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Optimization of the biofuel production by idealized fermentation of the animal manure, chicken wastes, and sewage sludge

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This study aims to optimize an economic procedure to produce biogas and bio-ethanol from different organic wastes such as sewage sludge (SS) and/or cattle dung (CD) and/or poultry manure (PM). The experiment was carried out at a wastewater treatment plant in Egypt. Each waste type was mixed with the starter, $CaCO_3$, and water then loaded in a fermenter and kept for 35 days at 35 °C under the anaerobic digestion. The evolved volume of the biogas and the content of methane CH4 were measured daily while the cellulase and protease enzymes were tested every four days. Results have indicated that the digester containing the SS has produced the greatest biogas volume (L) 27.45 L_b/D/d (liters biogas/digester/day), 0.61 L_b/D contents' volume/d, and cumulative 606.30 L_b/D during the 16th day. Significant CH_4 volume percentages produced during the 17th day were 72.07, 71.16, and 71.11% while the produced bio-ethanol alcohol was 2.47, 2.32, and 1.99% from the SS, CD, and PM, respectively. The procedure efficiency is prominent by the production of the biogases and *in-situ* activating enzymes all in one reactor that was periodically monitored for its reactants and product content. No need for the pre-treatment of wastes as raw materials or chemical additives and the fermented residue can be further tested for soil fertilization. These wastes can be promising for bio-energy production being economic and environment friendly.

Keywords: Anaerobic fermentation; Biofuel; Biogas; Methane; Organic wastes.

INTRODUCTION

A universal environmental request is to gradually replace petroleum-based energy sources with eco-friendly ones to decrease global warming and CO_2 gas emissions. Fossil fuels were responsible for around 93% of CO_2 emissions worldwide in 2021 (approximately 40% coal, 32% oil, and 21% gas)[1]. The continuously increased emissions of CO_2 and greenhouse gases are inhibiting many life conditions worldwide and initiating the earth planet impairment. Some developing countries should decrease their CO_2 emissions 5% below their 1990 level according to the Kyoto protocols recommendations[2]. In addition, since petroleum is the world's most widely handled energy source, beyond coal, natural gas, nuclear, hydrogen, and renewable energy, its global demand is predicted to rise by 40% in the year 2025 (FAO 2017). Thus, the limited fossil fuel supply and energy security have motivated many countries to create petroleum alternatives. For example, the energy sources in Egypt are almost dependent on fossil fuel and natural gas at a rate of 96%, coal at 1%, and renewable energy at 3%[3]. The development and optimization of renewable, biocompatible, and sustainable energy sources is a strongly recommended solution to meet the country's energy needs as clean energy and environment solutions are becoming increasingly necessary and urgent[4]. One of the solutions is to substitute the petrochemical fuels with neutral biofuels like bioethanol, biomethanol, and biodiesel as they are biodegradable, environment-friendly, and contribute to sustainability.

Biofuel is a liquid transport fuel capable to replace the petroleum fuel and is classified according to its technical specification and use, type of its raw-material source, and manufacture process. First-generation biofuel has been produced from carbohydrate-rich sources like sugar can, corn, beat and wheat via fermentation and distillation. Second-generation biofuel has been produced from lignocellulosic sources are not food crops like trees, agricultural wastes and wood processing residues as well as the municipal wastes. Third-generation types come from algal biomass[5].

Biogas-generating technologies are currently promising dual-purpose technologies since the biogas produced can meet many energy needs, while the organic residue can be used as a valuable fertilizer. Biofuel like bioethanol has been produced as a gasohol additive from sugar can or starch of maize or cassava as substrates. The lignocellulosic material is hydrolyzed to convert the cellulose and hemicellulose to monomeric sugars (saccharification step), which are then subjected to the fermentation step to produce the ethanol[6]. The saccharification and fermentation steps can be combined to operate simultaneously or separately. The simultaneous approach requires fewer reactors with greater yield of ethanol. The bioethanol has been produced from the rice straw via its chemical pre-treatment by alkali, acid followed by enzymatic treatment. Glucose was about 40% converted into bioethanol during three-day fermentation[2]. Biogas is a form of the clean and renewable energy produced from the decomposition of the animal and plant wastes[7, 8, 9, 6]. The biogas fuel can contain up to 63%–74% methane (CH_4) and with a percentage over 65% and carbon dioxide (CO_2). Methane is the highest component of the natural gas [10].

However, lignocellulosic materials processed to produce the biofuel are subjected to a number of preparation and pre-treatment steps including washing, grinding, etc., and leave several residues and byproducts either utilized or discarded that worsen the ecosystem[5, 11]. Organic wastes utilized as biodegradable source alternatives of the fossil fuels for energy aspects are eco-safe approach to discard wastes along with producing energy, terminating the natural carbon cycle and saving fossil fuels[9, 12, 13]. Conversion of organic wastes into valuable bioenergy

and bio-chemicals utilizing microorganisms is a bio-waste management attractive approach. For example, the Microbial Fuel Cell (MFC) is one of the perfect solutions treat wastewater while producing electricity. It is a bio electrochemical process that aims to produce electricity by using the electrons derived from biochemical reactions catalyzed by bacteria[14]. The complex organic compounds such as proteins, carbohydrates, and lipids are transformed into simple soluble molecules like sugars, amino acids, and fatty acids by association of microorganisms like microbes, yeast and bacteria strains producing the bioenergy and many valuable bio-chemicals[1].

The population increasing creates substantial quantities of foods and organic wastes that comprise complex carbohydrates like starch, cellulose, and hemicellulose resistant to degradation. Environmental policies encourage the organic wastes use and managing practices to produce energy and reduce greenhouse gas emissions. Valorization through anaerobic digestion, composting, and mulching to produce useful products (like ethanol) are possible managing practices. Anaerobic digestion is the decomposition of organic matter in absence of the oxygen, while composting is an aerobic decomposition of the organic matter. Depending on the composition of the organic waste, these strategies result in methane and organic products can be used as fertilizers. Some enzymes are produced that play essential roles in the decomposition of the cellulose, starch, and lignin to simple carbohydrates/sugars utilized by microbes for their growth and metabolic activities. During the fermentation and decomposition processes, the fungal/ bacterial cellulases hydrolyze cellulose into oligoglucans, and cellobiose is further converted into to glucose by β-glucosidase[15].

The biogas has been produced from the organic wastes through the anaerobic digestion that is the biological breakdown of organic matter under anaerobic conditions by the bacterial flora. It could be efficiently produced from the macro-algae biomass depending on the chemical composition of the biomass and algal taxonomy[16, 17]. The anaerobic digestion for the biogas production is carried out via four phases[18]: (i) *Hydrolysis*: the macromolecules are cut gradually into soluble monomers by extra-cellular enzymes (e.g. cellulases, hydrolases, amylases, etc.), (ii) *Acido-genesis*: the monomers formed by the hydrolysis are converted into organic acids and alcohols to release ammonium, CO_2, and hydrogen H_2, (iii) *Aceto-genesis*: the products of acido-genesis are converted into acetic acid, but CO_2 and H_2 are the main substrates of the methano-genesis, (iv) *Methano-genesis*: The final step is the formation of the CH_4 as two separate channels, the acetate and the mixture of H_2/CO_2[19]. The main steps are the pretreatment, enzymatic hydrolysis, and fermentation of sugars. Pretreatment and enzymatic hydrolysis are considered as major cost-centers and their optimization in cost-effective way is one of the major challenges in biofuel fabrication from the biomass. Future research seeks to optimize the pretreatment and enzymatic hydrolysis processes for specific types of organic and bio-wastes by microorganisms[1].

It has been found that the biogas produced by the anaerobic digestion of the two substrates (sewage sludge and landfill) is combustible, with a CH_4 content over 64%. The volume collected from the sludge wastes is ten times more than the volume of the biogas produced from the organic matter in the landfill. The volume of the produced biogas is always a function of the residence time of digestion and the concentration of organic matter in the experiment. The process depended on the microbial activities with the excretion of particular extracellular enzymes that break down the chemical bonds. The attachment of microbes to particle-producing enzymes in the particle region is the principal mechanism of hydrolysis, making the solubilization process possible. Incorporating the fermentation and anaerobic digestion into a bio-refinery can allow the generation of ethanol and biogas with improving the energy balance[20].

Thus, the main objective of this research is to evaluate the production biogas and bio-ethanol being the most widely utilized biofuels during the fermentation of different organic wastes including the sewage sludge (SS) and/or cattle dung (CD) and/or poultry manure (PM).

METHODS

Materials and tools

Fresh wastes were collected in Kafr El-Sheikh Governorate**: Sewage sludge (SS)** was from a wastewater treatment plant in Sakha, **Cattle dung (CD)** was gathered from an Animal Production Research Centre, while the **Poultry manure (PM)** was from a fattening poultry farm (PF) in Desouk. They were utilized as fresh as possible within 1–2 days. The raw materials' physical, chemical, and biological properties are shown in Table 1.

Table 1. Physical, chemical, and biological characteristics of the raw materials

Type of characteristics		Sewage Sludge (SS)	Cattle dung (CD)	Poultry manure (PM)
Physical Analysis	Moisture content (%)	90.0	75.0	82.0
	Total solid (%)	10.0	25.0	18.0
	pH	7.40	7.20	8.07
	EC (dS/m)	11.73	2.15	7.06
Chemical Analysis	Ammonia nitrogen ppm	741.0	214.0	1384.0
	Nitrate ppm	69.0	63.0	318.0
	Total Nitrogen %	2.01	1.64	2.28
	Organic matter %	58.46	77.56	42.78
	Organic carbon %	33.91	44.98	24.0
	Ash %	41.54	22.44	57.22
	Carbon / Nitrogen ratio	17:10	27:4:1	11:1
	Total Phosphorus %	0.90	1.03	1.22
	Total Potassium %	1.86	0.98	2.41
Biological Analysis	Total coliform bacteria (10^3 cfu/g)	40×10^3	70×10^3	105×10^3
	Fecal coliform bacteria (10^3 cfu/g)	25×10^3	44×10^3	73×10^3
	Salmonella, shigella (10^2 cfu/g)	13×10^2	23×10^2	$33x\ 10^2$

Starter: The starter was collected from an old operational biogas digester at the Agriculture Research Centre at Moshtohor, Kalubia Governorate, Egypt's Training Centre for Biogas and Recycling of Agricultural Residues (TCRAR).

Calcium carbonate ($CaCO_3$): It was used at the rate of 2.5% from initial total solids for pH buffering.

Digesters: Three units of Plastic anaerobic digesters were used in this experiment, as shown in Scheme 1. The first digester had a total volume of 60 litres and an active volume of 45. This digester was used in the anaerobic digestion of SS. The second and third digesters had a total volume of 45 litres with an active volume of 30 L for each, then they were used for the CD and PM anaerobic digestion.

Scheme 1. Digester units

Experimental and biogas reactor design

This experiment was set up at a wastewater treatment plant in Sakha, Kafr El-Sheikh Governorate, Egypt to evaluate the production biogas and bio-ethanol from different organic wastes sewage sludge (SS) and/or cattle dung (CD) and/or poultry manure (PM).

Three treatments of biogas mixtures were prepared as follows: The first digester: 33.750 L of SS + 11.250 L of starter + 112.5 g of $CaCO_3$. The second digester: 9 kg of CD + 7.500 L of starter + 13.500 L of water + 75 g of $CaCO_3$. The third digester: 12.500 kg of PM + 7.500 L of starter + 10 L of water + 75 g of $CaCO_3$.

The initial total solid reached 10% in all fermenters. The mixtures were loaded in fermenters and kept for 35 days under the condition of anaerobic digestion at a 35 °C temperature range. The evolved volume of biogas was measured daily, while their content of CH_4 was estimated every day throughout the experimental period. Also, all fermenters were tested for the cellulase and protease enzymes and ethanol percentage every four days.

Methods of analyses

Physical determination

Moisture content: The samples were dried to consistent weights at 105 °C [21]. Every material's moisture content was calculated as a percentage.

Total solids (TS) were estimated according to the[22].

pH Value: The pH values were directly determined in liquid samples or 1:10 distilled water extract in biogas manure utilizing a glass electrode of Orion Expandable ion analyzer EA920[23].

Electrical conductivity (EC): Electrical conductivity estimations were run in (1:10) biogas manure: water extract[24], utilizing EC meter I.C.M. model 71150.

Chemical determinations

Organic matter (OM) content of the compost materials was determined by glowing the biogas manure dried samples at 550 °C to a constant weight as suggested. **Ash**: Ash content was calculated using the following equation: % Ash = 100 – OM[21].

Organic carbon (OC) content was calculated by the equation: OM dry weight × 58%[25].

Ammoniacal and nitrate nitrogen: Soluble nitrogen forms NH_4^+ and NO_3, were estimated in samples according to the methods outlined[21].

Total nitrogen (TN): Total nitrogen in dried samples was determined utilizing the Kjeldahl digestion method. **Total phosphorus (TP)**: Total phosphorus was estimated utilizing a Spectrophotometer (model 670 SUV/VIS Jenway Company) in the acid solution of the digested samples using ascorbic acid and mixed reagent. **Total potassium (TK)**: Total potassium was estimated utilizing a flame photometer (model ILAE 201 Fisher Scientific Company) in the acid solution of the digested samples[25].

Cellulase Enzyme activity: Cellulase activity was measured using 5 g of sample was incubated with 20 mL of 2% carboxymethyl cellulose in 50 mM sodium acetate buffer (pH 5.5) at 30 °C for 24 h[26]. After incubation, the suspension was blended well and centrifuged for 20 min at 24148.8 Xg. The supernatant was targeted to reduce sugar analysis utilizing the dinitrosalicylic acid (DNS) method[27]. Exactly 2 mL of the supernatant were added to 2 mL of DNS solution (1 L of DNS reagent contained: 1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, and 1% NaOH) and boiled. Samples were immediately cooled, and the absorbance was estimated at λ = 575 nm. A calibration curve was set up with the various concentration of glucose (0–300 μg/mL), and the enzyme activity was expressed as μmole glucose/g sample/h.

Protease activity: Protease enzyme hydrolyses peptide bonds. When the protease enzyme digests casein (the pretentious substrate), the liberated free aromatic amino acids like tryptophan, tyrosine, and phenylalanine, corresponding to total amino acid content, are measured in the UV spectrum compared to standard tyrosine. Protease activity was determined[28] as follows: The reaction mixture was made of 1g of fresh soil and 1 mL of 1% casein solution in 50 μm Tris-HCI buffer (pH 8.1) and mixed. This mixture was incubated for one h at 37 °C, the reaction was terminated by adding 3 mL of cold (2 °C) 10% trichloroacetic acid, and the tubes

were allowed to stand for one h at 2 °C in a refrigerator to allow the undigested protein to precipitate. After precipitation, the mixture was centrifuged at 4000 rpm at room temperature for 30 min, and the supernatant was measured at λ = 280 nm. A standard curve was established using different concentrations of tyrosine. One protease unit was defined as the amount of enzyme that released 1.0 μM of tyrosine/g soil/h under assay standard conditions.

Determination of ethanol: Ethanol concentration was determined using the methods of Caputi et al.[29]. About 1 mL sample and 20 ml distilled water were mixed with 0.15 g NaOH in a 50 mL Erlenmeyer flask. The mixture is then raised to its boiling point and maintained for 2–3 minutes. Next, the ethanol solution was delivered to an Erlenmeyer flask containing 25 mL potassium dichromate reagent and incubated at 60 °C for 20 min. The absorbance was recorded at λ = 600 nm on a spectrophotometer (Perkin- Elmer Mod. 55E). The dichromate reagent was prepared by dissolving 34 g of potassium dichromate in 325 mL of pure H_2SO_4 and the final volume was adjusted to 500 mL with distilled water.

Microbiological determinations

Total and fecal coliform bacteria: As specified by Mac-Conkey, six plates were inoculated with 1 cc of the required dilution and poured with Mac-bile Conekey's salt medium[30]. Half of them were incubated for 24 h at 35–37 °C. Total coliform bacteria were counted on one plate, while fecal coliform bacteria were counted on another plate that had been incubated at 44.5 °C for 48 hours. Coliform group bacteria were red, pink, or practically colorless colonies with pink centers.

***Salmonella* and *Shigella* count**: The inoculated plates containing *Salmonella* and *Shigella* agar medium were incubated at 35–37 °C for 24 h. Black-cantered colonies were counted as *Salmonella* and *Shigella* microorganisms.

Gas determinations

Gas yield: biogas evolution was evaluated using a displacement approach that used acidified water to avoid carbon dioxide solubilization[31].

Methane content: it was determined daily by the *Multitec 540* strategy[32], which is a gas measuring device for analyzing gas mixtures formed in biological processes.

Statistical analysis

Every treatment (Data means) was repeated three times with control to see how different mixed percentages affected biogas output. A blank test was carried out in parallel with the assay bottle. Descriptive statistics were done by using Microsoft EXCEL version 14.0.4734 (2010).

RESULTS

Daily biogas and methane CH_4 production per digesters and cumulative production per day

During the experiment, a record of daily biogas volume production from the digesters was taken. The obvious results over the thirty-five days of experimental for the three feedstock materials are shown in Figure 1. During the experiment, the volume of biogas (L) per total volume of the digester (L_b/D/d) for each day and the number of biogas liters per litter digester (L_b/L_D/d) were determined. In the sludge treatment, the number of liters of biogas per day increased from the 15th to the 17th day (27.01 to 27.45 L_b/D/d), and the highest value was during the 16th day.

Meanwhile, the best values of L_b/D/d with CD and PM were during the 16th and 17th days when it was 12.95 and 13.39 L_b/D/d, respectively. On the other hand, the digester contents sludge was superior, where it was achieved at 27.45 L_b/D/d.

On the other hand, the maximum volumes (L) of biogas per L from digester contents (L_b/L_D/d) per day

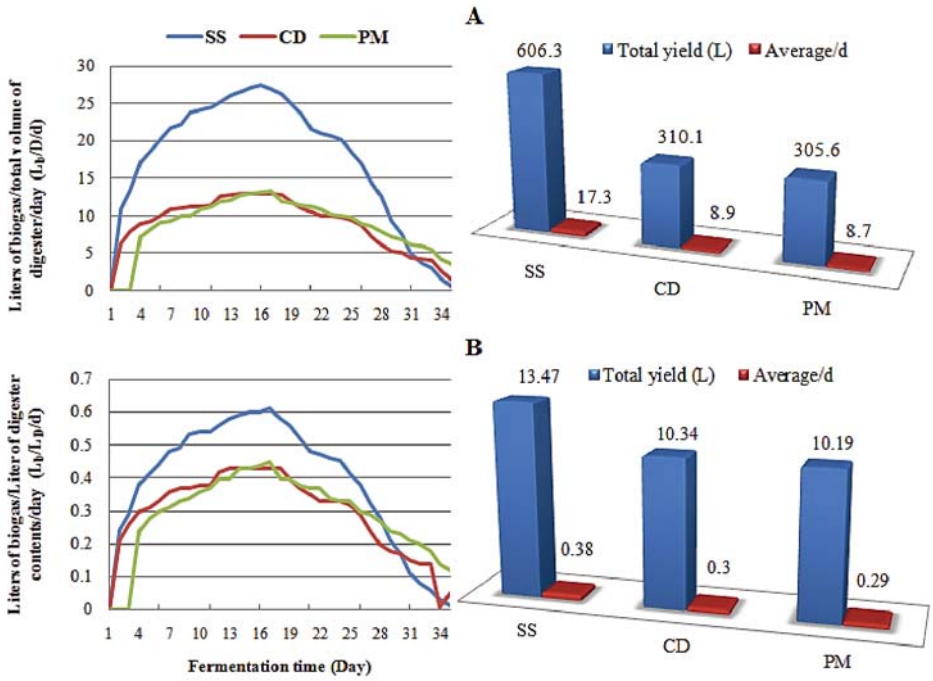

Figure 1. Biogas daily production during the anaerobic digestion of the sewage sludge (SS), cattle dung (CD), and poultry manure (PM). A – Quantity (L) of biogas/total volume of digester/day, B – liters of biogas/liter of digester contents/day, L_b/D/day: liter/digester /day L_b/L_D/ day: liter biogas/ liter digester/day. Each value is the mean of triplicate records

were achieved during the 16th day from the fermentation process, where it was 0.61, 0.43, and 0.45 L_b/L_D/d in digesters contained SS, CD, and PM respectively.

The results observed in Figure 2 revealed that the liters of CH_4/digester/day (L_m/D/d) reached maximum value during the 16th day fermentation process with both digesters content SS and CD; they recorded 19.78 and 9.22 L_m/D/d, respectively.

In contrast, the PM treatment during the 17th day was 9.52 L_m/D/d. Also, the same treatments with the same periods achieved the highest liter CH_4/litter digester/day (L_m/L_D/d), where they obtained 0.44, 0.31, and 0.32 L_m/L_D/d with the three digesters, respectively.

The accumulative biogas volume per day (Cumula L/Dig.) in Figure 3 was increased gradually until reaching the most significant values at the end of the fermentation of the biogas process. The highest values of accumulative volume (total yield) of biogas per day were 606.30, 310.14, and 305.58 Cumula. L/Dig. in the three digesters, respectively. The superior yield of L_b/D/d was in digester containing the sludge (606.30 L_b/D/d by an average17.32 L_b/day), followed by digesters containing the CD (310.14 by an average 8.86 L_b/day) while the minimum value was for the PM (305.58 by an average 8.73 L_b/day).

Also, data shows the total yield of L_b/L_D/ day was the best for the SS treatment (13.47 by average per day 0.38 L_b/day), while the PM showed lower values: 10.19 by average per day 0.39 L_b/day.

The digester contents SS was still superior in producing L_m/D/d and L_m/L_D/d. The accumulative liter of CH_4/digesters in Figure 3 was found to be 372.17, 185.05, and 177.82 Cumula L/Dig., in the mentioned treatments respectively. The treatment of SS recorded the highest total yield of L_m/D/d (372.17 by average/day 10.63 L_m/day) and L_m/L_D/d (8.27 by average/day 0.24 L_m/day). On the contrary, the treatment of PM was the lowest. Similar results have been reported[33]. The ratio 1:1 mix of PM and cow dung yielded a better CH_4 yield than each digested singly as a mono substrate. Also, it had been reported that the superior CH_4 yield from cow manure to the presence of unique micro-flora characteristics of the manure[34]. It was also found that CH_4 production

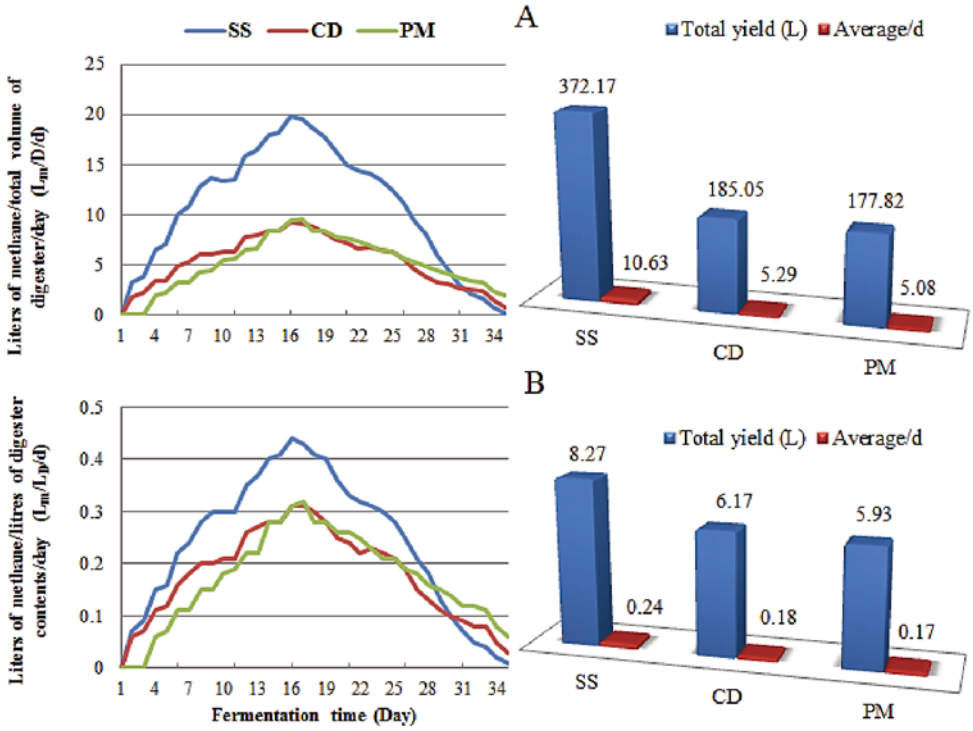

Figure 2. Methane daily production during the anaerobic digestion of the sewage sludge (SS), cattle dung (CD), and poultry manure (PM). A – Quantity (L) of methane/total volume of digester/day, B – liters of methane/liter of digester contents/day, L_m/D/day: liter/digester /day L_m/L_D/ day: liter methane/ liter digester/day. Each value is the mean of triplicate records

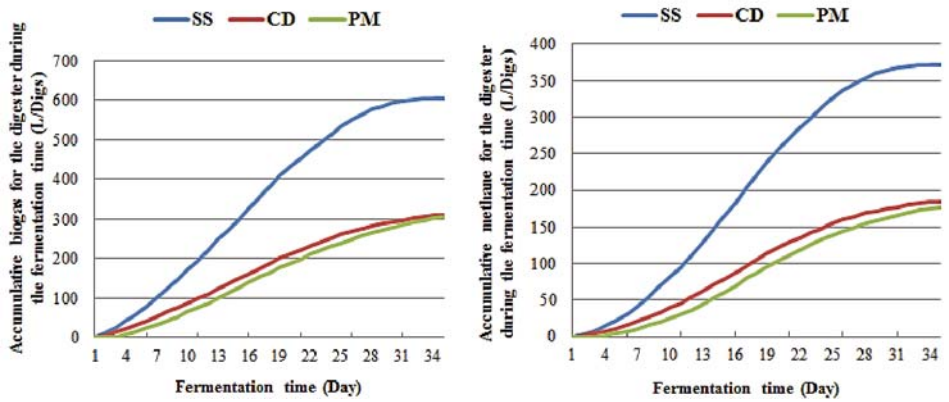

Figure 3. The accumulative production of the biogas and methane from the studied wastes during the fermentation time (days)

has slightly decreased with total solids concentrations increasing from 10 to 25% in batch anaerobic digestion of cardboard under meso-philic conditions.

The results agree with Afifi et al.[35]. They found that the most significant volume (L) of biogas yield was obtained during the fifth week of the fermentation process, with the optimal mixture treatment yielding 50.46 L of biogas per digester each week. Also, the final experiment's cumulative litter biogas/digester was 285.33, 300.54, and 329.95 in digesters containing CD, chicken manures, and mixture, respectively. Also, it has been found that the cumulative biogas and CH_4 were higher in digester "2" and digester "1" than in digester "3", and digester "4" contained household solid wastes at different rates of total solids [36]. The biogas and CH_4 production rates (L/kg) for volatile solids consumed were 953.73, 755.62, 755.62, and 213 for biogas; meanwhile, CH_4 was 498.88, 376.82, 25.30, and 13.44 in digesters B2, B1, B3, and B4, respectively.

Periodical CH_4 quantity percentage (%) of the produced biogas during anaerobic digestion of different feedstock

The results observed in Figure 4 cleared that the biogas production level differs significantly among different sources at different periods ($P < 0.05$) of the experiment. The highest level of CH_4 production was observed in sludge treatment on the 17th day as its level reached 72.07%, followed by period 19th day as its level reached 71.25%, followed by its level on period 21st day where its level went 69.14%. While the second level of CH_4 production was observed in PM as its higher level on the 17th day level reached 71.11%, followed by its level at 19 days as its level reached 70.19, followed by its level on the 21st day where level went 68.28%.

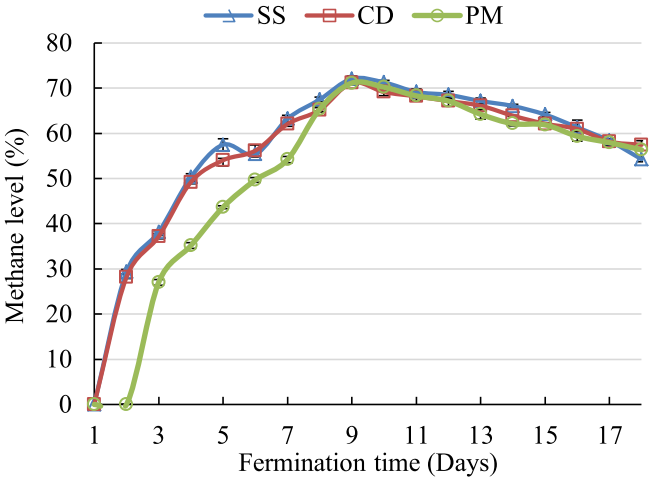

Figure 4. Periodical methane percentage (%) of the produced biogas during the anaerobic digestion of the sewage sludge (SS), cattle dung (CD), and poultry manure (PM). Error bars represent the standard deviation (SD)

The lower level of CH_4 in biogas production was observed in CD where its higher level was observed at 17 days as level reached 71.16%, followed by its level at 19 days of an experiment where its level got 69.13% and at 21 days as its level got to 68.17%. The best treatment of CH_4 of biogas production in sludge and the higher level of CH_4 of biogas production were observed from 17 to 21 days of the experiment.

It had been reported that different organic manures contain microorganisms that are important for the methanogenesis process[34]. Their combined influence may have led to the CH_4 output from the kitchen trash. Additionally, the growth of acetogenins and acidogenic bacteria, which transform hydrolysis products into by-products needed by methanogens bacteria during the CH_4-forming stage, is necessary for the survival of methanogens bacteria[37].

Effect of different treatments on the cellulase enzyme levels among different periods

The results observed in Table 2 showed that the cellulase enzyme level differs significantly among different sources at different periods of an experiment where the higher level of cellulase enzymes was observed in sludge for 12 days (1228.24 µ/mL) followed by its period at 16 days it reached to 1173.44 µ/mL then followed by its level at period eight day where it is obtained to 1056.24 µ/mL. The second level of cellulase enzymes was observed in the treatment of CD as its higher level was observed at 12 days as its level reached 1226.64 µ/mL, followed by its level at eight days as its level recorded 994.64 µ/ml, followed by its level at 24 days where its level recorded to 662.24 µ/mL. The lowest level of cellulase enzymes was observed in PM treatment where its higher level was observed at 16 days as its level reached 965.83 µ/mL, followed by its level at 12 days of an experiment where its level was recorded at 905.44 µ/mL and at 20 days as its level gone to 866.64 µ/ml. In general, the cellulase enzymes showed a higher level in sludge than in CD and PM treatments. This means sludge in the first fermentation process was the best raw material used for the biogas process and is faster than other materials. The highest level of the cellulase enzymes was observed in

Table 2. Changes in cellulase enzyme (µ/ml) during anaerobic digestion of the studied feedstock

Time (Days)	Cellulase enzyme (µ/ml) [Mean ± SD]		
	Sewage Sludge (SS)	Cattle dung (CD)	Poultry manure (PM)
0	595.44 ± 0.67 Ga	564.24 ± 99.77 Eb	527.84 ± 1.93 Hc
4	628.24 ± 1.48 Fb	641.04 ± 0.99 Fa	599.04 ± 0.55 Ec
8	1056.24 ± 1.59 Ca	994.64 ± 1.69 Bb	809.44 ± 0.59 Dc
12	1228.24 ± 0.64 Aa	1226.64 ± 1.30 Ab	905.44 ± 0.57 Ac
16	1173.44 ± 0.29 Ba	661.84 ± 0.60 Cb	965.83 ± 0.39 Bc
20	1047.04 ± 0.53 Ca	660.24 ± 0.96 Cc	866.64 ± 6.80 Cb
24	831.44 ± 0.35 Db	662.24 ± 0.63 Cc	854.64 ± 3.50 Ca
28	735.04 ± 0.60 Ea	594.24 ± 1.15 Db	554.67 ± 3.80 Fc
32	549.84 ± 0.49 Gb	567.04 ± 0.54 Ea	543.84 ± 0.65 Gc
35	411.17 ± 24.28 Hb	560.24 ± 1.97 Fa	297.61 0.56 Id

– Capital letters indicated that: Means within the same column of different litters are significantly different at (P < 0.05).
– Small litters showed that: Means within the same row of different litters are significantly different at (P < 0.05).
– Each value is the mean of triplicate records; SD: Standard division

12 days in both sludge and CD, while the period of 16 days was the best in the PM treatment.

Effect of different treatments on the protease enzyme levels among different periods

A higher level of protease enzymes was observed in PM at 16 days as its level reached 46.26 μ/mL followed by its level at 20 days as level reached 43.88 μ/mL, followed by its level at 12 days where its level reached 41.47 μ/mL. This may be due to PM's high nitrogen contents as well as high protein content[38].

The second level of protease enzymes observed in sludge was its higher level observed in it at 16 days as it reached 43.22 μ/mL, followed by its level at 20 days as level reached 41.88, followed by its level at 24 days, where its level went 37.84 μ/mL. On the contrary, a lower level of protease enzymes was observed in CD where its higher level was observed at 16 days as its level reached 34.57 μ/mL, followed by its level at 20 days of an experiment where its level reached to 32.36 μ/mL and at 24 days as its level reached to 29.13 μ/mL. The protease enzymes showed a higher level in the PM, sludge, and CD. A higher level of protease enzymes was observed from 16 days of the experiment, as observed in Table 3.

One frequently used indicator for the operational control of biological processes is enzyme activities. These enzymes catalyze the principal, additional, and alternative metabolic pathways. For example, several enzymes participate in the multi-step process of anaerobic digestion. Still, some of them can be used as indicators of the overall metabolic activity of the microbial community ex. dehydrogenase, cellulase, protease, and lipase[39, 40].

Effect of different treatments on ethanol production level

The results in Figure 5 indicate that the ethanol production level differs significantly among different sources at different periods ($P < 0.05$) of the experiment. The highest level of ethanol production was observed in the SS treatment after 16 days (2.47%) then after 20 days as it reached 2.23%, followed by the 24 days where its level reached 2.16%. The ethanol production in the CD treatment in the 16 days has reached 2.32%, and then it has reached 2.22% and 2.11% at the 24 days and 20 days, respectively. The PM has exhibited the lowest level of ethanol production that was 1.99% at 20 days, 1.97% at 16 days, and then it became 1.50% at 24 days.

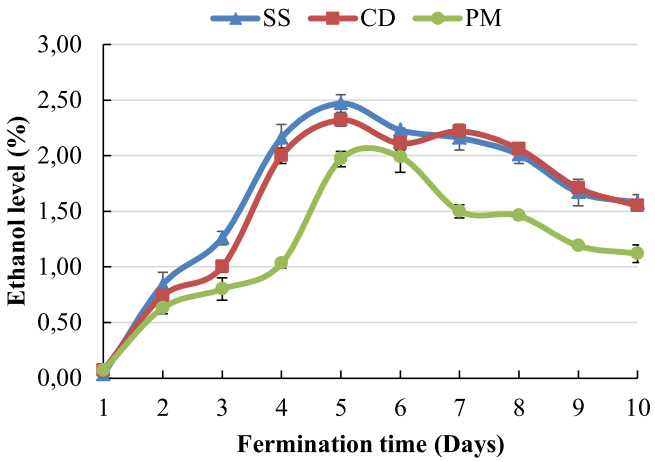

Figure 5. Ethanol percentage (%) produced during the anaerobic digestion of the sewage sludge (SS), cattle dung (CD), and poultry manure (PM). Error bars represent the standard deviation (SD)

DISCUSSION

Authors in this work have tried to assess the possibility of an economic bioethanol production during the biogas fermentation process being it is a by-product because bioethanol production is considered a costly--complicated technology[2, 6, 17, 9]. This study presents a low-cost procedure for the ethanol production during the fermentation process, which was followed at periods' intervals so that it is possible to limit the best amount of ethanol produced at a specific period. Then, the fermenter could be stopped at this time to collect the ethanol that can be further purified. Ethanol could be obtained during the biogas production process. An additional economic advantage is that one reactor or bio-refinery that incorporates both anaerobic digestion and fermentation enable the generation of both biogas and ethanol from one type of the feed stock waste[41]. All biochemical and enzymatic reactions that involved in the anaerobic digestion and fermentation processes are started and completed *in-situ* without any need for additional containers. The status of different chemical and biological phases could be monitored periodically as well as the daily and cumulative volumes of the different products including the methanol and ethanol gases. No need for a pre-treatment step for the wastes as reactants' raw materials and no need for chemical additives to complete the reactions.

Table 3. Changes in protease enzymes (μ/ml) during anaerobic digestion of the studied feedstock

Time (Days)	Protease enzyme (μ/ml) [Mean ± SD]		
	Sewage Sludge (SS)	Cattle dung (CD)	Poultry manure (PM)
0	27.81 ± 0.98 Ga	19.73 ± 0.55 Gb	28.79 ± 0.11 Ga
4	28.99 ± 0.91 Fb	21.54 ± 0.36 Fc	30.33 ± 0.55 Fa
8	35.00 ± 0.26 Da	23.42 ± 0.34 Ec	36.01 ± 0.39 Db
12	36.11 ± 0.12 CDb	27.03 ± 0.49 Dc	41.47 ± 0.37 Ca
16	43.22 ± 0.32 Ab	34.57 ± 0.31 Ac	46.26 ± 0.33 Aa
20	41.88 ± 0.08 Bb	32.36 ± 0.41 Bc	43.88 ± 0.34 Ba
24	37.84 ± 0.58 Ca	29.13 ± 0.89 Cc	36.03 ± 0.14 Db
28	33.30 ± 0.57 Ea	23.35 ± 0.52 Eb	33.37 ± 0.32 Ea
32	26.97 ± 0.30 Ga	19.95 ± 0.19 Gb	25.14 ± 0.17 Ha
35	19.70 ± 0.38 Ha	16.16 ± 0.82 Hc	17.54 0.36 Ib

– Capital letters indicated that: Means within the same column of different litters are significantly different at (P < 0.05).
– Small litters showed that: Means within the same row of different litters are significantly different at (P < 0.05).
– Each value is the mean of triplicate records; SD: Standard division

The hydrolysis of lignocellulose is the rate-limiting step in anaerobic digestion[6, 2]. Enzymatic catalysis by microorganisms or added during fermentation process promotes the lignocellulose hydrolysis, breaking it down to lower molecular weight substances, which are ready to be utilized by the methanogen's bacteria. It could be indicated by higher enzymatic solubility and fatty acid concentration in the hydrolytic bioreactor with the increased biogas production[15, 40, 42].

The cellulase enzymes showed a higher level in the SS than in CD and PM treatments. This means sludge in the first fermentation process was the best raw material used for the biogas process and is faster than other materials. The highest level of the cellulase enzymes was observed in 12 days in both sludge and CD, while the period of 16 days was the best in the PM treatment. Table 1 show that the difference between the chemical characteristics of the SS and CD and PM may be responsible for their processing efficiency for the biogas production being the SS an optimal raw material. The SS was 10% total solids' percentage, neutral pH and saline more than the CD and PM. It had middle percentage values of its nitrate, ammonia and total nitrogen, organic matter, organic carbon, ash, C/N ratio, and total potassium as well as minimum content of the total coliform and Fecal coliform bacteria (10^3 cfu/g) along with the Salmonella, shigella (10^2 cfu/g) content. These properties perhaps had offered a suitable biological and chemical medium for starting and terminating the enzymatic activities and chemical reactions necessary for the production of the biogases under estimation compared to the CD and PM.

CONCLUSIONS

Wastes' recycling in this study via the fermentation of the sewage sludge (SS), cattle dung (CD), and poultry manure (PM) can allow the clean energy production by utilizing the produced biogas and ethanol. The SS was the most efficient raw material for producing the biogas, methane CH_4, and bio-ethanol. Its production was ~ 606.3 L cumulative/digester, 72.07%, and 2.47%, respectively. This may be attributed to a more suitable biological and chemical medium offered by the SS for starting and terminating the enzymatic activities and chemical reactions necessary for the production of the biogases under estimation compared to the CD and PM. The suggested procedure was economically efficient as it utilize one reactor, free of chemical additives, no pre-treatment of raw materials and easily monitored throughout the different stages of biogas production. Compared to the lignocellulosic materials, the SS, CD, and PM are promising sources for the bioenergy production as they are economic, and their recycling is considered environment friendly.

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