Biotechnology Journal International
17(3): 1-13, 2017; Article no.BJI.30479
Previously known as British Biotechnology Journal
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Co-production of *Coprinus cinereus* (Schaeff.) S. Gray. s. lato Mushrooms and Biogas from Palm Oil Wastes in Tanzania

Mariam Said Mmanywa[1] and Anthony Manoni Mshandete[1*]

[1]Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences, University of Dar es Salaam, Uvumbuzi Road, P.O.Box 35179, Dar es Salaam, Tanzania.

Authors' contributions

This work was carried out in collaboration between both authors. Author MSM collected the study material, organized the compositions, carried out the mushroom cultivation and anaerobic digestion experiments, did the data analysis, drafting and writing the first draft of the manuscript. Author AMM edited the first draft of the manuscript, managed the literature searches, read and edited the final manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2017/30479
Editor(s):
(1) Standardi Alvaro, Department of Agricultural and Environmental Sciences, University of Perugia, Italy.
(2) Kuo-Kau Lee, Department of Aquaculture, National Taiwan Ocean University, Taiwan.
Reviewers:
(1) Eduardo Bernardi, Federal University of Pelotas, Brazil.
(2) Tonjock Rosemary Kinge, The University of Bamenda, Cameroon.
(3) Mutemi Muthangya, South Eastern Kenya University, Kenya.
Complete Peer review History: http://www.sciencedomain.org/review-history/17539

Original Research Article

Received 11th November 2016
Accepted 11th January 2017
Published 17th January 2017

ABSTRACT

Aims: The aim of this study was to investigate on the effect of: (a) mixed fractions of palm oil wastes on *Coprinus cinereus* edible and medicinal mushroom yield using solid state fermentation plastic bioreactor and (b) of spent mushrooms substrate (SMS) on the extent of methane yield in batch anaerobic bioreactors using cow dung manure as an inoculum. Only one research reported on pre-treatment of palm oil wastes and biogas production in Tanzania.
Study Design: Palm oil wastes were obtained from Bagamoyo district, Pwani Region, in Tanzania. *Coprinus cinereus* mushroom production was performed in solid state fermentation plastic bioreactor while biogas production was carried out in batch anaerobic bioreactors.
Place and Duration of Study: Department of Molecular Biology and Biotechnology, University of

**Corresponding author: E-mail: anthony.mshandete@yahoo.com, mshandete@amu.udsm.ac.tz;*

Dar es Salaam, between September 2012 and July 2013.
Methodology: Completely randomized design was used for this study where by nine different fractions of blended palm oil wastes were prepared, Spawn rate (mushroom seed of *Coprinus cinereus*) employed was 7% based on wet weight of the substrate (about 35 g per 500 g moist weight substrate), then placed in solid state fermentation plastic bioreactor and incubated in mushroom house. Time was recorded in days for the completion of growth of mycelium on substrates (/spawn running/vegetative growth), appearance of pinheads (pinning) and fruiting bodies (fructification). The data for the yield number, fresh weight of fruiting bodies and biological efficiency worked out against the dry weight of each substrate were also recorded. The substrates for biogas production used were biological treated palm oil wastes i.e. the spent mushroom substrates obtained after harvesting mushrooms and cow dung manure was as an inoculum. Anaerobic digestion of spent palm oil wastes mushroom substrates for biogas production was carried out in batch scale (500 ml E-Flask) under anaerobic condition at ambient temperature ranged from 28-30℃ and at various mixing palm oil wastes composition fraction in triplicates. Determining both biogas volume and methane content monitored the performance of the anaerobic digestion process in the batch anaerobic bioreactors.
Results: Mushrooms grew well in different composition of palm oil wastes and days for completion of spawn running to fruiting body formation ranged from 13 to 23 days; the best substrate formulation was 98% EFB (Empty Fruit Bunches) +1% S (Sediment)+1% P (Pome) which had highest mushroom yield (189 g fresh mushrooms/kg moist substrate) and maximum biological efficiency (35%). When the Spent Mushroom Substrates (SMS) were employed in batch anaerobic bioreactors for biogas production. The best results 0.43 and 0.49 CH_4 m^3/kg Volatile Solids (VS) added was obtained from pretreated (SMS) 98% EFB+1% S+1% and 39% MF (Mesocarp Fibers)+39% EFB+ (20% PK (Palm Kernel)+ 1% P+1% S palm wastes substrates formulation, respectively. The highest methane yield were 1.3-1.4 fold higher compared to methane yields 0.33-0.34 CH_4 m^3/kg VS registered from corresponding non-pre-treated palm oil wastes substrates formulations, mean methane content of the biogas obtained from treated SMS was 82%, which was slightly higher than 79% recorded from untreated palm oil wastes substrates formulations.
Conclusion: In conclusion it is technically feasible to co-produce both food in the form of *Coprinus cinereus* edible and medicinal mushrooms and bio-energy in the form biogas rich methane from palm oil wastes while at the same time reducing environmental pollution.

Keywords: Bioreactor; mixed palm oil wastes; co-production; anaerobic digestion; methane emission.

ABBREVIATIONS

BE: Biological Efficiency; CPO: Crude Palm Oil; EFB: Empty Fruit Bunches; FFB: Fresh Fruit Bunches; MBB: Molecular Biology and Biotechnology; MF: Mesocarp Fibers; MY: Mushroom Yield; P: Palm; PC: Palm Cake; PK: Palm Kernel; PKS: Palm Kernel Shell; PMF: Palm Mesocarp Fiber; POME: Palm Oil Mill Effluent; S: Sediments; SMS: Spent Mushroom Substrate; TS: Total Solids; UDSM: University of Dar e Salam; VS: Volatile solids.

1. INTRODUCTION

Cultivation of edible mushrooms with agricultural and agro-industrial residues as substrate is a value-added process to convert these materials, which are otherwise considered to be wastes, into valuable protein rich food and a cash crop of commercial interest [1]. Oil palm is a multi-purpose plantation and it is also an intensive producer of biomass. Accompanying the production of one kilogram of palm oil, approximately 4 kg of dry biomass are produced. Besides producing oils and fats, there are continuous interests in using oil palm biomass as the source of renewable energy. Palm oil constitutes about 10% of the total palm biomass produced in the plantation. The remainder (90%) consists of huge amounts of liquid and solid wastes [2]. The liquid waste (wastewater) generated from the extraction of palm oil comes mainly from separation processes [3]. A tone of fresh fruit bunches (FFB) processed into crude palm oil (CPO) generates solid waste including wet empty fruit bunches (22-23% of FFB), wet press palm mesocarp fibres (12-13.5% of FFB) and wet endocarp palm kernel shells (5-5.5% of FFB). The liquid waste comprises of high organic content mainly oil and fatty acids and wastewater, commonly known as palm oil mill effluent which accounts for 60% of FFB [4], some

of the solid wastes are used for household products like brooms, firewood and building material. Part of the waste is also used as fuel feedstock in oil palm plant operations and over 90% are discarded without recuperating the bioenergy contained in them causing serious environmental problem. These wastes make the palm oil industry truly attractive as a future source of renewable bioresources, which if exploited prudently, have the potential to contribute to the sustainability of the industry [5]. These wastes are renewable bioresources, which could fit well in the production of diverse value added bio products by employing the anaerobic digestion process [6].

But Palm oil production wastes are underutilized, disposed untreated and have been shown to have a detrimental impact on the environment [7] On the contrary, this waste has been identified as a renewable bioresources for biomethane production [8] and abatement of pollution problems through its utilization for biogas production. Similar to all other lignocellulosic biomass, palm oil wastes are composed of cellulose, hemicelluloses and lignin, among the three components, lignin has the most complex structure, making it recalcitrant to both chemical and biological conversion [9,10]. Pretreatment of palm oil wastes is therefore necessary to open its structure and increase its digestibility and subsequently the degree of conversion [11,12,13] In biological pretreatment, oxidizing enzymes and white-rot fungi were used to degrade the lignin content in one of the palm oil wastes [11,14]. Therefore, cultivation of mushroom on these wastes could alleviate pollution as well as provide protein food and income.

A white rot fungus *Coprinus cinereus* belongs to genus *Coprinus*, black-spored family *Coprinaceae* in division *Basidiomycota*. At maturity, they deliquesce i.e. go through an auto digestion from the bottom of the cap upwards, eventually turning into black ink [15] biogas production can be improved from palm oil wastes by pre-treating the blended palm oil wastes with *Coprinus cinereus* a white rot fungus and the pre-treated waste will then be used for biogas production using cow dung manure as an inoculum.

This study will contribute to knowledge on how to utilize the palm oil wastes effectively in Tanzania because palm oil industry still generates large volume of wastes rich in both lipids and lignocelluloses from the oil extraction process, and there are only few studies [16,17] done about this new convenient way of Biological pre - treatment of wastes in Tanzania and few about Quantitative assessment of palm oil wastes generated by Palm oil mills from West Africa [18].

2. MATERIALS AND METHODS

2.1 Fungal Source

Coprinus Cinereus (Schaeff.) S. Gray. s. lato a wild edible and medicinal mushroom isolate was obtained from strain bank of Department of Molecular Biology and Biotechnology (MBB), University of Dar es Salaam (UDSM).

2.2 Spawn Preparation

Spawn was prepared with intact sorghum grains brought from Kariakoo market, Dar es Salaam: following the procedure described by Mshandete and Cuff [19]. The grains were boiled for an average boiling time of 15-25 minutes. After draining excess water, 3% (w/w) of calcium carbonate ($CaCO_3$) was added, 3% of $NaSO_4$ was also added for pH adjustment and properly mixed into the grains before spreading them out on a clean plastic sheath. After air-drying for about 20 min, 150 g of the grains were packed in 330 ml wide mouth bottles (Kioo Ltd, Dar es Salaam) and sterilized in an autoclave (Koninklijke AD Linden JR. BN-Zwijinderect, Holland) at 121℃ and 1 atm for 1 hour. Each cooled bottle of sterilized grains was aseptically inoculated with an average amount of 2^{nd} generation *Coprinus cinereus* spawn obtained from MBB Bank. Inoculated bottles were shaken thoroughly by hand to distribute the spawn to the grains and placed in a room of temperature between 25-28℃.

2.3 Palm Oil Waste Substrates Preparation and Their Inoculation

Palm oil wastes (EFB, MF, PK and POME) were collected from Bagamoyo district, Pwani region, in Tanzania. Palm press cake was then obtained from pressing of uncovered palm kernels and all except POME were sun dried for 5 days. EFB and MF were chopped into 0.5-2 cm lengths using a locally made manual chopper. The dried palm kernel shells, chopped empty fruit bunches and mesocarp fibers were soaked in water for 1 hours to moisten them and then stalked on the floor to remove the excessive moisture and maintain 65-72% moisture level. Completely randomized design was employed where by

wastes were blended into different fraction (Table 1) to make a total of 500 g. per rectangular plastic containers bioreactor in triplicate and subjected to sterilization at 121℃ for two hours. Sterilized wastes in plastic bioreactor were inoculated with the spawn rate of 7% based on wet weight of the substrate (about 35 g per 500 g moist weight substrate, after inoculation, these bioreactors were incubated for spawn running in a spawn running room as: suggested by Mshandete and Cuff [19].

2.4 Spawn Running (Vegetative Growth), Pinhead Initiation and Fruit Body Formation

Spawn running was followed by direct observation of the inoculated substrates until the substrates were completely invaded with mycelia. The number of spawn running days for mycelia to colonize the substrate was recorded. The conditions during spawn running in the room were 28±2℃ and relatively air humidity 78±2%. Once the mycelia of *Coprinus cinereus* strain had grown throughout the whole substrate, the substrate was keen observed because there is a thin line between full colonization pinhead formation and full growth of mushrooms. When necessary, the moisture was maintained with the use of mist sprayers. Data were recorded periodically during the growing season namely, first flush, second flush and third flush as follows; time was recorded in days for the completion of growth of mycelium on substrates, appearance of pinheads and maturation of fruiting bodies. The data were also recorded for the yield number and fresh weight of fruiting bodies and biological efficiency worked out against the dry weight of each substrate.

2.5 Harvesting and Determination of Biological Efficiency, Mushroom Yield and Mushroom Size

Harvesting of *Coprinus cinereus* fruit bodies was done when young or pre-capping stage, firm and freshly (immature/button stage) as recommended by Mshandete and Cuff [19]. During harvesting, fresh mushroom bodies were counted and weighed. Three aspects of mushroom crop yield and productivity were evaluated according to procedure described by Royse and cooworkers [20].

(i) Mean mushroom size was determined as follows: total weight of fresh mushrooms harvested/total number of mushrooms harvested.

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

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

2.6 Biogas Production

Anaerobic co-digestion of palm oil wastes for biogas production was carried out in batch scale (500 ml E-Flask) under anaerobic condition at ambient temperature ranged from 28-30℃ and at various mixing waste composition fraction in triplicate. The raw material used as a substrate were the biological treated palm oil wastes (spent mushrooms substrates) and cow dung manure as inoculum; biogas loading volume varied accordingly with respect to the value of TS and VS of the mixer composition fraction. Biogas production for both control and experimental design was monitored and measured until biogas production reduced significantly, produced gas was measured at 5 days' interval for 40 total of days, the obtained data were used to present the three important aspects for the evaluation of substrate producing biogas;

I. Total methane production (ml) of each of the substrate fraction
II. Percentage increment
III. Methane yield (m^3/kg) of each of the substrate fraction

2.7 Process Performance Monitoring

The performance of the anaerobic digestion process in the batch bioreactors was monitored by determining both biogas volume and methane content. The methane content was estimated according to procedures described by Erguder and coll [5] and Mshandete and coworkers [21] by the concentrated alkaline absorption method. Each bioreactor was manually shaken by swirling for 1minute prior to biogas volume measurement. Biogas volume was measured using a graduated 100 ml gas-tight plastic syringe with a sample lock, according to [21]. A needle plugged at the tip of the graduated 100 ml gas-tight plastic syringe was pierced through the air-tight n-butyl stopper in gas sampling septum and then the syringe plunger was pulled to draw the gas from the bag. The gas volume readings were taken on the graduated mark corresponding to the end of the plunger, which is a point that resists soft

Table 1. The nine blended substrate fractions used

No	MF	EFB	PC:PK	PC	PK	POME	Sediment
1	98%=490 g	-	-	-	-	1%=5 g	1%=5 g
2	-	98%=490 g	-	-	-	1%=5 g	1%=5 g
3	49%=245 g	49%=245 g	-	-	-	1%=5 g	1%=5 g
4	44%=220 g	44%=220 g	10%=50 g	-	-	1%=5 g	1%=5 g
5	39%=195 g	39%=195 g	20%=100 g	-	-	1%=5 g	1%=5 g
6	44%=220 g	44%=220 g	-	10%=50 g	-	1%=5 g	1%=5 g
7	39%=195 g	39%=195 g	-	20%=100 g	-	1%=5 g	1%=5 g
8	44%=220 g	44%=220 g	-	-	10%=50 g	1%=5 g	1%=5 g
9	39%=195 g	39%=195 g	-	-	20%=100	1%=5 g	1%=5 g

gentle pulling of the piston. The lock was opened and the plunger pushed to withdraw the gas from the syringe into the storage bag. The process was repeated until the bag was empty. The gas measurements were done at ambient temperature. Total methane production was determined by cumulative sum of daily gas collection for entire experimentation period. Methane yield was determined as the volume of methane produced per unit weight of fresh weight, total solids and volatile solids in the substrate.

2.8 Statistical Analysis

Data were analyzed using the data analysis toolbox in excel software. All the experiments were carried out in triplicates to ensure reproducibility. Moreover, the data were expressed as mean ± S.D. The data for mushroom size, mushroom yield, B.E. and methane yield were subjected to one way Analyses of Variance (ANOVA) at the 5% level (significant different at $p<0.05$) using Graph-Pad in Stat 3.10, 32 bit for Windows, created July 9, 2009 (GraphPad Software, Inc., San Diego, California, United States).

3. RESULTS AND DISCUSSION

3.1 Mushroom Cultivation

3.1.1 Spawn running, pinheads and fruiting bodies

The mycelium spread well through the palm oil wastes in only 3 to 5 days after spawning. Five days after mycelia colonization contaminants from genus *Trichoderma* were observed in Fig. 1(a). The colonization rate then abruptly ceased and it took more 8 days for the mycelia to compete with the contamination. Then afterwards minute fruit bodies appeared on 14^{th} to 16^{th} days after inoculation while it took 1 to 2 days for mature mushrooms to be ready for harvesting and the mushrooms were harvested when young and immature as shown in Fig. 1(b). The mushrooms were harvested when young and immature so that they can be used for food while leaving the treated substrates ready for further processes. The days to completion of spawn run (vegetative growth), pinhead formation and fruiting body formation (reproductive phase) as represented in (Table 2) fall within days reported previously by [19,22], while working with *Coprinus cinereus* using sisal wastes as substrates supplemented with chicken manure, cow dung manure and human urine.

3.1.2 Biological efficiency

BE was calculated to determine how the mushrooms utilized nutrients present in the substrates efficiently. The BE percentage of mushroom production from palm oil wastes substrate formulations were significantly different ($p<0.05$) (Fig. 2).

The BE for *Coprinus cinereus* mushroom have been reported to be influenced by type of spawn carrier, type of technology employed, environmental conditions, type of substrates utilized either composted or non-composted, type and nutrient contents of supplements/additives used to enrich the substrates and variety of strains employed [23-26]. BE was calculated to determine how the mushrooms utilized nutrients present in the substrates efficiently, this is important because the ability of the mushroom to utilize nutrients well determine the production of biogas in the final process. The substrate number two composed by 98% EFB +1%S +1% P and the substrate number nine made with 39% MF +39%EFB +20%PK +1%POME + 1% S had highest biological efficiency of 35 and 31, respectively implying their high nutrient utilization, whereas least substrate composition was 39%MF +39%EFB +(20%PC) + 1%P +1%S (Fig. 2). The BE obtained in this study are in the

range of 2-119% previously reported by other researchers using *Coprinus cinereus* grown on sisal wastes mushroom substrates with and without supplements [22].

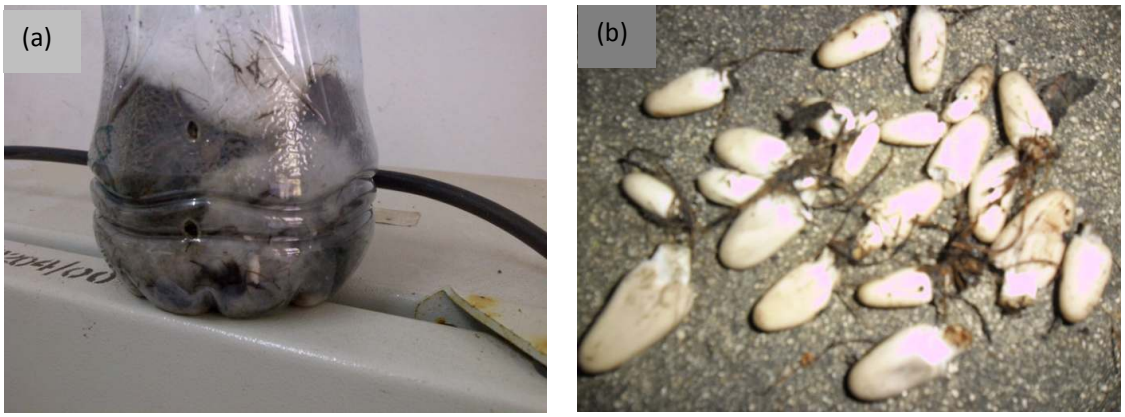

Fig. 1. (a) Full colonization and primordial initiation (b) Harvested immature mushrooms

Table 2. Days for completion of spawn running to fruiting body formation

No	Substrate formulation	Days for completion of spawn running	Days for pinhead formation	Days for fruiting body formation
1	98%MF+1%S+1%P	17	19	21
2	98%EFB+1%S+1%P	13	15	17
3	49%EFB+49%MF+1%S+1%P	16	18	20
4	44%MF+44%EFB+(10%PC:PK)+1%P+1%S	14	17	18
5	39%MF+39%EFB+(20%PC:PK)+1%P+1%S	16	18	20
6	44%MF+44%EFB+(10%PC)+1%P+1%S	14	17	19
7	39%MF+39%EFB+(20%PC)+1%P+1%S	19	21	23
8	44%MF+44%EFB+(10%PK)+1%P+1%S	13	15	17
9	39%MF+39%EFB+(20%PK)+1%P+1%S	13	15	17

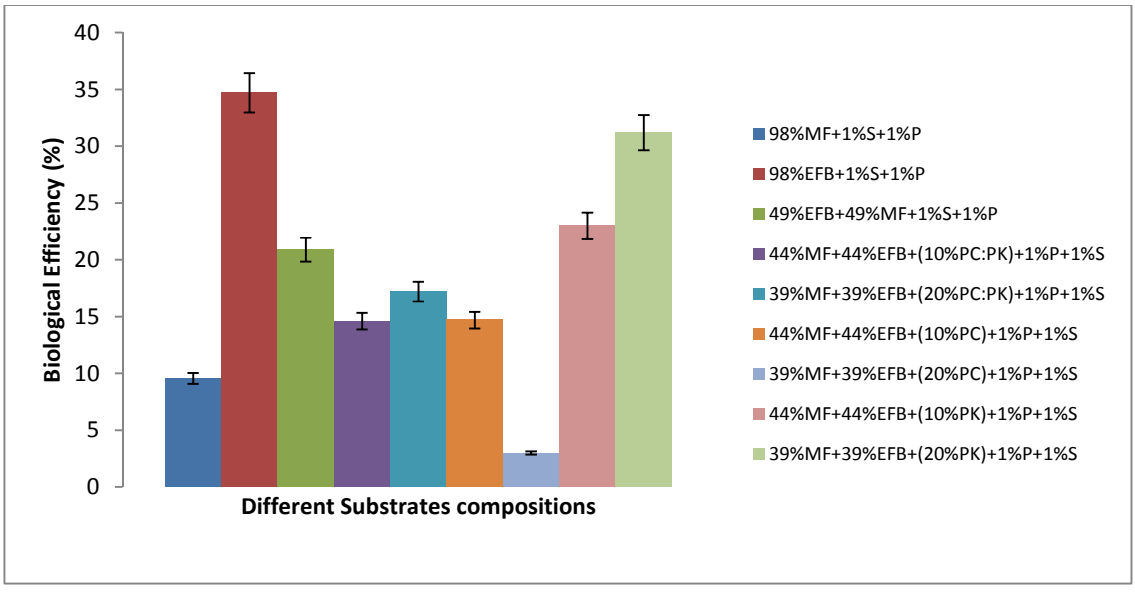

Fig. 2. Biological efficiency from different substrates proportions formulations
Error bars indicate standard error of the mean of the replicates

3.1.3 Mushroom yield

The crop of *Coprinus cinereus* was harvested in three flushes. Across all substrates, the maximum yield was obtained in first flush than the second and third flushes. The lowest quantity of mushrooms was harvested in the third flush (Fig. 3). Results show that mushroom yields were directly related to the types of substrates formulation. Substrate formulation of 98%EFB+1%S +1%P had highest yield of 189 g fresh mushroom/kg wet substrate. Mushroom yield in the range of range of 23-381 have been reported by [19, 22] to which the present highest mushroom yield falls within. Mushroom yield is important as it helps in determination of best substrate for mushroom cultivation which yield high number of mushrooms in a short period. The 98%EFB+1%S+1%P formulation is the best for *Coprinus cinereus* mushroom cultivation as it can guarantee maximum mushroom yield and the possible reason to its maximum yield could be the spacing between substrate and low content of oil on it which allow for better mycelia penetration and growth. Also the substrate formulation of 39%MF +39%EFB +20%PK +1%P +1%S had better mushroom yield of 155 g fresh mushroom/kg wet substrate. However, the substrate formulation of 44%MF +44%EFB +10%Pk +1%P +1%S had the lowest mushroom yield possibly due to high oil content both from mesocarp fiber palm kernel. It has been reported that the luxuriance and rapidity of growth of a certain mushroom partly depend on the appropriate culture medium used in its cultivation, strain used, duration of cropping period, which consequently affect mushroom yield [27].

3.1.4 Mushroom size

The mean size of the mushroom is essential for market purpose. Biological efficiency enhanced the utilization of the substrates and accumulation of the biomass into mushroom fruiting bodies and thus improved individual mushroom size Mushroom size of fresh harvested mushrooms occupies a significant role during grading, packaging, distribution and market quality of mushrooms. For example, big size and button unopened *Coprinus* mushrooms attract highest return in the market place [25,28]. In this study the relatively largest mean mushroom size 0.32 centimeter was obtained from mushrooms harvested on palm oil wastes of substrate formulation of 39%MF +39% EFB + 20%PK +1% P +1% S) and substrate formulation of 98% MF + 1%S +1%P had the average mushroom mean size compared to other formulations. However, mushroom size varied in response to different

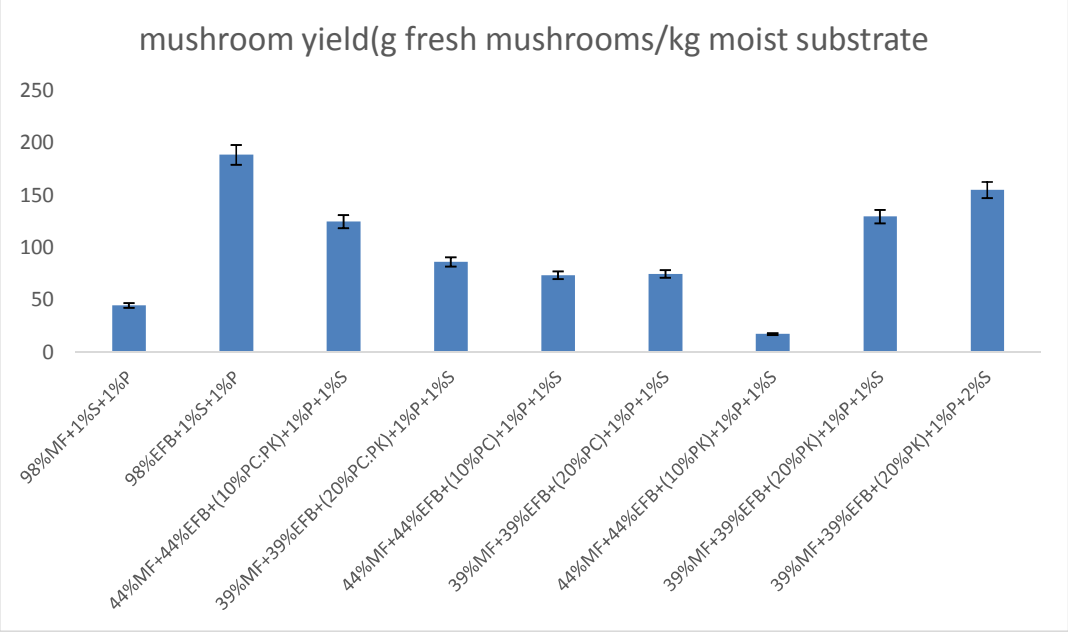

Fig. 3. Mushroom yield (g fresh mushrooms/kg moist substrate) from different substrates proportions formulations

Error bars indicate standard error of the mean of the replicates

wastes formulation as shown in Fig. 4. It has also been recently reported by Reyes and coworkers [25], Praphant [28], Kües [29] and Kurtzman [30] that variations of mushroom sizes in *Coprinus cinereus* is a common phenomenon since fruiting body development process is very complex and the induction formation of the fruiting bodies is affected by complex interactions of environmental factors such as temperature, humidity, light, ventilation and nutrients in mushroom growth substrate. This results section proved that *Coprinus cinereus* mushroom could grow on palm oil wastes and revealed the favorable substrate composition of palm oil wastes for the growth of *Coprinus cinereus* mushroom.

3.2 Biogas Results

3.2.1 Baseline biogas from untreated palm oil wastes substrate formulation

In order to evaluate the effect of pre-treatment on the methane productivity, it was necessary to carry out baseline data to establish the methane volume, methane content and methane yields from various palm oil waste formulation. Results presented in Table 2 shows that the methane yield ranged from 0.20-0.42 CH_4 m^3/kg VS added). The highest methane yield was obtained from 44% MF+ 44% EFB+ (10%PK) +1%P+1%S substrate formulation while the lowest was recorded from 39%MF+39%EFB+(20%PC:PK) +1% P + 1% S substrate formulation. The methane content of the biogas was good and ranged between 75-85% regardless of substrate formulation used.

3.2.2 Biogas from pre-treated palm oil wastes substrate (SMS) formulation

Total methane production shows the increase of methane produced in intervals, it also provides the information of the best substrate composition. Methane yield of each substrate composition shows which substrate composition is best for methane production, this provides a best choice of a substrate composition for maximum methane production. Results presented in Table 3 showed that the methane yield ranged from

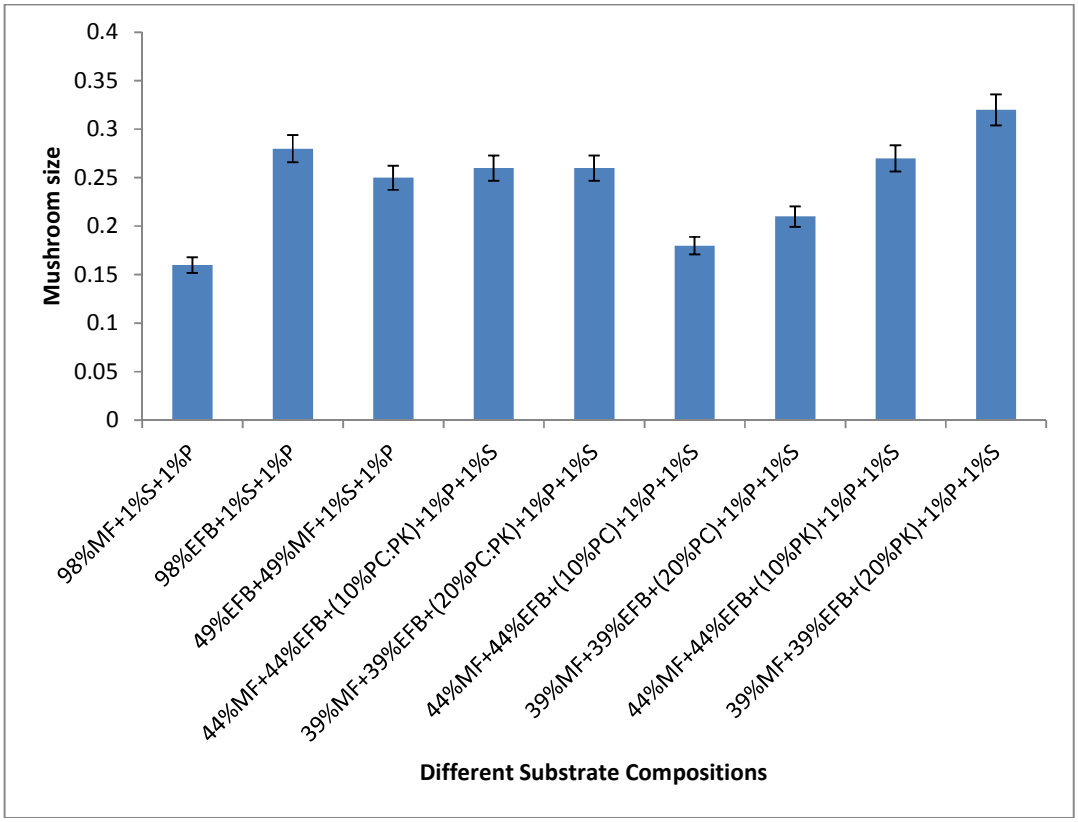

Fig. 4. Mushroom size (cm) from different substrates proportions formulations
Error bars indicate standard error of the mean of the replicates

Table 2. Methane content, total volume and methane yield from untreated palm oil wastes substrate formulations (control baseline data)

No.	Substrate formulation	Methane volume (ml)	Methane content (%)	Methane yield (m^3/kg fresh wt.)	Methane yield (m^3/kg TS)	Methane yield (CH_4 m^3/ kg VS)
1	98%MF+1%S+1%P	676	85	0.19	0.26	0.29
2	98%EFB+1%S+1%P	778	77	0.17	0.3	0.33
3	49%EFB+49%MF+1%S+1%P	899	84	0.27	0.33	0.38
4	44%MF+44%EFB+(10%PC:PK)+ 1%P+1%S	683	79	0.22	0.28	0.29
5	39%MF+39%EFB+(20%PC:PK)+ 1%P+1%S	478	79	0.13	0.18	0.20
6	44%MF+44%EFB+(10%PC)+1% P+1%S	668	83	0.20	0.24	0.29
7	39%MF+39%EFB+(20%PC)+1% P+1%S	889	77	0.24	0.34	0.38
8	44%MF+44%EFB+(10%PK)+1% P+1%S	694	75	0.25	0.36	0.42
9	39%MF+39%EFB+(20%PK)+1% P+1%S	783	75	0.26	0.3	0.34

Table 3. Methane content, total volume and methane yield from treated palm oil wastes substrate formulations (SMS data)

No	Substrate formulation	Methane volume (ml)	Methane content (%)	Methane yield (m^3/kg fresh wt.)	Methane yield (m^3/kg TS)	Methane yield (m^3/kg) VS
1	98%MF+1%S+1%P	877	82	0.16	0.33	0.37
2	98%EFB+1%S+1%P	1024	82	0.22	0.39	0.43
3	49%EFB+49%MF+1%S+1%P	828	84	0.19	0.32	0.36
4	44%MF+44%EFB+(10%PC:PK)+ 1%P+1%S	1043	83	0.17	0.29	0.33
5	39%MF+39%EFB+(20%PC:PK)+ 1%P+1%S	400	81	0.076	0.18	0.20
6	44%MF+44%EFB+(10%PC)+1% P+1%S	587	80	0.085	0.16	0.19
7	39%MF+39%EFB+(20%PC)+1% P+1%S	681	83	0.14	0.25	0.43
8	44%MF+44%EFB+(10%PK)+1% P+1%S	508	80	0.094	0.17	0.19
9	39%MF+39%EFB+(20%PK)+1% P+1%S	837	83	0.21	0.43	0.49

0.19-0.49 (CH_4 m^3/kg VS added). The highest was obtained from 39%MF+39%EFB+(20%PK) +1%P+1%S substrate formulation while the lowest were observed from 44% MF + 44% EFB + (10%PC) + 1%P + 1%S and 44% MF + 44%EFB+(10%PK) +1%P+1%S substrate formulations. The methane content of the biogas was good, which ranged between 80-84% regardless of substrate formulation used.

3.2.3 Effect of pretreatment on methane yield of palm oil wastes fractions

Percentage increment describes the effect biological treatment of wastes on methane yield from treated wastes in comparison with methane produced from untreated waste. The effects on methane yield (m^3 CH_4/kg VS of palm oil wastes added) potential (increase or decrease) of

different fractions compared to control (untreated) are presented in Fig. 5. An increase (positive increment) in methane yield potential of between 13.15-44.11% was registered while negative/decrease of methane yield potential of -5.26 to -54.76 was also observed from treated (SMS) compared to untreated (control). It was obvious that the vegatative growth and mushroom production from different palm waste substrate formulation had effects (positive or negative) when the SMS (substrate left after mushrooms were harvested) was employed as substrates in batch anaerobic bioreactors for production of biogas. The best results 0.43 and 0.49 CH_4 m^3/kg volatile solids (VS) added obtained from pretreated (SMS) 98%EFB+1%S+1%P and 39%MF+39%EFB+(20%PK)+1%P+1%S palm wastes substrates formulation, respectively where as the lowest results 0.19 CH_4 m^3/kg volatile solids (VS) added obtained from pretreated 44% MF + 44% EFB + (10% PC) + 1% P + 1% S and 44% MF + 44% EFB + (10% PK) + 1% P + 1%S respectively.The highest methane yield were 1.3-1.4 fold higher compared to methane yields 0.33-0.34 CH_4 m^3/kg VS registered from corresponding non-pre-treated palm oil wastes substrates formulations. Also the mean methane content of the biogas obtained from treated (SMS) was 82%, which was slightly higher than 79% recorded from untreated palm oil wastes substrates formulations. Further data analysis by statistical functions of excel 2016 was performed, where a paired-samples t-test was conducted to compare mean value of biogas produced after biological pre- treatment of palm oil wastes fraction and biogas from non-pre-treated palm oil wastes conditions. There was no statistical significant difference in the scores for biogas from pre-treated palm oil wastes (M=0.332, V=0.012) and non-pre-treated (M=0.324, V=0.0039) conditions; t (8) =-0.203, p = 0.05. and the statistical results suggest that pre- treatment might not have improved biogas production.

Results obtained in this demonstrated feasibility of the utilization of palm oil extraction wastes by applying an innovative bio refinery approach of integrating production of edible mushrooms and biogas rich methane thus adding more value to the bio-resource. The substrates used for biogas loading was the biological treated palm oil wastes and the inoculum used was the cow dung manure, the aim of treating the wastes biologically was to degrade the lignin content from the palm oil wastes. It was obvious that the vegatative growth and mushroom production from different palm waste substrate formulation had effects (positive or negative) when the SMS (substrate left after mushrooms were harvested) was employed as substrates in batch anaerobic

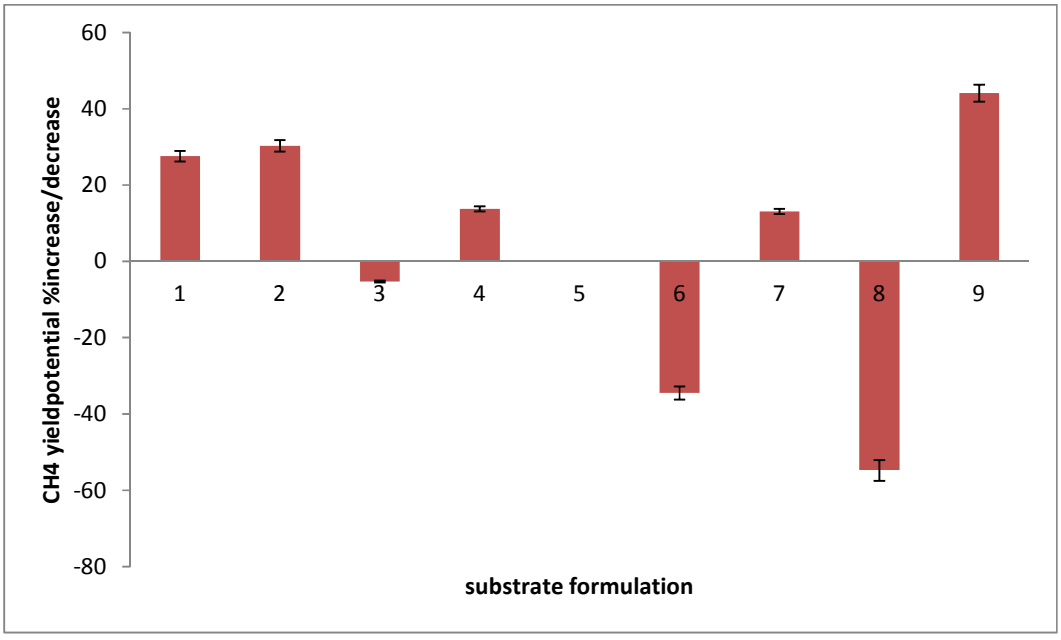

Fig. 5. Effect of pretreatment on methane yield of treated SMS palm oil wastes fractions in comparison with untreated wastes

Error bars indicate standard error of the mean of the replicates

bioreactors for production of biogas. The best results 0.43 and 0.49 CH_4 m^3/kg VS added obtained from pretreated (SMS) 98% EFB + 1%S + 1% P and 39% MF + 39% EFB + (20% PK) + 1% P + 1% S palm wastes substrates formulation, respectively. The highest methane yield registered 30-44% methane yield increase compared to methane yields 0.33-0.34 CH_4 m^3/kg VS recorded from corresponding non-pre-treated palm oil wastes substrates formulations. Similarily, tendency of fungal biological pretreatment to enhanced biogas production from lignocellulosic substrates has been recently reported. Nevertheless, the pretreatment was limited only to vegetative growth only i.e. without formation of fruiting bodies (mushrooms) as in the case presented in this study [31]. Sisal wastes solid state fermentation with a ligninolytic CCHT-1 strain and *Trichoderma reseei* at different inoculation rates and incubation periods singly and combined to improve methane production were investigated. Sisal wastes pre-treated with CCHT-1 for 4 days at an inoculation rate of 10% (wet weight inoculant/ SLDR) gave methane yield of 0.203±0.019 m^3 CH_4/kg VS added while pre-treatment of SLDR with *T. reseei* for 8 days at an inoculation rate of 25% (wet weight inoculant/sisal wastes) gave methane yield of 0.192±0.024 m^3 CH_4/kg VS added [30] This was an increment of between 24 to 30% in methane yield, compared to 0.145±0.015 m^3 CH_4/kg VS added obtained for the untreated samples [30]. On the other hand, pretreatment of sisal wastes prior to its anaerobic digestion (AD) was investigated using a two-stage pre-treatment approach with two fungal strains, CCHT-1 and *Trichoderma reseei* in succession in anaerobic batch bioreactors. gave a methane yield of 0.292 ± 0.04 m^3 CH_4/kg volatile solids (VS) added, which was higher than when fungal strains used separately [30]. Generally, an increment in the range of 30–101% in methane yield in comparison to the un-treated sisal wastes was obtained. The results in this study confirmed the potential of *Coprinus cinereus* fungus pre-treatment of palm waste to first produce edible mushroom crop prior to anaerobic digestion of spent mushroom substrates (SMS) to achieve significant improvement in biogas production.

4. CONCLUSION

This study proved the hypothesis that biogas yield from palm oil wastes could be improved by pre biological treatment of the wastes. The most suitable substrate formulation for *Coprinus* mushroom production, most suitable substrate formulation for methane and the most suitable formulation for co-production of *Coprinus* mushrooms and biogas were determined. Those substrates formulations could further be investigated to optimize mushroom and biogas production. Other supplements like sources of Nitrogen should also be investigated on their potential to improve mushroom yield and hence biogas production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhang R, Li X, Fadel JG. Oyster mushroom cultivation with rice and wheat straw. Bioresour Technol. 2002;82:277-84.
2. Basiron Y. Palm oil production through sustainable plantations. Eur J Lipid Sci Technol. 2007;109:289-95.
3. Alimon AR. The nutritive value of Palm Kernel Cake for animal feed. Palm Oil Dev. 2004;40:12-4.
4. Najafpour GD, Zinatizadeh AAL, Mohamed AR, Hasnain IM, Nasrollahzadeh H. High-rate anaerobic digestion of palm oil mill effluent in an up flow anaerobic sludge-fixed film bioreactor. Process Biochem. 2006;41:370-9.
5. Erguder JF, Tezel U, Guven E, Demirer GN. Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors. Waste Manage. 2001;21:643-50.
6. Sheil D, Casson A, Meijaard E, Van Nordwijk M, Gaskell J, Sunderland-Groves J, et al. The impacts and opportunities of oil palm in Southeast Asia: What do we know and what do we need to know? Occasional paper no. 51, CIFOR, Bogor, Indonesia; 2009.
7. Izah SC, Angaye CNT, Ohimain EI. Environmental impacts of oil palm processing in Nigeria. Biotechnol Res. 2016;2(3):132-41.
8. Rupani PF, Singh RP, Ibrahim HM, Norizan SE. Review of current palm oil mill effluent (POME) treatment methods: Vermicomposting as a sustainable practice. World Appl Sci J. 2010;11: 70-81.
9. Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol

production. Bioresour Technol. 2002;83:1-11.

10. Taherzadeh MJ, Karimi K. Pre-treatment of lignocellulosic wastes to improve ethanol and biogas production: A review. Bioresources. 2007;2:707-38.
11. Bjornsson L, Mshandete A, Mattiasson B. Pre-treatment methods for enhanced biogas production from sisal waste In: Ahring BK, Hartmann H, editors. Proceedings of the 4th International Symposium on Anaerobic Digestion of Solid Waste; Copenhagen, Denmark. 2005;116-23.
12. Bensah EC, Mensah M. Chemical Pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. Int J Chem Eng. 2013;21.
13. Shen S, Nges IA, Yun J, Liu J. Pretreatments for enhanced biochemical methane potential of bamboo waste. Chem Eng. J. 2014;240:253-59.
14. Müller HW, Trösch W. Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. Appl Microbiol Biotechnol. 1986;24:180-5.
15. Raymond P, Mshandete AM, Kajumulo KA. Production of oxidative and hydrolytic enzymes by *Coprinus cinereus* (Schaeff). Gray from sisal wastes supplemented with cow dung manure. Biotechnol Res Int. 2015;24:1-9.
16. Temu SG, Mshandete AM, Kajumulo KA. Tanzania palm oil industry: Auditing and characterization of oil palm wastes potential bioresource for valorization. J Chem Biol Phy Sci. 2014;4:804-11.
17. Temu SG, Moshi AP, Nges IA, Mshandete AM, Kivaisi AM, Mattiasson B. Mixed palm oil waste utilization through integrated mushroom and biogas production. Br Biotechnol J. 2016;11(4):1-12.
18. Koura TW, Kindomihou V, Dagbenonbakin G, Janssens M, Sinsin B. Quantitative assessment of palm oil wastes generated by mills in Southern Benin. Afr. J. Agric. Res. 2016;11(19):1787-96.
19. Mshandete AM, Cuff J. Cultivation of three types of indigenous wild edible mushrooms: *Coprinus Cinereus, Pleurotus flabellatus* and *Volvariella volvocea* on composted sisal decortications residue in Tanzania. Afr J Biotechnol. 2008;7(24): 4551-62.
20. Royse DJ, Rhodes TW, Ohga S, Sanchez JE. Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. Bioresour Technol. 2004; 91:85-91.
21. Mshandete A, Björnsson L, Kivaisi AK, Rubindamayugi ST, Mattiasson B. Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment. Water Res. 2005;39:1569-75.
22. Mwita LN, Lyantagaye SL, Mshandete AM. The effect of the interaction of varying chicken manure supplement levels with three different solid sisal wastes substrates on podocarp cap lengths and diameters, stipe lengths and diameters and dry weights of *Coprinus cinereus* (Schaeff) S. Gray s.lato. Afr J Biotechnol. 2011;10(7): 1172-80.
23. Chaiyama V, Petcharat V, Kritsaneepaiboon P. Some morphological and physiological aspects and cultivation of *Coprinus comatus* (O.F. Müll.) Gray. Warasan Songkhla Nakharim. 2007;29(2): 261-74.
24. Chen JT, Li WS, Wu KT. Effects of culture media and casing materials on fruiting characteristics of *Coprinus comatus*. J Taiwan Agric Res. 2007;56:316-26.
25. Reyes R, Lopez L, Kumakura K, Kalaw SP, Kikukawa T. *Coprinus comatus*, a newly domesticated wild nutraceutical mushroom in the Philippines. J Agric Technol. 2009;5(2):299-316.
26. Siwulski M, Sobieralski K, Pawlak R. Utilization of wild forms of the mushroom *Coprinus comatus* (O.F. Müll.) S. F. Gray *Coprinus* in breeding of commercial strains. J Veg Crop Prod. 2001;7(1):3-7.
27. Frimpong-Manso J, Obodai M, Dzomeku M, Apertorgbor MM. Influence of rice husk on biological efficiency and nutrient content of *Pleurotus ostreatus* (Jacq. ex.Fr.) Kummer. Int Food Res. 2010;17:59-64.
28. Praphant O. Mushroom for a Living: *Coprinus* cultivation in Thailand. Mushroom growers handbook 2 shiitake cultivation. Haeng-oon Bldg. 150-5 Pyungchang-dong, Jongno-gu, Seoul 110-846, Korea Mushroom World. 2005;199-207.

29. Kües U. Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev. 2000; 64:316-53.
30. Kurtzman J. Ventilation for mushroom cultivation: The importance of the needs of mushrooms and of the gas laws. Micol Aplicada Int. 2010;22(2): 63-78.
31. Muthangya M, Mshandete AM, Kivaisi A. Enhancement of anaerobic digestion of sisal leaf decortication residues by biological pre-treatment. ARPN J Agric Biol Sci. 2009;4:66-73.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17539