ANAEROBIC CO-DIGESTION OF BIOLOGICAL PRE-TREATED NILE PERCH FISH SOLID WASTE WITH VEGETABLE FRACTION OF MARKET SOLID WASTE

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ABSTRACT

Anaerobic co-digestion of various organic wastes has been shown to improve biogas yield of fish wastes. This paper presents the effect of pre-treating Nile perch fish solid waste (FSW) using CBR-11 bacterial culture (CBR-11-FSW) and commercial lipase enzyme (Lipo-FSW), followed by batch anaerobic co-digestion with vegetable fractions of market solid waste (VFMSW) in various proportions, using potato waste (PW) and cabbage waste (CW) as co-substrates either singly or combined. Results indicated that CBR-11 pre-treated FSW co-digested with PW or CW in 1:1 ratio (substrate: inoculum) had positive effect on methane yield, while Lipo pre-treated FSW had negative effect on methane yield. Using CBR-11-FSW:PW the highest yield was 1.58 times more than the untreated FSW. Whereas, using Lipo-FSW:CW the highest yield was 1.65 times lower than un-treated FSW. Furthermore, the optimal mixture of CBR-11 pre-treated FSW and PW and CW co-substrates resulted into higher methane yield of 1, 322 CH_4 ml/gVS using CBR-11-FSW (10):PW (45):CW (45) ratio. The ratio enhanced methane yield to 135% compared to control. In conclusion, results demonstrates that optimal mixture of CBR-11 pre-treated FSW with both PW and CW as co-substrates enhanced methane yield and provide base line data for potential application in continuous anaerobic bioreactors investigation.

Keyword: Nile perch waste, CBR-11 bacteria, anaerobic co-digestion, cabbage waste, lipozyme, potato waste.

INTRODUCTION

The current higher consumption and consequently the generation of large amount of waste are the result of the rapid economic growth and increasing population in the world. Anaerobic digestion (AD) of organic wastes has been promoted in order to avoid the emissions of CH_4 resulting from the uncontrolled anaerobic decomposition of organic waste. Fish waste consists of 40-70% dry matter content. The remaining portion contains protein, fats, collagen, bones, heads, viscera, skin and some muscles tissues. Anaerobic digestion is the suitable option for treatment of fish wastes due to its high protein, lipid content and also high concentration of organic matter, which could lead to high methane production potentials. However, the methane yield may be reduced due to too high protein and lipid contents (Thirumurugan and Gopalakrishnan, 2012). Since proteins and lipids digestion of fish waste tend to slow down the AD process and can cause the process to collapse due to the production of ammonia, volatile fatty acids (VFAs) and long chain fatty acids (LCFAs) at inhibitory concentration (Diaz *et al.*, 2011; Karlsson *et al.*, 2011). Free ammonia, is toxic to methane forming bacteria and results in the inhibition of methane production. LCFAs are inhibitors of methanogenic microorganisms. Besides, operational instabilities related to sludge flotation and washout are also reported) (Nges *et al.*, 2012; Regueiro *et al.*, 2012). Anaerobic co-digestion of various organic wastes either un-treated or pre-treated has been shown to overcome the inhibitory technological problems and improve biogas yield from proteins and lipids rich fish wastes.

The VFMSW collected from food market, essentially contain sugars and hemicelluloses which are highly biodegradable with 60-82% volatile solids (VS) content. The high biodegradability of the VFMSW encourages the rapid production of volatile fatty acids (VFAs), which will lead to a rapid pH drop, consequently results into inhibitions of the methanogenic activity. Moreover, VFMSW is defined as low nitrogen and phosphorus containing materials. Thus the methane yield that can be obtained from VFMSW would be lower than that from waste with higher nitrogen content (Callaghan *et al.*, 2002). Therefore, mixing with other wastes, such as animal manure, sludge and abattoir wastes/animal wastes, that have high nitrogen content is preferable, since in this way the acidification of the system can be avoided (Garcia-Pena *et al.*, 2011). To that effect anaerobic digestion of VFMSW for methane production have been reported by different researchers and has been reviewed on aspects such as hydrolysis; kinetics, modeling, process aspects (performance, two- and single-phase systems, wet and dry technologies) pre-treatment, co-digestion, digestion enhancement etc (Gunaseelan, 1997; Malta-Alvarez, *et al.*, 2000; Gunaseelan, 2004; Gunaseelan, 2007).

Co-anaerobic digestion or co-digestion is anaerobic digestion performed on a mixture of at least two different but complimentary substrates (Cuetos, *et al.*, 2011). There is abundant literature about the utilization of co-digestion, such as co-digestion of organic fraction of municipal solid wastes and agricultural residues, organic solid wastes and sewage sludge or more specific wastes such as cattle slurry and waste milk (Malta-Alvarez, *et al.*, 2000; Callaghan *et al.*, 2002). Co-digestion offers many possible ecological, technological and economical benefits (Alvarez and Liden, 2008). Bioenergy production in biogas plants could be enhanced by 80-400% by using organic wastes and by-products as co-substrates (Weiland, 2010). Despite the well known reported co-digestion benefits, such as optimum humidity, buffering capacity and C/N ratio or inhibitory substances dilution (Malta-Alvarez, *et al.*, 2000), it is not clear whether some co-substrates have adverse impact when they are co-digested with another waste in particular if there is synergisms or antagonisms among the co-digested substrates and if several co-substrates of similar biochemical composition can be co-digested (Malta-Alvarez, *et al.*, 2000; Callaghan *et al.*, 2002). Therefore, it is critical to obtain an optimal mixture of the available co-substrates as well as the optimum operating conditions, which allow high biogas yields without compromising the stability of the process (Alvarez, *et al.*, 2010; Thirumurugan and Gopalakrishnan, 2012).

Anaerobic digestion for biogas production from VFMSW, different animal manures, agro-industrial waste, sewage sludge, weeds, kitchen wastes, animal wastes singly or co-digested has been reported by different researchers at laboratory, pilot and full scales (Gunaseelan, 1997; Mata-Alvarez *et al.*, 2001; Gunaseelan 2004; Gunaseelan, 2007). However, there is scarce information in the literature about AD of fish waste and enhancement by various strategies such as pretreatments, co-digestion etc. This is of special interest in the operation of pilot and full-scale anaerobic digesters, where the feed co-substrates composition may be variable. Nevertheless successful biogas production from solid wastes removed from fish farm effluents, sludge from saline fish, salmon molt hatching, solid fish waste with or without co-digestion have been reported (Lanari and Franci, 1998; Gebaur, 2004; Mshandete *et al.*, 2004; Gebaur and Eikebrokk, 2006; Salam *et al.*, 2009). On the other hand, very little have been reported on enhancement by chemical, physical and biological pre-treatment prior to anaerobic digestion of marine and fresh water fish wastes with or without co-digestion (Lanari and Franci, 1998; Gumisiriza *et al.*, 2009; Thirumurugan and Gopalakrishnan, 2012; Nges *et al.*, 2012; Regueiro *et al.*, 2012). McDermott *et al.* (2001) reported enhanced AD of aquaculture effluent by ultrasonication pre-treatment method. Pre-treatment using anaerobic microbial inoculum on solubilization of fish market wastes reduced the time taken for AD of the waste and generated methane yield of 0.36 m^3/kg VS added which was 1.3 times more than the control (Thirumurugan and Gopalakrishnan, 2012). Gumisiriza *et al.* (2009), reported enhancement of AD of Nile perch processing wastewater by co-digestion, physical and biological pretreatments. It was found that co-digestion of fish processing wastewater (FPW) with 10% gVS of brewery wastewater enhanced methane yield to a highest increment of 66%. LCFAs removal prior AD enhanced methane yield to an increment of 52% at LCFAs removal of 8%. Furthermore, pretreatment of FPW with aerobic microbial cultures isolated from a fish waste stabilization pond enhanced methane yield to an increment of 60% after 18 h, 68% after 15 h and 76 % after 12 h of incubation, respectively, for strains CBR-11, BR 10 and a mixture of the two (CBR-11 + BR10) (Gumisiriza *et al.*, 2009). Information on enhancement of methane yield from Nile perch fish solid waste by combined biological pre-treatment and co-digestion with vegetable fraction of market solid waste does not exist in the literature. Therefore, the objectives of this study were first to evaluate the co-digestion of microbial pre-treated FSW (CBR-11-FSW) with vegetable fraction of market solid waste Potato waste (PW) and Cabbage waste (CW) compared to untreated FSW at ratio of 1:1 (inoculum to substrate). The second objective was to establish the optimal mixture of CBR-11 pre-treated FSW and PW and CW co-substrates on volatile solids VS (g) basis.

MATERIALS AND METHODS

Substrate and inoculum preparation

Two different types of substrates were used in this study. 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.

Vegetable fractions of market solid waste (VFMSW) comprised of two types of vegetable wastes, potato waste (PW) and cabbage wastes (CW) were obtained from Buguruni and Ilala local markets, in Dar es Salaam Metropolitan. The quantities generated are shown in Table-1. Potato waste consisted of potato peels and rotten potatoes from chips vendors while CW consisted of spoiled cabbage leaves left-over in the market place. After collection, PW and CW were taken to the laboratory and stored in -20^{o}C. Before use PW and were chopped down to 20 mm using kitchen knife (Super Cut stainless steel, Germany) and shredded in a mechanical meat mincer to ensure particle size < 12 mm and homogeneity. Finally the minced VFMSW (PW and CW) were packed separately in plastic containers and stored at -20^{o}C for later use. An active inoculum used in this experiment was obtained from anaerobic wastewater stabilization pond located at Vic-fish, fish processing factory in Mwanza. The inoculum was stored in 25-liter plastic containers with anaerobic headspace to ensure degradation of easily degradable organic matter still present in the inoculum.

Source of bacterial strain and commercial lipase

The bacterial strain (CBR-11), 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 CBR-11 bacterial strain is local isolate which has been primarily characterized and reported (Gumisiriza *et al.*, 2009). Lipozyme TL IM commercial name for lipase enzyme was generously provided as a gift from Novozyme A/S Krogshoejvej, Denmark.

Microbial and commercial lipase enzyme pre-treatment of fish solid waste

Prior to pre-treatment of FSW with bacterial strain, CBR-11 bacterial strain was first grown on 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. Then the culture was incubated at 30^{o}C in a shaking incubator (Orbital Incubator S150, Stuart Scientific, UK) shaking at 120 rpm and grown to an optical density of 2.0, measured spectrophotometrically at 650nm wavelength (Thermo Spectronic Helios Gamma, England). Then 3% (w/w) of CBR-11 bacteria strain was added to a plastic bottle containing Nile Perch FSW, mixed thoroughly and incubated for 12 hours at room temperature (27-33^{o}C). After pre-treatment, the pre-treated FSW (CBR-11-FSW) was analyzed for total solids (TS) and volatile solids (VS) which were used to calculate the amount of gVS of CBR-11-FSW to be used in co-digestion experiment. For the purpose of comparison, commercial lipase enzyme Lipozyme TL IM was also used to pre-treat FSW, whereby 1% (w/w) Lipozyme was weighed and mixed with FSW and incubated as in CBR-11 bacterial strain and used as substrate for co-digestion experiment. For each pre-treatment triplicates samples were used.

Batch anaerobic bioreactor

The effect of co-digestion of pre-treated FSW (CBR-11-FSW) and (Lipo-FSW) with PW and CW on methane yield were conducted in a 500 ml conical flasks with a working volume of 300 ml. Building and operation of batch anaerobic bioreactors (BAB) were done as previously described by Mshandete *et al.* (2004).

In the co-digestion study, concentration of pre-treated CBR-11-FSW and Lipo-FSW at which the highest methane yield was obtained (9% TS) was kept constant in the preparation of all the proportions used in this experiment. The proportions of pre-treated FSW (CBR-11-FSW or Lipo-FSW) PW, CW 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.

Experimental set-up

Co-digestion of pre-treated FSW (CBR-11-FSW) and (Lipo-FSW) with PW and CW.

The set-up involved loading of pre-treated FSW (CBR-11-FSW), PW and CW individually (singly) at (substrate:inoculum) ratio of 1:1 and co-digestion of (CBR-11-FSW:PW); (CBR-11-FSW:CW) and (CBR-11-FSW:PW:CW) of substrate: inoculum ratio of (1:1) for the first two mixture and substrate: substrate: inoculum ration of (1:1:1) for the mixture. On the other hand, Lipo-FSW involved loading of pre-treated FSW (Lipo-FSW-PW and Lipo-FSW-CW at 1:1 substrate: inoculum ratio. The set-up for this part consisted of 56 batch bioreactors. Control bioreactors contained inoculum only (without substrate) was included and the biogas produced was substracted from experimental bioreactors. All the digestions were run in triplicates for 42 days. The experiments were terminated when less than 50 ml biogas production was recorded for each experimental bioreactor over two weeks period.

Optimization of co-digestion of pre-treated FSW (CBR-11-FSW) with PW and CW as co-substrates

To optimize co-digestion of pre-treated FSW (CBR-11-FSW) with PW and CW a total of 48 batch bioreactors were set-up. Control biorectors contained inoculum only (without substrate) were also included and used as above stated. To establish the optimal mixture proportions for co-digestion of pre-treated FSW (CBR-11-FSW) with PW or CW as co-substrates individually or in mixed form were set-up on different volatile solids weight (gVS) basis. Co-digestion ratios of CBR-11-FSW:PW or CBR-11-FSW:CW at (10:90), (20:80), (30:70), (40:60) and (50:50) on gVS basis were set-up. Co-digestion ratios of CBR-11-FSW with both PW and CW at (10:45:45), (20:40:40), (30:35:35), (40:30:30) and (50:25:25) on volatile solids weight (gVS) basis were also set-up. The optimal mixture was established based on highest methane yield obtained.

Analytical methods

Biogas volume and composition was measured and analysed after every 7th day for 42 days. Measurement of biogas volume was performed using 100 ml gas-tight glass syringe with a gas lock (Fortuna®, Poulten and Graf GmbH, 97877 Wertheim, Germany). The gas composition was estimated by KOH concentrated absorption method (Erguder, *et al.,* 2001). 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 average methane produced by each set and the difference was divided by the weight of volatile solids (VS) (gVS) in the substrate fed to the digestor. Alkalinity, volatile fatty acids (VFAs) and pH of the substrate before loading and at the end of anaerobic digestion (AD) were measured by titration method (Lahav and Morgan, 2004). Total organic carbon (TOC), total solids (TS), total nitrogen (TN), volatile solids (VS) and chemical oxygen demand (COD) were measured as described in Standard Methods (APHA, 1998). Total organic matter (TOM) was determined by multiplying TOC (%) by a factor of 1.80 (Iglesias-Jimenez and Perez-Garcia, 1992).

Statistical analyses

All experiments analyses were carried out at least in triplicates to ensure reproducibility and all data were expressed as mean ± standard deviations (S.D.) The data for biogas content and methane yield were subjected to analyses of variance (one-way ANOVA) at the 5% level (significant different at $p<0.05$) using the Statistical Package for Social Sciences (SPSS) Program Version 15.0 (SPSS, 2006).

RESULTS AND DISCUSSIONS

Composition of Nile perch fish solids wastes (FSW) and vegetable fractions of market solid waste (VFMSW)

The pH of the inoculum was 7.83±0.43, total solids (TS) % fresh) was 0.64 ±0.07 while VS (% of TS) was 39.03 ±0.02. The characteristics of FSW and VFMSW are summarized in (Table-1). Biomethanation of waste potential depends on the concentration of the three main organic components: proteins, lipids and carbohydrates. Hence substrate characterization is required to predict methane production and establish co-substrate mixing ratios (substrate:inoculum ratio) to be investigated to avoid inhibition and failure of anaerobic digestion (AD). Results in (Table-1) illustrated the analyzed parameters for three substrates FSW, PW and CW which, was significant different at ($p<0.05$).

Table-1. Characteristics of fish solid waste (FSW) and vegetable fractions of market solid waste (VFMSW) used in anaerobic co-digestion experiment.

Parameter	**FSW**	**VFMSW** (PW)	**VFMSW** (CW)
pH	7.10±0.02	5.66±0.06	3.55±0.01
TS (%WW)	37.4±0.03	23.41±0.11	6.33±0.05
VS (%TS)	82.37±0.28	92.37±0.12	89.69±0.02
MC (%)	62.6 ±0.45	76.59 ±0.32	93.67 ±0.09
TOC (%TS)	48.26±0.26	51.14±0.38	52.42±0.50
TOM (%TS)	86.87±0.47	92.06±0.68	94.05±0.91
TKN (%WW)	2.78±0.12	0.46±0.32	0.54±0.01
NH4-N (mg/l)	8.86±0.25	33.00±0.92	14.27±0.68
C:N	17.16	109.65	97.22
Alkalinity ($gCaCO_3/l$)	5.23±0.3	*	*
VFA (g/l)	121.06±0.21	*	*
SCOD (gO_2/l)	31.19±6.15	15.77±0.15	37.33±0.45
SCOD (gO_2)/gTS	83.39	67.64	589.73
Total lipids (%WW)	20.09±0.24	ND	ND

MC = Moisture Content; ND = Not determined; SCOD = Soluble Chemical Oxygen Demand;
*= Alkalinity and VFA was not determined by Titration method since the Initial pH was lower than the destination pH (5.75).

Results demonstrated that the three substrates were relatively rich in organic matters, which demonstrated that they could be amenable to anaerobic digestion with or without co-digestion. However, each substrate should be considered individually depending on the composition. pH value in the anaerobic bioreactor is an important parameter to imply the anaerobic digestion process stability. pH for PW and CW was acidic, which ranged between 3.5-6.5 and were devoid of buffering capacity while the pH for FSW was neutral 7.0 with significant buffering capacity measured as $CaCO_3$ of 5 g/l. On one hand, total nitrogen for PW and CW was low, which ranged between 0.4-0.5% coupled with high C:N ratio range of 97-110. On the other hand, FSW had high nitrogen content of 2.78% which was 5.6 times more compared to that recorded on average from PW and CW but it was coupled with low C:N ratio of 17 which was 6 times lower on average than that recorded for PW and CW. The low C:N in FSW implied that they contain a large quantity of nitrogen, mainly in organic forms, such as proteins. It is well established that vegetable wastes to which PW and CW belongs are highly biodegradable causing fast pH decrease due to rapid acidification (rapid production of VFAs), which could inhibits the methanogenic activity if used as sole feedstock without pH and buffering adjustments (Garcia-Pena, *et al.*, 2011). Moreover, vegetable wastes are also defined as low nitrogen containing materials (Callaghan, *et al.*, 2002) such previous observation was supported by low nitrogen content recorded for PW and CW (0.4-0.5%) in this study. It has been reported that methane yield that can be obtained from vegetable waste such as PW and CW would be lower than that from waste with higher nitrogen content due to the fact that vegetable wastes digestion as sole substrate is not technical and biologically feasible as limited nitrogen would be available for microbial populations (Callaghan, *et al.,* 2002; Robra, *et al.,* 2010). Therefore, mixing of PW and CW with other wastes, such as FSW that have high nitrogen content is preferable, since this way the acidification of the system can be avoided (Garcia-Pena, *et al.,* 2011). Also FSW is a great potential co-substrate because of its high buffering capacity ($CaCO_3$ of 5 g/l). High buffering capacity protect the system as the result of an increase of VFAs and decrease in pH. The carbon to nitrogen (C:N) ratio refers to the relative amounts of carbon and nitrogen present in the organic wastes. Nevertheless it depends on the composition of raw materials. The low C:N ratio of 17 in FSW was similar to those low C:N ratio found for human excreta, animal manure and sewage sludge (Polprasert, 2007). The high C:N ratio of up to 110 obtained in this study seemed to confirm what has been reported in other works in agro-industrial waste, sawdust, wood chips etc (Mshandete, *et al.,* 2004; Polprasert, 2007). FSW need to be co-digested with the addition of substrates with lower alkalinity and higher C:N ratios such as PW and CW. Thus the combination of substrates with low and high C:N ratios is preferable to obtain the optimum and improved biogas production. Generally, bacteria take up carbon 25-30 times faster than nitrogen (Polprasert, 2007). Thus the C:N ratio should be below 20 and up to 30 maximum as several researchers had reported but it all depends on the chemical characteristics of the mixed wastes and their ratios, which are optimal for methanogenic performance (Mshandete, *et al.,* 2004; Polprasert, 2007). Results in (Table-1) showed that while PW and CW were devoid of lipids, FSW had high lipid content of 20% (wet/weight) (w/w). Therefore FSW could be ideal feedstock for biogas production, because it is rich in proteins and lipids, and also contains high organic matter content (Table-1). Nevertheless it has been widely reported that high LCFAs concentrations can destabilize anaerobic digesters due to inhibition of methanogenic bacteria by possible damage to cellular membrane (Pereira, *et al.,* 2003). This shows that mono/single digestion of FSW alone is likely to face inhibition of the process due to the very high LCFAs and free ammonia concentrations that will likely occur in bioreactors (Pereira, *et al.,* 2003; Karlsson, *et al.,* 2011; Thirumurugan and Gopalakrishnan, 2012; Nges, *et al.,* 2012; Reguiro, *et al.,* 2012). Therefore, co-digestion of FSW with PW and CW would overcome the technical limitation of LCFAs and free ammonia leading to suitable C:N ratio for anaerobic digestion process hence enhanced methane yield.

Co-digestion of biological pre-treated FSW with PW and CW

Comparing the source of enzyme used to pre-treat FSW prior to co-digestion, crude enzymes from cultured CBR-11 bacterial culture gave the highest methane production and methane yield than commercial lipase enzyme (Lipozyme TL IM) (Figures 1 and 2). This could probably be due to some of the commercial lipase enzyme used lost its activity through binding to solid matrix, active site inactivation, loss of co-factors, reversible and irreversible inhibition (Parawira, 2011).

Co-digestion of CBR-11pre-treated FSW with PW and CW

Co-digestion is the simultaneous AD of a mixture of two or more substrate and act as a mechanism for improved AD process. The total methane production, methane yield and methane content from the co-digestions of pre-treated CBR-11-FSW with PW or CW as co-subtrates at 1:1 substrate to inoculum ratio are shown in (Figure-1). Analysis of variance for total methane production, methane yield and methane content for the substrates combinations showed that there was significant differences among the combination tested ($p<0.001$). Results indicated that CBR-11- FSW co-digested with PW and CW in 1:1 ratio (substrate to inoculum) ratio had positive effect on total methane production and methane yield. Results in (Figures 1 A and B) showed that the total methane production and methane yield obtained at 1:1 ratio varied between 256-346 ml and 658-887 ml CH_4/gVS added, respectively compared to control (untreated Nile Perch FSW) (with 219 ml for total methane production and methane yield 562 CH_4/gVS added, respectively). The highest methane yield of 887 CH_4 ml/g VSadded was obtained from CBR-11-FSW:PW combination, which gave methane yield increment of 58% compared to untreated FSW control (562 CH_4 ml/gVS). It was followed by 807 CH_4 ml/gVS added obtained from CBR-11-FSW:CW with methane yield increment of 44% compared to control (untreated Nile perch FSW). The lowest methane yield 658 CH_4 ml/gVS was obtained from CBR-11-FSW:PW:CW with 17% methane yield increment compared to control (untreated Nile perch FSW). On the other hand, the highest methane yield obtained from CBR-11-FSW:PW was 87% and 181% of the theoretical methane yield of lipids (1014 ml/gVS added) and proteins (490 ml/gVS added), respectively (Nges, *et al.*, 2012). The lowest methane yield obtained from co-digestion of pre-treated FSW (CBR-11-FSW:PW:CW) was 65% and 134% of the theoretical methane yield of lipids and proteins, respectively. The observed enhanced methane yield could be due to positive synergism in co-substrates, supply of additional nutrients from co-substrates, improved C:N ratio, dilution of inhibitory materials in co-substrates and increased buffering capacity by co-substrates (Mshandete, *et al.,* 2004; Hartmann and Ahring, 2005; Monou *et al.,* 2008; Li *et al.,* 2009; Luste and Luostarinen, 2010). Unfortunately data on the methane yield was not available in the literature so as to compare with methane yield obtained from microbial pretreted FSW co-digested with VFMSW. Therefore direct comparision of the methane

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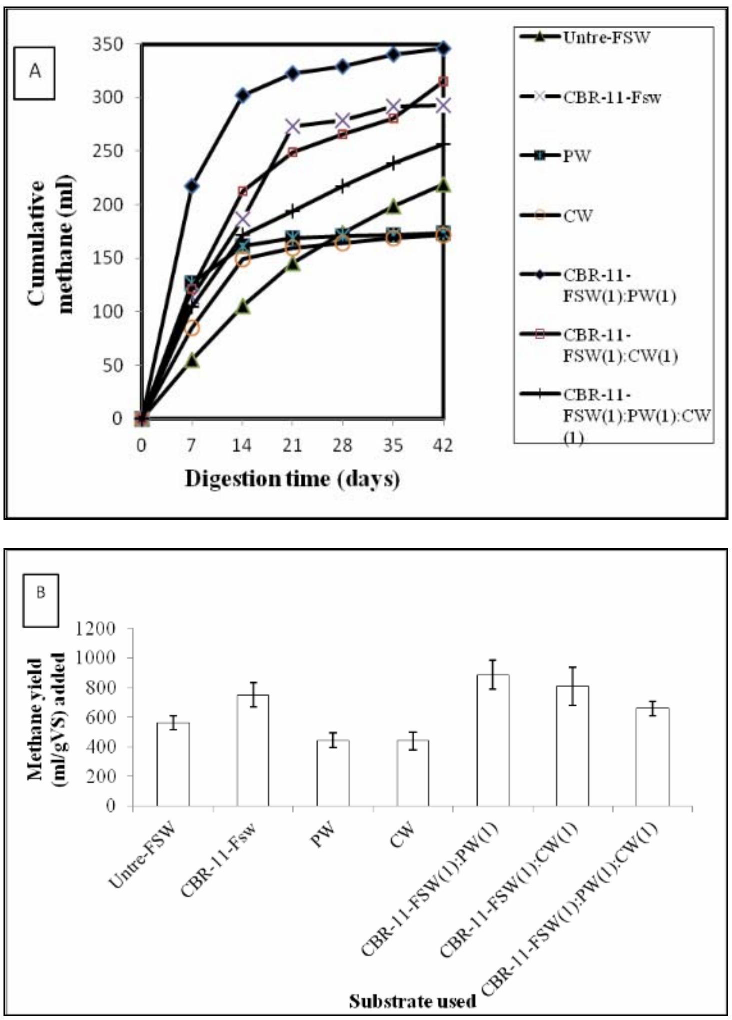
yield data in the present study cannot be made. Nevertheless, the tendency of methane yield increment due to fish wastes co-digestion with other organic wastes with or without pre-treatment have been reported in the literature even though very little. Co-digestion with 33% of fish waste and 67% of sisal pulp waste resulted into maximum methane yield of 0.62 m^3 CH_4/kg VS added. That co-digestion ratio enhanced methane yield to an increment of 59-94% compared to methane yield of sisal pulp waste (0.32 m^3 CH_4/kg VS added) and fish waste (0.39 m^3 CH_4/kg VS added) alone (Mshandete, *et al.*, 2004). Pre-treatment using anaerobic microbial inoculum for solubilization of fish market wastes generated methane yield of 0.36 m^3/kg VS added, which corresponded to methane yield increment of 30% compared to methane yield (0.277 m^3/kg VS) obtained from control (Thirumurugan and Gopalakrishnan, 2012). Co-digestion of fish processing waste (FPW) with 10% gVS of brewery wastewater enhanced methane yield to a highest increment of 66% (compared to control raw FPW methane yield of 0.56 m^3/kgVS). Long chain fatty acids (LCFA) removal of 8% prior AD enhanced methane yield to an increment of 52% compared to raw FSW control. Pretreatment of FPW with aerobic microbial cultures isolated from a fish waste stabilization pond enhanced methane yield to an increment of 60% after 18 h, 68% after 15 h and 76.0% after 12 h of incubation, respectively, for strains CBR 11, BR 10 and a mixture of the two (CBR 11 and BR10) compared to control raw FSW (Gumusiriza, *et al.*, 2009). The average CH_4 content of the biogas from co-digestion in this study ranged between 60-80% (Figure-1C). The highest methane content 80% in biogas was recorded from CBR-11-FSW: CW co-digestion while the lowest methane content of 60% was recorded from PW and CW mono-digestion.



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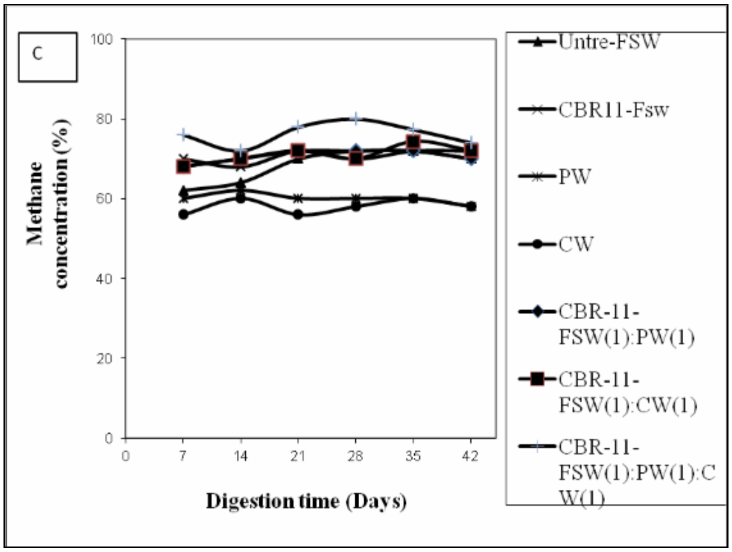

Figure-1. Total methane production (A) methane yield (B) and methane content (C) from AD of Nile perch FSW (9%) pre-treated by CBR-11 bacterial culture and co-digested with potato waste (PW) and Cabbage waste (CW) singly or combined FSW: VFMSW (1:1 ratio). Error bars indicate standard error of the mean of the replicates.

Co-digestion of Lipozyme TL IM pre-treated FSW with PW and CW

Pre-treatments whether physical, chemical or biological or combined for problematic substrates such as fish wastes prior to anaerobic co-digestion with other organic wastes aims to ensure maximum methane output (Gumisiriza, *et al.*, 2009). Nevertheless the potential inhibition and benefit that the co-substrate can bring into the system must be considered. Co-substrates that introduce the lowest level of inhibition to anaerobic bioreactors must be favored against those responsible for elevated inhibition leading to antagonisms ultimately resulting in reduction in methane production (Malta-Alvarez, *et al.*, 2000; Callaghan, *et al.*, 2002; Nges, *et al.*, 2012; Long, *et al.*, 2012). Results in (Figure-2) indicated that Lipo pre-treated FSW co-digested with PW and CW at 1:1 ratio had negative effect on total methane production and methane yield. Methane yield of 339, 408, 540 CH_4 ml/gVS were obtained from Lipo-FSW:PW, Lipo-FSW:CW, Lipo-FSW:PW:CW: respectively, which were 1.04-1.65 times lower than untreated FSW control (methane yield 562 CH_4 ml/gVS). These results demonstrated presence of antagonism provided by evidence of antagonism by comparison of the methane yield produced in co-digestion of Lipozyme TL IM Lipase pre-treated FSW with PW or CW or both and un-pretreated FSW. Furthermore, results meant that there was no enhancement of AD when Lipo-FSW was digested alone or co-digested with VFMSW (PW or CW) singly or in combination. This could be explained that pre-treatment of FSW by the lipase enzyme should have degraded the complex lipid-rich compounds in FSW into hydrolysis by-products which are primary constituents in lipid-rich organics such as LCFAs or re-crystallization compounds which might have subsequently inhibited methanogenesis and methane formation. Also there could have been an assortment of unknown enzymatic mechanisms and interactions with FSW protein that mighty resulted into high concentration of free ammonia inhibitory to AD. Many researchers have reported that high LFAs and free ammonia concentrations released from protein and lipids rich substrate during AD can contribute to inhibition of the digestion process and a reduction of methane production (Chen, *et al.*, 2008; Luste, *et al.*, 2009; Palatsi, *et al.*, 2011; Luste, *et al.*, 2011; Long, *et al.*, 2012). The results obtained in the current study indicated that the pre-treatment of FSW by commercial lipase enzyme did not effectively improved the methane production consequently the methane yield from FSW co-digestions with PW or CW hence should be avoided in enhancement investigation employing FSW as a substrate. The average CH_4 content of the biogas from co-digestion in this study ranged from 60-84% (Figure-2C). The highest methane content 84% in biogas was recorded from LIPO-FSW:CW co-digestion at (1:1) ratio while the methane content of 60 and 64% was recorded from PW and CW mono-digestion, respectively (Figure-2C).

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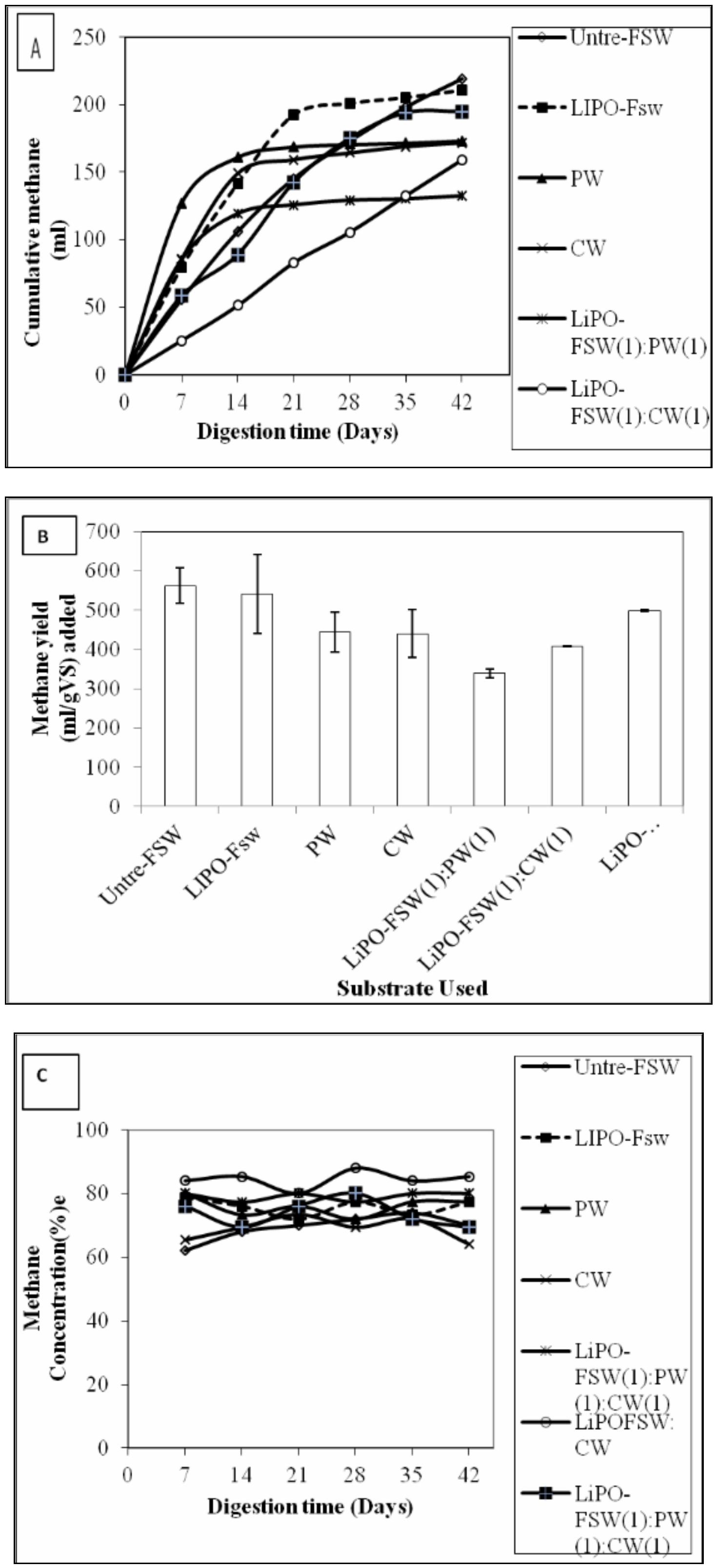
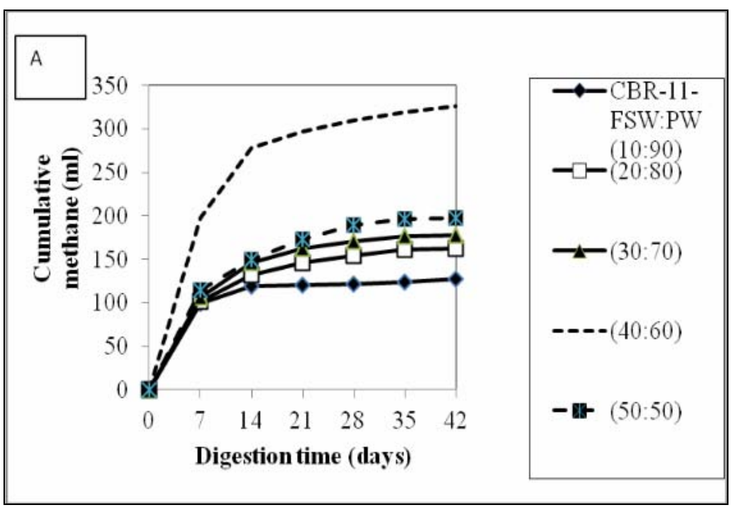


Figure-2. Total methane production (A) methane yield (B) and methane content (%) (C) from AD of Nile perch FSW (9%) pre-treated by LIPOZYME commercial lipase enzyme and co-digested with a mixture of potato waste (PW) and cabbage waste (CW) singly or combined FSW: VFMSW 1:1 ratio. Error bars indicate standard error of the mean of the replicates.

Optimization of co-digestion of pre-treated FSW (CBR11-FSW) with PW and CW

Pre-treatments advancements in co-digestion research using a variety of organic wastes as co-substrates aims to ensure that co-digestion produces the greatest volumetric methane yield possible while ensuring that the full potential of co-substrates is achieved (Luste, *et al.*, 2009; Gumisiriza, *et al.*, 2009; Parawira, 2011; Palatsi, *et al.*, 2011; Luste, *et al.*, 2011; Long, *et al.*, 2012; Luste, *et al.*, 2012). Therefore, establishment of optimal mixture, ratio of co-substrates, which should be digested together are necessary in order to achieve higher methane yield. Co-digestion of pre-treated FSW (CBR11-FSW) with VFMSW (PW and CW) at 1:1 substrate:inoculum ratio showed that methane yield could be enhanced to much higher yield compared to untreated Nile Perch FSW (Figure-1). This however did not show at what proportions of CBR-11-FSW and either PW or CW could be required for the much higher methane yields desired. To that effect, a study was designed in which the co-substrate were divided in five groups with different substrate percentage proportions based on gVS loaded in bioreactors as follows (CBR11-FSW: PW or CW) (10:90; 20:80; 30:70; 40:60; and 50:50). Results for PW in (Figures 3 A and B) showed that the highest total methane production and methane yield of 325 ml and 835 ml CH_4 /gVS added were obtained from CBR-11-FSW: PW (40:60) substrate ratio proportions. The highest methane yield of 835 ml CH_4 /gVS added obtained was 82% and 170% of the theoretical methane yield of lipids (1014 ml/gVS added) and proteins (490 ml/gVS added), respectively (Nges, *et al.*, 2012). The reasons for such enhanced methane yield possibly were due nutrients availability in favourable proportions between CBR-11 and PW. Potato waste nutrient are dense waste known to contain a number of vitamins such as vitamin A, B1, B2, B6, B12, Niacin, folate, riboflavin, tryptophan, vitamin C and E (www.wisegeek.com/what is the nutritional; www.whfoods.com/genpage.php?tname=foodspice&dbid =48, 2012). Minerals in potato waste include Ca, P, N, Mg, Fe, Mn, Zn Ni,Co,S and K (www.wisegeek.com/what nutritional value 2012; www.whfoods.com/genpage.php?tname=foodspice&dbid = 48, 2012). All these minerals and vitamins are necessary for better functioning of anaerobic microbes and hence higher methane yield (Gerald, 2003; Nges, *et al.*, 2012). The lowest total methane production and methane yield of 127 ml and 326 ml CH_4 /gVS added was obtained from CBR-11-FSW: PW (10:90) substrate ratio proportions, which were only 32% and 66% of the theoretical methane yield of lipids (1014 ml/gVS added) and proteins (490 ml/gVS added), respectively (Nges, *et al.*, 2012). Methane content ranged from 68 to 80% (Figure-3 C). The highest methane content 80% in biogas was recorded from 10:90 (CBR-11-FSW:PW) co-digestion while the lowest methane content of 68% was recorded from 20:80 (CBR-11-FSW:PW) co-digestion.



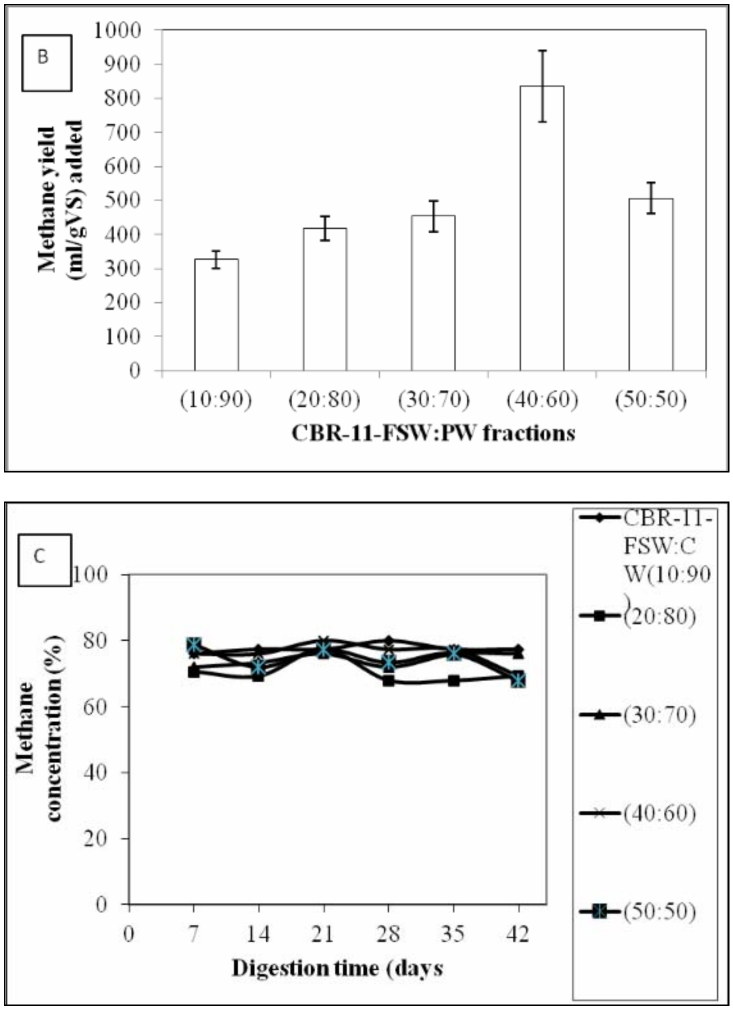


Figure-3. Total methane production (A) methane yield (B) and methane content (%) (C) from AD of Nile perch FSW (9%) pre-treated by CBR-11 bacterial culture and co-digested with potato waste (PW) at different percentage proportions. Error bars indicate standard error of the mean of the replicates.

Results for CW in (Figures 4 A and B) showed that the highest total methane production and methane yield of 458 ml and 1176 ml CH_4 /gVS added were obtained from CBR-11-FSW: CW (30:70) substrate ratio proportions. Total methane production and methane yield were 1.5 times more than those obtained from CBR-11-FSW:CW co-digested at 1:1 ratio (substrate to inocolum ratio). The highest methane yield of 1176 ml CH_4 /gVS added obtained was 116% and 240% of the theoretical methane yield of lipids (1014 ml/gVS added) and proteins (490 ml/gVS added), respectively (Nges, *et al.*, 2012). Below CBR-11-FSW: CW (30:70) substrate ratio proportions total methane production and methane yield increased slightly with an increase of substrate concentration. Beyond CBR-11-FSW: CW (30:70) substrate ratio, total methane production and methane yield decreased with an increase in substrate concentration probably due to increasing in inhibitory substances. The better yield CBR-11-FSW:CW (30:70) substrate ratio proportions could be attributed to better buffering, low inhibitory substances and easily availability of nutrients to microbes. The cabbage waste are known to contain a number of vitamins such as vitamin A, B_6, B_{12} E, C, K, Thiamin, Riboflavin, Niacin, Pantothenic Acid and Folate (http://www.veganpeace.com/nutrient_information/nutrient_content_tables/displaytables/vegetables/vegetables3.htm, 2012). Cabbage waste is also rich in Beta-carotene (provitamin A) which is essential for the proper functioning of enzymes in anaerobes (www.whfoods.com/genpage.php?tname=foodspice&dbid = 48, 2012). Cabbage waste is also known to contain Calcium, Manganese, Iron, Selenium, Magnesium, Phosphorus, Zinc Potassium, Sodium and Copper. While Potassium is rich in Cabbage (35-77/100g), it is very low in Sodium (1-10/100g) http://www.veganpeace.com/nutrient_information/nutrient_content_tables/displaytables/vegetables/vegetables3.htm, 2012). High concentration of sodium can be detrimental to anaerobic microbes and could results in low methane yield (Gerald, 2003). The lowest total methane production and methane yield of 149 ml and 383 ml CH_4 /gVS added was obtained from CBR-11-FSW:CW (10:90) substrate ratio proportions, which were only 38% and 78% of the theoretical methane yield of lipids (1014 ml/gVS added) and proteins (490 ml/gVS added), respectively (Nges, *et al.*, 2012). Methane content ranged from 68 to 80% (Figure-4 C). The highest methane content 80% in biogas was recorded from CBR-11-FSW: PW (40:60) co-digestion while the lowest methane content of 68% was recorded from CBR-11-FSW:PW (50:50) co-digestion.

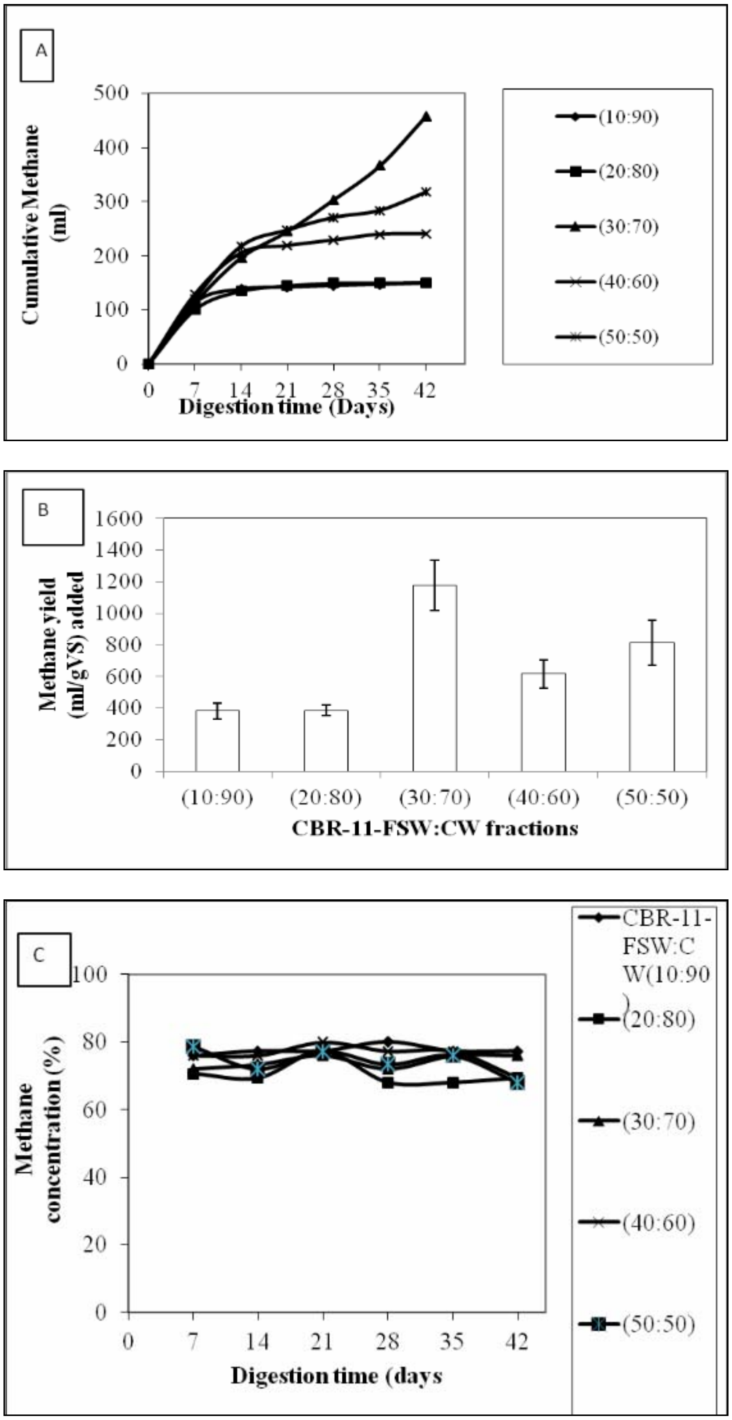


Figure-4. Total methane production (A) methane yield (B) and methane content (%) (C) from AD of Nile perch FSW (9%) pre-treated by CBR-11 bacterial culture and co-digested with cabbage waste (CW) at different percentage proportions. Error bars indicate standard error of the mean of the replicates.

In AD, synergy can occur when two or more substrates are co-digested together and the methane yield obtained from co-digestion process is greater than when substrate are digested alone. Methane yield results in (Figure-5A) provided evidence of synergy when compared to the methane yield produced in co-digestion presented in (Figures 1-4). Results (Figure-5A) showed that optimal mixture of CBR-11 pre-treated FSW and PW and CW co-substrates resulted into higher methane yield. The highest total methane production and methane yield of 515 ml and 1322 ml CH_4 /gVS added, respectively were obtained from ratio of CBR-11-FSW (10):PW (45):CW (45) on volatile solids (gVS) basis, which enhanced methane yield to an increment of 135% compared to control (562 CH_4 ml/gVS) added. The highest methane yield of 1322 ml CH_4 /gVS added obtained was 130% and 270% of the theoretical methane yield of lipids (1014 ml/gVS added) and proteins (490 ml/gVS added), respectively (Nges, *et al.*, 2012). The methane yield increase trend for optimal anaerobic co-digestion mixture in this study have been reported previous elsewhere. Callaghan *et al*. (2002) achieved an increase in the methane potential of 23% on VS basis with a mixture of 70% cattle manure, 20% fish offal and 10% inoculum. On the other hand, when pig manure was blended with 5-30% fruit waste on VS basis, methane yield increase of 131- 406% was achieved (Alvarez, *et al.,* 2010). Recently Li, *et al.* (2012) reported 10% methane yield enhancement achieved from thermo-chemically pre-treated fats, oils and grease (FOG) co-digestion compared to FOG co-digestion without thermo-chemical pre-treatment. In this work, taking into account the methane yield on VS basis an increase between 49% for CBR11-FSW(30):(70)CW and 135% CBR11-FSW(10): PW(45):CW(45) compared to untreated Nile Perch FSW was achieved.

The total methane production like wise methane yield decreased progressively with an increase in concentration of CBR-11-FSW up to CBR-11-FSW (30):PW (35):CW (35) where methane yield was still higher compared to untreated Nile Perch FSW (control). Further increase of CBR-11 treated FSW from CBR-11-FSW (40):PW (30):CW (30) up to CBR-11-FSW (50):PW (25):CW (25) decreased the methane yield. The lowest methane yield of 389 and 355 ml CH_4 /gVS added, were obtained from CBR-11-FSW (40):PW(30):CW(30) and CBR-11-FSW (50):PW (25):CW (25) ratios, respectively. Those methane yields from CBR-11 pre-treated FSW were 1.45-1.58 times lower than methane yield of (562 CH_4 ml/gVS) added obtained from untreated Nile Perch FSW (control). The lower methane yield was probably due to poor buffering capacity of the system caused by increasing amount of free ammonia and possibly long chain fatty acids (Guangxue, *et al.,* 2009; Parawira, 2011; Nges, *et al.,* 2012). The average methane content of the biogas produced in this combination was 60 - 80%. The highest methane content 76% in biogas was recorded from CBR-11-FSW (50):PW (25):CW (25) ratios co-digestion while the lowest methane content of 56% was recorded from CBR11-FSW(10): PW(45):CW(45) ratios co-digestion.

The best methane yield obtained from optimal mixture of CBR-11 pre-treated FSW ratio of CBR-11-FSW (10):PW (45):CW(45) compared to methane yields obtained from mono-digestion and/or co-digestion trials (See Figures 1-4) clearly demonstrated a picture of synergy obtained in batch anaerobic bioreactors. However, there was a lack of published research literature providing an accurate picture of synergy as the identification of this effect is often the result of comparison methane yields of mono-digestion and co-digestion trials on different methodologies batch or semi-continuous/different operational conditions. The synergy picture demonstrated by the optimal CBR-11-FSW (10):PW (45):CW(45) was possibly as the result of more balanced nutrient composition, additional element, minerals or co-factors within the optimal mixture, additional carbon to the system due to PW and CW which brought the C:N ratio closer to the optimal for AD. Additionally PW and CW improved moisture content, which subsequently improved mixing within the system. High buffering capacity from FSW acted as an excellent co-substrate.

In this study the range of methane content between 60-80% obtained in Figures 1C, 2C, 3C,4C and 5C was within and slightly above the range of 60-65% reported by other workers on anaerobic co-digestion of fish offals and sisal wastes in batch anaerobic bioreactors (Mshandete, *et al.,* 2004). The differences in compositions, type and nature of the substrate used blending/co-substrates employed, carbon sources and the nature of the inoculum employed were responsible for the methane content differences.

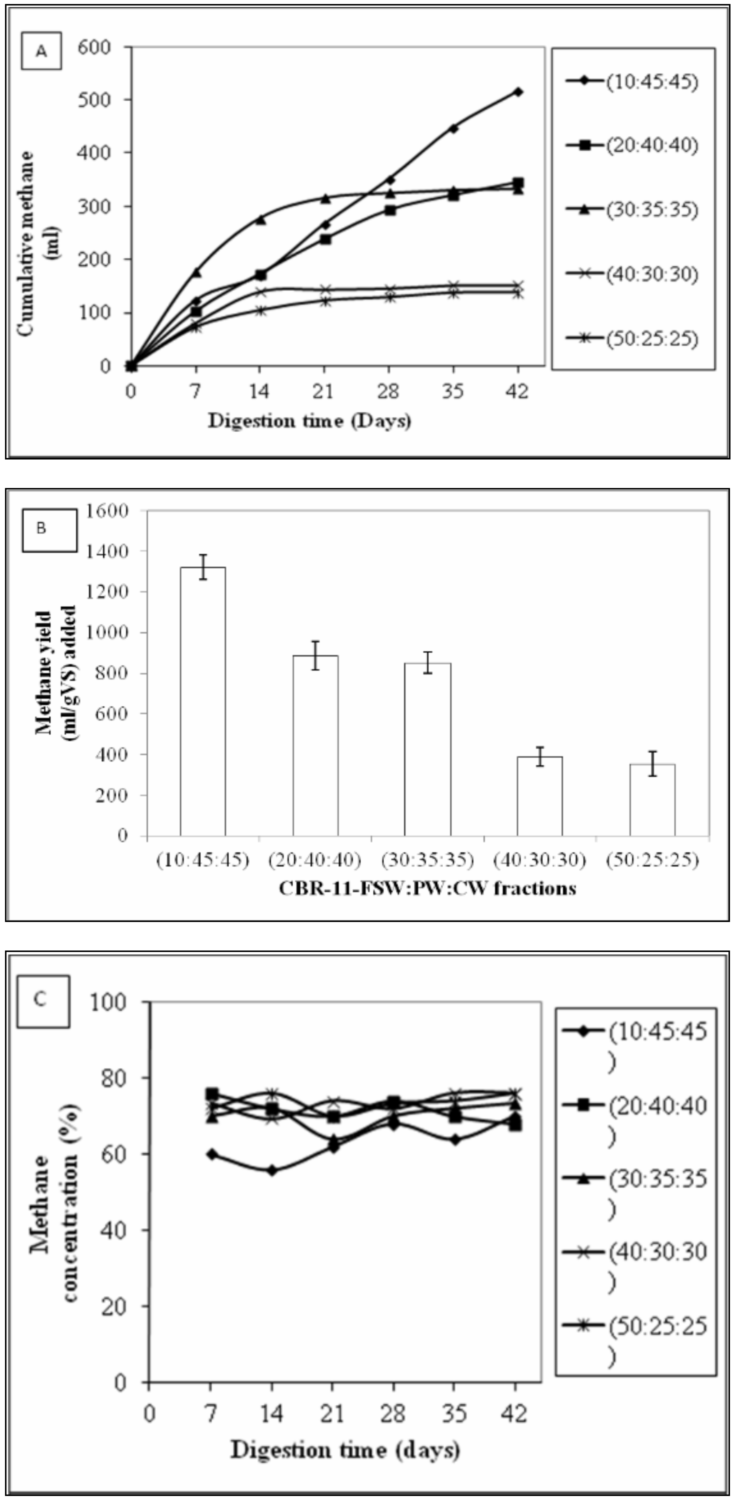


Figure-5. Total methane production (A) methane yield (B) and methane content (%) (C) from AD of Nile perch FSW (9%) pre-treated by CBR11 bacterial culture and co digested with a mixture of Potato waste (PW) and cabbage waste (CW) in different percentage proportions. Error bars indicate standard error of the mean of the replicates.

CONCLUSIONS

This is the first scientific report on enhancement of biomethanation of FSW by anaerobic co-digestion of biological pre-treated Nile perch fish solid waste with vegetable fraction of market solid waste (PW and CW). As indicated in the results FSW co-digestion with PW and/or CW was technically feasible. Actually results in this study demonstrated that pre-treatment of Nile Perch FSW by CBR-11 prior to co-digestion effectively resulted into increased methane production and hence greater methane yield. The ideal co-substrates, optimum mixing ratios of CBR-11 pre-treated FSW and PW and CW were identifed based on methane yield. Co-digestion of pre-treated FSW (CBR-11-FSW(30) with CW(70) and CBR-11-FSW(10):PW(45):CW(45) ratios gave the highest methane yield of 1176 and 1322 ml CH_4 /gVS added, respectively, resulting into methane yield increment in the order of 49-135% compared to methane yield (562 ml CH_4 /gVS added) obtained from untreated Nile Perch FSW at 9% of TS (control). Optimum mixing ratios CBR-11 pre-treated FSW with PW and CW as co-substrates established in this study provided important information/base line data for potential application in continuous anaerobic bioreactors investigation. The positive results could provide valuable information and original contribution to justify full-scale investigation in a continuing research program and to the field of research on anaerobic co-digestion of microbial (CBR-11) pre-treated Nile Perch FSW with vegetable fraction market solid wastes in particular potato and cabbage wastes.

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