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COMBINED THERMO-MICROBIAL PRE-TREATMENTS METHODS FOR ENHANCED BIOGAS PRODUCTION FROM NILE PERCH FISH SOLID WASTE IN TANZANIA

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Abstract

Anaerobic digestion (AD) of fish waste is difficult mainly due to high protein and lipid content which inhibit the process. Pre-treatments methods are option to improved AD of fish wastes for biogas production. Effects of heating, pasteurization separately and combined with CBR-11 bacteria strain culture (internal code) pre-treatment on biogas production from fish solid waste (FSW) was investigated in batch anaerobic bioreactors (BAB). The two thermo pre-treatment methods had a positive effect with best results 311 and 361 CH_4 ml/g volatile solids (VS) $_{added}$ obtained from heated FSW for 90 seconds at 130^oC and pasteurised FSW for 60 minutes at 70^oC, respectively. The highest methane yield were two fold compared with non-pre-treated FSW (163 CH_4 ml/gVS_{added}). Heated and pasteurised FSW each separately combined with CBR 11 bacterial culture pre-treatment prior to AD had a maximum methane yield of 697 and 491 CH_4 ml/gVS_{added}, respectively. Resulting in three to four fold increases in methane yield compared with non-pre-treated FSW (163 CH_4 ml/gVS_{added}). The methane content of the biogas ranged between 70-81% regardless of pre-treatment methods employed. In conclusion combined thermo-microbial pre-treatment is a novel method for enhanced biogas production of FSW. However, remain to be tested in continuous anaerobic digester with or without co-digestion.

Keywords CBR-11-bacterial strain**,** Fish Solid Waste, Heating, Pasteurization

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Introduction

Fish processing industries generate liquid and solid waste, that are the potential valuable resource, from which high-value products could be obtained [1]. In East Africa Nile Perch fish processing along Lake Victoria forms an important segment of the economy producing up to 52800 tons of Nile Perch fish fillets generating up to 210 million Euros [2]. With 31 fish processing factories in place and average capacity of processing 60 – 140 tones of fish per day, they generates up to 36,000 tons of fish solid waste (FSW) and 1,800,000 m^3 of liquid waste annually [3]. Fish solid waste is constituted with heads (44%), skeleton and fins (30%), skins (10%), offals (5%), scales (5%), fat solid and liquid (4%), and fish bladder (moals) 2%. Worldwide the fish processing industries often encounter problems with the handling and disposal of fish waste generated due to increased cost of waste treatment plants, particularly with regard to energy [4]. In East Africa fish wastes are not properly disposed or treated leading to environmental problems such as eutrophication of the lake, which result in changes in species composition, species loss and health related problems [5,6]. Therefore, a major drawback of fish industry is its negative impact on the water bodies environment in the form of organic/inorganic pollution [4]. To deal with such problems and comply with regulations, the effluent from the fish processing must be treated. Because of the high content of protein and lipids, fish wastes are energy-rich feedstock, which makes them interesting substrate for AD [7]. The AD is the method of treating fish waste and at the same time produces energy in the form of biogas rich methane and effluents as biofertilizer for application on agricultural fields for nutrient recovery [4,8,9]. However, fish wastes pose a particular technological problem in anaerobic bioreactors/systems for both treatment and production of bioenergy in the form of biogas rich methane. The high content of protein and lipids may cause inhibition of the AD process as they release high levels of ammonia and free long chain fatty acids (LCFA) concentration when digested [1]. Therefore fish wastes are generally regarded as difficult substrates for AD mainly because of their typical high protein and lipid contents are known to inhibit the methanogenesis [10]. This is due to their adsorption onto the cell wall/membrane and interfering with the transport of soluble substrates to the biomass and consequently causing the conversion rate of substrates to decrease [1,11,12]. Ammonia is a product of urea hydrolysis or anaerobic degradation of protein-rich substrates such as fish waste. Ammonia concentrations below 200 mg/l is beneficial to anaerobic process but when present at high concentrations, ranging from 0.1 to 1.1 kg/Nm^3, inhibits anaerobic microorganisms [11, 12]. Free ammonia is reported to be the main cause of inhibition since it is highly membrane-permeable and can therefore diffuse passively into the cell causing proton imbalance, and/or potassium deficiency [1]. Additionally, lipids may cause inhibition problems in AD due to their tendency to promote floating scum and possible accumulation of inhibiting degradation intermediates such as long- chain fatty acids (LCFAs) [13]. During anaerobic degradation, lipids in forms of triglycerides are hydrolyzed to glycerol and LCFAs and later to volatile fatty acids. These compounds are potentially attractive for biogas production due to their high potential in methane yield. Long chain fatty acids and delicate balance between syntrophic acetogenesis and syntrophic methanogenesis inhibit methanogenesis and hence inhibit lipid own hydrolysis [14]. The inhibition mechanism is related to the adsorption of the surface-active acids onto the cell wall, which exert an acute toxic effect on the microorganisms involved in the β-oxidation and methanogenic pathways [11]. Furthermore, low degradability of triglycerides may hinder AD process and thus cause serious problems such as sludge floatation, loss of biomass from bioreactor, toxicity of intermediates, formations of films of oil and grease on the surface of bioreactors, degrading efficiency of the anaerobic process and generating malodorous gases [15,16,17]. Inhibition of methanogenic Archaea has been

reported to hinder methane generation [15,16,18,19]. Despite all these operational problems, AD is widely used in treating lipid-rich wastes [1,19,20,21,22]. Pre-treatment methods are good option to achieve satisfying stability and efficiency in anaerobic digestion process [1,4]. Pasteurization has been recommended for waste treatment before AD process as a pre-treatment method for treating different types of biological contaminants to reduce risk of pathogen spread [23,24]. Furthermore pasteurization of animal by-product at 70^{o}C for 1 hour has been found to increase biogas yield to four fold (1.14 l/gVS) compared with non-pasteurized animal by-products (0.31l/gVS) [25] . Also methane production has been found to increase above organic loading rate (OLR) applied (from 2.1 to 3.7kg VS/m^3 day) when pasteurization (hygenization) was used [24]). However, pre-treatment (pasteurization: 70^{o}C for one hour, autoclaving at 133^{o}C, 300 kPa for 20 minutes and alkali hydrolysis (sodium hydroxide, 50 or 100 g NaOH/kg VS) of five different types of pig slaughterhouse by-products; fat, blood, raw waste (meat, fat and bones), intermediate product (pressed raw waste) and bone flour prior to AD using batch and semi-continuously fed bioreactor at 55^{o}C was found to have no effect on methane yield [26]. Literature on pre-treatment (pasteurization: 70^{o}C, or heating 130^{o}C and addition of microbial culture) of Nile Perch fish solid waste for improved anaerobic digestion for biogas production is totally non-existent [8]. The objective of this study therefore was to evaluate the feasibility of pasteurization/heating pre-treatments singly or in combination with microbes as pre-treatment methods suitable for enhancing biogas production from anaerobic digestion of Nile Perch FSW.

Material and Methods

Substrate and inoculum preparation

Fish solid waste (a mixture of fish scales, viscera, fish scrap, fat solids, proteins and fish rejects) produced during fish processing was obtained from Tan-perch, Vic-fish and Mwanza Nile Perch fish processing factories in Mwanza, Tanzania. The FSW from different fish processing factories were mixed and stored at -20^{o}C until used. Before use in a frozen condition FSW was chopped to reduce particle sizes down to
20 mm using kitchen knife (Super Cut stainless steel, Germany). Thereafter, chopped FSW was shredded in a mechanical meat mincer to ensure particle size < 12 mm and homogeneity. Finally the minced FSW was packed in plastic containers and stored at -20^{o}C for later use. An active inoculums used in this experiment was obtained from anaerobic wastewater stabilization pond located at Vic-fish, fish processing factory in Mwanza. Inoculums was stored in 25- liter plastic containers with anaerobic headspace to ensure degradation of easily degradable organic matter still present in the inoculums and remove dissolved methane. The characteristics of FSW and inoculums are summarized in (Table.1)

Source of bacterial strain

The bacterial strain (CBR-11, KS10 and BR10), which have been found to express lipolytic activity without proteolysis was obtained from strain bank at the department of Molecular Biology and Biotechnology, University of Dar es Salaam, Tanzania. The BR 10 and CBR-11-bacterial strain are local isolate which has been primarily characterised and reported [27]. Bacterial strain KS10 is also a local isolate which has not been characterised yet. Lipozyme TL IM commercial name for lipase enzyme was generously provided as a gift from Novozyme A/S Krogshoejvej, Denmark.

Experimental set-up

The effect of pre-treatment method and time on the methane yield were determined in a 500 ml conical flasks with a working volume of 300 ml. Setting–up of batch anaerobic bioreactors (BAB) were done as previously described [28]. Loading of pre-treated FSW was done at inoculum: substrate ratio of 1:1. This investigation was divided into two experimental parts.

investigation on optimal pre-treatment time

In order to examine the effect of pre-treatment on the performance of AD of FSW two different pre-treatment methods were employed. The first method was pasteurization, where FSW was subjected to 70^{o}C in the oven (Gallenkamp, Sanyo OMT Oven, UK) for 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes. The second method was heating (boiling) in which FSW was subjected to boiling temperature of 130^{o}C in an oven (Gallenkamp, Sanyo OMT Oven, UK) for 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 seconds. After each pre-treatment, pre-treated FSW was left to cool and TS and VS was analysed prior to loading of bioreactor. The proportions of pre-treated FSW and distilled water appropriate to achieve 300 ml working volume mixture were calculated according to TS and VS obtained with fixed amount of inoculum of 200 ml. Distilled water was used to fill-up to the working volume.

The experiment set-up consisted of 36 batch anaerobic bioreactors for pasteurized Nile Perch FSW, 30 batch anaerobic bioreactors for heated Nile Perch FSW, 3 batch anaerobic bioreactors for non-pre-treated Nile Perch FSW and 3 bioreactors for inoculum without Nile perch FSW which, brings to a total of 75 batch anaerobic bioreactors. Each batch anaerobic bioreactor was sealed with a gas-tight rubber stopper which was equiped with gas sampling septum for withdrawing biogas samples, and gas tight bag for collecting biogas. After loading and closing tightly the bioreactor was run for 55 days. In this experiment the performance of pre-treated Nile Perch FSW was compared to non-pre-treated Nile Perch FSW.

Investigation on the effect of Nile Perch FSW pre-treatment by pasteurization/heating with CBR-11-bacterial strain.

The experimental set-up were as in experiment 1 above. The experiment was designed to investigate on the effect of pre-treating pasteurized/heated FSW with CBR-11- bacterial strain. CBR-11- bacterial strain was isolated from stabilization pond treating high strength fish processing wastewater and exhibited lipolytic activity when grown on tributyrin agar plates [27]. Prior to bacterial pre-treatment CBR-11 strain was first inoculated in sterilized tributyrin broth media (pH 7.0) containing 0.25% peptone meat, 0.25% peptone casein, 0.30% yeast extract and 1.0% tributyrin as the sole carbon source. Inoculated tributyrin broth was incubated at 30^{o}C in a shaking incubator (Orbital Incubator S150, Stuart Scientific, UK) shaking at 120 rpm and left to grow to an optical density of 2, measured spectrophotometrically at 650 nm wave length (Thermo Spectronic Helios Gamma, England). For pre-treatment purpose whole microbial culture was used in this experiment. Pre-treatment of pasteurized/heated Nile Perch FSW with microbial culture was done by adding 3% (w/w) of CBR-11 strain to the pasteurized/heated FSW, mixed thoroughly and incubated for 12 hours at room temperature (27 - 33^{o}C). After pre-treatment time, the pre-treated substrate was analyzed for total solids (TS) and VS which were used to calculate the amount of gVS of FSW to be loaded. After loading bio-reactors were run for 45 days. At the end of experiment TS, VS, volatile fatty acids (VFAs) and alkalinity was analysed. For each treatment triplicates samples were used.

Lipase enzyme activity assays

Lipase enzyme activity in the crude enzyme extract from CBR-11 bacterial strain was determined by titration method already described previously [29]. According to this procedure, 18 ml of an emulsion of arabic gum (5% w/v) and olive oil (5% w/v) (prepared in 50 mM phosphate buffer, pH 7.0) were mixed with 2 ml enzyme extract and allowed to react for 1 hour at 37^{o}C in a shaking incubator (Orbital Incubator S150, Stuart Scientific, UK). The reaction was then stopped by adding 20 ml of 1:1 acetone/ethanol mixture. The reaction was further agitated for 10 minutes to facilitate total extraction of fatty acids. Extracted fatty acids was titrated against 0.05 N NaOH in a pH-stat (Boeco, pH meter BT-600, Germany) until end-point 11.0. Blank assays were done by adding the acetone/ethanol mixture prior to the enzyme sample. One unit of lipase activity was defined as the amount of enzyme, which produced 1 µmol of fatty acids equivalent per minute under the assay conditions.

Total Lipid extraction from CBR-11 bacterial strain/Lipozyme pre-treated Nile Perch FSW

Crude fish oil from CBR-11 and Lipozyme pre-treated Nile Perch FSW was extracted using Folch method as previously described [30] with some modifications. The method used extraction ratio of 20 parts 2:1 chloroform/methanol to 1 part FSW tissue as follows: 15 g FSW tissue was homogenized with 600 ml 2:1 chloroform/methanol. The mixture was filtered and then washed several times with 2:1 chloroform/methanol, and 0.88% NaCl in water was added to the combined filtrate at a final ratio of 8:4:3 chloroform/methanol/water. The final biphasic system was centrifuged at 6000 x g for 10 minutes and the entire lower phase was collected into a pre-weighed glass tube and evaporated to dryness in an oven (Gallenkamp, Sanyo OMT Oven, UK) set at 80^{o}C until all traces of solvent in the sample tube was completely removed.

Analytical Methods

Biogas volume and composition was measured and analysed after every 7th day for 55 days in experiment 1 and 45 days in experiment 2. Measurement of biogas volume was performed using 100 ml gas-tight glass syringe with a gas lock (Fortuna®, Poulten & Graf GmbH, 97877 Wertheim, Germany). The gas composition was estimated by KOH concentrated absorption method [31]. In this method only methane is determined while other biogas components such as CO_2 and H_2S are dissolved in the KOH solution. Methane yield was calculated by taking the average difference of the methane produced by the control from the the average methane produced by each set and the difference was divided by the weight of volatile solids (gVS) in the substrate fed to the digestor. Alkalinity, VFAs and pH of the digestate at the end of AD were measured by titration method [32]. Total organic carbon (TOC), total solids (TS) and volatile solids (VS) were measured as described in Standard Methods [33]. Total organic matter (TOM) was determined by multiplying TOC (%) by a factor of 1.80 [34]. Total lipids were determined by Folch method [30]. Analysis of fatty acids composition was done after trans-esterification of lipids into fatty acid methyl esters (FAME) using sodium methoxide [35]. FAME analysis was carried out using a Varian 3400 GC system equipped with a flame ionisation detector, Supelcowax 10 capillary column (58 m x 0.32 mm x 25µm film thickness; Supelco, Bellefonte, PA, USA) was used to separate FAME. The carrier gas was helium at 1.79 x 10^{-1} MPa. Temperature programme for separation was: initial temperature of 50^{o}C was held for 5 min, increased to 220^{o}C at 25^{o}C/min and held for 21 min and then increased to 240^{o}C at 15^{o}C/min and held for 10 minutes. Injector temperature was maintained

at 60°C for 0.1 min, and then was further increased to 250°C at 60°C/min and held for 2 min. Detector temperature was kept constant at 250°C.

Results and Discussion.

Proximate compositions of Nile Perch FSW

In Eastern Africa fish industry is dominated by Nile Perch, which 30-40% of the fish that is processed for human consumption comprises solid waste [36]. The proximate composition of fish waste from fish varies considerably with fish species, tropical or temperate fish, fresh water or saline fish, size, season, fishing ground, fish deffating, fish processing, type of fish waste and analytical methods employed [37]. Considering those facts, proximate composition data of Nile Perch FSW presented in (Table. 1) could be considered fair representative of characteristics of fish wastes analyzed. In this study analysis of Nile Perch FSW was carried out on fish waste comprised of the mixtures of fish scales, viscera, fish scrap, fat solids, proteins and fish rejects. In the literature data on composition of solid fish wastes are very scanty [1,8]. Nevertheless, the proximate composition values obtained in this study for the composition of Nile Perch FSW were within those values reported for solid fish waste [28,37,38]. The ash content (13% of TS) indicated that Nile Perch FSW was relative good source of minerals while fat content was also relatively high (20% of TS). Moreover, FSW contained high supply of protein (31% of TS). Furthermore, the Nile perch FSW also contained a very high organic matter measured as TOM (85% of TS), VS (87% of TS, TOC (47% of TS). Organic matter content measures the nutritional value (lipids, proteins and carbohydrates). These values indicated that most solids in the Nile Perch FSW were of organic origin and have high-energy production potential if efficiently biodegraded through anaerobic digestion [27]. Additionally, the average pH 6.5 and alkalinity about 4 g/l were more indicative values that the Nile Perch FSW could be amenable through anaerobic digestion.

Table 1. Characteristics of Nile Perch fish solid waste used in anaerobic degradation experiment.

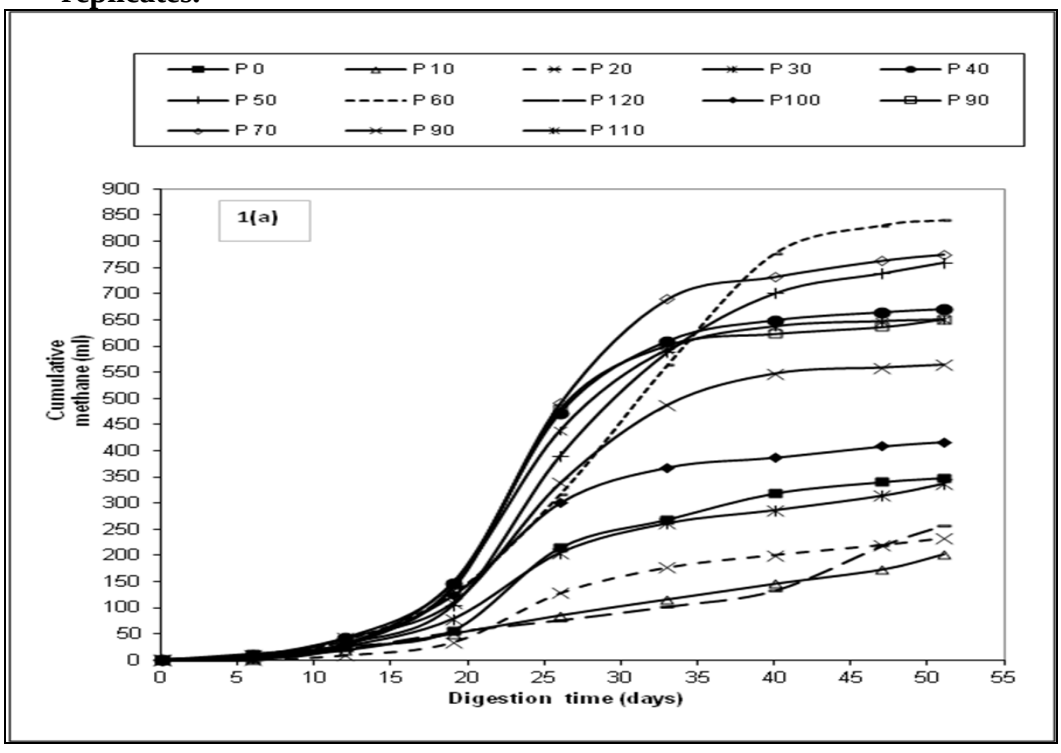
pH	6.54±0.01
Moisture content (% of fresh)	62.34±0.32
TS (% of fresh)	37.66±0.45
VS (% of TS)	86.61±0.15
Ash content (% of TS)	13.39±0.04
TOC (% of TS)	47.02 ±0.16.
TOM (% of TS)	84.64±3.12
TKN (% of TS)	4.93±0.43
Crude protein (TKN%x6.25)	30.81±1.04
Total lipids (% of TS)	20.09±0.02
COD (mg O2/gVS)	2031±50.76
C:N	9.54 ±1.54
VFA (mg/l)	121.06±5.0
Alkalinity (mg/l)	4048 ±150.54

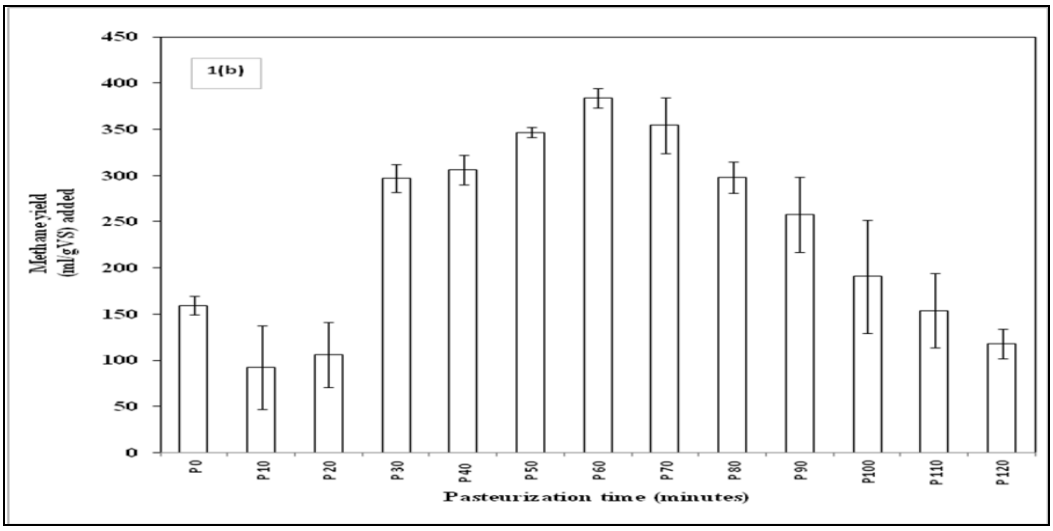
Values are average of triplicate analyses ±SD

The effect of pre-treatment method and time variations on methane production and methane yield of Nile Perch FSW

Fish waste and fish sludge can be expected to have very high methane yields due to their lipid and proteineous nature. The theoretical yield for lipids is about 1014 m^3 CH4/ton VS while the theoretical yield for protein is about 490 m^3 CH4/ton VS [39].The methane production and methane yield of Nile Perch FSW varied depending on the pre-treatment method and exposure time regimes investigated. Fig. 1(a) and 2(a) showed the methane production pattern values obtained was similar to the ones reported previously for oil and fats [12] and for pure lipids [14] which were obtained from batch anaerobic digestion. Comparing pre-treatment methods used, pasteurization of FSW at 70^{o}C for 60 minutes gave the highest methane production and methane yield of 840 ml CH4 and 384 ml CH4/gVS_{added}, respectively (Fig. 1a). The methane yield was equivalent to 59% increase compared to unpasteurized FSW (159 CH_4 ml/gVS_{added}) (Fig.1b). On the other hand, the maximum methane yield was 38% and 78% of the theoretical methane yield of lipids (1014 ml/gVS_{added}) and proteins (490 ml/gVS_{added}), respectively [39]. The lowest methane production and methane yield from pasteurized FSW was 202 ml CH4 and 92 ml CH4/gVS_{added}, respectively (Fig. 1a) and was recorded for Nile Perch FSW pasteurized for 10 minutes. This was equivalent to about 42% lower in CH4 yield compared to untreated Nile Perch FSW (159 CH_4 ml/gVS_{added}) and was 9% and 19% of the theoretical methane yield of lipids and proteins, respectively.

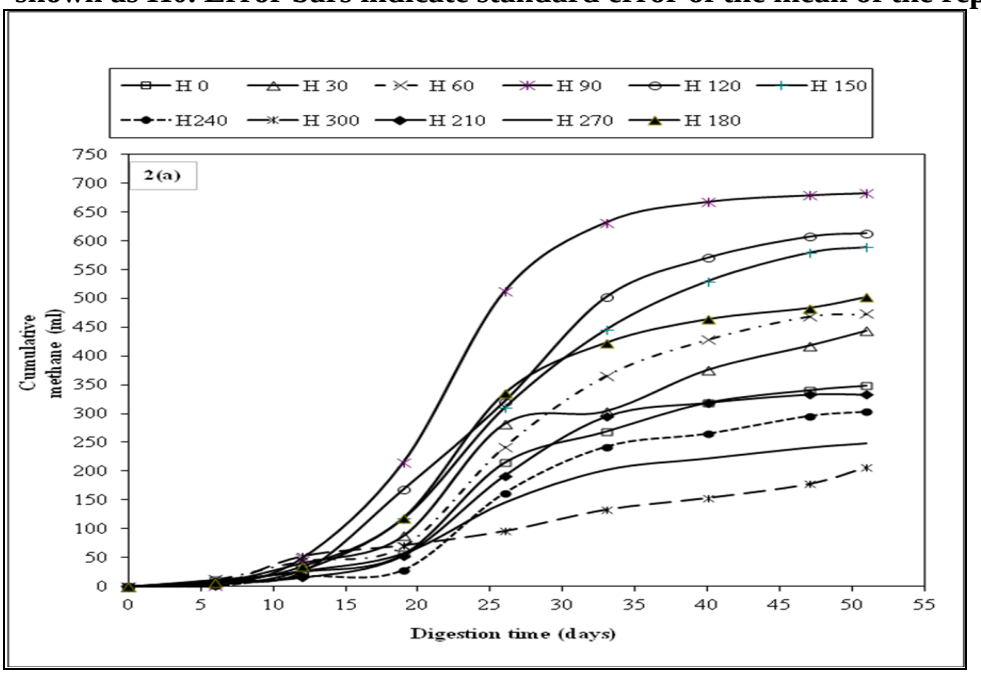
Fig.1 (a). Cumulative methane production and (b) methane yield from AD of Nile Perch FSW pre-treated by pasteurization at different time intervals. Untreated FSW is shown as P0. Error bars indicate standard error of the mean of the replicates.

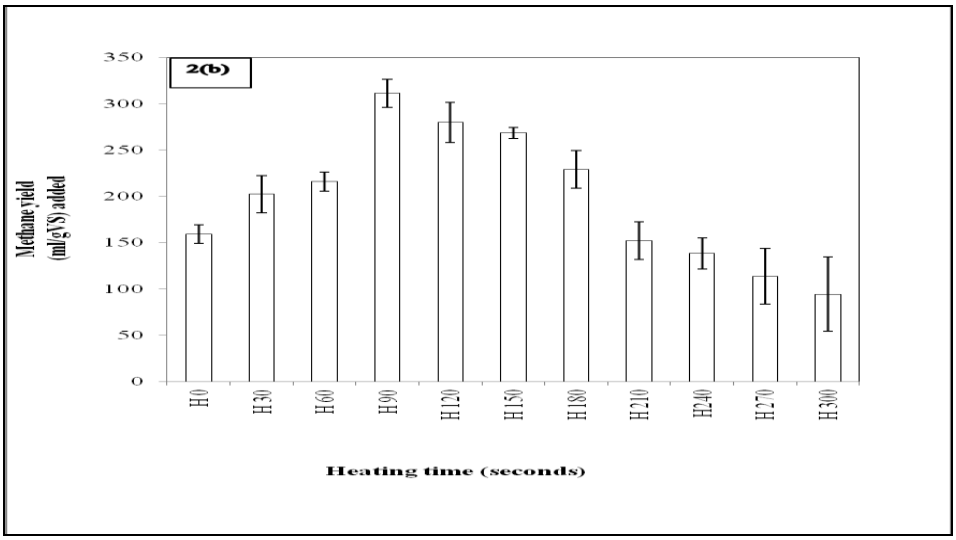




Heating was the second pre-treatment method, which was used to pre-treat Nile Perch FSW. In this method, Nile Perch FSW was heated briefly for few seconds, and the heated FSW was used for biogas production. Highest methane production and methane yield were 682 mlCH4 and 311 ml $CH4/gVS_{added}$, respectively were obtained at 90 seconds pre-treatment period. The maximum methane yield was equivalent to 51% increase compared to unheated Nile Perch FSW (159 CH_4 ml/gVS_{added}) (Fig. 2a,b). On the other hand, the maximum methane yield was 31% and 63% of the theoretical methane yield of lipids (1014 ml/gVS_{added}) and proteins (490 ml/gVS_{added}), respectively [39]. The lowest methane yield from 300 seconds heated FSW was 154 $mlCH4/gVS_{added}$ (Fig. 2a,b), which, was 3% lower compared to unheated Nile Perch FSW (159 CH_4 ml/gVS_{added}).

Fig.2 (a). Cumulative methane production and (b) methane yield from AD of Nile Perch FSW pre-treated by heating at different time intervals. Untreated FSW is shown as H0. Error bars indicate standard error of the mean of the replicates.



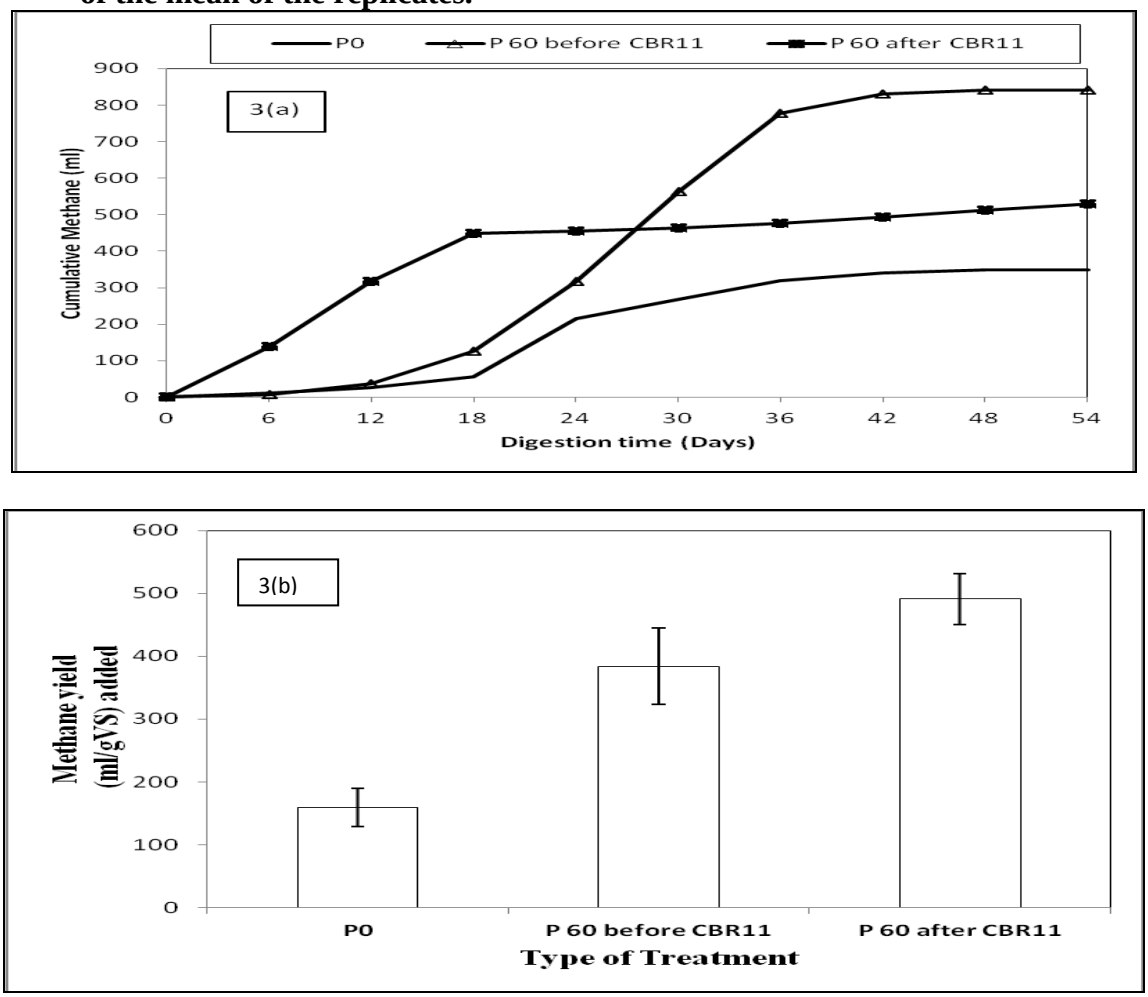


Results in (Fig. 1a,b and Fig. 2a,b) showed that pasteurization at 70^{o}C for 60 minutes and heating at 130^{o}C for 90 seconds resulted in a two fold increase in both cumulative and methane yields compared to untreated Nile Perch FSW. The findings on enhanced methane yield by pasteurization at 70^{o}C for 60 was in agreement with [25] working with mixed minced animal by-products they found that pasteurization of animal by-products from slaughterhouses had a fourfold increase in methane yield. However, increased methane yield by heating at 130^{o}C found in the present investigation was in contrast to [26], who found that pre-treatment of mixed pork waste by sterilization at at 133^{o}C, 300 kPa for 20 minutes had no effect on methane yield. Nevertheless, it only improved methane production per kg VS waste added due to an increase of VS content caused by water evaporation. Results in this study demonstrated that pre-treatment of Nile Perch FSW by heating at (130^{o}C) prior to AD actually resulted into increased methane production and hence methane yield. The differences in the extent of production and methane yield compared to previous findings [26] could be most probably be due to the differences in compositions, type and nature of the substrate used, the nature of the inoculum, the type of the reactor used and the mode of feeding employed.

The effect of thermal pre-treatment methods combined with CBR-11 bacterial strain pre-treatment on the extent of methane production and methane yield from Nile Perch FSW.

The biogas quality (methane content) ranged between 70-80% regard less of pre-treatment method used. Pre-treatment Nile Perch FSW by pasteurization at 70^{o}C for 60 minutes followed by bacterial strain CBR-11 pre-treatment improved AD process resulting in maximum total methane and methane yield of 530 ml CH4 and 491 ml CH4/gVS added, respectively (Fig. 3 a,b). The maximum methane yield obtained was higher by 36% compared to pre-treatment by pasteurization of Nile Perch FSW at 70^{o}C for 60 minutes only (361ml $CH4/gVS_{added}$) i.e. without being followed by bacterial strain CBR-11 pre-treatment.

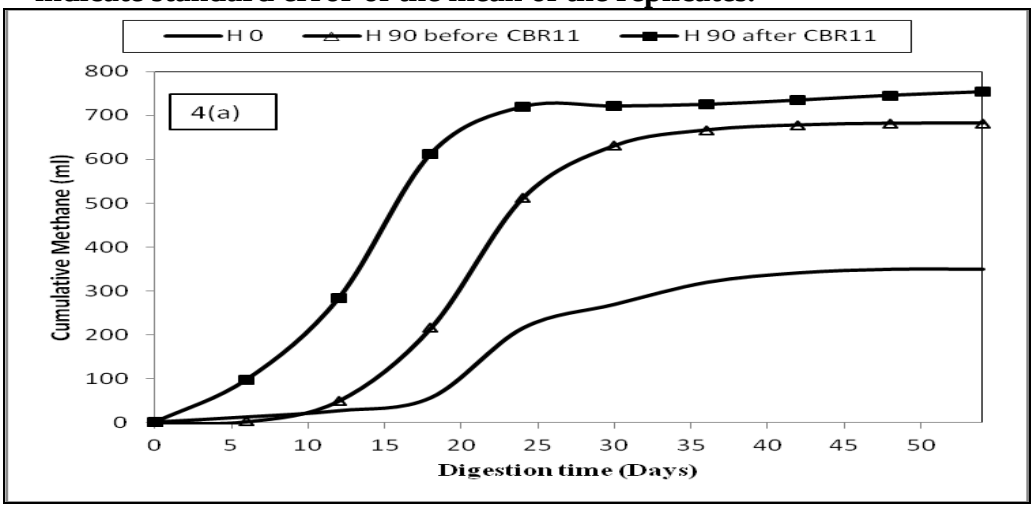
Fig. 3. Cumulative methane production (a) and methane yield (b) from AD of Nile Perch FSW pasteurized at 60 minutes followed by CBR-11 bacterial strain pre-treatment. Untreated FSW is shown by PO. Error bars indicate standard error of the mean of the replicates.

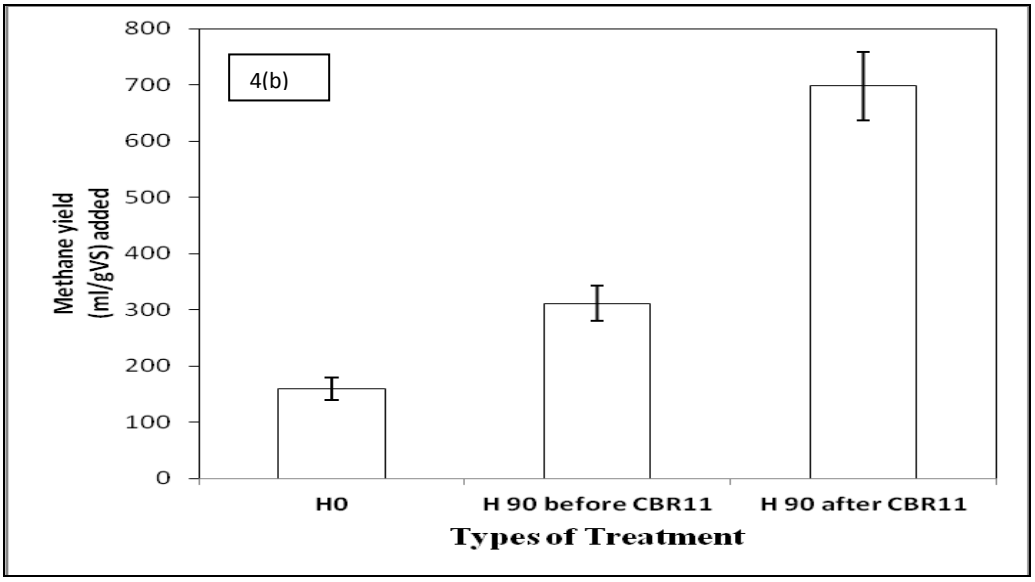


On the otherhand pre-treatment of Nile Perch FSW by heating at 130 ^{o}C for 90 seconds followed by bacterial strain CBR-11 significantly improved the subsquent AD process. It gave highest total methane and methane yield of 753 ml and 697 ml CH4/gVS added, respectively (Fig.4 a,b). The highest methane yield obtained was higher by 124% compared to the best results (311 ml CH4/gVS_{added}) obtained from heating pre-treatment of Nile Perch FSW at 130^{o}C for 90 seconds only i.e. without being followed by bacterial strain CBR-11 pre-treatment. The methane yield obtained from heated Nile Perch FSW at 130^{o}C for 90 seconds followed by the bacterial strain CBR-11 pre-treatment was 69% of the theoretical methane yield of lipids (1014 ml/gVS_{added}) while was higher by 142% compared to proteins theoretical value methane yield of (490 ml/gVS_{added}). It was evident that addition of CBR-11 bacterial strain to thermal pre-treated Nile Perch FSW prior to AD enhanced the biodegradation and biogas process [27]. The enhancement of methane yield could be due to pre-adapted microbial strain (bacterial strain CBR-11) which, has been reported to reduce the inhibition caused by LCFA and ammonia present in Nile Perch FSW through utilizing them in their metabolic activities [27]. Also thermal pre-treatements of Nile Perch FSW)

(pasteurization at 70 ^{o}C and heating at 130 ^{o}C) prior to AD somehow through evaporation lowered the concentration of long chain fatty acids, which are inhibitory to anaerobic digestion process leading to enhanced methane yield [40,41].

Fig. 4. Cumulative methane production (a) and methane yield (b) from AD of Nile Perch FSW heated at 90 seconds pre-treatment times followed by CBR-11 bacterial strain pre-treatment. Untreated FSW is shown as H0. Error bars indicate standard error of the mean of the replicates.



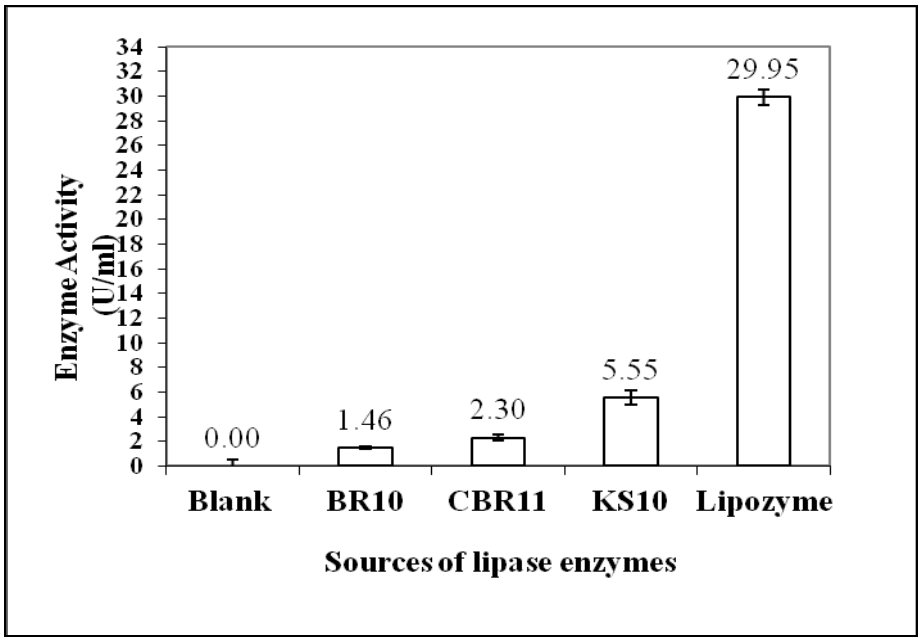


Lipolytic enzyme activity of CBR-11 bacterial strain used for pre-treatment of Nile Perch FSW

Microbial enzymatic extract preparation from microbes such as *Pseudomonas aeruginosa* for pretreatment of high fat content anaerobic substrates by lipase is a new and promising application for lipases to hydrolyze and dissolve fats [42]. It has been found recently to improve the biological degradation of high fatty substrates, accelerating the process and reducing time of anaerobic digestion as well as doubling biogas production [42]. CBR-11 bacterial strain combined with thermal pre-treatments (pasteurization at 70^{o}C and heating at 130 ^{o}C) at optimal parameters demonstrated novel methods to enhance methane yield from Nile Perch FSW. In particular heating at 130 ^{o}C for 90 seconds combined with CBR-11

bacterial strain hold potential for scaling up methane production from Nile Perch FSW. Therefore it was neccessary to carry out experiments to establish the ability of CBR-11 bacterial strain to produce lipolytic enzyme extracellulary. Enzyme activities of three local strains and commercial enzyme were tested for their activities (Fig.5). Local strain, CBR-11 and BR10 and KS10 were also tested for their enzyme activity just to compare their activities with that of commercial enzymes. The later two strains (KS10 and BS10) were not used in pre-treatment experiments. The whole cell culture were used to prepare the reaction mixture which was used for pre-treatment of Nile Perch FSW. Highest lipase activity (29.95 U/ml) was observed in Lipozyme (commercial name for lipase) followed by strain KS10, CBR11 and BR10 with 5.55, 2.51 and 1.46 U/ml, respectively. The lipase activity observed in this experiment was in agreement with that of other researchers [29]. While working with *Penicillium restrictum* the highest lipase activity was 30.3 U/g initial dry weight. Enzymatic extract preparation from *Pseudomonas aeruginosa* KM110 under accession No. HQ730879 with lipase activity (0.3 U/ml) was used to perform enzymatic hydrolysis pretreatment of a synthetic dairy wastewater with 1000 mg/l total fat content [42]. Bacterial product Liquid Certizyme 5^{TM} has been found to increase soluble chemical Oxygen Demand (COD-sol) of digestive tract content from meat processing industry (43). Lipase activities of the three local strains indicated that the local bacterial strains were actually releasing their enzyme extracellulary to digest the lipids during pre-treatment experiment prior to AD of Nile Perch FSW.

Fig 5. Lipolytic enzyme activities of indigenous strains isolates compared to commercial lipase. Error bars indicate standard error of the mean of the replicates.



Effect of fatty acids content on the extent of methane yield from Nile Perch FSW

The main reason for enhanced methane yield for the case of heated and pasteurized Nile Perch FSW presented in (Fig.1,2,3 and 4) compared to untreated Nile Perch FSW was type and content of fatty acids. The fatty acid analysis of Nile Perch FSW presented in (Table 2) revealed the dominance of unsaturated fatty acids for heated and pasteurized FSW while

saturated fatty acids dominated the unpre-treated Nile Perch FSW. In general, results showed that compositions of unsaturated fatty acids, oleic (C18:1) and palmitoleic acid (C16:1) and saturated fatty acids, palmitic acid (C16:0) were dominant. Palmitic acid was dominant in untreated Nile Perch FSW while palmitoleic acid dominated the heated and pasteurized Nile Perch FSW with low palmitic concentration. Free long chain fatty acid have been reported to severely inhibit methanogenesis [44]. The inhibition is normally caused by adsorption of LCFA to bacterial biomass, causing floatation and precipitation, which hinders the transfer of substrate and products causing delay in methane production [40]. Pre-treatment of Nile Perch FSW by heating or pasteurization probably had an effect in the accumulation of Oleic, Palmitoleic and palmitic acid. In anaerobic degradation of LCFA, it has been observed that the increasing accumulation of the palmitic acid can be due to an inhibition of palmitic acid biodegradation caused by oleic acid accumulation [40]. Transformation of of oleic acid to palmitic acid is a fast and non-limiting step in oleic degradation while the degradation of palmitic acid to acetate is a slow process. Accumulation of palmitic acid in the anaerobic bioreactors therefore cause temporary inhibition hence reduced methane production. More methane production and higher methane yield observed in pasteurised and heated Nile Perch FSW was probably due to low concentration of palmitic acid as compared to unpre-treated Nile Perch FSW (Table 2). This observation is in agreement with other investigators in which palmitic acid was found to be dominant fatty acid 3 to 52 times higher than oleic acid in a reactor containing activated sludge [41]. On the contrary in the inactivated sludge *(*autoclved at 121^{o}C for 15 minutes) the main fatty acid detected was oleic acid which was 8 times higher than palmitic acid [41]. This suggested that during anaerobic degradation of Nile Perch FSW accumulation of palmitic acid in bioreactors was responsible for the low methane production as observed in (Fig. 3 and 4).

Table 2. Fatty acid content in Nile Perch fish solid waste Untreated (Un-FSW), Heated (130^{o}C for 90 seconds, H-FSW) and Pasteurized (70^{o}C for 60 min, P-FSW).

Fatty acid content	**Chemical structure**	**Fatty acid (%) in Nile Perch FSW**		
		Un-FSW	**H-FSW**	**P-FSW**
Myristic	C 14:0	18.91	11.08	16.10
Pentadecanoic	C 15:0	0.59	0.72	0.75
Palmitic	C 16:0	23.43	16.12	9.35
Palmitoleic	C 16:1	14.12	20.83	20.93
Stearic	C 18:0	6.28	7.09	7.12
Oleic	C 18:1	20.59	21.98	24.97
Linoleic	C 18:2	1.32	1.62	0.10
α-Linolenic	C 18:3	1.38	1.77	1.69
γ-Linoleic	C 18:4	0.12	0.12	0.07
Eicosanoic	C 20:0	0.12	0.29	0.32
Eicosatetraenoic	C 20:4	1.47	1.86	1.74
Eicosapentaenoic	C 20:5	1.98	2.81	2.57
Docosanoic	C 22:1	0.06	0.12	0.10
Docosatetraenoic	C 22:4	0.71	1.06	2.89
DPA	C 22:5	3.36	4.50	4.17
DHA	C 22:6	5.55	8.02	7.13

Conclusions

This is the first scientific report on enhanced biogas production from lipid rich Nile Perch FSW by pasteurization, heating pre-treatments combined with microbial pre-treatments. Pre-treatment of Nile Perch FSW by pasteurization at (70^{o}C, 60 minutes) or heating at (130^{o}C, 90 seconds) was successful in treating lipid-rich FSW leading to enhanced methane yield with two fold increase in methane yield compared to un-pretreated Nile Perch FSW. Furthermore, pre-treatment of pasteurized and heated Nile Perch FSW with bacterial strain CBR-11 resulted into more methane yield, which suggest the suitability of the combined pre-treatment methods to enhance methane production from Nile Perch-FSW. In particular, heating pre-treatment of Nile Perch FSW at 130^{o}C for 90 seconds followed by bacterial strain CBR-11 pre-treatment. Analysis of fat content after microbial pre-treatment showed that anaerobic degradation of oleic acid to palmitic acid and finally to acatate occured without inhibition for pre-treated Nile Perch FSW samples as compared to the situation without pre-treatment. Despite the high yields of methane observed for thermal and microbial pre-treated Nile Perch FSW in batch anaerobic bioreactors there are limitations of using fish waste for biogas production in continuous anaerobic digester due to high content of lipids and proteins. These compounds are known to inhibit the methanogenesis. Anaerobic co-digestion could alleviate such limitations and can increase methane production of digesters by 50 to 200%, depending on the operating conditions and the co-substrates used. Co-digestion of thermal-microbial pre-treated Nile Perch FSW with complimentary co-substrates such as vegetable wastes could be more promising for continuous anaerobic bioreactors. However, the concept remains to be tested at laboratory scale and at up-scale continuous anaerobic bioreactors.

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