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THE ANAEROBIC DIGESTION OF CATTAIL WEEDS TO PRODUCE METHANE USING AMERICAN COCKROACH GUT MICROORGANISMS

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ABSTRACT

The objective of this study was to characterize anaerobic batch digestion of cattail weeds botanical fractions singly and in combination using American cockroach gut microorganisms as an inoculum. The effects of increasing concentration of cattail weeds expressed as percentage of total solids (TS) from 5, 15, to 30 and 60% on methane yield was investigated at ambient temperature of 29±1°C. The results showed that highest methane yields were obtained at 5% TS for both individual and mixed cattail botanical fractions. A maximum methane yield of 447 CH_4 ml/g volatile solids (VS) and 288 ml/gVS added were obtained from leaves and whole cattail weeds (comprised of spikes, leaves, stems rhizomes and roots each 20% wet weight), respectively. An average methane content in the range of 72 and 79% was obtained in the biogas produced at 5 to 60% TS for both separate and mixed cattail botanical fractions. Lower TS % led to faster onset of biogas production and higher methane productivity. Blending of cattail botanical fraction at 5 to 60%TS in general did not improve the methane yield compared to that obtained from separate botanical fractions. Methane yield obtained from whole cattail weed was less by a factor of 1.6 compared to that of leaves at 5%TS. It was concluded, that anaerobic digestion of cattail weed is feasible and could serve the dual roles for producing biogas, a clean renewable energy and reducing the weed as part of its management.

Keywords: weeds, cattail, methan production, biogas, botanical fractions, blends, alternative energy, cokroach gut culture, inoculum.

INTRODUCTION

The search for alternative energy sources is an ongoing effort throughout the world. Developing countries are trying to reduce their dependence on imported fossil fuels. At the same time various developed countries are also trying to move away from the use of hazardous and waste producing energy non-renewable sources. Renewable organic material (ROM) energy sources which originate from plant, animal and microbial biomass, such as organic residues and waste from all human activities and from energy crops are often prioritized in efforts to mitigate the greenhouse effect and eventually achieve a completely sustainable energy supply. Anaerobic digestion (AD) is one of the biological processes that has received a new fillip in recent years since the energy crisis of the early 1970s, especially following the Gulf war and energy price rises (D.R. Kashyap, K.S. Dadhich and S.K. Sharma, 2003). The AD process has captured many imaginations because it turns organic matter into a valuable source of renewable energy. During AD organic matter is degraded in the absence of oxygen. The multi-step process results in a biogas. Biogas units, when both biogas and biofertilisers are produced in a proper way, can positively affect waste and water management, sanitation, cultivated soils, food production, bioenergy utilisation, job creation ('green jobs' i.e. jobs supporting sustainability) etc. These advantages together with the growing shortage of firewood and rising cost of fossil fuels has made AD increasingly interesting world wide. These benefits of the process might make it well suited for use in developing countries like Tanzania since treating organic waste to yield biogas while recycling nutrients constitutes a sustainable cycle. Aquatic weed can help fulfill the biomass requirements for biogas production. However, their use as potential source of biomass for bioenergy production is rather a recent concept, but research results from around the world for aquatic weeds as a source of inexpensive energy supply via AD are promising and is gaining attention throughout the world (S.A. Abbasi and P.C. Nipaney, 1991; V. Singhal and J.P.N. Rai, 2003).

Cattails such as *Typha latifolia linn* and *Typha domingesis* Pers found in or near water in marshes, ponds, lakes and in depression areas. They occur around the world and are potential resource for biotechnogical applications. They are ubiquitous in distribution aquatic obligate wetland indicator plant species, which are emergent weeds and have rapid growth rate. Therefore they become monotypic culture and eliminate other native plant species when the hydrology, salinity or fertility changes. *Typha domingesis* Pers had covered 65-80% of 30 km^2 of the lake Jipe in Northern Tanzania. This has resulted into ecological, environmental and economic problems including shrinkage of the lake and reduction of fish yield thus affecting the local communities' economic stability. The lake drying is implicated to the climatic and/or human induced phenomenon (P.Z. Yanda, 2007). Since removal of water weeds involves time and cost, pleas have been made to direct research towards finding uses for aquatic macrophytes instead of concentrating efforts on eradication and destruction. Therefore, AD of a lignocellulose cattail resulting in production of biogas a carbon neutral renewable energy rich methane gas has attracted universal attention recently (P. Thanakoses, A.S. Black and M.T. Holtzapple, 2003). However, cattail weed (*Typha spp*) is one of the biomass with little published information on AD. Most recent reported studies on cattail aimed at optimization of acidogenesis of cattail to produce volatile fatty acids (VFAs) by rumen cultures and AD of cattail with rumen culture in the presence of heavy metals

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(Z.H. Hu, and H.Q. Yu, 2006; Y. Zheng-Bo, Yu. Han-Qing and W. Zhi-Liang, 2007). AD of cattail weed for biogas production using American cockroach *Periplaneta Americana* L. Gut contents as an inoculum (culture/starter seed) in anaerobic bioreactors is yet to be investigated.

AD takes place in methonogenic environments such as fresh and marine water sediments, the rumen, the intestinal tract of animals and insects, landfills and anaerobic bioreactors. AD takes place almost everywhere in nature, such as in human and animal intestines, in garbage bins, in wet soils, at landfill sites, in bioreactors. All anaerobic bioreactors have one factor in common that they all apply natural anaerobic consortia of microorganisms for biodegradation and biotransformation processes. *P. Americana* had its origins in Africa and through commerce it spread virtually worldwide. The microbial ecology of the digestive tract of *P. americana* has been investigated in several studies (Cruden, D.L. and Markowetz, A.J., 1987; Kane, M.D. and Breznak, J.A., 1991). The colon rumen is anaerobic and carries a dense and extremely varied microbial population which consists of both facultative and obligate anaerobes such as methanogenic archaea which, are endosymbionts and free living (Gijzen, H.J., Zwart, K.B., van Gelder, P.T. and Vogels, G.D., 1986; Gijzen, H.J. and Barughare, M., 1992; Zurek, L. and Keddie, B.A., 1998). Furthermore, measurements of methane emissions of 35 l/l/d from the cockroach *P. Americana* has been reported to be even higher than from the rumen microbial system (Gijzen, H.J. and Barughare, M., 1992). With such volumetric methane production the cockroach hindgut appears substantially more efficient than any anaerobic bioreactor currently available (Gijzen, H.J., 2002). Numerous species of microorganisms have been demonstrated in different regions of the cockroach digestive tract and were regarded as part of the normal gut flora (Cruden, D.L. and Markowetz, A.J., 1987; Zurek, L. and Keddie, B.A., 1998). Anaerobic digestion process in *P. Americana* has been at work in the digestive tract as a natural process for years (Gijzen, H.J., 2002). However, an analysis of the literature revealed that no study, however, seems to have been made so far on the utilization mixed cultures from American cockroach gut flora as an inoculum during AD of different botanical fractions and blends of cattail weeds in anaerobic bioreactors. This therefore is the first study, which could prompt more investigations on the exploitation of *P. Americana* microbial systems with the ultimate aim of developing efficient high rate anaerobic bioreactor based on the microbial processes of the cockroach digestive tract.

MATERIALS AND METHODS

Site description and sampling

The *Typha domingensis* used in study were collected from lake Jipe, Mwanga district, Kilimanjaro region (Figure-1). The lake is situated astride the Kenya-Tanzania border, just to the east of the northern Pare Mountains of Tanzania. Tsavo West National Park of Kenya borders the southern portion of the lake while Mt Kilimanjaro dominates the horizon some distance to the northwest. The lake is a small, shallow lake (maximum length 12 km, area 30 km^2 with average depth less than 3m). The lake is located at Latitude: 3° 34' 60 S, Longitude: 37° 45' 0 E. Anaerobic digestion of cattail weeds was carried out in biogas laboratory at the Department of Molecular Biology and Biotechnology, University of Dar es Salaam, Tanzania.

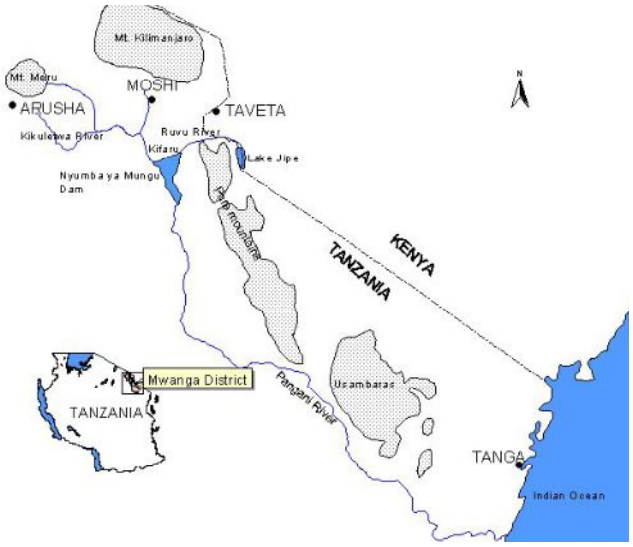


Figure-1. Location of lake Jipe.
Source: GISE Unit-NEMC (May 2004)

Estimation of cattails quantity

Quantification and characterisation of the cattails was necessary to make approximations of how much biomass could be available for biogas production. Using a small boat the potential quantity of cattails was estimated using a $1m^2$ quadrat dropped randomly at 12 different locations of the lake Jipe at Makuyuni landing beach. The quantity of the cattail found in $1m^2$quadrat was established. After harvesting the samples, whole cattail was weighed and the weights recorded. After these five botanical fractions of cattail namely; spikes, leaves, stems, rhizomes and roots were cut and weighed separately and their weight recorded.

Inocula collection and preparation

Availability of anaerobic inocula for bioreactor has been cited as one of the bottle neck in the bioreactor start up during implementation of biogas technology in developing countries (Poggi-Varaldo, H.M., Valdés, L., Esparza-García, F. and Fernandez-Villagómez G., 1997). Sediments from lakes, oceans, rivers, ponds, rice fields etc are known to harbour a diversity of anaerobic microorganisms implicated for methane emission from those habitats.

Two type of sediments were collected namely; lake Jipe sediment (LJS), sisal residues stabilization ponds sediment (SRS) were collected from lake Jipe at Makuyuni village fish landing beach and Kisangara sisal leaf decortications factory Mwanga district, respectively. The sediments were collected at a depth of about 3m using

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VANDORN grab sample and were filtered through a mesh (2 mm-pore size) to remove large particles such as stones, grits, leaves and pieces of roots, fish scales wastes etc. The filtered sediment was transferred in 5 litres plastic gallons and was firmly shut. Adult American cockroaches *P. americana* were used in this investigation (Gijzen, H.J. and Barughare, M., 1992). The cockroaches were collected from Tandale in Dar es Salaam city. The place is known for stocking various food stuff therefore there is plenty availability of food for cockroaches to survive in abundance. A total of 36,000 adult *P. americana* weighing between 1.5 and 2.5 g were caught and kept in containers which are used for rearing cockroaches in the laboratory. Cockroaches were chilled and cut open by dorsal longitudinal incision to expose the digestive tract. The gut contents were pooled and weighed. The average gut content was about 0.5 g. Fresh cockroaches gut contents collected were cut into pieces and mixed with degassed water in 1:1 ratio, then filled 2/3 of six 20 L gallons. The gallons were firmly shut and connected to the gas bags and were kept for three months. After this period it was used an inoculum and was designated as American cockroach gut culture (ACGC). LJS had total solid (TS) of 2.3% out of which 56.38% being VS and organic carbon of 22.28 % (of TS), SRS had TS of 3.14% out of which 16.74% being VS and organic carbon of 18.62% (of TS). On the other hand, ACGC had TS of 2.47% out of which 88.7% being VS and organic carbon of 25.18% (of TS).
Methanogenic activity test

Methanogenic activity test

Preparation of methane calibration standard

Methane calibration standard was prepared by injecting 1 ml of pure methane from the cylinder using a 1 ml needle syringe, into 120 ml serum bottle sealed with rubber septa and aluminium cap. In the stock bottle, 5 ml of air was added to create an overpressure. From the stock bottle, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of the diluted methane were withdrawn one after another and injected into 120 ml serum bottles. The gas samples (0.2 ml) from each bottle were taken by 1ml needle syringe and analyzed on a HP 5890 II gas chromatography (GC) equipped with a Flame Ionization Detector (FID) and a column packed with Hayesep Q, 60-80 mesh. The carrier gas was nitrogen at 20 ml/min and oven, detection and injection port temperatures were 100 °C, 175 °C and 150 °C, respectively. The area for each peak after injection was used to obtain standard methane calibration curve through plotting area as a function of amount of methane. The standard equation obtained in the methane standard calibration curve was used for calculations of the amount of methane produced.

Methane production measurement from the inocula

The three LJS, SRS and ACGC inocula collected were tested for methanogenic activity using three methanogenic substrates; acetate (60.05 g/mol), formate (46.0254 g/mol) and methanol (32.04 g/mol) in 120 ml serum bottles. For each inocula prepared above, 10 ml were dispensed into nine serum bottles (120 ml), 9 for LJS, 9 for SRS and 9 for ACGC. Then 1 ml of 40µmol of the each substrate was added separately for each three inocula i.e. each 9 bottles for each inoculum had 3 bottles acetate, 3 bottles formate and 3 bottles methanol. Each bottle was then closed with rubber stoppers and aluminium caps. The bottles were evacuated and the atmosphere was replaced with 80% N_2: 20% CO_2 v/v at 0.5 atmosphere overpressure to maintain anaerobic conditions (Lyimo, T.J., Pol, A. and Op den Camp, H.J.M., 2002a). The bottles were incubated at 30°C while shaking (125 r.p.m). Methane production measurements were done by sucking 0.5 ml of gas from each bottle and then injected into a GC through an injector port for analysis. The control in this analysis was autoclaved distilled water. Same procedures as above were done and incubation was at 30 °C under shaking (125 r.p.m). Methane measurement was monitored at an interval of one hour for the first 6 hours and at an interval of two hours for the following 15 hours. At day 2 and 3 of incubation methane measurement was done at regular time twice in day. From day 4 up to day 46 measurements was done at regular time once every day until methane production was insignificant. In total the methane measurement lasted after 1105 hours. Calculation of the amount methane (CH_4) in nanomoles (nmol) was based on the methane standard calibration curve using the standard equation. The methane production rate (MPR) from each inoculum was expressed as nmol CH_4/hr/g formula weight (fw) of the substrate used according to Lyimo, T.J., Pol, A. and Op den Camp H.J.M (2002b). Each test for methanogenic activity was triplicated and three measurements were made from each serum bottle. The average and standard deviation for the three measurements were then calculated. The control in this analysis was autoclaved distilled water without added substrates. Same procedures as above were done and incubation was at 30 °C under shaking (125 r.p.m). The best in terms of methane production rate (MPR) based on measurements of methane accumulated in test vials during incubations of inocula samples with a three methanogenic substrates was selected as an inoculum for the subsequent AD of cattail weeds in batch anaerobic bioreactors (BAB).

Bioreactor configuration and operation

Anaerobic digestion of cattail weeds using American cockroach gut microorganisms as an inoculum experiments were conducted in 0.5 l Erlenmeyer flasks with a working volume of 0.3 l. Anaerobic conditions were established by flushing the flasks content mixtures with nitrogen:carbon dioxide mixture (80:20%) for 5 minutes to replace air (oxygen). Then the mouth of each flask was sealed immediately with butyl rubber stoppers. An outlet in the stopper was used for collecting biogas in gas-tight aluminium enforced polyethylene bags. Each biogas bag was fitted with a gas sampling septa closed with n-butyl stoppers and sealed with aluminium caps. The batch anaerobic bioreactors were kept at an ambient

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temperature of 29± 1°C and were shaken manually for 1 min twice daily to mix their content.

Experimental design

The amount of fermentable material of feed in a unit volume of slurry is defined as solid concentration. The concentration of cattail weeds expressed as percentage of TS in the bioreactors and the inoculum-to-substrate ratio (ISR) are parameters that have considerable effects on the cost, performance and reliability of the fermenters and the digestion process. The different proportions of ACGC inoculum and cattail weeds singly and in combination investigated are presented in Table-1. Eight runs containing cattails weeds of 5, 15, 30 and 60% of TS were performed. The ACGC inoculum-to-substrate ratio on VS basis (ISR) ranged between 0.65 and 35.

Table-1. Amounts of cattail weed fractions and inoculum, expressed as % of TS, used in the various batch anaerobic digestion experiments. The inoculum-to-substrate ratio on VS basis (ISR) is also given.

Fraction					Fraction				
Spike (%TS)	5	15	30	60	Leaves (%TS)	5	15	30	60
Inoculum (% TS)	95	85	70	40	Inoculum (% TS)	95	85	70	40
ISR	16	5.3	2.4	0.66	ISR	35	7	2.4	0.68
TS % of the final mixture	7.8	8.6	10	16	TS % of the final mixture	7.6	8.3	9.9	15
Fraction					Fraction				
Stems (%TS)	5	15	30	60	Rhizomes (%TS)	5	15	30	60
Inoculum (% TS)	95	85	70	40	Inoculum (% TS)	95	85	70	40
ISR	22	5.4	2.3	0.66	ISR	20	5.6	2.2	0.65
TS % of the final mixture	7.7	8.5	9.9	15	TS % of the final mixture	7.7	8.5	10	15
Fraction					Fraction				
Roots (%TS)	5	15	30	60	Spikes, stems and leaves (%TS)	5	15	30	60
Inoculum (% TS)	95	85	70	40	Inoculum (% TS)	95	85	70	40
ISR	31	7.6	2.9	0.83	ISR	19	5.4	2.3	0.66
TS % of the final mixture	7.7	8.5	10	16	TS % of the final mixture	7.7	8.6	10	15.5
Fraction					Fraction				
Rhizomes and roots (%TS)	5	15	30	60	Whole cattail (%TS)	5	15	30	60
Inoculum (% TS)	95	85	70	40	Inoculum (% TS)	95	85	70	40
ISR	18.5	6.0	2.5	0.74	ISR	17	5.6	2.4	0.69
TS % of the final mixture	7.8	8.6	10	16	TS % of the final mixture	7.8	8.6	10	15.8

The digestion mixtures had final TS concentrations ranging from 7% to 16% TS. The experimental set-up for five cattails botanical fractions alone consisted of 60 BAB. On the other hand, combinations of cattails botanical fractions were digested in 36 BAB. Three mixtures on wet weight basis were used; 33.3%:33.3%:33:3% (spikes: stems: leaves), 50:50% (rhizomes: roots) and whole cattail 20%:20%:20%:20%:20% (spikes: stems: leaves: rhizomes: roots). Sodium hydrogen carbonate (1.5 g/l) was added to increase the initial buffering capacity of the bioreactors. All the anaerobic digestions of cattails weeds botanical fractions both alone and in combinations were run in triplicates for 60 days. These experiments were terminated when insignificant methane yield of about 2mls CH_4 per gVS added was observed. Controls in triplicates containing only inoculum (ACGC) were included in order to measure the background methane production from the inoculum and this was subtracted from the total biogas production from tested cattail botanical fractions singly and in combinations.

Analytical methods

The volume of biogas collected in the gas-tight aluminium enforced polyethylene bags was measured using a 100-ml gas-tight plastic syringe with a gas lock. The gas composition of 5 ml samples of the biogas was estimated by the absorption of carbon dioxide and hydrogen sulphide in concentrated KOH solution (20 g/L) as described by Ergüder, T.H., Tezel, U., Güven, E., Demirer, G.N (2001). In this method, only CH_4 is determined and other biogas components such as CO_2 and H_2S are dissolved in the KOH solution. Methane yield was calculated by subtracting the amount of methane produced by the control (average production from three controls) from the methane production of each substrate and dividing the difference by the mass of volatile solids in the substrate fed to the BAB. The concentrations volatile fatty acids (VFAs) of acetate, proprionate and butyrate were analyzed by using a Hewlett-Packard gas chromatograph (type HP 5890) according Mshandete, A., Kivaisi, A.K., Rubindamayugi, M., Mattiasson, B (2004). Glass column (1.8 m long and 2 mm internal diameter) filled with 10%

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SP1200/1% H_3PO_4 on 80/chromosorbWAW was used. Nitrogen was used as a carrier gas at a flow rate of 40 ml/min. Oven, injection and detection temperatures were 130, 170 and 175°C, respectively. Partial alkalinity (PA), total alkalinity (TA) and pH, were measured as previously described by Björnsson L., Murto, M., Mattiasson, B (2000) using a TIM titration manager with anABU 901 Autoburette (Radiometer, Copenhagen, Denmark). TS, VS, ash content and total Kjeldahl nitrogen (TKN) were determined according to standard methods American Public Health Association., (1998). The organic carbon was determined by the rapid dichromate oxidation method previously described by Nelson, D.W. and Sommer, L.E (1996).

RESULTS AND DISCUSSIONS

Yield and composition of cattails

To estimate how much biogas can be produced by AD of cattail weeds it was necessary to make approximations of how much biomass can be available. The estimation of the relative abundance of cattails weeds Typha domingensis Pers and its botanical fractions which had covered 65-80% of 30 km^2 of the lake Jipe in Northern Tanzania is presented in Table-2. However, since readings were taken from 12 various sites it can be stated that the estimates were a fair indication of the yield at those particular sites at Makuyuni landing beach. Biomass yield (ton/hactare) of entire *Typha domingensis* Pers was estimated as 193.3 tons (approximately 42.46 tons dry matter/hactare at 21.97%TS). This dry matter biomass is comparable to the estimated 40 tons/hactare reported for cultivated *T. latifolia*, *T. angustifolia* (Wild, U., Kamp, T., Lenz, A., Heinz, S. and Pfadenhauer, J. 2001).

Table-2. Estimated relative quantity of cattails and its botanical fractions (Mean values n = 3).

	Spike	Stems and leaves	Rhizomes and roots	Whole (total) cattail
Area covered by cattails (19.5 km^2) tons	6,435	234,000	136,500	376,935
Area covered by cattails (24 km^2) tons	7,920	282,000	168,000	463,920
Fresh biomass yield tons/hectare	3.3	120	70	193.3
Dry biomass yield tons/hectare	0.76	27	15.44	42.46

The chemical constituents of cattails and its botanical fractions alone and in combination are listed in Table 3. The values recorded for chemical constituents of cattails botanical fractions and combination differed significantly ($p<0.05$). This was not unexpected since the distribution of nutrients resources in plants is allocated differently in botanical fractions. Similar observation have also been reported for chemical composition of entire and botanical fractions of water hyacinth (*Eicchornia crassipes*) (Aboud, A.A.O., Kidunda, R.S. and Osarya, J., 2005). The moisture content of the fresh entire cattail was within ranges of 76 to 90% reported for 19 emergent species including *T. latifolia linn* (Banerjee, A. and Matai S., 1990). The VS (% of TS) 86% was close to the range of 88.8-93.5% reported for *T. latifolia linn* (Zheng-Bo, Y., Han-Qing, Yu. and Zhi-Liang, W., 2007). The crude protein values of obtained for the entire cattail of 24.56% was similar to 24% reported in the literature for emergent plants (Banerjee, A. and Matai, S., 1990). The range of values of 8-28% ash content recorded for the entire cattail and its botanical fractions were comparable to 8-25% ash content ranges reported for aquatic plants (Banerjee A. and Matai, S., 1990, Aboud, A.A.O., Kidunda, R.S. and Osarya, J., 2005; Zheng-Bo, Y., Han-Qing, Yu. and Zhi-Liang, W., 2007). The organic carbon 47% of TS reported in this study for cattail entire plant is comparable to a range of 43-47% reported for plant materials (Allen, S.E., 1989). The high organic matter (fraction) measured as VS and organic carbon as well as total nitrogen demonstrated that cattail weeds represent an abundant resource for producing renewable energy.

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Table-3. Chemical composition of cattail and its botanical fractions alone and in combinations (Mean values n = 3).

	CSP	CST	CLE	CRH	CRO	SSL	SL	RR	CEP
Total solids (% fresh sample)	23.07	16.37	17.04	18.11	29.66	19.00	22.63	22.06	21.97
Moisture content (%)	76.93	83.63	82.96	81.89	70.34	81.00	77.37	77.94	78.03
Volatile solids (% of TS)	87.90	91.16	88.38	91.14	72.32	89.70	90.47	79.82	86.09
VS/TS ratio	3.82	5.56	5.18	5.03	2.43	4.72	3.99	3.61	3.91
Ash (%TS)	12.0	8.84	11.62	8.86	27.68	10.3	9.53	20.18	13.91
Total nitrogen (% of TS)	2.4	2	2.83	1.92	4.17	3.81	1.7	4.67	3.93
Crude protein (%TKN x 6.25)	15	13	17.69	12	26.06	23.81	10.63	29.19	24.56
Organic carbon (% of TS)	52.23	44.98	45.01	43.27	48.40	57.53	39.52	46.89	53.79
Carbon:Nitrogen ratio (C:N)	21.76	22.49	15.90	22.53	11.60	15	23.24	10.04	13.68

CSP = cattail spike, CST = cattail stem, CLE = cattail leaves, CRH = cattail rhizomes, CRO = cattail roots, SSL = spike, stem and leaves (SSL), SL = stem and leaves, RR = rhizomes and roots, CEP = cattail entire plant

Methanogenic activity of the inocula

Methanogenic activity measurements still remain significant tools for sorting out the importance of different microbial physiological groups, which exist in methanogenic environments (Lyimo, T. J., Pol, A., Op den Camp, H.J.M., Harhangi, H.R. and Vogels, G.D., 2000). The quality and quantity of inoculum is critical to the performance and stability of biomethanogenesis during AD in bioreactors. It is also possible to reduce retention period by addition of inoculum (Yadvika, S., Sreekrishnan, T. R., Kohli, S. and Rana, V., 2004; Tomei, M.C., Braguglia, C.M. and Mininni, G., 2008). Samples of inocula from three sources LJS, SRS and ACGC were examined for the presence of methanogenic activities by enrichment cultures using acetate, formate and methanol as sole carbon sources. Methanogenic activities were detected in all three inocula, which implied that the microorganisms could use all the three substrates added. However, methane production rate (MPR) expressed as nmoles/gfw/h differed significantly ($p < 0.05$) among the three inocula sources and the three carbon sources employed in this investigation (Figure 2). The MPR for acetate ranged from 0.95-1.22 nmoles/gfw/h while MPR for formate ranged from 1.40-2.13 nmoles/gfw/h and that of methanol was between 0.96-2.42 nmoles/gfw/h. The highest MPR was obtained from ACGC followed by SRS and the least was from LJS. The MPR obtained from ACGC was higher than that of LJS and SRS by 9-83% depending on the methanogenic substrate used. These results suggest that among the three inocula sources investigated, ACGC was the best starter (inoculum) and was used for the subsequent AD of cattails weeds botanical fractions alone and in combination.

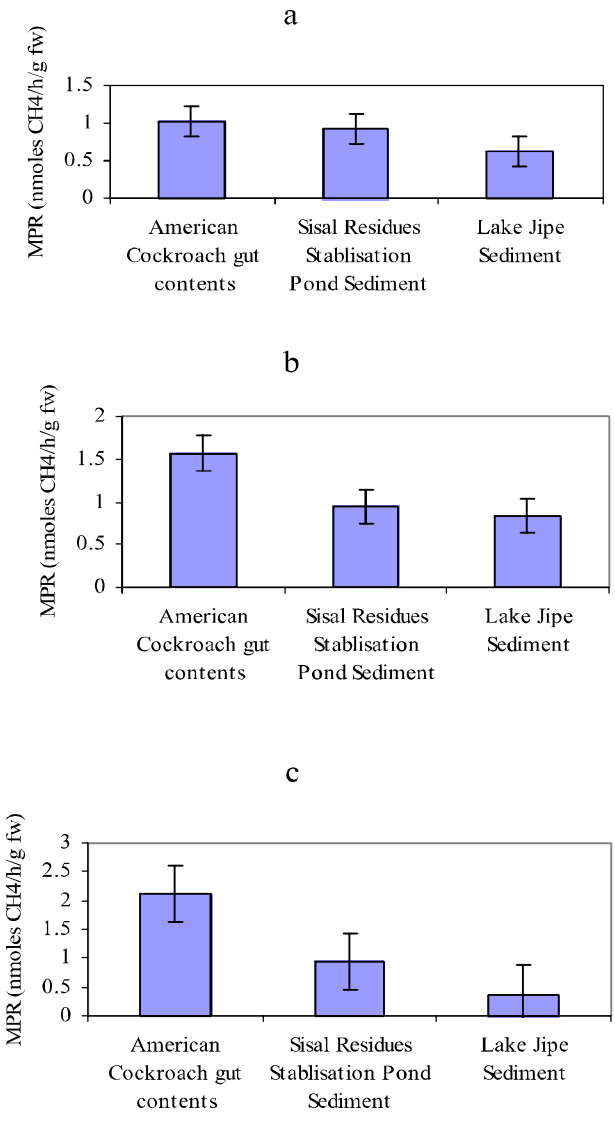


Figure-2. Methanogenic activity expressed as MPR of the three inocula usingthree different methanogenic substrates (a) Acetate (b) Formate and (c) Methanol.

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Composition of biogas

The AD of cattail weeds botanical fractions singly and in combination using ACGC as the inoculum in BAB produced biogas. The biogas produced in the first four days of digestion comprised of low methane content, which ranged between 20-40%. However as digestion time progressed the proportion of methane steadily increased (data not shown). This is not unexpected as it is common phenomena, which have been reported by other researchers. Badger, D. M., Bogue, M.J. and Steward, D. J (1979) have observed that biogas produced during the initial stages of plant biomass AD in batch cultures contain mainly carbon dioxide. This has been considered to occur because preliminary AD in batch cultures is similar to that in the rumen of animals. In this study, the overall average methane content (%) in the biogas produced by the end of the experiment of 60 days; regardless of the concentration of cattails weeds fractions added singly or in combination is presented in Figure 3. The highest methane content of 78.87 ±2.7% was obtained at substrate concentration of 5 % TS followed by 77.90±1.6% obtained at 15%TS and that of 76.49±2.5% was obtained at 30% TS while the least 72.04±2.49% was obtained at 60%TS. The methane content reported in this study falls within the range of 70-84% reported for potato waste concentration of 10-80 %TS in batch cultures (Parawira, W., Murto, M., Zvauya, R. and Mattiasson, B., 2004).

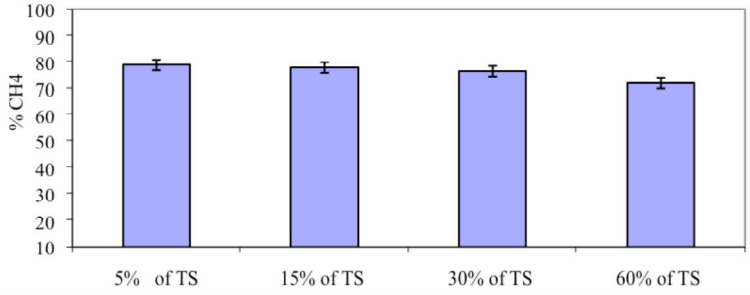


Figure-3. The overall average biogas composition in terms of methane content at different solid concentrations of botanical cattails weeds fractions singly and in combinations.

Methane yield from separate cattail weeds botanical fractions

A wide range of biomass has been considered as potential sources for methane production. Biomass yield is one of the parameter that makes biomass to methane conversion economically and technically feasible. Thus methane yield is an important economic factor in AD. The single and mixtures of cattail botanical fractions were studied with regard to methane production in batch assays for a period of 60 days. The methane yield during AD of cattails weeds botanical fractions alone at different concentrations is shown in Figure 4. These results showed that methane yield of separate botanical fractions were inversely proportion to increasing cattails weed solids concentration from 5% to 60% of TS. The methane yields from cattails separate botanical fractions irrespective of their solid concentration differed significantly ($p < 0.05$). The highest methane yields for separate cattail weeds botanical fractions were obtained at 5% TS. Methane yield at 5%TS solids concentration from botanical cattail weed fractions alone ranged from 150-447 CH_4 ml/gVS added. The separate cattail weed fraction displayed the following order of the quantity of methane produced per VS added: leaves > stems > roots > rhizomes > spikes. The highest 447 CH_4 ml/gVS added and the lowest methane yields of 150 CH_4 ml/gVS added were obtained from leaves and spikes, respectively. A critical analysis of literature revealed no studies performed on the anaerobic batch digestion of solid cattails weeds separate botanical fractions were found in the literature. Therefore, preclude rigorous comparisons of methane yields values obtained in this work were not possible. However, rough comparable range of 38 to 410 CH_4ml/gVS added has been reported from different botanical fractions of aquatic macrophytes weeds species such as Eichhornia, Pistia, Azolla, Salvinia, Lemna and Ceratopteris. Pistia has been found to be an excellent substrate for biogas production with range of 350-410 CH_4ml/gVS (Gunaseelan, V.N., 1997). Nevertheless, methane yields from different plant parts have been reported to produce biogas at different rates and give different yields (Gunaseelan, V.N., 1997; Gunaseelan, V.N., 2004). Also, it has been postulated that methane yields and kinetics in leaves are generally higher than in stems (Gunaseelan, V.N., 1997). The methane yield data obtained in this study at 5%TS for leaves was higher by 1.4% than that obtained for stems, which confirmed the above concept.

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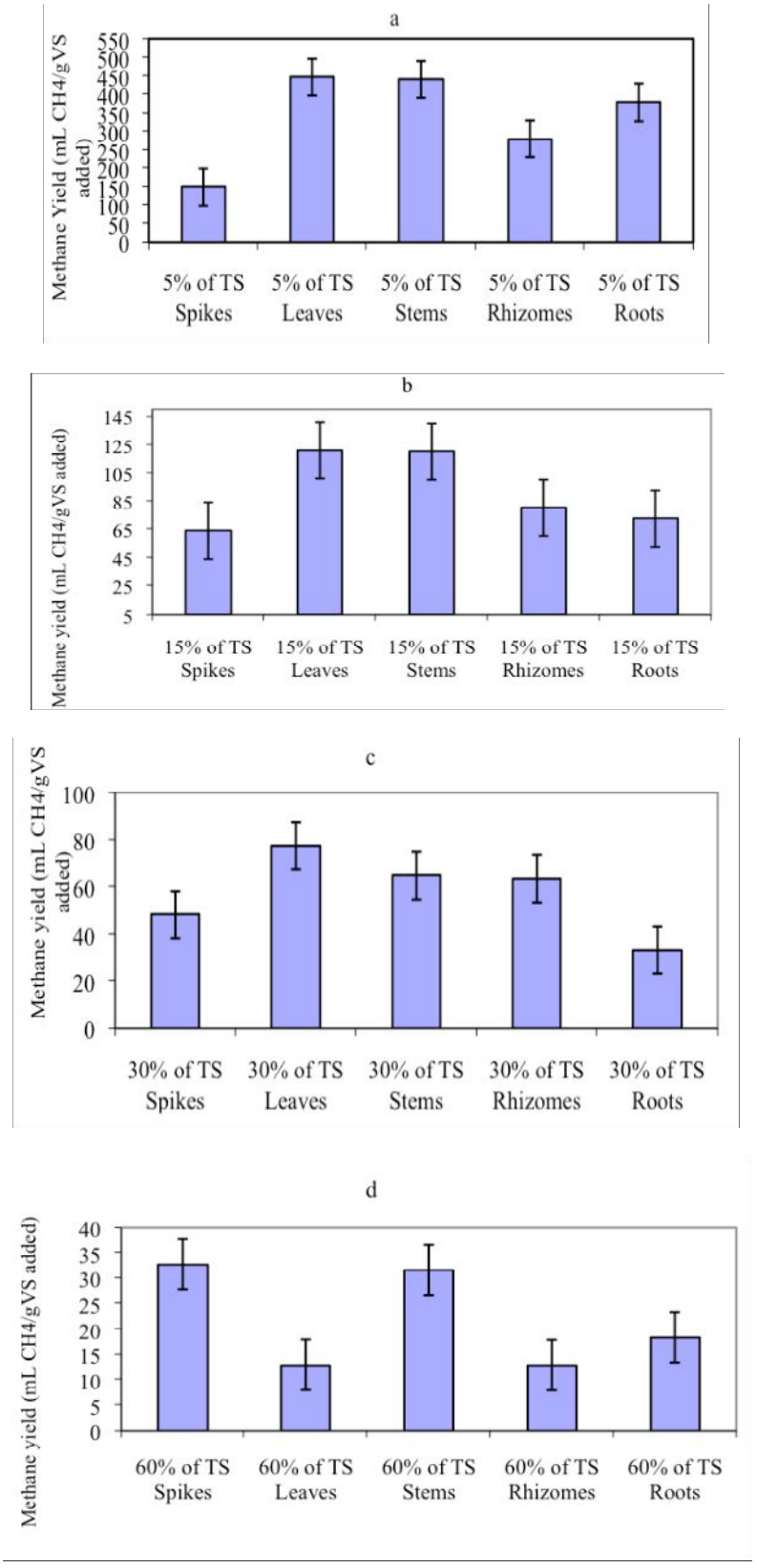
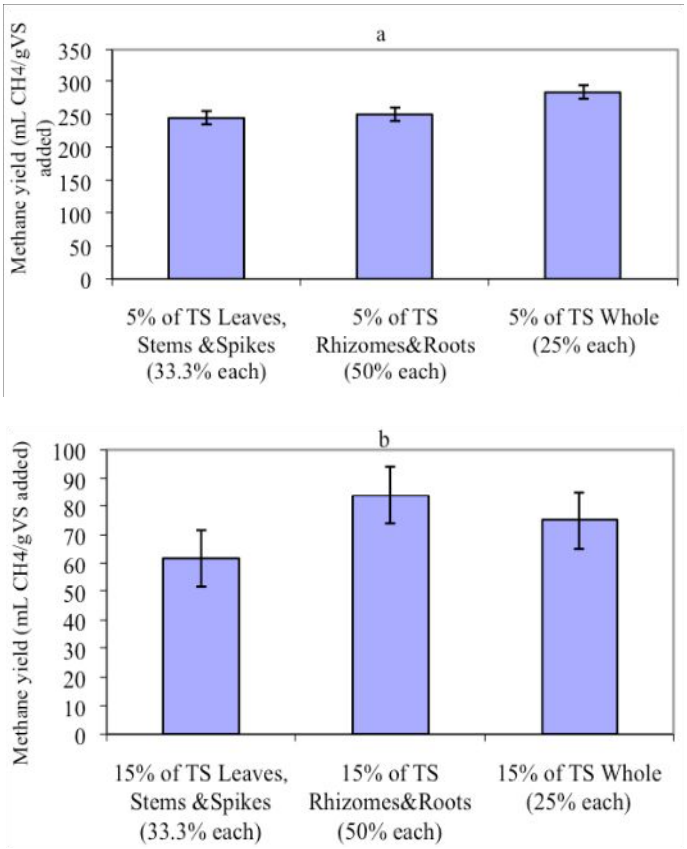


Figure-4. Methane yields during batch anaerobic digestion of cattail weeds separate botanical fractions at different solid concentrations (a) 5%, (b) 15%, (c) 30% and (d) 60%.

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Methane yield from blended cattail weeds botanical fractions

Examination of Figure-5 indicates that methane yields during batch AD of cattail weeds mixed botanical fractions increased with decreasing solid concentrations from 60% to 5%. Analysis of variance (ANOVA) for methane yield for the cattails weeds mixed botanical fractions showed that there were significant differences among the blends tested ($p < 0.05$). The highest methane yield was obtained at 5%TS solids concentration and ranged from 246-285 CH_4 ml/gVS added. The direct comparison of methane yield from spike, stem, leaves, rhizomes and roots blends was not possible, since no data on AD of those fractions blends were available in the literature. The highest and lowest were obtained from whole cattail (spikes, leaves, stems rhizomes and roots each 20% wet weight) and shoot which comprised of spikes, leaves and stems (33.3%:33.3%:33.3% wet weight), respectively. The methane yield obtained in this study from whole cattail weed of 285 CH_4 ml/gVS added was 2.7 times higher than the highest methane yield of 106 CH_4 ml/gVS added reported recently; during AD of cattail with rumen culture in the presence of heavy metals (Zheng-Bo, Y., Han-Qing, Yu. and Zhi-Liang, W., 2007). The possible differences in the methane yields obtained in this study and that from literature could be possibly due to differences in the feedstock (chemical composition, pretreatment), in the experimental conditions (temperature and retention time, addition of metal additives) as well as the inocula used. In this study American cockroach gut culture was used as an inoculum while that reported in the literature was rumen fluid obtained from a fistulated goat. Addition of inoculum and acclimatisation of the inoculum to the feedstock during AD in bioreactors has been reported to affect both the biogas yield and methane content in biogas (Gunaseelan, V.N., 1997; Yadvika S, Sreekrishnan, T.R., Kohli, S. and Rana, V., 2004).



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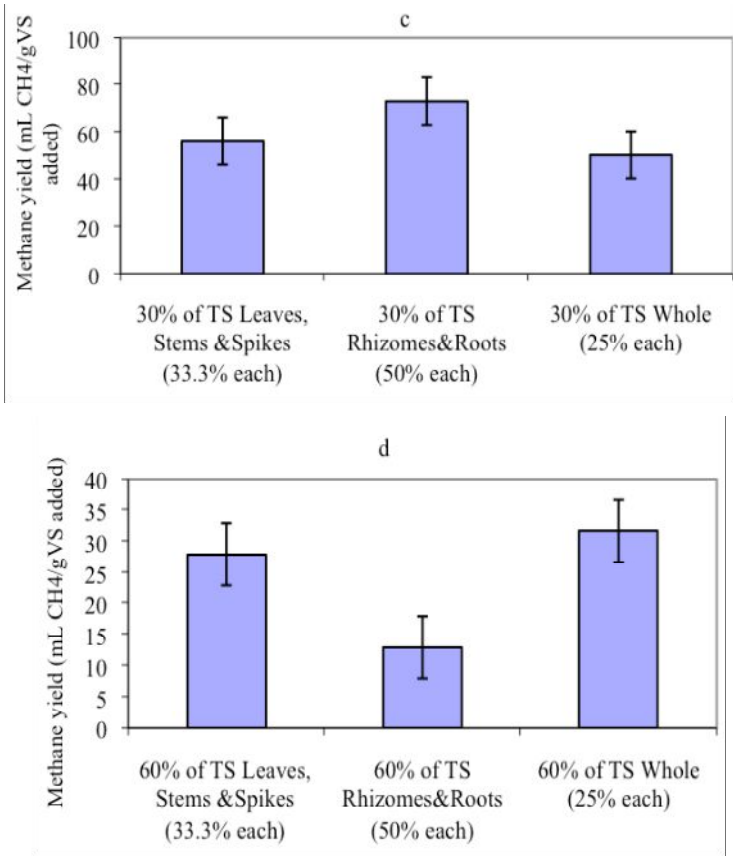


Figure-5. Methane yields during batch anaerobic digestion of cattail weeds mixed botanical fractions at different solid concentrations (a) 5%, (b) 15%, (c) 30% and (d) 60%.

Effects of blending cattail weeds botanical fractions on methane yield

It was interesting note that blending of different cattail botanical fraction at 5 to 60%TS substrate concentration in general did not improve the methane yield compared to that obtained from separate botanical fractions with exception of that obtained from spikes which was lower than that obtained from cattail blends. The highest methane yield obtained at 5%TS from cattail leaves was higher by 57-82% compared to highest methane yield obtained from mixed botanical fractions at 5%TS (Figures 4 and 5). It seemed that the marked decrease in the methane yield could be due to negative synergism established in the digestion medium and possibly there was no supply of additional nutrients by blending different cattail botanical fractions. An interesting option for improving yields of AD of solid wastes is co-digestion. To enhance methane yield from cattail weeds mixed fraction the work is underway to investigate the effect of co-digesting cattail weeds with nitrogen rich fish wastes and/or animal manures which are available around the lake Jipe.

C:N ratio and methane productivity

It is necessary to maintain proper C:N ratio in the composition of the feedstock for efficient biogas production. Often the C:N ratio is used as an index of the suitability of organic feeds for methane production. However, there is no agreement in the literature on the ideal C:N ratio. The ratio proposed as ideal C:N for AD varies from less than 10 to 72 (Itodo, I. and Awulu, J.O., 1999, Ward. A.J., Hobbs. P.J., Holliman, P.J. and Jones D.L., 2008). Although, in the present study, the C:N range of 10-23 of separate and mixed cattail weed botanical fractions (Table 3) fell within the ideal range of C:N ratio, they showed widely disparate methane production (data not shown) and methane yields as seen in (Figure 4 and 5). This supports the argument that several factors, besides C:N ratio, influence biogas production and some studies also suggested that C:N ratio varies with temperature (Yadvika S., Sreekrishnan, T.R., Kohli, S. and Rana, V., 2004). From the results obtained in this study on methane productivity, it can be pointed out that utilization of cattail weed for biogas production could be advantageous because of its high water content, available organic matter and a favorable C:N ratio (10:1-23:1).

PH, alkalinity and VFAs

During all the experiments, there were no indication of failure such as low pH, insufficient alkalinity and the accumulation of VFAs in any of the BAB fed by both separate and mixed cattail botanical fractions at different solid concentrations of 5%, 15%, 30% and 60%. The initial pH ranged between 7.6 and 7.8 while the final pH values range was 7.4-8.4 (data not shown) which suggest that acidification of bioreactors was not occurring

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during AD of separate and blended cattail botanical fractions investigated. The pH values obtained in this study were within the range of 6.8-7.2, which is ideal for AD and normal functioning anaerobic bioreactors (Ward. A.J., Hobbs. P.J. Holliman, P.J. and Jones, D.L., 2008). The growth rate of methanogens is greatly reduced below pH 6.4 due to toxic effects of the hydrogen ions, which are believed to be closely related to the accumulation of VFAs (Anderson, G.K. and Yang, G., 1992). Low pH is indicative of a depletion of the bicarbonate buffering capacity and is often considered to be a sign of a failed AD process system. The initial partial alkalinity ranged between 2500 and 3500 mg CaCO3/l while that observed at the end of AD period was 3600-3800 mg CaCO3/l (data not shown). Jenkins, R.S., Morgan, J.M., Zhang, X (1991) reported partial alkalinity above 1200 mg CaCO3/l as being typical for properly operating anaerobic bioreactors and for stable AD process operation. In this study the initial buffering capacity of the system was enhanced by addition of $NaHCO_3$ to 1500 mg CaCO3/l. Addition of inoculum has been attributed to contribute to good buffering capacity of anaerobic bioreactors (Yadvika, S., Sreekrishnan, T.R., Kohli, S. and Rana, V, 2004). This implies that American cockroach gut inoculum used also contributed to buffering of the system. Furthermore there was also contribution from soluble carbon dioxide in biogas converted to bicarbonate during AD process (Guwy, A.J., Hawkes, F.R., Wilcox, S.J. and Hawkes, D.L., 1997). In a well balanced AD process, VFAs levels are low. In this study AD of both separate and mixed cattail weed botanical fractions investigated at different TS substrate concentration showed lower levels of VFAs in their digested slurry which ranged between 4.3 and 6.4 mM for acetic acid, 0.01–0.09 mM for propionic acid and 0.008–0.05 mM for butyric acid. The initial propionic acid to acetic acid (P/A) ratio was well below the critical limit of 1.4. It has been previously reported by other researchers that during AD an increase in P/A ratio greater than 1.4 and a build-up of acetic acid and butyrate to above 200 mM as well as 100 mM of proprionate, can explain process inhibition and ultimate anaerobic bioreactor failure (Hill, D.T., Cobb, S.A. and Bolte, J.P., 1987; Ahring, B.K., Sandberg, M. and Angelidaki, I., 1995).

CONCLUSIONS

- The present study, reports for the first time the utilization of microbial population available in American cockroach gut as a seeding material in anaerobic digestion of cattail weeds botanical fractions singly and in combination to produce methane.
- The methane yield reported in this study provides an extensive database on the extent of methane production from cattail weeds botanical fractions alone and in combination.
- This study has demonstrated that substantial differences were observed in the methane yields of different cattail weed botanical fractions singly and blended at different TS concentration.
- Among the cattail weed botanical fraction tested for both single and blended; best results were obtained at 5% different TS concentration. Leaves, stems, roots, rhizomes and whole cattail weed showed methane yields greater than 280 ml/gVS added and thus could be considered in terms of renewable energy sources in Tanzania and elsewhere and can be carried out at a decentralized local level. The biogas can be used directly for heating, cooking on site thus reduces energy imports. The surplus energy can be easily converted into electricity or be exported as piped or compressed gas.

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