anaerobic digestion process

Table 1 depicts banana leaves characteristics before fungal pre-treatment. The TS and VS (%TS) contents were 24.5 and 84.3%, respectively, indicating that banana leaves are

Table 1 Characteristics of banana leaves waste before fungal pretreatment (Mean \pm standard deviations of three replicates)

Component	Percentage (%)
Total solids (TS)	24.5 \pm 0.4
Volatile solids (%TS)	84.3 \pm 0.5
Moisture contents (MC)	75.5 \pm 0.4
pH	8.2 \pm 0.1
Carbon to Nitrogen ratio (C/N)	18.8 \pm 0.3
Cellulose	28.9 \pm 0.9
Hemicelluloses	23.5 \pm 1.1
Lignin	18.9 \pm 0.5

suitable for fungal treatment. The C/N and pH of the BL were within acceptable ranges for FP as reported by some authors. Bellettini et al. (2016) reported that the substrates with the C/N ratio range between 15:1 and 25:1 and pH range between 6.5 and 7.0 are well suited for FP. The moisture content was adjusted to 75% as per Mustafa et al. (2016), who indicated that FP with *P.ostreatus* is most effective at 75% MC. The lignocellulosic components: cellulose, hemicellulose and lignin were also similar to the reported values for un-pretreated banana leaves as reported by some authors. Fernandes et al. (2013) reported the cellulose, hemicellulose and lignin for semi-dried banana leaves to be 26.7%, 25.8% and 17%, respectively. In comparison with the biomass compositions of other substrates such as rice straw, beach wood, and palm midrib pre-treated with *P. ostreatus*, it seems hemicellulose and lignin contents which are mostly affected by *P. ostreatus* ranged from 11.2 to 22.9% and 13.1 to 22.9%, respectively (Bari et al. 2015; Metri et al. 2018; Mustafa et al. 2016; Owaid et al. 2017). Therefore, the biomass compositions of the banana leave analyzed in this study fall within acceptable limits for the fungal treatment. The pH in anaerobic digestion process for untreated and treated BL were 7.90 ± 0.02 , and 7.40 ± 0.00 , respectively, and were within acceptable ranges for the AD process (Richard et al. 2019). According to Vögeli et al. (2014), substrates with more than 60% VS are suitable for resources recovery such as biogas production. In the current study, the VS for untreated and treated BL were 78.2 and 77.10%, respectively, these were above 60% and, therefore, acceptable for biogas production.

Fungal treatment of banana leaves wastes using *Pleurotus ostreatus*

Table 2 indicates the number of days taken for spawn running, pinhead formation, the formation of fruiting bodies, number of clusters, yields and biological efficiency for ten mushroom bags used in the experiment. The spawn running took about 29 days; followed by an average of 3 days of pinhead formation. The complete fruit body formation and the mushroom harvest took about 36 days. The average weight of the mushroom harvested per 2 kg of BL bag was 181 ± 19 g which was slightly lower than those reported by other authors (Amirta et al. 2016). The percentage of biological efficiency (BE) in this study was 37%. The varying biological efficiency results with *Pleurotus ostreatus*, when cultivated in different substrates, have been reported in the literature. Yang et al. (2013) indicated that the BE with *Pleurotus ostreatus* when cultivated with sterilized rice and wheat straws supplemented with wheat bran (20%) were found to be 53.9 and 51.3%, respectively. Vieira and de Andrade (2016) studied the effect of the cultivation of oyster mushroom (*P. ostreatus*) on different potential materials namely; decumbens grass, brizantha grass, sugarcane bagasse and wheat straw with nitrogen supplementation. The results indicated that BE increased with nitrogen supplementation with BE ranging from 86.4% (wheat straw) to 123.9% (brizantha grass). In comparison to these findings, the BE value obtained in this study was low. However, the results obtained in this study was slightly higher than the results obtained by Girmay et al. (2016) who indicated the BE of *P. ostreatus* when cultivated in paper waste and wheat straw to be 34.2% and 35.9%, respectively. Therefore, the difference in BE results with *P. ostreatus* is attributed to many factors including the spawn rate used, strain type, number of times the mushroom were harvested, and optimization conditions just to mention a few. Table 3 presents the results of the compositions of dry BL after fungal treatment. The VS of banana leaves after the fungal treatment was 75.7%—which indicates the loss of about 10% VS from non- treated BL. The decreased VS means that part of the organic matter of the pretreated substrates was incorporated into fruiting body formation. The pH of the BL decreased approximately by two units after the fungal treatment. Rouches et al. (2016) indicated that the pH drop during fungal treatment is

Table 2 Mushroom formation, number of clusters, fruit bodies, yield, and biological efficiency

Spawn running (day)	Pinhead formation (day)	Mushroom harvest (day)	Clusters (Nos)	Fruit bodies (Nos)	Yield (g of fresh mushroom/2 kg of the substrate)	Biological efficiency (%)
29 \pm 3	32 \pm 3	36 \pm 3	3 \pm 1	32 \pm 8	181 \pm 19	37 \pm 4

Results comprise of the mean of ten mushroom bags \pm standard deviations

Table 3 Composition of banana leaves after fungal treatment

Component (%)	Fungal treated	% decrease after treatment
pH	6.4 ± 0.1	–
Volatile solids (%TS)	75.7 ± 0.8	10
Cellulose	26.9 ± 0.7	7
Hemicelluloses	17.9 ± 0.7	24
Lignin	16.9 ± 0.6	10

probably caused by the release of acetyl groups during the delignification process. The biomass compositions, cellulose (CE), hemicelluloses (HCE) and lignin (LIG) decreased by 7%, 24% and 10%, respectively, after the fungal treatment. This result suggests that hemicellulose and lignin were more degraded by *P. ostreatus* as compared to the cellulose and, therefore, in comparison to results reported in most studies which indicated cellulose was less degraded by *P. ostreatus* compared to lignin and hemicellulose (Bari et al. 2015; Metri et al. 2018; Owaid et al. 2017). The reduced lignocellulosic components mean that the insoluble fibres were hydrolyzed into soluble components which is beneficial for fermentative microorganisms activities during the recovery of biogas (Budzianowski 2016). In addition to the loss of the organic matter, Table 4 indicates that the trace elements (Fe, Mn, Co, Ni and Mo) in un-treated banana leaves was significantly higher than the treated banana leaves ($P < 0.05$) as tested by T-test in regression analysis of Ms excel. The reduced trace elements in treated banana leaves indicate that mushrooms bio cumulates the trace elements from the cultivated substrates.

Anaerobic digestion process of fungal treated and un-treated banana leaves wastes

Figure 2a indicates the daily biogas yield for untreated and treated banana leaves. Both reactors showed the quick release of biogas after day 1, and gradually decreased before

Table 4 Trace elements compositions in banana leaves before and after fungal treatment

Trace elements	Before treatment	After treatment
Fe (%)	0.653 ± 0.005	0.399 ± 0.004
Mn (%)	0.054 ± 0.007	0.034 ± 0.004
Mo (ppm)	20.833 ± 6.714	17.967 ± 1.674
Co (ppm)	8.03 ± 0.058	6.50 ± 0.000
Ni (ppm)	1.67 ± 0.058	1.60 ± 0.000

Results comprise of the mean of three replicates ± standard deviation (Mushroom bio cumulates the trace elements from the cultivated banana leaves)

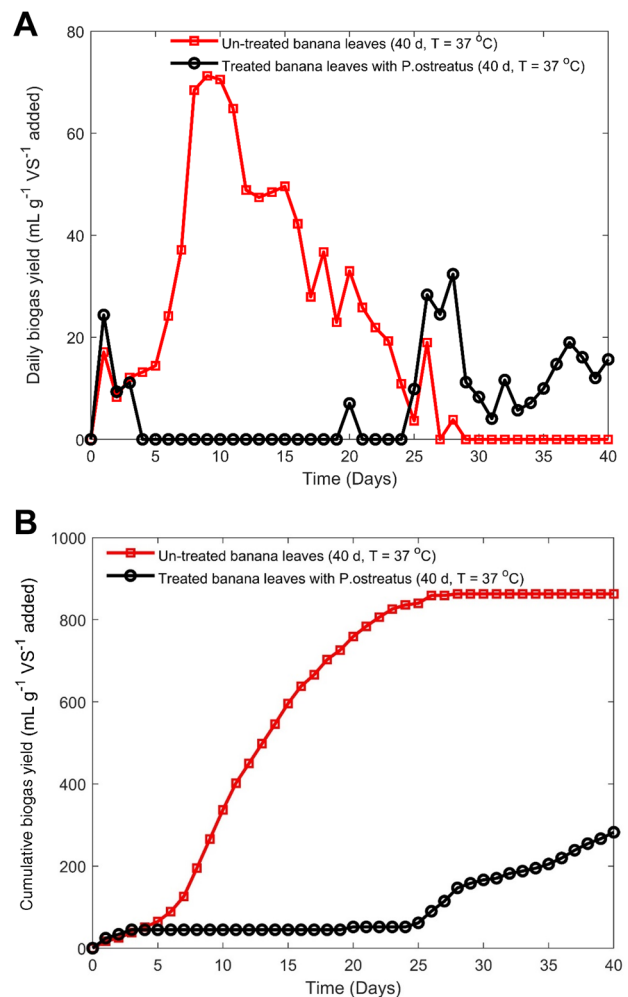


Fig. 2 a Daily biogas yields of un-treated and fungal treated banana leaves by *P. ostreatus*, data are means of the two replicates. b Cumulative biogas yields of un-treated and fungal treated banana leaves by *P. ostreatus*, data are means of the two replicates

it started to increase again. Within the first 2 days, the biogas yield in a fungal treated BL was slightly higher than that in un-treated BL but decreased to zero in day 4–19 before it started to increase again. The quick-release of biogas in a fungal treated BL may be attributed to reduced lignocelluloses components after fungal treatment with *Pleurotus ostreatus*. The peak daily biogas yield in fungal treated BL was 32.36 mL g⁻¹ VS⁻¹ day⁻¹ which was observed in day 28.

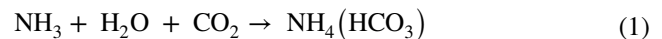
On the other hand, during the day 40 of the experiment the biogas yield in the un-treated BL was significantly higher ($P < 0.05$) as compared to the biogas yield in fungal treated BL. The un-treated BL reactor showed a stable biogas production and a peak daily biogas yield of 71.26 mL g⁻¹ VS⁻¹ day⁻¹ which was observed in day 9. Figure 2b indicates the cumulative biogas yields for un-treated BL and fungal treated BL. After 40 days of the AD process, the cumulative biogas yield in the fungal treated BL was 282 mL g⁻¹

VS⁻¹ which was 3 times lower than that of the un-treated BL (863 mL g⁻¹ VS⁻¹). Nevertheless, the biogas yield trend results in Fig. 2 shows that the difference in the biogas yields between the un-treated and fungal treated BL could be reduced if the experiment would continue in more than 40 days (This experiment was terminated in day 40, due to necessary stoppage of all lab activities and the closure of the university due to COVID 19 pandemic diseases outbreak).

The quick stable biogas yield in un-treated BL reactor during day 40 of the experiment may be attributed to the higher essential elements in un-treated banana leaves, as indicated in Table 4. In accordance to Banks et al. (2012), the addition of trace elements in the anaerobic digestion systems plays significant roles in improving enzyme activities, growth of methanogens and stability of the anaerobic digestion process. Therefore, according to this study, an improvement in biogas yield stability from fungal treated banana leaves with *P. ostreatus* would require among others the trace elements supplementation. The biogas production obtained by fungal treatment of banana leaves with *P. ostreatus* in a comparison with other pre-treatment techniques of banana leaves is discussed below. The findings from Chanakya and Sreeshha (2012) indicated that retting as a pre-treatment of banana leaves in a plug flow digester at the retention time of 30 d resulted in a biogas yield of 400 mL g⁻¹ TS⁻¹ at room temperature of 28 °C after 40 days of digestion. This biogas yield was considerably higher than the biogas yield of 282 mL g⁻¹ VS⁻¹ (corresponding to 214 mL g⁻¹ TS⁻¹) observed in our study. In another study, Jena et al. (2020) investigated the influence of FeCl₃ addition as the means to improve the biogas production from semi-dried banana leaves. The addition of FeCl₃ resulted in the cumulative biogas production of 2105 mL (when the production by inoculum is subtracted) which was higher than the results obtained in the current study (743 mL). However, in another study by Kamdem et al. (2013), the biogas yield of 126 mL g⁻¹ TS⁻¹ was obtained from physical treatment through size reduction of banana leaves which was lower in comparison with 214 mL g⁻¹ TS⁻¹ obtained in this study. The reason for the lower biogas yield as indicated in their study was high lignin concentration in banana leaves which affected the digestibility. In our study, the fungal treatment enhanced lignin degradation by 10%. Generally, this study shows that *Pleurotus ostreatus* can be cultivated on banana leaves wastes to produce Oyster mushrooms which result to the enhancement of the lignocellulose digestibility of the banana leaves wastes for the biogas recoveries, and this creates a sustainable means to manage un-utilized banana leaves wastes.

The pH after the AD process was 7.18 and 7.3 for fungal treated and untreated BL, respectively, which was slightly alkaline and suitable for the methanogenic process (Zhao et al. 2019). After 40 d of the AD process, the volatile solid

removal efficiencies were 4.1% and 5.6% for fungal treated and un-treated BL, respectively, and this suggests that the experiment would require more time for more organic matter removal in the process. The total alkalinity increased after the AD process from 2247 to 434 mg CaCO₃ L⁻¹ and from 2189 to 4974 mg CaCO₃ L⁻¹ for un-treated and fungal treated BL, respectively. The increase of the total alkalinity is mainly attributed to the breakdown of protein and amino acids which generate ammonia. The ammonia generated help to contribute to the formation of NH₄ (HCO₃) buffer when combines with CO₂ and H₂O as per Eq. 1 resulting to the process stability of the batch reactors (Shen et al. 2016). The volatile fatty acids accumulation decreased after 40 days of the AD process from 2415 to 613 mg L⁻¹ and from 1440 to 633 mg L⁻¹ for fungal treated and un-treated BL, respectively. These VFAs concentrations were still significantly high and indicate that the total conversion of organic matter was not yet achieved. According to Maragkaki et al. (2018), the negligible or absence of VFAs in digestates of AD process indicates the total conversion of the organic matter to biogas. The VFA/Alkalinity ratio after the AD process were 0.12 and 0.13 for untreated and treated BL, respectively. The low VFA/Alkalinity ratios indicate that the methanogenic stage was stable and was not disturbed by VFAs in the AD process



Conclusions

Banana leaves wastes may be fungal treated using *Pleurotus ostreatus* to produce edible mushrooms and biogas. About 181 ± 19 g of edible mushrooms per wet 2 kg of BL wastes and biogas yield of 282 mL g⁻¹ VS⁻¹ from fungal treated BL were obtained in this study. It was also observed that as mushrooms grow the digestibility of the banana leaves is enhanced but mushroom growth caused the reduction of the important trace elements in the treated banana leaves (Fe, Mn, Mo, Co and Ni) necessary for the improvement of the anaerobic digestion process. Thus to improve biogas production from fungal treated banana leaves using *P.ostreatus*, it is very important to supplement trace elements in the anaerobic digestion process. Generally, this study shows that one of the sustainable means to manage un-utilized banana leaves wastes is through the production of edible mushrooms for food and biogas production for energy.

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Author contributions All authors read, helped in discussion and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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