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Evaluation of Pre-treatment Methods and Anaerobic Co-digestions of Recalcitrant Melanised Chicken Feather Wastes with other Wastes for Improved Methane and Electrical Energy Production

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Abstract

Poor utilisation of abundant and highly recalcitrant melanised chicken feather (MCF) as a substrate for biological process has led to its accumulation in the environment. This study investigated the possibility of using MCF and its hydrolysates as a substrate for methane and electrical energy (EE) generation. A two-phase system which involves pre-treating MCF and subsequent co-digesting of the pre-treated MCF or its hydrolysates with other wastes was employed for each process. For methane production, MCF hydrolysates obtained from biodegradation of untreated or pre-treated MCF and inocula of cow dung, poultry slaughterhouse and abattoir wastes were used in anaerobic digester (AD). EE was generated through degradation of MCF with and without rice waste water by *Pseudochrobactrum* sp. IY-BUK1 in a microbial fuel cell (MCF). Results showed that pre-treated MCF were degraded about 3 fold faster than untreated, and produced 4 times the amount of keratinase and soluble proteins. MCF pre-treated with $Ca(OH)_2$ and co-digested with cow dung resulted in significant improvement in biogas production over those pre-treated with NaOH, steam and bacteria and co-digested with poultry slaughterhouse and abattoir wastes. *Pseudochrobactrum* sp. strain IY-BUK1 degraded about 70% of 5% (w/v) pre-treated MCF in MFC with resultant 67.3 U/ml keratinase. Similar to methane yield, $Ca(OH)_2$ pre-treated MCF were degraded faster in MFC inoculated with IY-BUK1 and produced a maximum voltage, power, and current density corresponding to 350.6mV, $37.77x10^4$mW/m2 and 0.112mA/m^2 respectively in 8 days when compared with un-treated feathers, that produced maximum of 174mV after 13 days. The use of rice water waste in the MFC as supplement produced 167mV more voltage than when only IY-BUK1 was used. The study revealed that recalcitrant MCF pre-treated with $Ca(OH)_2$ and co digested with cow dung and rice water waste can be successfully employed as a cheap substrate for biogas and electricity production.

Keywords: melanised, feathers, pre-treatment, methane, microbial fuel cells, keratinase, *Pseudochrobactrum* sp. IY-BUK1

1. Introduction

As meat consumption increases globally, generation of wastes from slaughterhouses and abattoirs continues to grow and millions of tons are generated yearly (Peng *et al*., 2019). In third world countries, especially in Africa, the feather wastes are poorly managed and underutilized and, therefore, serve as source of nuisance to humans, animals, and the environment. They are often burned or deposed to landfill, which are non-ecofriendly means of management (Pandian *et al*., 2012; Yusuf *et al*., 2016). The recalcitrant nature of melanised feathers over non-melanised has been the reason for their degradation by soil microbes that utilize feather as substrates to produce enzymes and protein rich hydrolysates (Grande *et al*., 2004; Gunderson and Frame, 2008; Gurav *et al*., 2016). Meanwhile, recycling of feather can provide a cheap and alternative source of protein feed stuff, energy, fertilizers, low cost substrates for production of other value added microbial products and can function as a means of clearing the environment of unwanted wastes (Khardenavis *et al*., 2009; Lasekan *et al*., 2013; Paul *et al*., 2013; Yusuf *et al*., 2015). Cheap and innovative methods within the capacity of our under-equipped research facilities are therefore needed to reach the objectives of alternative poultry feather utilization.

Furthermore, the persistent release of pollutants to environment by fossil fuels and the frightening rate at which it is getting exhausted has been scientists' great concern over years (Rahimnejad *et al.*, 2012; Parkash, 2016). Searching for cheap and clean alternative sources of energy is currently the biggest challenge for governments and scientists wanting to meet up with the ever increasing demand for cleaner energy (Nair *et al.*, 2013). The use of chicken feather waste (especially the white feather) to produce methane gas has been widely studied by many scientists through anaerobic digestion (AD) (Forgacs *et al*., 2011; Forgács *et al*., 2014; Mézes and Tamás, 2015). In order to improve methane yield from feather protein close to the theoretical methane yield of 0.496 Nm3/kg VS (volatile solids) (Davidsson, 2007), scientists have adopted different pre-treatment methods such as chemical, enzymatic, physical and biological ones to improve feather digestibility and biogas yield. Forgacs *et al*. for instance, reported improved methane yield from 0.18 Nm3/kg VS to 0.40 Nm3/kg VS) from feather pre-treated with recombinant *Bacillus megaterium* strain. Similarly, enzymatic and alkaline pre-treatments of feathers increased the methane by 21% and 32% according to

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Salminen *et al*. (Salminen *et al*., 2003) through anaerobic digestion. However, pre-treatment of melanised chicken feathers (MCF), which is believed to be more recalcitrant to microbial or enzymatic degradation, as well as co-digesting it with other wastes for improved biogas production has not been documented yet.

Microbial fuel cell (MFC) approaches employ use of microorganisms, specific substrates and conditions for electricity generation (Zhou *et al*., 2013; Parkash, 2016). In each waste, the organic compound is harnessed and utilised by single or groups of microorganism to produce useful products (Reiche and Kirkwood, 2012; Chaturvedi and Verma, 2014). The high content of different organic compounds including varying amount of carbon and nitrogen compounds in chicken feathers has made them a suitable substrate for electricity generation. Studies have attempted various types of organic compounds and wastes (both industrial and agricultural) as substrate in MFC (Zhou *et al*., 2013; Parkash, 2016), but only few have reported the use of feathers to produce electrical energy through MFCs (Chaturvedi and Verma, 2014). A study by Chatuverdi and Verma (2014) employed the use of *P. aeruginosa* strain SDS3 in MFC to degrade white feather to generate maximum voltage of 141 mV in 14 days. However, no study has attempted to use MCF to generate electricity. Due to their melanin pigment content and fibrous and insoluble structural proteins joint by disulphide bond linkage, hydrogen bond and hydrophobic interaction, direct usage in MFC and AD may take longer period. Since feather hydrolysates is the efficient substrate to use for biogas production in AD, pre-treating the MCF with either heat, chemical and/or biological agents may improve feather hydrolysate productions by suitable feather degrading bacteria. Similarly, pre-treating MCFs may also improve the potentiality of the feathers of being an easy-to-use substrate for microbes involved in electrical energy production. *Pseudochrobactrum* sp. IY-BUK1 is a novel keratinolytic bacteria that shows profound proteolytic activity against both melanised and non-melanised feathers (Yusuf *et al*., 2019b).

The aim of this study was, therefore, to evaluate the possibility of using raw MCFs and hydrolysates produced from its biodegradation as a substrate for electrical energy and biogas generation. Furthermore, the study aimed to assess the effectiveness of different pre-treatment methods on MCFs for suitable utilisation by single (*Pseudochrobactrum* sp. IY-BUK1) or groups of bacteria as substrate for improved production of biogas and electrical energy.

2. Materials and Method

2.1. Sample Collection and Processing

Black (melanised) chicken feather (MCF) wastes were procured from chicken abattoir in Kano, Nigeria. The collected feathers were spread on clean surface and other non-feather parts were handpicked. The feathers were then washed with water and detergent extensively, followed by defatting in solution containing 200ml of deionizer water, methanol and chloroform in ratio 1:1:10 and finally rinsed in distilled water. The washed feathers were oven dried at 80^{o}C for 6 h until constant weight was obtained (Yusuf *et al*., 2016; Yusuf *et al*., 2019a).

2.2. Isolation of feather degrading bacterium and preparation of culture media

The melanised feather degrading bacterium (MFDB) used in this study was isolated from chicken manure of local chickens reared in a wooden cage. Isolation was carried out in feather meal agar and broth as previously described by (Yusuf *et al*., 2016, 2019b). The identity of the bacterium was identified as *Pseudochrobactrum* sp. IY-BUK1 (Genbank accession number: MK024282) using morphological, biochemical and molecular characteristics (Yusuf *et al*., 2019b). The strain showed an enhanced preference for biodegradation of black feathers than white feathers with enhanced production of keratinase and protein hydrolysates.

Feather meal broth (FMB) which contained 10.0 g/L of feather, 0.5 g/L of NaCl, 0.7 g/L of K_2HPO_4, 1.4 g/L of KH_2PO_4, and 0.001 g/L of $MgSO_4 \cdot 6H_2O$ pH 7.5 was prepared as described by Yusuf *et al*. (2016, 2019a,b) and was used for propagation of the bacterium in an orbital shaker operated at 150 rpm at 30 °C.

2.3. Pre-treatment of melanised chicken feathers using physical, chemical and biological methods

MCFs were pre-treated using physical (steam and boiling), biological (bacteria) and chemical methods. Steam (thermal) pre-treatment was performed by autoclaving 5 g of whole MCFs suspended in 100 ml of distilled water at 121^{o}C, 21psi for 30 min. MCF preheated with boiled water for 30 min was also used. Chemical pre-treatments were carried out using three chemicals, NaOH, Na_2SO_3, and $Ca(OH)_2$. Pre-treatment of MCFs with NaOH and Na_2SO_3 was carried out according to method of Laba and Szczekala, (2013) using 100mM concentrations. In brief, MCFs were suspended in solutions of 100mM (1 g feathers per 100ml) Na_2SO_3 and NaOH. Pre-treatment with $Ca(OH)_2$ employed the method of Fargacs *et al*., 2013, where 1.0 g of MCF (TS) was suspended in 2 % $Ca(OH)_2$ solution for 1 hour to give a final concentration of 100 g $TS_{feather}$/L. The MCFs in both cases were then separated, precipitated (in case of $Ca(OH)_2$), and washed several times with distilled water. Biological pre-treatment was carried out according to the method of Laba and Szczekala, (2013) by exposing 5 g of MCFs to short-term culture of *Pseudochrobactrum* sp. IY-BUK1 in 250ml flask containing 100 ml FMB. After 5 days of continual shaking at 150 rpm at 30 ^{o}C, the partially digested raw MCFs were filtered using Whatman no.1 filter paper. The partially digested MCFs were then washed with distilled water and allowed to dry.

Five grams of different pre-treated MCFs were then placed in 100mL of FMB and autoclaved at 121^{o}C, 21psi for 15 min. Untreated raw MCFs were used as control. Inoculation of the FMB containing appropriate MCFs was done with 5 ml (3.15 x 10^6 cell/mL) of *Pseudochrobactrum* sp. IY-BUK1 except for the control experiments. Volatile solid (VS), and Total solid (TS) of the feathers were determined according to APHA standard methods (APHA, 2005).

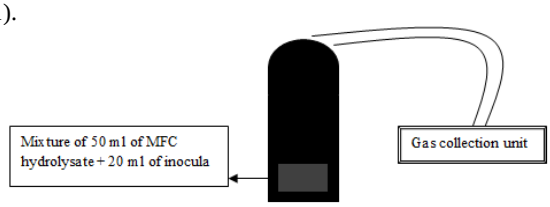
2.4. Determination of the Protein Concentration, keratinase activity and feather degradation

An aliquot of 10ml of culture was collected from FMB every 24 h and centrifuged at 10,000 rev/min for 15 min. The cell-free supernatant fluid was used for the

measurement of soluble protein by spectrophotometer (Schimazhu, Japan) with Lowry method (Lowry and Rosebrough, 1951). 1 absorbance was considered as 1 mg/mL protein using bovine serum albumin as standard. Similarly, an aliquot of the cell-free supernatant was used to determine the level of keratinase produced using azokeratin as a substrate as described by *Joshi et al*. (Joshi *et al*., 2007). Feather degradation (%) was estimated by subtracting the final weight of MCFs in the flask from the final weight of the MCFs from the control. Degradation degree (%) calculation was based on method described by Yusuf *et al*. (2016).

2.5. Methane digester design and batch anaerobic digestion assay

Methane production using pre-treated and untreated MCFs were carried out in batch digestion experiments in duplicates using feather hydrolysates. The AD was constructed similar to the type employed by Forgács *et al*. (2014) but with some modifications. ADs were constructed using 150 ml plastic containers. Each of the containers was channeled tightly to a gas collection unit (reservoir) (Fig. 1).



Figuer 1. Anaerobic digester setup for methane production by the various pre-treatment melanised feathers

For each AD, 50 mL of hydrolysates obtained from either untreated or pre-treated MCFs were placed as a substrate. The inocula used were cow dung, poultry slaughterhouse waste, and abattoir wastes. Twenty millilitre (20 ml) of the inocula was placed in each AD. Each reactor was then capped and sealed with butyl rubber props, and anaerobic condition was ensured by flushing with a gas mixture and incubated at 50 °C for 30 days**.** The plastic bottles were shaken in the incubator every day. Gas samples of 0.25 ml were regularly taken from the headspace using a pressure-tight syringe**.** Geo tech 5000 Biogas analyser was used for the analysis of the biogas harvested. The analyser computes the percentage composition of methane (CH_4).

2.6. MFC construction and its operation

The MFC was constructed as described by Chaturvedi and co-worker (Chaturvedi and Verma, 2014) with slight modifications. A dual chambered H-type locally made MFC was designed consisting of two identical plastic containers (700ml each with an operating volume of 500ml and a head space of 200ml). The cathode and anode were connected by a salt bridge. The salt bridge was prepared by adding 3% NaCl (w/v) into 100ml of dH_2O and mixed properly. Then, 1.6% Agar (w/v) was added to the salt solution and was boiled for 3 min. It was then filled into a half inch PVC pipe sealed with an aluminium foil paper from one side. Two identical carbon rod electrodes and copper wires, with an apparent surface area of 49.2cm^2, were used as anode and cathode, to which a copper wire was connected and fixed with PVC gum, and the copper wires were passed through a thin hole on the plastic container cover, respectively (Fig. 2). An external circuit of 66Ω was used in the experiment. The anolyte solution was FMB which contains 0.5% (w/v) of appropriate pre-treated MCF at pH 8.0. Additional sources of carbon (sucrose, glucose, rice wastewater) were added at 1% concentration in another MFC setup. The catholyte solution is 100mM phosphate buffer (pH 7.0).

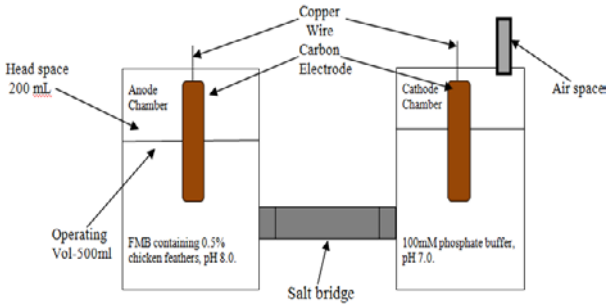


Figure 2. Schematic design of MFC connected with salt bridge

The experiment was carried out in batch mode. During the start up the anolyte was inoculated with 25ml of 18 h old culture of *Pseudochrobactrum* sp. IY-BUK1. The voltage reading was taken after 24 h time interval with digital multi meter (DT-830 model).

2.7. Statistical analysis

Results were presented in Tables and Figures. Statistical analysis was performed using SPSS statistical package (SPSS Inc., Chicago, IL, USA). Chi-square test was used to compare differences between different pre-treatment methods. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effect of pre-treatment methods of MCF on Protein Concentration, keratinase activity and feather degradation

Different pre-treatments were applied to the MCFs to enhance their digestibility prior to methane and bio-energy production by strain IY-BUK1.

The results of effect of each pre-treatment method on the overall rate of degradation, keratinase production and soluble protein yield are summarized in Table 1. It was found that MCF pre-treated with $Ca(OH)_2$ resulted in faster feather degradation and highest keratinase yield, followed by NaOH, then bio-thermal method ($p<0.05$). In line with this, MCFs pre-treated with $Ca(OH)_2$ resulted in about 4 fold increase in soluble protein concentration when compared with untreated MCF in 5 days. Pre-treating MCFs with strain IY-BUK1 for 5 days was less effective in increasing MCF digestibility by the strain after final incubation, as only 64% of MCF was degraded and only 98.2 U/ml keratinase was released. While degradation of $Ca(OH)_2$ treated MCF started after 2 days of incubation, that of boiled MFC was be observed only after 4 days (data not shown). As degradation progressed from day 2, MCF residues decreased considerably, resulting in a colour change in the FMB from colourless medium to completely black fermentation broth at the end of 5 days of incubation in flask containing $Ca(OH)_2$, NaOH and bio-thermal treated MCFs. Bacterial hydrolysis of Na_2SO_3 treated MCF generated a lower amount of keratinase and soluble proteins when compared with other chemicals [NaOH and

$Ca(OH)_2$]. Feather hydrolysates obtained by centrifuging the FMB at 4°C and 15,000 ×g for 15 min was stored at -20 °C and used for biogas production.

Table 1. Comparing effects of different pre-treatment methods on feather degradation, keratinase production and soluble protein released by *Pseudochrobactrum* sp. IY-BUK1 after 5 days of incubation

Type of pre-treated MCF (N=5g/L)	Degradation (%)	Keratinase activity (U/ml)	Concentration of soluble protein (mg/ml)
	Values in parenthesis are % difference from untreated MCF		
Untreated MCF	26	32.6±1.0	86.2±14.6
Bio-thermal	100 (74)	122.3±0.3	321.0±11.2
Boiled	64 (38)	98.2±1.8	195.4±2.3
Steam	96 (70)	114.4±1.4	295.3±9.8
Chemical (Na_2SO_3)	89 (63)	84.3±1.2	245.8±10.1
Chemical (NaOH)	100 (74)	133±0.1	302.4±12.1
Chemical $Ca(OH_2)$	100 (74)	148.2±2.2	333.5±7.5

3.2. Batch anaerobic digestion assays

In the batch anaerobic digestion experiment, biogas production from protein rich hydrolysates obtained from the selected pre-treated MCFs and that of untreated MCFs were compared, and the result was shown in Fig. 3. After 30–days incubation, $CaOH_2$ treated MCF produced 0.26 Nm^3/kg VS methane which is slightly higher when compared with other treated MCFs and untreated samples (0.125 Nm^3/kg VS).

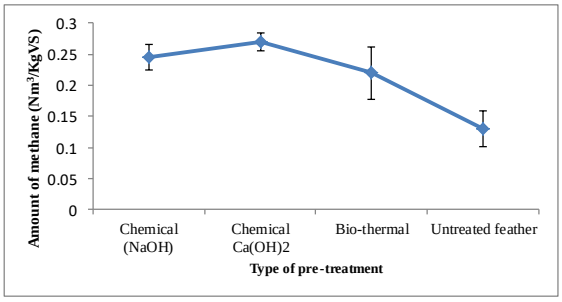


Figure 3. Methane production using untreated and pre-treated melanised feathers after 30 days of incubation

The methane produced in untreated MCF co-digested with cow dung, poultry manure and abattoir waste was 0.195 ± 0.021, 0.105± 0.021, and 0.121± 0.021 Nm3/ kg VS respectively, while that pre-treated chemically with $Ca(OH)_2$and as well co-digested with cow dung produced the highest methane of 0.396 Nm3/kg VS which is equivalent to 79.8% of the theoretical value from feather proteins (Davidsson, 2007) (Fig. 4).

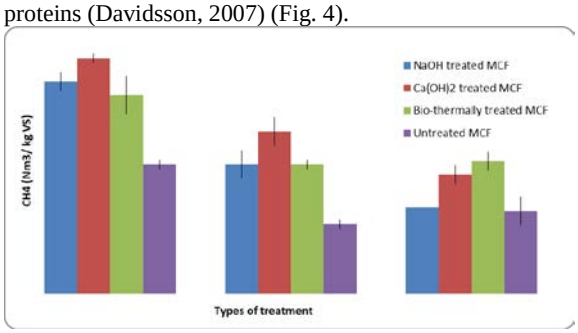


Figure 4. Methane production using hydrolysates co-digested with cow dung, poultry slaughterhouse and abattoir wastes after 12 days of incubation.

In contrast, pre-hydrolysed MCF by poultry slaughterhouse waste showed the least methane yield after 12 days of incubation. Bio-thermally treated MCF yielded 0.195 Nm3/kg VS methane when it was co-digested with cow dung, the amount that is significantly higher than the methane yield from hydrolysates collected in the same MCFs but co-digested with poultry and abattoir wastes.

3.3. Electrical energy generation in MFC fed with pre-treated MCF

Voltage generation in MFC fed with three types of pre-treated MCF showed no significant difference but showed marked difference with untreated MCF. However, $Ca(OH)_2$ treated MCF yielded maximum voltage of 355mV in 8 days and was relatively stable up to 14th day. The MCF pre-hydrolysed with IY-BUK1 fed into MFC recorded an initial potential of 38mV within 24 h, and after 8 days of incubation, a voltage of 315mV was achieved; the voltage reached its peak (347mV) at 11th days of incubation, and it was stable up to 15th day and thereafter showed gradual decrease (Fig. 5).

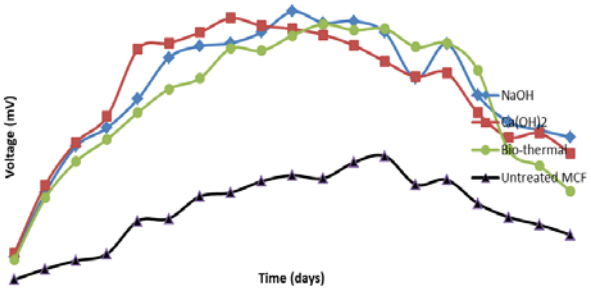


Figure 5. Voltage/day generation in MFC using pre-treated and untreated MCFs in 19 consecutive days

Only approximately 100mV of voltage was produced when hydrolysates obtained from the MCFs after 30 days was used (data not shown). The current and power densities recorded in MCF treated with $Ca(OH)_2$ followed same pattern with voltage, and reached maximum of 0.112mA/m^2 and 37.77x10^{4}mW/m^2 respectively after 9 days of incubation (Fig. 6).

Addition of rice water waste to the FMB as extra source of carbon significantly improve the voltage to 467mV in 7 days (Fig. 7)

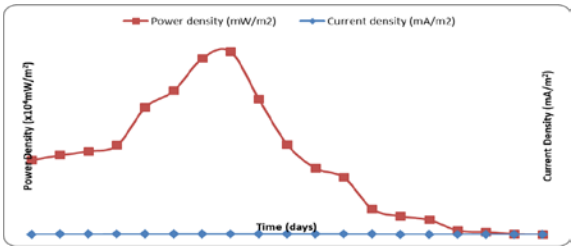


Figure 6. Power and current densities per day in MFC using MCF pre-treated with $Ca(OH)_2$

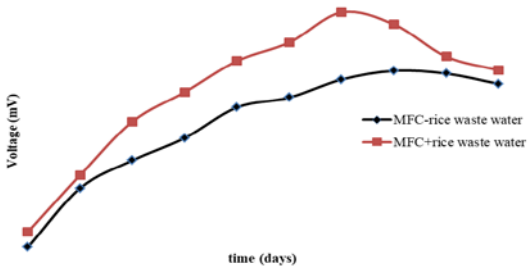


Figure 7. Effect of rice waste water on voltage generation in MFC containing $Ca(OH)_2$ treated MCF.

4. Discussion

This study has dealt with evaluating the effectiveness of different pre-treatment and co-digestion methods in improving degradability and subsequent utilization of melanised chicken feather waste by microbes for methane and electrical energy production. Prior studies have confirmed that presence of melanin together with compact and resistible structure of melanised feathers are responsible for its low degradability by microbes or their proteases (Gunderson and Frame, 2008; Okoroma *et al*., 2012) as well as low methane production (Salminen *et al*., 2003; Forgács *et al*., 2014). However, degradability of white (non-melanised) feathers has been reported to have improved when pre-treated with either chemical, heat or microbes (Salminen *et al*., 2003; Łaba *et al*., 2013; Forgács *et al*., 2014; Sinkiewicz *et al*., 2017) prior to anaerobic digestion and has also been proved to improve methane yield (Forgacs *et al*., 2011; Forgács *et al*., 2014). Reports of pre-treatment of melanised feathers for any purpose is scarce. In this study, biodegradation of pre-treated MCFs by *Pseudochrobactrum* sp. IY-BUK1 was approximately 75% higher than untreated feathers and feathers pre-treated with microwave-alkali (Lee *et al*., 2016). In addition to weakening keratin bonds in feather, involvement of heat in all the pre-treatments is done in order to eliminate any pathogenic microbe that might be present in the feathers, in fulfilment of EU legislation of handling of slaughterhouse waste prior to use in anaerobic digestion (EC by-product regulation, 2002). The pre-treatments resulted in cracking the stabilizing disulfide bonds in MCFs in similar way to non- MCFs, which resulted in faster degradation and earlier and higher production of keratinase and hydrolysates rich in soluble proteins. Complete degradation of 5 g/L (w/v) MCF pre-treated with $Ca(OH)_2$ was completed in 8 days and 333.5±7.5 mg/mL soluble proteins was obtained, which is one and half times higher than that obtained using MCF pre-treated with boiling. This agrees with the findings of Junoh *et al*. (2016) who reported efficiency of $Ca(OH)_2$ pre-treatment of biological waste. The higher soluble protein in hydrolysates that resulted from MCFs pre-treated with $Ca(OH)_2$, NaOH over or compared with white feathers indicated its suitability as substrate for biogas production.

Higher concentration of soluble proteins in FMB containing MCFs in comparison with FMB containing equal amount of non-MCF may be partly due to melanin residue, and may indicate its suitability as substrate for biogas production. Use of feather hydrolysates resulting from microbial degradation of non-MCFs in biogas production has been reported (Forgacs *et al*., 2011). In addition, several studies have reported that co-digesting the substrate with other organic wastes (mostly animal waste) has successfully improved gas yield. In this study, there is no significant difference ($p>0.05$) in amount of methane produced in ADs containing hydrolysates from $Ca(OH)_2$, NaOH and bio-thermal treated MCFs which have close concentration of soluble proteins. However, the pre-treatments resulted in improved biogas production to 43% over the theoretical methane yield from proteins. This is comparable to results reported by Forgacs *et al*. who reported rapid increase in rate of degradation and subsequent biogas production when non-melanised feathers were pre-treated biologically with a recombinant *Bacillus megaterium* strain prior to biogas production. Biogas produced in ADs supplemented by cow dungs improved methane production by 52.7% over non-supplemented ADs and about 40% and 30% over hydrolysates supplemented with poultry manure and abattoir wastes respectively.

Higher and faster yield of soluble protein and keratinase from chicken feather biodegradation has been reported in the presence of additional sources of carbon and nitrogen (Thangam and Rajkumar, 2000; Bernal *et al*., 2003; Lo *et al*., 2012; Yusuf *et al*., 2016). Complete degradation of 5g/L of treated and untreated MCFs by strain IY-BUK1 in FMB supplemented by 1g/L of sucrose and rice waste water was achieved in significantly lesser time than non-supplemented FMBs (data not shown) in similar way with feather degrading bacterium *Alcaligenes* sp AQ05-001 (Yusuf *et al*., 2016). This was applied in MFC by feeding the anodic chamber with FMB containing 0.5% of MCF and 1% of additional carbon source (rice water waste). The overall effect of MCF pre-treatment was also remarkable in MFC, where untreated MCF produced highest voltage of 174mV much later (13^{th} day), compared with pre-treated MCF which produced an average of 175mV in day 3. The slight efficiency of $Ca(OH)_2$ pre-treatment over NaOH and bio-thermal treatment was also expressed in MFC, where the highest voltage of approximately 355mV was achieved in 8^{th} days but achieved in the 11^{th} day with MCF fed with NaOH treated MCF.

The highest amount of voltage generated in this study was higher than 141mV reported by Chaturvedi and Verma, 2014 when white feathers were degraded by feather degrading bacterium-*P. aeruginosa* strain SDS3 (Chaturvedi and Verma, 2014). The early rise of voltage, current and power densities and subsequent stability of voltage prior to decline indicated the feasibility of electricity generation using MCF, as success of MFC depended partly on rapid onset and duration of stationary phase (Chaturvedi and Verma, 2014). While stationary phase was more prolonged in MFC fed with untreated MCF than pre-treated, supplementation with rice waste water further reduced the ascending and stationery phases, which resulted in higher power generation that lasted for short time. The slow rate of degradation of untreated MCF in MFC as compared to rapid degradation of pre-treated MCFs may then have some advantage, since slow rate of feather degradation will prolong stationary phase of MFC, but with lower voltage.

In summary, biogas and electric energy production from MCF was possible in amount comparable with non-MCF and other wastes especially when pre-treated with $Ca(OH)_2$. The use of MCFs in biogas and MFC will help in fulfilling the need for achieving hygiene conditions in environment and also offering an environmental, friendly, and economically feasible method for the utilization of MCF waste to produce renewable energy.

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Conflict of Interest:

The authors declare that they have no conflict of interest

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