PRODUCTION OF BIOGAS FROM GROUNDNUT SHELL (*Arachis hypogaea*) CO-DIGESTED WITH COW DUNG THROUGH ANAEROBIC DIGESTION

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Production of Biogas from Groundnut Shell (*Arachish ypogaea*) Co-Digested with Cow Dung through Anaerobic Digestion

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By

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APPROVAL SHEET

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DEDICATION

This thesis manuscript is dedicated to my dear Families

STATEMENT OF THE AUTHOR

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BIOGRAPHICAL SKETCH

The author was born on January 23, 1983 (GC) in Bisidimo 01 Kebele, from his mother W/Ro Halima Musa and his father Ato Debebe Cherinet in Babile Woreda, Estern Hararge Zone,Oromia Regional State. He attended his elementary and junior school education in Bisidimo and secondary and preparatory school in Harer Senior Secondary School .Then; in 2002 he joined Addis Ababa University and graduated in June 2005 (GC) with B.Sc. degree in Biology. Then, after serving as a secondary school teacher for two years, he also worked as a head of Woreda Education Office and as an advisor for EsternHararge Zone Education Office for nine years. Then after he joined postgraduate program of Haramaya University to pursue his M.Sc. study in Biotechnology at the School of Biological Science and Biotechnology.

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ACRONOMYS/ABBREVIATIONS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
CD	Cow Dung
MC	Moisture Content
GS	Groundnut Shell
MDS	Mass of Dry Solids
TS	Total Solids
VS	Volatile Solid
SRT	Solid Retention Time
HRT	Hydraulic Retention Time

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ABSTRACT

Biogas is an alternative eco-friendly renewable energy source that produced by methanogenic bacteria through anaerobic digestion of organic materials. In this research, biogas production from co-digestion of the cow dung (CD) and groundnut shell (GS) with five mix ratios (T1 100%CD,T2 75%CD+25%GS,T3 50%CD+50%GS,T4 25%CD +75%GS,T5 100%GS) was evaluated under mesophilic conditions (38ºC) using batch fermentation for 21 days. The substrates were prepared with different total solid percentage such as 4, 6, and 8%. In all the treatments, physico-chemical parameters such as total solid (TS), volatile solid (VS), % organic carbon, nitrogen and pH were measured before and after digestion. The daily biogas production was subsequently measured by water displacement method for 21 days. The results were revealed that all measured physico-chemical parameters were significantly varied between before and after AD. As per the experimental results 8 % total solid with mixed ratio of 75%CD + 25%GS showed the maximum reduction of volatile solids and total solid content was observed. There was a significant difference in % organic carbon reduction was observed in between before and after AD. On the other hand, in all the treatments % of organic nitrogen content were increased after anaerobic digestion. In addition to this, C/N ratios were analyzed, the results were revealed that highest C/N ratio was observed in 8% total solid with 100% CD and lowest value in 8% total solid with 100%GS. Among the different proportion of the total solid, 8%, total solid with mix ratio of 25%GS+75%CD showed highest production of biogas compared with all other treatments. Over all the results of this study indicate that the increase in biogas yield and reduction in volatile solids and total solids can be significantly enhanced when cow dung (CD) is co-digested with groundnut shell in 75%:25% mix ratio.

Key Words: Biogas, Co-digestion, Eco-friendly, Groundnut shell.

1. INTRODUCTION

Energy is one of the most important factors for human development and global economic growth. About 80% of the world's energy consumption still originates from combusting fossil fuels (Goldenberg and Johansson, 2004). The prime challenge for the country is to provide the minimum energy services to allow the rural people to achieve decent standard of living. Forecasting future energy demand is one of the most important policy tools used by the decision makers all over the world (Ediger and Akar, 2007). The overdependence on fossil fuels as primary energy source has led to global climate change, environmental pollution and degradation, thus leading to human health problems. In the year 2040, the world population as predicted to be is 9 – 10 billion people and must be provided with energy and materials (Okkerse and Bekkum, 1999). Biomass is any organic matter typically agricultural crops wastes, grasses etc that can give energy directly by being burnt or indirectly by being changed into other forms like bio fuels such as biogas or ethanol. If correctly used, biomass could contribute significant amount of energy for any country. For instance, a recent study shows that biomass could produce 17-28% of US electricity by 2020 (Fountoulakis *et al*., 2008).

Biogas is an alternative and renewable energy source produced through anaerobic digestion process, which is a natural-biological process in which an interlaced community of bacteria cooperates to obtain a stable fermentation through assimilation, transformation and decomposition of the organic matter present in waste into biogas. This is a complex multistep process in terms of microbiology, where the organic material is degraded to result in methane gas under the absence of oxygen. The end product of this anaerobic digestion is production of mainly the combustible gas, methane (CH4) and a liquid effluent (Rilling, 2005).

Anaerobic fermentation of organic materials has long been used to generate useful resources which have been harnessed for the use of mankind (Uri, 1992; US Environmental Protection Agency, 2001). As early as the 18th century, anaerobic process of decomposing organic matter was known, and in the middle of the 19th century, it became clear that anaerobic bacteria are involved in the decomposition process.

Biogas technology offers a very attractive route to utilize certain categories of biomass for meeting partial energy needs. In fact, proper functioning of biogas systems can provide multiple benefits to the users and the community resulting in resource conservation and environmental protection (Santosh *et al.,* 2004). But what makes biogas distinct from other renewable energy is its importance in controlling and collecting organic waste material and at the same time producing fertilizer and water for use in agricultural irrigation. Unlike other forms of renewable energy, biogas neither has any geographical limitations and required complex technology for producing energy and it is neither complex nor monopolistic.

Biogas consists primarily of utilizable methane (CH_4) and carbon dioxide (CO_2), which are both colorless and odorless. However, depending on the source of the organic matter and the management of the anaerobic digestion process, small amounts of other gases may be present (Arogo *et al.,* 2009; Madu and Sodeinde, 2001). Methane has 20 times more greenhouse gas potential than carbon dioxide, so the capture and burning of methane significantly reduces the greenhouse gas effect (Atkins *et al.,* 2008). Biogas is produced by the microorganisms during the anaerobic fermentation of biodegradable materials. Anaerobic fermentation is a biochemical process in which particular kinds of bacteria digest biomass in an oxygen-free environment resulting in production of CH_4, CO_2, H_2 and traces of other gases along with decomposed mass.

In Ethiopia, biomass in the form of wood, charcoal, crop residues, animal dung and agro-industrial wastes accounts for more than 93 percent of the national energy supply (World Bank, 1984) and 98 percent of the rural household energy use (Sheriff, 1987).It is, therefore, obvious that biomass represents an important part of the raw materials necessary for the satisfaction of energy needs in many developing countries including Ethiopia. This role of biomass continues to grow in Africa, where the effects of the present energy crisis are particularly acute. The ever increasing human population, the decreasing availability of fuel wood, coupled with the ever-rising prices of kerosene, natural gas in Ethiopia, draw attention of the need to consider alternative sources for

domestic and cottage level industrial use in the country. Such energy sources should be renewable and should be accessible to poor.

Groundnuts (*Arachis hypogaea*), also known as peanuts are the edible seeds of a legume or bean family (Fabaceae) plant that grow to maturity in the ground. Cultivated in nearly 100 countries, over 90% of which are in developing countries.The groundnut is a staple food and valuable cash crop for millions of households (CGIAR, 2004-2005, cited in Pazderka and Emmott, 2010). They can be consumed directly (roasted and salted), processed into oil or cake/meal, or further processed into confectionary products or snack food. In Ethiopia, groundnut is the second important lowland oilseed of warm climate next to sesame (Dawit and Samuel, nd). The lowland areas of Ethiopia have considerable potential for increased oil crop production including groundnut.

After its first introduction to Eretria in the 1920 and then to Harer (EARO, 2000 cited in Dawit and Samuel, nd), groundnut is grown in many lowland areas of Ethiopia. It is mainly grown in eastern Harerghe, with immense potential in Gamogofa, Illubabor, West Gojam, North Shoa, North and South Wello, East and West Wellega, and Western Tigray (CSA 2010, cited in Dawit and Samuel, nd). In the Eastern lowland areas of Ethiopia have considerable potential for increased oil crop production including groundnut. Particularly areas such as Babile,Gursum and Darolabu are the major producers of groundnuts for local and commercial consumption (Getnet and Nugussie, 1991; Chala *et al*., 2012).

Previously different researchers (Animut Assafa, 2013; Misgana Lami, 2014. and Gizachew Mebratu, 2015) have evaluated biogas production from different agro wastes. However, no research has been done to evaluate biogas production potential from groundnut shell in sole or combined with cow dung. This research was, therefore, designed to assess biogas production potential of this agro waste with the following general and specific objectives.

General objective

- ✓ To determine the amount of biogas that can be produced from Groundnut shell co-digested with cow dung or alone through anaerobic digestion.

Specific objectives:

- ➢ To characterize groundnut shell (GS) and cow dung (CD) in terms of total solids (TS), volatile solids (VS), organic carbon, carbon/nitrogen ratio and pH before and after anaerobic digestion;
- ➢ To evaluate the average and daily cumulative biogas production from solo and mixture of groundnut shell and cow dung combined in different proportions.

2. LITERATURE REVIEW

2.1. Energy

Our economy depends on the availability of energy most of which has come from the combustion of fossil fuels. Fossil fuels include coal, natural gas, and a variety of liquid fuels, such as gasoline, diesel fuel, and heating oil are derived from petroleum. These are not renewable (at some point they will become depleted) and increasingly costly. Unlike fossil fuel combustion, renewable energy sources provide energy without depleting fossil fuel reserves and with much lower overall carbon dioxide emissions (Smil and Vaclav, 2003).

2.2. Biogas

Biogas is a biological gas which originates from bacteria in the process of biodegradation (fermentation) of organic material (from plants, animals and sometimes human origins) under anaerobic (oxygen free) conditions. Biogas is also an alternative and renewable energy source produced through anaerobic conversion of organic matter into a combustible biogas rich methane (CH4) and liquid effluent. (Ogejo *et al.*, 2009). Methanogens (methane producing bacteria) are the last link in a chain of microorganisms which degrade organic material and return the decomposition products to the environment.

2.3. Biogas Composition

Biogas obtained after fermentation is a mixture of gases among which methane (CH4) is useful. However, depending on the source of the organic matter and the management of the anaerobic digestion process, small amounts of other gases such as ammonia (NH3), hydrogen sulfide (H_2S) and water vapour (H_2O) may be present (Ogejo *et al*., 2009).

2.4. Physical Characteristics of Biogas

According to its composition, biogas is a gas appreciably lighter than air and is colorless; it produces twice as less energy by combustion with equal volume of natural gas. Like those of any pure gas, the characteristic properties of biogas are dependent on pressure

and temperature. They are also affected by the moisture content (Uri, 1992; Ogejo *et al*., 2009).

2.5. Feed Stocks (Substrates) for Biogas Production

2.5.1. Cow dung

Due to low energy efficient during combustion of cow dung, small particles and noxious gases are emitted which are one of the most threatening evitable risk factors (Smith, 2002). However, it can be anaerobically digested to give energy in the form of biogas which is the combination of mainly methane and carbon dioxide. For biogas production cow dung properties may depend on diet types. However, in comparison of cow dung, manure from pig and poultry have a higher biogas potential. This is because ruminants already have some anaerobic digestion in their first stomach (Teame*et al*, 2014).

2.5.2. Groundnuts

Groundnuts (*Arachi shypogaea*), also known as peanuts are the edible seeds of a legume plant that grow to maturity in the ground. Cultivated in nearly 100 countries, over 90% of which are developing countries, the groundnut is a food staple and valuable cash crop for millions of households (CGIAR, 2004-2005, cited in Pazderka and Emmott, 2010). They can be consumed directly (roasted and salted), processed into oil or cake/meal, or further processed into confectionary products or snack food. As a legume, groundnut fixes atmospheric nitrogen in soils and thus improves soil fertility and saves fertilizer costs in subsequent crops. This is particularly important when considered in the context of the rising prices of chemical fertilizers which makes it difficult for small scale farmers to purchase them. In livestock farming communities, groundnut can be used as fodder for livestock and increases productivity as the groundnut haulm and seed cake are rich in digestible crude protein content (Simtowe *et al.*,nd).

2.6. Anaerobic Digestion

The anaerobic fermentation of organic materials has long been used to generate useful resources which have been harnessed for the use of mankind (Uri, 1992; US

Environmental Protection Agency, 2001). As early as the 18th century, anaerobic process of decomposing organic matter was known, and in the middle of the 19th century, it became clear that anaerobic bacteria are involved in the decomposition process. Anaerobic digestion provides some exciting possibilities and solutions to such global concerns as alternative energy production, handling human, animal, municipal and industrial wastes safely, controlling environmental pollution, and expanding food supplies (Uri 1992; Ofoefule and Uzodinma, 2006). As demand for energy is increasing astronomically, and the fossil based fuels become scarce and more expensive, and carbon dioxide emission levels become of greater concern; Biogas a by-product of anaerobic fermentation and a renewable energy source have currently been recognized globally as a means of solving the problem of rising energy prices, waste treatment /management and creating sustainable developments.

2.7. Microbiology of Anaerobic Digestion Process

The anaerobic digestion process involves a large number of microorganisms, which convert the feedstock to the methane and carbon dioxide rich biogas through a series of biochemical reactions that can be described by four steps, viz. hydrolysis, acidogenesis, acetogenesis and methanogenesis.

2.7.1. Hydrolysis

In the first step, the hydrolysis, the big molecules of carbohydrates, proteins and lipids are split into smaller components (sugars, fatty acids and amino acids) by the help of extra-cellular enzymes secreted by microorganisms which most of them are obligate anaerobic. A complex consortium of microorganisms participates in the hydrolysis and fermentation of organic material (Rojas *et al*., 2010).

2.7.2. Acidogenesis

Once complex organics are hydrolyzed, the hydrolysis products which are relatively small soluble compounds can diffuse inside the bacterial cell through cell membrane. Acidogenic (acid-forming) bacteria convert sugars, amino acids and fatty acids to smaller

organic acids, hydrogen, and carbon dioxide. The communities of bacteria responsible for acid production include facultative anaerobic bacteria, strict anaerobic bacteria, or both (Zinder, 1988).

2.7.3. Acetogenesis

In the third step, acetogenesis, the products of the acidification are converted into acetic acids, hydrogen, and carbon dioxide by acetogenic bacteria. Acetogenic bacteria such as *Syntrobacter wolini* and *Syntrphomonas wolfei* convert volatile fatty acids (e.g. propionic acid and butyric acid) and alcohol into acetate, hydrogen, and carbon dioxide, which are used in methanogenesis (Zaher *et al*., 2007).

2.7.4. Methanogenesis

The formation of methane, which is the ultimate product of anaerobic digestion, occurs by two major routes. Formic acid, acetic acid, methanol and hydrogen can be used as energy sources by the various methanogens. The primary route of methane produced (two-thirds of methane gas) is the fermentation of the major product of the acetogens phase, acetic acid, to methane and carbon dioxide (Zaher *et al*., 2007). Bacteria that utilize acetic acid are acetoclastic bacteria (acetate splitting bacteria).The remaining methane is generated from H2 and CO2 by the hydrogenotrophic methanogens. Mackie and Bryant (1981) reported that acetate synthesis form H2 and CO2 accounts for only 1-2% of total acetate formation at 40 °C and 3-4% at 60 °C in a cattle waste digester.

2.8. Factors Influencing Biogas Production

2.8.1. Temperature

Anaerobic microorganisms are temperature sensitive and temperature specific. In general, methane production increases with increase in temperature. The three temperature regimes used in anaerobic digesters are psychrophilic, mesophilic, and thermophilic with optimums temperature ranges for the growth of methane forming bacteria of 41 to 77%, (Hao *et al*., 2011). The U.S Environmental Protection Agency (2001) reported that two temperature ranges are of interest for anaerobic treatment the mesophilic range and the thermophilic range, the mesophilic range extends from 29^0C to 38^0C usually chosen as the optimum temperature.

2.8.2. pH

Maintaining an acceptable pH in the digester is important for the system to work well. Acid-forming bacteria prefer a pH above 6.2.While methaneforming bacteria live best under neutral to slightly alkaline conditions. Once the process of fermentation has stabilized under anaerobic conditions, the pH will normally take on a value of between 7.0 and 8.5 (Uzodinma and Ofoefule, 2009). Due to the buffer effect of carbon dioxide-bicarbonate (CO2 = HCO3) and ammonia –ammonium (NH_3-NH_4+), the pH level is rarely taken as a measure of substrate acids and/or potential biogas yield. According to Ogejo *et al*., (2009), most anaerobic bacteria will perform well in the pH range of 6.8 to 7.2. The pH of the substrate decreases initially when organic material is first loaded into the digester and volatile acid are produced. However, the pH of the digester will increase and then stabilize, when methane producing bacteria consume the acids (due to alkalinity produced).

2.8.3. Retention Time

The retention time is the number of days the organic material stays in the digester. There are two significant retention times in anaerobic digester, solid retention time (SRT) and hydraulic retention time (HRT). The SRT is the average time the bacteria (solid) are left in the anaerobic digester. The HRT is the time the liquid is in the anaerobic digester. SRT is the most important retention time, and should be determined correctly because it indicates the potential of bacteria washout. If a significant washout of bacteria occurs, the digester fails (Igoni *et al.*, 2007).

2.8.4. Particle size

The production of biogas is also affected by particle size of the substrate. Too big particle size is problematic for microbes to digest and it can also result in blockage in the digester, whereas small particle size gives a large surface area for substrate adsorption and thus allows the increased microbial activity followed by increase in the production of gas (Yadvika *et al*., 2004).

2.8.5. Water Content

Bacteria take up the available substrates in dissolved form. Therefore, biogas production and the water content of the initial material are interdependent. Rilling (2005) reported that when the water content is below 20% by weight, hardly any biogas is produced. Optimum moisture content has to be maintained in the digester and the water content should be kept in the range of 60-95% (Demetriades, 2008).

2.8.6. Volatile solids.

The weight of organic solids burned off when heated to about550^0C is defined as volatile solids. The biogas production potential of different organic materials can also be calculated on the basis of their volatile solid content. The higher the volatile solid content in a unit volume of fresh dung, the higher the gas production For example, a kg of volatile solids in cow dung would yield about 0.25 m3 biogas (Sathianathan,

1975).According to Santana and Pound (1980) biogas production increase 1inearly with increasing total solids concentration.

2.8.7. Volatile Acid

The concentration of volatile fatty acid (VFA) serves as an indicator during anaerobic digestion process. An increase in volatile acid concentration suggests that either the organic loading to the system has been increased or that the methane farming bacteria are inhibited from performing their normal functions. A decrease in volatile acids indicates either that the loading has been reduced or that the system is acclimatizing (adjusting) to new conditions (Uzodinma and Ofoefule, 2009).

2.8.8. Feeding Quality

All feeding materials consisted of solid matter and water while the solid matter alone consists of volatile solid (VS) and non-volatile solid or fixed solid (FS). Volatile solid are part of the total solids, consist of the substrate that can be converted in to bio gas (Jan *et al.*, 2010), but fixed solids are part of the solid content of the substrates that remain in the form of residue which can be important bio-slurry for compositing. Before feeding the digester, the extract, especially the cow dung, has to be mixed with water in the ratio of 1:1 on the unit volume base However if the dung is dry form water has to increase accordingly to arrive at the desired consistency of substrate. The dilution should be made to maintain a total content from 7 to 19 percent.

2.8.9. Salts

Salt is important for the function of all micro-organisms the salt contain essential building blocks for the micro-organisms, such as sodium, potassium and chloride. These substances are available in any substrate and do not need to be added to the biogas process separately. However some wastes have high concentration or releasing excess salt which can inhibit micro-organisms from biogas production process. Salt and sugars generally have a preservative effect that is why they inhibit bacterial growth. Too much salt or sugar causes the cell to pump out water and lose both form and function (Chaban

et *al.,* 2006). Wastes from the food and fisheries industries or different type of protein-rich materials that lead to release of ammonia could lead to increasing salt concentration in biogas production

2.9. Percentage of Methane in Biogas

Biogas is the mixture of gas mainly composed of 60 to 70 percent methane, 30 to 40 percent carbon dioxide and low amount of other gases like water vapour, hydrogen sulphide, hydrogen, and nitrogen. Marchaim (1992) stated that typical values for methane content are in the range of 50 to 60 % for animal manure and up to 75 % for feedstock containing fats. The proportion of CH4 to CO2 in the gas depends on the substrate and is slightly affected by temperature, pH and pressure. Barnett *et al*., (1978) reported that anaerobic digestion of cow dung, chicken manure, pig manure, farm wastes, sewage sludge and elephant grass produced gas that contained, 65 to 70, 67 to 70, 68, and 60 percent of methane respectively. Neelakantan *et al*., (1978) stated the anaerobic digestion of plant materials vis., berseem, oat, dhub grass, and paddy straw, hybrid Napier with and without 2.5 percent nitrogen as ammonium sulphate. The biogas produced comprised of 58 percent methane, 28 percent carbon dioxide and 14 percent of carbon monoxide, oxygen, nitrogen, and hydrogen Neelakantan *et al*., (1978). Oba *et al*., (1981) studied anaerobic digestion of agricultural wastes and cattle wastes as methane generator. The gas produced was found to contain 60 to 84 percent methane.

3. MATERIALS AND METHODS

3.1. Design of the Experiment

The experiments were arranged in a completely randomized factorial design with three replications. All experiments were carried out at mesophilic temperature (38^0C) using five sets of digesters corresponding to the five-substrate mix-ratios in three types of adjusted percent of total solids

3.2. Sample Collection and Preparation of Substrate for Anaerobic Digestion

Groundnut shell (GS) and Cow Dung (CD) were used as feedstock's for AD process of biogas production.GS was collected from Eastern Harerghe zone from Babile district and CD was collected from the Haramaya University cattle farm. Both GS and CD were oven dried and crushed using all-purpose high speed smashing machine to break into smaller particles to ensure consistency of the mix. Taking equal amounts of each dried fresh substrate (e.g. 10g), total solid was determined before AD. Thereafter, the two substrates were combined in different proportions so as to have five substrate treatments. These were T1 (100% CD), T2 (75% CD+25%GS), T3 (50%CD: 50%GS), T4 (25%CD: 75%GS) and 100% GS. Based on their original %TS, the substrates were adjusted to have TS of, 4, 6 and 8 by diluting in appropriate amount of distilled water including 100ml of fresh rumen fluid used to facilitate the start of AD (Sunarso *et al*., 2012). This helps to understand the effect of varying %TS in biogas production. Fresh rumen fluid was collected from the nearby slaughterhouse of Haramaya University and filtered through a cloth of 0.5 mm sieve diameter to separate the solid content from slurry and transported by plastic bottle and properly handled. Anaerobic digestion of the substrates was done in 0.5 L digester under mesophilic temperature (38^oC) using five sets of digesters corresponding to the five-substrate mix-ratios each with different %TS. The pH of the slurry was maintainedwithin the pH range for optimal biogas production, by adding sodium hydroxide and hydrochloric acid to the organic substrate .i.e. about neutral (Yadvika *et al*., 2004) after the initial pH is measured. The experiment was arranged in a

completely randomized factorial design with three replications of five types in three TS concentrations.

3.3. Digester Configuration and Setup for Biogas Production

Digesters were setup in order where the digester containing the slurry was connected to the 0.5L plastic bottle with acidified brine solution that was connected to an empty bottle meant for collecting the brine solution that was expelled out from the second container. The acidified brine solution was prepared by dissolving NaCl in distilled water with few drops of sulphuric acid until a supersaturated solution is formed to prevent the dissolution of biogas in the water. All the three containers were interconnected with plastic tubes having a internal diameter of 1 cm. The tube connecting the first bottle to the second was fitted just above the slurry in the headspace of the first bottle to help gas collection. Thus, the biogas produced by fermentation of the slurry was driven from the first bottle to the second bottle that contained a brine solution so as to displace a volume of the brine solution equivalent to the volume of biogas produced. The lids of all digesters were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas.

3.4. Analyses of Physico-chemical Characteristics of Substrates

Both CD and GS were analyzed for total solids (TS), volatile solids (VS), moisture content and pH before and after AD process based on the Standard Methods for the Examination of Water and Wastewater (APHA, 1999).

3.4.1. Total Solids

First a clean evaporating dish was oven-dried (at 105 ºC for 1hour), cooled in a desiccator and weighed immediately before use. Sample of substrate (10 g) was placed on the evaporating dish and put in an oven (contherm 260M) at 105 °C using a crucible to evaporate for 24 hours. After 24 hours, the crucible was taken out from the oven, cooled in desiccators and weighed using electronic balance (PB602). Thereafter, the percentage of TS was calculated using the following formula (APHA 2540 B, 1999).

$$\%TS = \frac{MDS}{mFS} X100$$

Where, MDS = mass of dry sample, mFS = mass of fresh sample.

3.4.2. Volatile Solids (VS)

The total solid obtained was ignited at 550^{o}C in a muffle furnace for 3hrs to determine the volatile and fixed solids of the sample. Then volatile solid content in the sample was determined using the formula indicated in APHA (1999).

$$\%VS = \frac{mDS - m(ash)}{mDS} \times 100$$

Where,

% VS = percentage of volatile solids

mDS = mass of dry solids in gram

m (ash) = remaining mass after ignition =fixed solid in grams.

3.4.3. Moisture content determination

To determine the percentage of moisture content (MC) in the samples, 10 g of fresh substrate was dried in an oven (contherm 260M) at 105 °C for 24 hours and reweighed. The moisture content was then calculated as follows (APHA 2540 E, 1999).

$$\%MC = \frac{W-D}{W} X100$$

Where,

MC = moisture content

W = initial weight of sample in grams,

D = weight of sample after drying at 105 °C in grams

3.4.4. C: N Ratio

In order to determine the C: N ratio, the amount of organic carbon was first determined by Walkley-Black method while the N was determined using macro-kjedahl method. Thereafter, C: N ratio of each substrate was determined (Walkley – Black, 1934). For the

determination of organic carbon, 2gm dried organic waste was weighed and transferred to a 500-mL Erlenmeyer flask. About 10ml of 0.167 M $K_2Cr_2O_7$ was then added by means of a pipette and 20mL of concentrated H_2SO_4 was added by means of a dispenser and was swirled gently to mix thoroughly, (avoiding excessive swirling that would result in organic particles adhering to the sides of the flask out of the solution).This mixture was allowed to stand for 30 minutes.

The flasks were placed on an insulation pad during this time to avoid rapid heat loss. The suspension was diluted with 200mL of water to provide a clearer suspension for viewing the endpoint. Then 10mL of 85% H_3PO_4 and 0.2g of NaF were added using a suitable dispenser, (The H_3PO_4 and NaF were added to complex Fe^{3+} which would interfere with the titration end point). Finally, 10 drops of ferroin indicator was added. (The indicator was added prior to titration to avoid deactivation by adsorption).

The mixture was then titrated with 0.5 M $FeSO_4$ to a burgundy end point. The color of the solution at the beginning was yellow-orange but turned to dark green at the endpoint (the change in color depends on the amount of un-reacted $Cr_2O_7^{-2}$ remaining, which shifts to a turbid grey before the endpoint and then changes sharply to a wine red at the end point). Use of a magnetic stirrer with an incandescent light made the endpoint easier to see in the turbid system (fluorescent lighting gives a different endpoint color).

$$\text{Calculation} \quad \%C = \frac{(\mathrm{B}-\mathrm{S})\times \mathrm{N}\times 0.39\times \mathrm{mcf}}{\mathrm{Wo}}$$

Where:

B = ml of $FeSO_4$ solution used to titrate blank

S = ml of $FeSO_4$ solution used to titrate sample

N= Normality of $FeSO_4$ (0.5N)

0.39= mill equivalent weight of C in g

mcf= moisture correction factor

Wo= dray sample weight in g.

The total nitrogen in the sample was determined using the Kjeldahl method. This method has three main steps. These are digestion, distillation and titration. One gram of sample and 6 ml of concentrated H_2SO_4 were added into a test tube and mixed carefully. Then

3.5 ml of H_2O_2 was added step by step. Violet color due to reaction was observed. As soon as the violent reaction was ceased the tube was shaken by hand. After adding 3g catalyst mixture the sample was allowed to stand for 5 to 15 minutes in the test tube rack before digestion. Then the digester was allowed to wait until its temperature reached 370°C. As the digester reached the temperature 370°C, the digestion process was continued for about 4 hours until a clear solution was observed. After the digestion process, tube was transferred to the fume hood for cooling. About 50 ml of distilled water was added and shaken by hand to avoid sulphate precipitation in the solution. At this time 25 ml of 40% NaOH solution was added into the digested and diluted solution. Then 250 ml of conical flask containing 25 ml of boric acid, 25 ml of distilled water and an indicator solution was placed under the condenser of the distiller with its tip immersed into the solution and the distillation continued for about 8 minutes until the total volume became between 200 ml to 250 ml. Finally the solution was titrated using 0.1N H_2SO_4 to a reddish color and %Nitrogen was calculated using the following formula:

$$\%\mathrm{N} = \frac{\mathrm{V} \times \mathrm{N} \times 0.014 \times 100 \times \mathrm{mcf}}{\mathrm{Wo}}$$

Where,

V = Volume H_2SO_4 in ml consumed during titration

N= Normality H_2SO_4 (0.1N).

0.014 = mill equivalent weight of nitrogen in g

mcf = Moisture correction factor

Wo = Sample weight on dry matter in g

Finally, the ratio of carbon to nitrogen was calculated as:

$$\frac{\%C}{N} = C{:}N$$

3.4.5. Determination of pH

The pH values were determined using digital pH meter (HANNA HI 8314) before and after AD. During the experimental period the optimal pH was 6.7 to 7.2. In the case of before AD, an electrode was inserted into samples of substrate that was diluted using distilled water before inoculation of rumen fluid and after pre-treatment. pH measurement after AD was done using pH electrode which was inserted into samples of substrate that was digested for about 21 days in AD process.

3.5. Evaluation of the Amount of Biogas

Biogas was collected by water displacement method. In order to prevent the dissolution of biogas in the water, acidified brine solution was prepared following the method suggested by Elijah *et al*. (2009). As biogas production commenced in the fermentation chamber, it was delivered to the second chamber, which contained the acidified brine solution. Since the biogas was insoluble in this solution, a pressure built-up to provide the driving force for displacement of the solution. Thus, the displaced brine solution was measured to represent the amount of biogas produced daily starting from first day of inoculation.

3.6. Data Analysis

One-ay analysis of variance (one-way ANOVA) was used to analyze mean difference between treatments, whereas data paired samples T-test was used to investigate statistical significance within a bio-digester for values between before and after AD. P-value <0.05 was considered as a statistically significant. Data were analyzed using SPSS version 19.

4. RESULTS AND DISCUSSION

4.1. Characterization of physico-chemical parameters of the substrates

4.1.1. Comparison of pH before and after AD

As the result shown in table 1, the pH values of all treatment types before AD ranged from 6.3-7.2, this is within optimum range for fermentation in biogas production. The pH value of each substrate type did not significantly vary between the three %TS levels both before and after AD. The pH value of the different substrate types, however, varied for all %TS levels adjusted. That is, pH was found to slightly decrease with increasing proportion of ground nut shell. In all treatment cases, pH value decreased after AD (Table 1). The decrement could be due to the accumulation of volatile fatty acids produced by the activity of hydrolytic acidogenic bacteria (Gomec and Speece, 2003). , According to Verma (2002), the low pH value resulting after AD is not suitable for most methanogenic bacteria. This could explain perhaps the decline in the daily biogas production observed in this study with increasing hydraulic retention time (Figure 1A, B and C).

Table 1.Comparison of pH values and %MC before and After Anaerobic Digestion (AD).Of substrates with adjusted %TS of 8, 6 and 4 %.(Values are mean ± SE, n= 3).

Adjusted % of TS for each Treatments	Parameters			
	Before pH	After pH	Before % MC	After % MC
For 8% T1	7.22±.010	5.54±.060	10.00±.000	28.00±.001
T2	7.13±.020	6.34±.051	9.03±.057	26.00±.001
T3	7.01±.010	5.35±.050	8.20±.010	24.00±.001
T4	6.82±.020	5.03±.110	6.99±.000	22.001±.001
T5	6.51±.026	4.88±.043	6.00±.000	20.002±.001
For 6% T1	7.08±.015	5.03±.035	10.0±.000	35.001±.001
T2	6.91±.015	5.61±.015	9.03±.057	34.002±.003
T3	6.84±.052	4.96±.060	8.20±.010	32.002±.001
T4	6.67±.020	4.60±.020	6.9±.000	29.003±.002
T5	6.60±.020	4.46±.030	6.0±.000	28.003±.002
For 4% T1	7.04±.045	5.28±.015	10.00±.000	40.002±.003
T2	6.65±.030	6.30±.100	9.10±.005	39.003±.003
T3	6.52±.058	5.20±.100	8.20±.005	38.00±.004
T4	6.33±.310	4.76±.118	6.99±.000	37.00±.002
T5	6.39±.020	4.41±.020	6.00±.001	36.00±.004

Means followed by different small letters in row are significant at $p<0.05$ (paired samples for within treatment) Means followed by different capital letters in column are significant at $P<0.05$ (one-way ANOVA for between treatments). T1=100%CD, T2=75%CD+25%GS, T3=50%CD+50%GS, T4=25%CD+75%GS and T5=100%GS.

4. 1.2. Analysis of TS and VS Values of Substrate before and after AD

The values of %VS ranged from 83.1 to 85.6 for all substrates types at all %TS level. Statistical comparison showed that the value of %VS of each substrate type did not vary between the three %TS levels. However, %VS of all the three %TS levels was found to be higher in substrates with ≥50% GS in the mix. After AD, %VS of all substrate types at all %TS level decreased significantly (Table 2). These reductions might be due to conversion of the substrates into biogas through AD (Rafique *et al*., 2010). However, the maximum decrement (from 83.43±.05 to 68.0033±.005) of volatile solid was observed in substrate made of 75%CD: 25%GS with its TS adjusted to 8%. total solid concentration under the treatment T2 contain suggesting more digestion of substrates by bacteria for either production of biogas or their own metabolic use.

The maximum value of TS reduction was also recorded from the treatment T2 that contain 75% CD and 25% GS, the reason might be highly digested by methanogenic bacteria and converted into biogas and other products. In this studied the maximum decrement of TS and VS after digestion has been directly related to maximum biogas productions. In biodigester T_2 (75% CD and 25% GS) the maximum TS and VS was decreased as shown (Table 2) and in this biodigester also the maximum biogas production was record.

Table 2. Comparison of %VS values and TS before and After Anaerobic Digestion (AD) of substrates with adjusted %TS of 8, 6 and 4%. (Values are mean ± SE, n= 3).

Treatments Adjusted % of Ts	Parameters Before %TS	After %TS	Before % VS	After% VS
For 8% T1	90.30±.100	65.03±.057	83.3033±.005	72.31±.010
T2	90.63±.321	60.00±.005	83.4367±.055	68.00±.005
T3	90.83±.321	60.01±.010	84.4033±.005	71.01±.015
T4	92.83±.115	59.86±.055	85.50±.005	70.98±.010
T5	94.10±.100	55.03±.026	85.60±.005	70.90±.001
For 6% T1	90.10±.100	65.46±.055	83.13±.147	72.14±.066
T2	90.46±.152	63.19±.105	83.25±.083	68.60±.010
T3	92.00±.264	61.20±.100	84.39±.010	71.00±.001
T4	92.80±.100	59.92±.026	85.50±.010	71.8±.010
T5	94.13±.060	55.40±.100	85.60±.010	72.01±.010
For 4% T1	90.00±.000	65.50±.015	83.06±.109	72.01±.010
T2	90.40±.100	63.30±.10	83.12±.026	68.81±.100
T3	91.86±.152	62.0±.100	84.19±.010	71.00±.002
T4	92.66±.152	59.9667±.02082	85.23±.049	72.00±.002
T5	94.00±.208	55.5000±.10000	85.29±.005	71.01±.010

Means followed by different small letters in row are significant at $p<0.05$ (paired samples for within treatment) Means followed by different capital letters in column are significant at $P<0.05$ (one-way ANOVA for between treatments). T1=100%CD, T2=75%CD+25%GS, T3=50%CD+50%GS, T4=25%CD+75%GS and T5=100%GS.

4.1.3. Comparison of Organic Carbon and Nitrogen between before and After AD

Percentage of organic carbon, nitrogen and carbon to nitrogen ratio were analyzed and the results were indicated in table-3. The percent of organic carbon were significantly reduced after anaerobic digestion in all bio-digester, this indicates that organic carbon might have been consumed by bacteria to support their metabolic activities or converted in to biogas (Devlin *et al.*, 2011). The highest organic carbon was recorded from 100%CD it suggesting the high content of biodegradable materials (Abdel- Hadi and El-Azeem, 2008). Moreover, highest carbon reduction was recorded in 8% total solid with mix ratio of 75%CD+25%GS (from 31.22±.001 to 24.05±.044) andlowest organic carbon degradation in 100% GS (from 30.00±.005 to 27.42±.004). The organic carbon reduction after AD implies the more degradation process was takes place in the bio-digester (Gerardi, 2003).On the other hand, in all the treatments % of organic nitrogen content was increased after anaerobic digestion.

The highest carbon to nitrogen ratio was recorded in 100%CD and least in 100% GS. However, the balance of carbon to nitrogen in a feed material is important for anaerobic fermentation process. It is often suggested that an optimum C: N ratio was between 20:1 and 30:1 (Marchaim, 1992). In line with this most of the digester within the suggested range of C: N ratio. If the C: N ratio was very low; nitrogen was liberated and accumulated in the form of ammonia (NH_3). The increased concentration of NH_3 would increase

Table 3**.**Comparison of Percent of Organic Carbon (%OC) and Nitrogen (N_2) between before and After Anaerobic Digestion (AD) for the various substrate (values are mean ± SE, n= 3) for three replication in three types of adjusted total solid in one table. This helps to understand the effect of varying %TS in biogas production.

Treatments		Parameters				
Adjusted % of TS		Before %C	After %C	Before N_2	After N_2	C:N Before AD
For 8%	T1	31.60±.004^{Aa}	26.05±.053^{Bb}	1.3±.004^{Aa}	1.5±.004^{Bb}	31.6:1.3^{Ca}
	T2	31.22±.001^{Ba}	24.05±.044^{Db}	1.2±.004^{Aa}	1.4±.004^{Bb}	31.22:1.2^{Aa}
	T3	30.63±.003^{Ca}	25.00±.034^{Cb}	1.2±.004^{Aa}	1.8±.004^{Ab}	30.63:1.2^{Ba}
	T4	30.20±.000Da	25.04±.001^{Cb}	1.4±.004^{Aa}	1.6±.004^{Ab}	30.2:1.4^{Da}
	T5	30.00±.005Da	27.02±.015^{Ab}	1.4±.004^{Aa}	1.9±.004^{Ab}	30:1.4^{Da}
For 6%	T1	31.41±.006^{Aa}	26.5±.006^{Bb}	1.3±.004^{Aa}	1.8±.004^{Ab}	31.4:1.3^{Ca}
	T2	31.10±.001^{Ba}	24.55±.008^{Db}	1.2±.004^{Aa}	1.4±.004^{Bb}	31.1:1.2^{Aa}
	T3	30.42±.000^{Ca}	25.60±.031^{Cb}	1.2±.004^{Aa}	1.5±.004^{Bb}	30.4:1.2^{Ba}
	T4	30.10±.005Da	25.44±.025^{Cb}	1.4±.004^{Aa}	1.9±.004^{Ab}	30.1:1.4^{Da}
	T5	29.91±.008^{Ea}	27.92±.066^{Ab}	1.4±.004^{Aa}	1.7±.004^{Ab}	29.9:1.4^{Da}
For 4%	T1	31.33±.004^{Aa}	26.7±.003^{Bb}	1.3±.004^{Aa}	1.8±.004^{Ab}	31.3:1.3^{Ba}
	T2	31.05±.003^{Aa}	24.9±.001^{Db}	1.2±.004^{Aa}	1.6±.004^{Ab}	31:1.2^{Aa}
	T3	30.60±.004^{Ba}	26.30±.000^{Cb}	1.2±.004^{Aa}	1.8±.004^{Ab}	30.6:1.2^{Aa}
	T4	30.20±.001^{Ca}	26.84±.002^{Bb}	1.4±.004^{Aa}	1.9±.004^{Ab}	30.2:1.4^{Ca}
	T5	28.99±.000Da	27.42±.004^{Ab}	1.4±.004^{Aa}	1.9±.004^{Ab}	28.99:1.4^{Da}

Means followed by different small letters in the row are significant at *P< 0.05* probability levels for paired samples T-test within treatment while means followed by different capital letter in column are significant at 5% level of significance between treatments for one way ANOVA. T1=100%CD, T2=75%CD+25%GS, T3=50%CD+50%GS, T4 =25%CD+75%GS and T5=100%GS.

4.2. Determination of Average Daily and Cumulative Biogas Production from Solo and Co-Digestion of the Selected Substrates

Biogas production was monitored for 21 days of hydraulic retention time for all substrate types having 8, 6 and 4% TS. Though there was variation between substrate types, gas production was noticed starting from the first day of incubation, suggesting the presence of microbial activity acting on some organic materials introduced with inoculums. In all %TS levels, gas production progressively increased up to about 9th day of incubation in all substrate types, but declined thereafter until no production was observed on the 21st day of incubation (Fig.1A, B and C). This could be due to depletion of degradable components of the substrates or fermentation inhibitory effects of some compounds, accumulation of volatile acids, for example. Accumulation of volatile acid after AD is shown by pH measurement as indicated above. Gomec and Speece (2003) and Verma (2002) reported that the low pH value resulting after AD is not suitable for most methanogenic bacteria. Comparison between substrate types showed that substrate type with 75%CD and 25%GS yielded high amount of biogas in all %TS levels followed by 50%CD: 50%GS and 25%CD: 75%GS mix ratios (Fig. 1A, B and C). This higher biogas production from these three treatments might be due to positive synergistic effect of CD to GS.Teame*et al*.(2014) reported mixing of different substrates may balance fermentation conditions including pH, organic carbon etc. than using substrates in sole. Thus, biogas production is a function of the feedstock's organic content and its biodegradability (Macias-Corral *et al*., 2008).

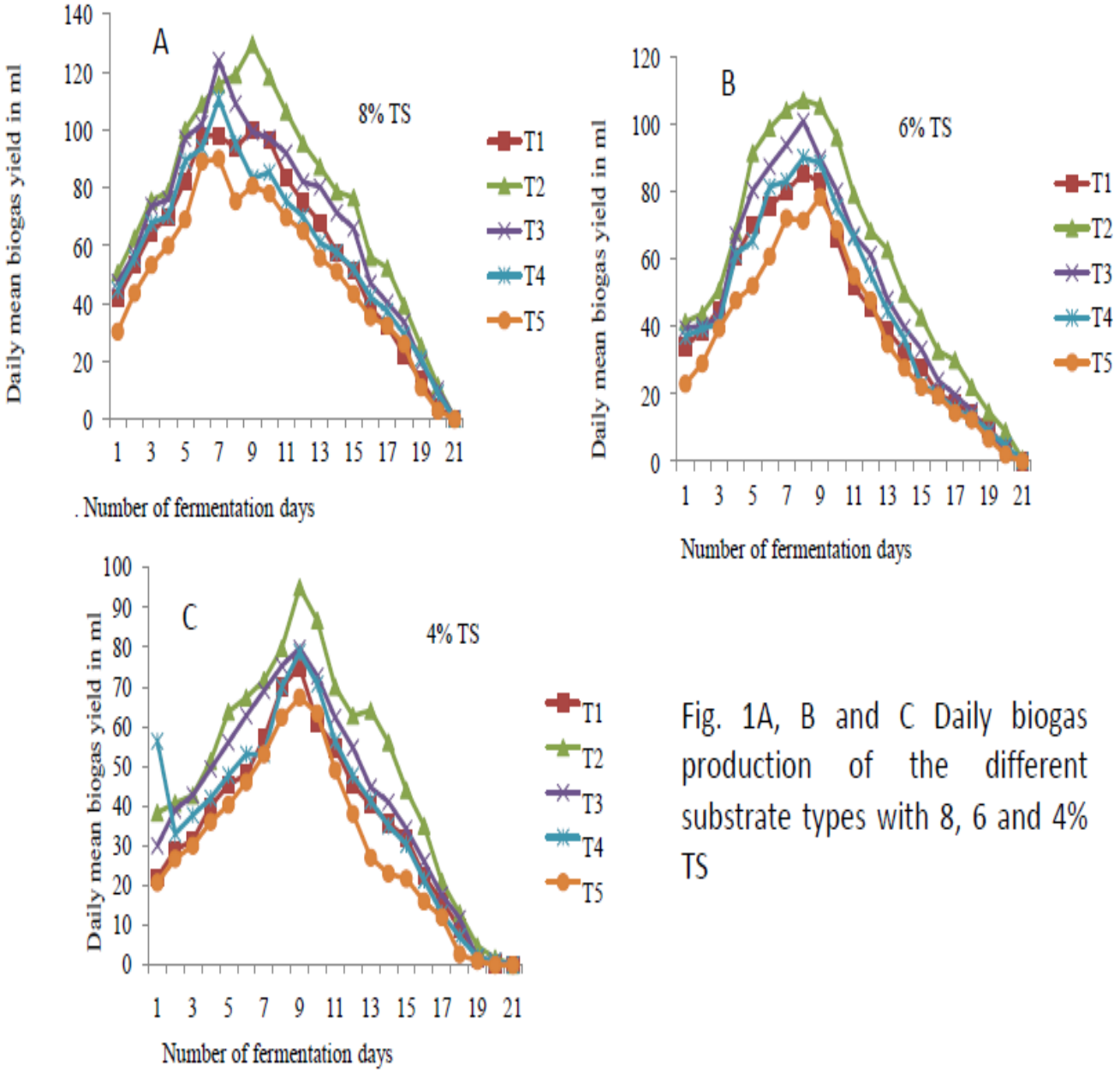

Fig. 1A, B and C Daily biogas production of the different substrate types with 8, 6 and 4% TS

Cumulative biogas yield of the three %TS of this substrate mix was compared and result showed that this substrate mix with its initial %TS adjusted to 8% was superior to 6 and 4% TS adjusted ones.

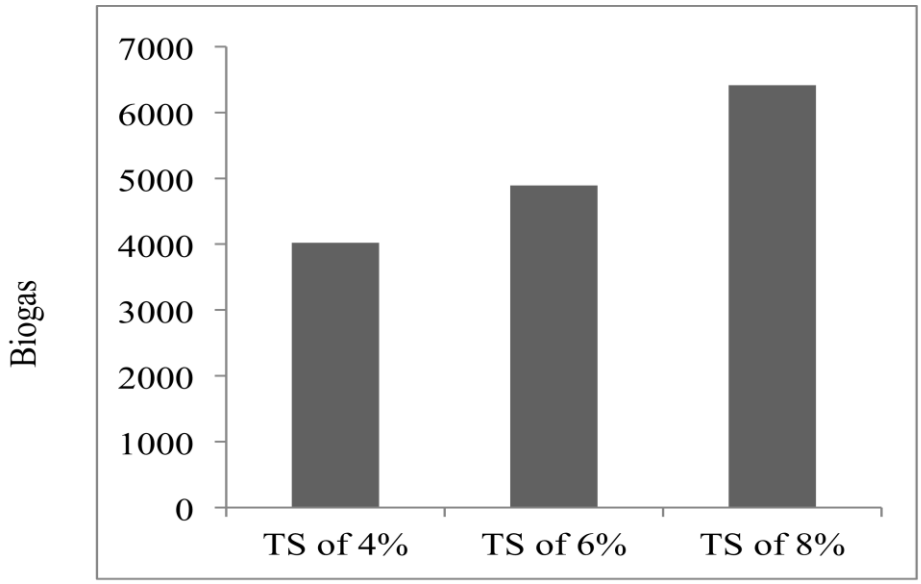

Figure 2. Total cumulative biogas yields from different concentration of TS (4, 6 and 8%)

Table 4.Total biogas production in each bio-digester for three types of adjusted percent of TS.

Bio-digester	Mix Ratio	Total Biogas for TS of 8%	Total Biogas for TS of 6%	Total Biogas for TS of 4%
T1	100%CD+0%GP	1146.2	897.9	713.6
T2	75%CD+25%GP	1532.1	1218.1	1008
T3	50%CD+50%GP	1425.4	1040.2	869
T4	25%CD+75%GP	1250.5	949.6	796
T5	0%CD+100%GP	1060.6	784	634
Total		6414.8	4889.8	4020.6
Average		1282.96	978	804.1

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

The production of biogas from agricultural, municipal and industrial wastes through anaerobic digestion can contribute to sustainable energy production. Accordingly, in this research evaluation of biogas production from groundnut shell (GS) co-digestion with cow dung (CD) with five mixed ratio (T1 (100% CD), T2 (75% CD+25%GS), T3 (50%CD: 50%GS), T4 (25%CD: 75%GS) and T5 (100% GS) under mesophilic conditions (38ºC). In all treatments, TS, VS, organic carbon, and pH were analyzed before and after digestion.

In this study, the pH was found to increase significantly with increasing of cow manure proportion in the mix, suggesting that cow manure helps to maintain the pH to meet the optimum required. The comparison of pH values between before and after AD showed that pH values are significantly decreased for all the treatment. In addition to this, maximum reduction of total solid (TS) and volatile solid were observed in T2 bio-digester (75% CD and 25% GS) after anaerobic digestion. The percent of organic carbon were significantly reduced after anaerobic digestion in all bio-digester; the highest organic carbon was recorded from 100%CD. However, C: N ratio of most of the treatments was found in between 20:1-30:1 which was suitable condition for methanogenic bacteria to reproduce and produce optimum biogas.

The daily biogas production was measured by water displacement method for 21 days. Comparison between substrate types showed that substrate mixed ratio with 75%CD and 25%GS yielded high amount of biogas in all %TS levels followed by 50%CD: 50%GS and 25%CD: 75%GS.

5.2. Conclusion

The general outcome of this study suggested that the groundnut shell co-digested with cow manure improved the biogas potential compared to cow manure alone.The highest biogas production was observed in 8% total solid with mix ratio of 75%CD and 25%GS. Therefore, it can be concluded that, this mix ratio is an optimum substrate concentration for co-digestion of ground nut shell with cow dung for biogas production

5.3. Recommendations

- ✓ Awareness and skill development training should be given on sustainable use of groundnut shell as a co-substrate for biogas production.
- ✓ Since this investigation was done at mesophilic temperature (38^{o}C), it is recommended that further study be carried out at room temperature (20^{o}C) and at thermophilic conditions (55^{o}C).
- ✓ Efforts should be made to measure the biogas quality by using gas Chromatography.

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7. APPENDIXS

Table 1: pH and % MC between Before and After AD.

Adjusted % of TS for each Treatments	Parameters			
	Before pH	After pH	Before % MC	After % MC
For 8% T1	7.22±.010	5.54±.060	10.00±.000	28.00±.001
T2	7.13±.020	6.34±.051	9.03±.057	26.00±.001
T3	7.01±.010	5.35±.050	8.20±.010	24.00±.001
T4	6.82±.020	5.03±.110	6.99±.000	22.001±.001
T5	6.51±.026	4.88±.043	6.00±.000	20.002±.001
For 6% T1	7.08±.015	5.03±.035	10.0±.000	35.001±.001
T2	6.91±.015	5.61±.015	9.03±.057	34.002±.003
T3	6.84±.052	4.96±.060	8.20±.010	32.002±.001
T4	6.67±.020	4.60±.020	6.9±.000	29.003±.002
T5	6.60±.020	4.46±.030	6.0±.000	28.003±.002
For 4% T1	7.04±.045	5.28±.015	10.00±.000	40.002±.003
T2	6.65±.030	6.30±.100	9.10±.005	39.003±.003
T3	6.52±.058	5.20±.100	8.20±.005	38.00±.004
T4	6.33±.310	4.76±.118	6.99±.000	37.00±.002
T5	6.39±.020	4.41±.020	6.00±.001	36.00±.004

Table 2: Percent (%) of TS and VS between before and After AD.

Treatments	Parameters			
Adjusted % of Ts	Before %TS	After %TS	Before % VS	After% VS
For 8% T1	90.30±.100	65.03±.057	83.3033±.005	72.31±.010
T2	90.63±.321	60.00±.005	83.4367±.055	68.00±.005
T3	90.83±.321	60.01±.010	84.4033±.005	71.01±.015
T4	92.83±.115	59.86±.055	85.50±.005	70.98±.010
T5	94.10±.100	55.03±.026	85.60±.005	70.90±.001
For 6% T1	90.10±.100	65.46±.055	83.13±.147	72.14±.066
T2	90.46±.152	63.19±.105	83.25±.083	68.60±.010
T3	92.00±.264	61.20±.100	84.39±.010	71.00±.001
T4	92.80±.100	59.92±.026	85.50±.010	71.8±.010
T5	94.13±.060	55.40±.100	85.60±.010	72.01±.010
For 4% T1	90.00±.000	65.50±.015	83.06±.109	72.01±.010
T2	90.40±.100	63.30±.10	83.12±.026	68.81±.100
T3	91.86±.152	62.0±.100	84.19±.010	71.00±.002
T4	92.66±.152	59.9667±.02082	85.23±.049	72.00±.002
T5	94.00±.208	55.5000±.10000	85.29±.005	71.01±.010

Table 3. Daily mean biogas yields from sole alone and co-digestion with CD and GS ± SE (ml) (n=3), for TS of 8%

Days	8%T1	8%T2	8%T3	8%T4	8%T5
1	41.66±2.081	50.66±1.527	47.3±2.516	44.7±1.527	30.3±8.962
2	53.33±3.511	62.6±3.05	57.3±2.516	55.67 ±2.516	43.6±3.055
3	64.66±3.214	75.6±3.055	73.7±3.055	67.7±1.527	53.3±4.041
4	70.00±2.645	78.6±1.52	76.0±1.732	70.3±1.527	60.0±7.937
5	82.33±2.516	100.0±10.00	97.0±2.645	89.3±3.055	69.0±6.557
6	98.00±2.000	109.0±6.55	102.0±2.645	93.7±3.511	89.0±4.582
7	98.00±2.000	115.6±4.04	124.00±4.511	110.7±7.57	90.0±13.228
8	93.66±3.214	119.0±2.64	109.0±5.291	95.3±5.033	75.3±5.033
9	100.00±12.16	129.6±4.50	99.3±2.081	83.3±4.041	80.6±7.637
10	96.33±4.72	118.3±1.52	97.0±2.000	85.3±2.516	78.0±8.544
11	83.33±11.93	106.3±4.50	92.0±3.605	75.3±4.041	69.7±1.527
12	75.00±5.56	95.3±2.08	82.0±5.567	70.0±1.000	65.0±2.000
13	68.00±7.00	87.3±3.78	80.3±5.507	61.0±2.000	55.7±4.041
14	57.66±3.21	78.6±7.76	71.3±1.527	57.7±2.516	51.0±2.645
15	51.33±1.52	76.6±4.93	66.0±4.000	52.0±4.000	43.3±1.527
16	38.33±3.05	56.0±4.00	47.0±2.000	42.3±3.214	35.3±5.507
17	32.33±3.05	52.3±2.51	40.3±3.055	37.3±1.527	32.3±3.214
18	22.33±2.51	39.3±3.05	33.6±3.511	29.3±2.081	26.0±3.000
19	13.66±5.50	25.3±4.16	19.6±1.527	21.0±2.000	11.0±2.000
20	4.33±3.05	11.6±3.05	10.3±1.527	9.00±1.000	3.0±2.000
21	.00±.000	.33±.288	.33±.2886	.17±.288	.0±.000
total	1146.2 ml	1532.1 ml	1425.4 ml	1250.5 ml	1060.9 ml

Table 4. Daily mean biogas yields from sole alone and co-digestion with CD and GS ± SE (ml) (n=3), for TS of 6%

Days	**6%T1**	**6%T2**	**6%T3**	**6%T4**	**6%T5**
1	34.00±4.582	41.3±4.041	39.3±1.1547	37.0±1.000	23.0±2.64
2	38.6±1.527	43.7±3.055	40.3±1.523	39.3±2.081	29.3±2.081
3	45.0±2.000	50.7±3.055	42.3±3.055	40.7±3.785	39.3±2.081
4	60.6±2.081	68.0±2.645	67.0±2.000	61.3±1.527	47.7±2.516
5	70.0±3.000	91.3±1.527	80.3±4.509	65.3±4.509	52.0±2.645
6	75.6±2.516	98.7±3.214	87.3±7.637	81.3±3.214	60.7±5.033
7	80.0±1.000	104.0±6.245	93.7±6.110	83.0±2.000	72.0±3.000
8	85.3±3.511	107.0±10.44	100.7±2.081	90.0±1.000	71.3±3.214
9	82.7±5.507	105.3±4.509	89.7±3.511	88.3±4.725	78.3±7.637
10	65.7±5.507	96.0±5.291	80.0±1.000	75.3±5.033	68.7±3.214
11	51.7±3.785	79.7±1.527	67.3±3.055	66.3±5.6862	55.0±5.000
12	45.3±4.509	68.3±7.637	61.3±8.144	55.3±4.509	47.7±2.516
13	38.7±1.527	62.7±6.429	48.0±2.645	44.7±2.081	34.7±4.041
14	32.7±2.5166	49.7±4.509	39.7±1.527	36.3±3.511	27.7±2.516
15	28.0±2.645	42.7±2.516	33.3±2.516	22.7±2.516	22.0±2.000
16	20.0±1.000	32.7±2.516	24.0±2.645	20.7±2.081	19.3±2.516
17	17.3±2.516	30.0±1.000	19.7±1.527	15.3±3.511	14.3±3.055
18	14.0±1.000	22.0±2.645	15.0±2.000	13.0±2.645	12.3±1.527
19	10.0±1.000	14.7±1.527	8.0±1.000	9.0±2.000	6.7±1.527
20	4.0±1.000	9.0±2.000	4.0±1.000	4.7±.577	2.0±1.000
21	.0±.000	.7±.577	.17±.288	.17±.288	.00±.000
total	897.9 ml	1218.1 ml	1040.2 ml	949.6 ml	784 ml

Table 5. Daily mean biogas yields from sole alone and co-digestion with CD and GS ± SE (ml) (n=3), for TS of 4%

Days	**4%T1**	**4%T2**	**4%T3**	**4%T4**	**4%T5**
1	22.0±2.000	38.3±2.081	30.0±2.000	56.3±2.081	20.7±.577
2	29.0±2.645	40.7±2.081	39.0±1.000	33.0±2.645	26.7±4.163
3	31.3±1.52	42.7±2.516	42.7±2.516	37.7±2.516	30.0±1.000
4	40.0±1.000	51.3±3.214	49.3±2.081	42.3±2.516	36.0±3.605
5	45.3±1.527	63.7±3.511	56.0±6.082	47.7±2.516	40.3±.577
6	48.0±2.000	67.3±2.516	62.7±2.516	53.0±2.645	46.0±1.000
7	57.0±2.645	71.7±2.081	69.0±1.000	53.0±2.645	53.0±2.645
8	70.0±2.000	79.7±1.527	75.3±1.527	70.0±2.000	62.3±2.081
9	75.0±5.000	95.0±5.000	79.7±2.516	78.7±3.214	67.3±3.055
10	61.3±8.082	86.7±6.110	72.7±3.785	70.7±2.886	63.3±4.163
11	54.6±5.033	70.0±8.000	62.3±3.214	56.3±2.081	49.0±1.000
12	45.3±3.055	62.7±7.023	54.7±3.055	47.7±1.527	38.0±2.000
13	40.3±1.527	64.0±2.000	44.7±3.055	41.3±1.527	27.0±1.000
14	35.6±1.527	56.0±2.000	41.0±1.000	35.0±1.000	23.0±2.645
15	32.0±2.000	44.0±2.000	34.3±1.527	30.3±2.51	21.7±2.516
16	22.3±1.527	35.0±1.000	26.0±1.000	21.3±1.52	16.0±2.645
17	16.0±1.00	21.0±1.000	17.7±1.527	13.0±1.00	12.0±1.000
18	9.3±1.527	13.0±1.000	11.7±2.081	7.0±2.000	2.7±1.527
19	1.6±1.154	4.7±2.516	1.7±1.154	2.0±1.000	1.0±.000
20	.3±.5773	1.7±1.154	1.0±.000	.83±.288	.0±.0000
21	.0±.000	.0±.00000	.0±.000	.00±.000	.0±.00000
total	713.6 ml	1008 ml	869 ml	796 ml	634 ml

Table 6.Total biogas production in each bio-digester for three types of adjusted percent of TS.

Bio-digester	Mix Ratio	Total Biogas for TS of 8%	Total Biogas for TS of 6%	Total Biogas for TS of 4%
T1	100%CD+0%GP	1146.2	897.9	713.6
T2	75%CD+25%GP	1532.1	1218.1	1008
T3	50%CD+50%GP	1425.4	1040.2	869
T4	25%CD+75%GP	1250.5	949.6	796
T5	0%CD+100%GP	1060.6	784	634
Total		6414.8	4889.8	4020.6
Average		1282.96	978	804.1

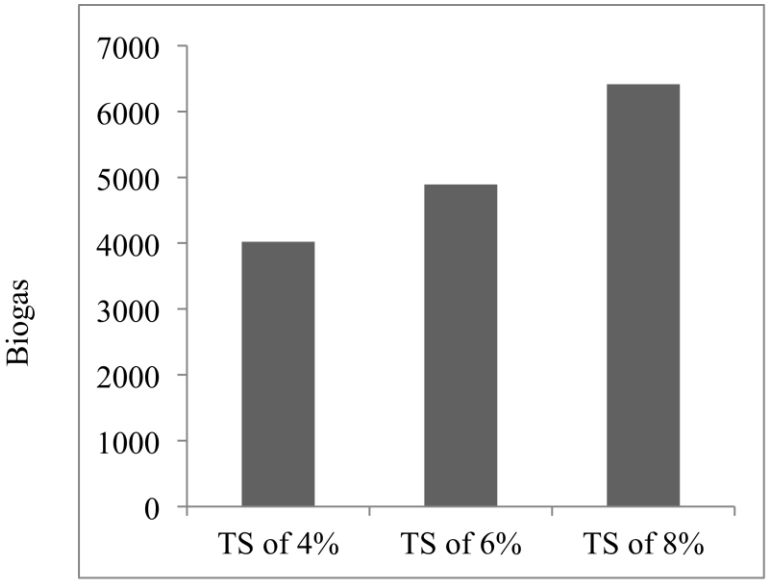

Figure 1. Total cumulative biogas yields from different concentration of TS (4, 6 and 8%)