

Evaluation and Optimization of Methane Production from Different Manure Types

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Abstract

Animal manure represents an important biomass source for the production of renewable energy. In Jordan, thousands of tons of animal manure are produced annually from cows, sheep and chickens. This study aims at studying the production of methane from cow, sheep, and chicken manure to determine which type of manure gives the highest yield of methane. This study is also aimed at evaluating the effects of incubation temperature, initial pH, and trace-element supplementation (nickel, iron, zinc, copper, cobalt, manganese and boron) on the production of methane. Finally, the methane yield under optimal growth conditions will be evaluated. Methane was successfully produced from cow manure in tightly-closed stainless steel digesters that yielded the highest methane production (10 %) after thirty-five days of incubation compared to other types of manure. No methane was detected in the case of chicken manure, and only 0.1 % was detected in the case of sheep manure. The production of Methane from cow manure was then optimized using the one-factor-at-a-time method. The Methane yield increased to 47 % when incubation was done at 40°C compared to the room temperature incubation (22.9 %) and 30°C (26 %). It was also found that methanogenesis was high at acidic pH (pH 4; 42 %) when compared to other pH values (pH 5 or 6). In other experiments, the effect of trace-element supplementation was positive with the methane yield being three times greater in the presence of trace-element supplementation than that without supplementation. Finally, methane was produced under optimized conditions (40°C, pH 4, with trace-element supplementation) with the yield being higher than any other experiment (68.5%). This study highlights the feasibility of methane production from local manure types and the optimized conditions under which methane can be overproduced.

Keywords: Methane production, Optimization, Cow manure, Jordan

1. Introduction

The need for energy to assure the continuity of various human activities is obviously expanding (Owusu and Asumadu-Sarkodie, 2016). Energy can be generated from different sources that are classified into renewable and nonrenewable sources. Biomass, hydropower, wind, and solar energies are few examples of renewable energy sources, whereas petroleum, coal, and uranium are examples of the nonrenewable sources. Being a renewable energy source, Biomass is believed to have a high potential for satisfying a significant part of the global energy needs (Demirbas, 2009).

Studies have indicated that there are about 1.4 billion people in the world still lacking access to electricity especially those living in rural areas (Kaygusuz, 2012). As a result, the number of rural communities depending on energy from biomass is predicted to rise from 2.7 billion nowadays to 2.8 billion in 2030 (Owusu and Asumadu-Sarkodie, 2016; Kaygusuz, 2012). Biomass used for energy production is quite versatile and includes animal manure, municipal solid waste, waste from food

processing, agricultural crops and their waste byproducts, wood and wood wastes, and aquatic plants and algae (Demirbas *et al.*, 2009).

In Jordan, there are different biomass-sources that can be utilized for energy production including animal waste. It is estimated that more than 65511 tons of animal manure are produced annually from three types of animals (cows, sheep, and chicken); there are about 96100 cows, 2.5 million sheep, and 3.77 million chickens, producing about 6809, 55300, and 3402 tons of manure per year, respectively (Al-Momani and Shawaqfah, 2013). From an environmental and health point of view, this considerable amount of manure is hazardous being a major source of fresh water and groundwater pollution, nutrient leaching, ammonia emission and pathogen release if it remained untreated (Al-Momani and Shawaqfah, 2013).

Animal manure can be used for biogas production. For instance it can be produced from cows (Onwuliri *et al.*, 2013) and sheep (Broucek, 2014). The resulting economic and environmental benefits of energy production from animal wastes are substantial because energy is produced, organic raw materials are converted to high-quality

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fertilizers, and the hygienic conditions are significantly improved (Sakhawat *et al.*, 2013, Amankwah, 2011).

Biogas is produced by the anaerobic digestion of organic raw materials by anaerobic microorganisms such as *Bacteria* and *Archaea* (Amankwah, 2011). Biogas is usually a mixture of the gases of methane, carbon dioxide, and traces of other gases like hydrogen sulfide and hydrogen (Prabudessia *et al.*, 2014). Methane is the main component that makes biogas flammable. The concentration of methane in biogas is known to be largely dependent on several factors including the organic carbon source, the number and activity of methane-producing *Archaea* (methanogens), and the fermentation rate (Jorgensen, 2009). Methane production (methanogenesis) is also affected by several environmental conditions. In nature, methanogens favor warm temperatures as in the case of rumen's warm and constant temperature (39°C) (Madigan *et al.*, 2012). Likewise, large-scale methane production is usually run at mesophilic or thermophilic conditions (Boe and Angelidaki, 2010). The microorganisms in the anaerobic digester are also sensitive to pH and trace-element supplementation (Mussoline *et al.*, 2013; Jorgensen, 2009).

This study is aimed at producing methane from three different manure types (cows, sheep, and chicken manure) to determine which type gives the highest methane yield. This study is also aimed at evaluating the effects of different incubation conditions, namely, temperature, pH, and trace-element supplementation (nickel, iron, zinc, copper, cobalt, manganese and boron) on the production of methane using the one-factor-at-a-time method. Finally, an experiment under optimal growth conditions was carried out for the sake of this study through which the methane yield was evaluated.

2. Materials and Methods

2.1. Manure Sample Collection

Three manure types were used for methane production (chicken, cow, and sheep manure). Manure was collected from animal farms in the northern part of Jordan during January of 2015. The selected region is known to have intensive animal farming at the national level. The manure samples were collected in clean plastic bags, and were transported directly to the laboratory.

2.2. Methane Production and Evaluation

The production of methane was carried out in stainless steel digesters (20 L) according to the general procedures published in the literature (Recebli *et al.*, 2015; Onwuliri *et al.*, 2013). Two kilograms of fresh manure were mixed with 4 L of water to give a ratio of 1:2 (w/v) and were transferred to the digester. The digesters were incubated at uncontrolled room temperature for twenty-one and thirty-five days without changing the initial pH and without the addition of trace elements. The digesters were tightly closed and a rubber (cascade rubber) was used to ensure an intact sealing. The digesters have two openings on the top to collect the gas and to measure the accumulated pressure. During the gas analysis time, the sampling line for gas analysis was connected with the gas analyzer (Geotechnical Instruments, England). The percent of the methane volume as well as the percent of other gases were

determined at the Royal Scientific Society in Amman, Jordan.

2.3. Effect of Incubation Conditions on Methane Production

The effect of incubation conditions (temperature, initial pH, and trace-element supplementation) on the methane yield was studied using the one-factor-at-a-time method. In respect to the incubation temperature, there were three different constant incubation temperatures (40°C, room temperature, and 30°C). A closed incubator with the constant desired temperature was used and an internal thermometer was also used to monitor the temperature in the digester. After incubation, biogas was collected and analyzed as mentioned earlier (Section 2.2). The effect of initial pH on methane yield was carried out at three different initial pH values (4, 5 and 6). The manure and water mixture was adjusted to the required initial pH before starting the incubation. Regarding the effect of trace-element supplementation, six mL of mineral solution were added to ensure an additional source of iron, zinc, manganese, boron, cobalt, copper, nickel, and molybdenum (Scherer, 1983). The mineral solution was prepared by dissolving 1.5 g of FeCl₂.4H₂O, 70 mg of ZnCl₂, 100 mg of MnCl₂.4H₂O, 6 mg of H₃BO₃, 190 mg of CoCl₂.6H₂O, 2 mg of CuCl₂.2H₂O, 24 mg of NiCl₂.6H₂O and 36 mg of Na₂MoO₄.2H₂O in 990 mL of distilled water and 10 mL of HCl (25 %) without heating. The experiment was carried out as mentioned earlier. All experiments were done in triplicates.

2.4. Methane Production under Optimized Conditions

After the determination of the individual optimal conditions (conditions that revealed the highest methane concentration) for methane production, an experiment was carried out under optimized conditions, and methane was evaluated as mentioned earlier in this study (Section 2.2).

3. Results

3.1. Methane Production from Different Manure Types

Three types of animal manure (chicken, cow, and sheep manure) were collected from animal farms in the northern part of Jordan to be used for the production of methane. After twenty-one and thirty-five days of incubation at uncontrolled room temperature, methane was produced, and its volume percent was determined. It was found that the cow manure produced the highest percent of methane volume when incubated either for twenty-one days (Table 1) or thirty-five days (Table 2) compared to other types of manure. Moreover, as expected, a higher methane content was obtained after thirty-five days (10%) (Table 2). No methane was detected from chicken manure, whereas only 0.1% was detected from the sheep manure, (Table 2). Therefore, cow manures is regarded as the most suitable manure for methane production.

Table 1. Composition of biogas produced from sheep, cow and chicken manures after 21 days. All experiments were done in triplicates.

| Manure Type | CH ₄ | CO ₂ | O ₂ | Other gases |
|-------------|-----------------|-----------------|----------------|-------------|
| Sheep | 0.1±1.6* | 34.8±4.0 | 0.5±0.3 | 64.5±4.3 |
| Cow | 2.1±0.2 | 24.1±0.8 | 0.4±0.1 | 73.4±0.9 |
| Chicken | 0.0±0.0 | 53.1±49.9 | 6.5±6.6 | 40.4±36.5 |

*Mean of volume percent ± SD.

Table 2. Composition of biogas produced from sheep, cow and chicken manures after 35 days. All experiments were done in triplicates.

| Manure Type | CH ₄ | CO ₂ | O ₂ | Other gases |
|-------------|-----------------|-----------------|----------------|-------------|
| Sheep | 0.1±1.6* | 54.2±4.7 | 0.3±0.2 | 45.3±5.0 |
| Cow | 10.0±1.9 | 43.2±3.5 | 0.2±0.1 | 46.1±5.5 |
| Chicken | 0.0 ±0.0 | 87.6±6.4 | 0.1±0.05 | 12.1±6.4 |

*Mean of volume percent ± SD.

3.2. Effect of Incubation Conditions on Methane Production

The Effect of incubation conditions (temperature, initial pH, and trace-element supplementation) on the methane production from cow manure was studied. Three different temperatures (room temperature, 30°C, and 40°C) were used in this study. The results showed that the highest percent of methane volume (47%) was obtained at 40°C, whereas the lowest value was obtained at the room temperature (23%) (Figure 1).

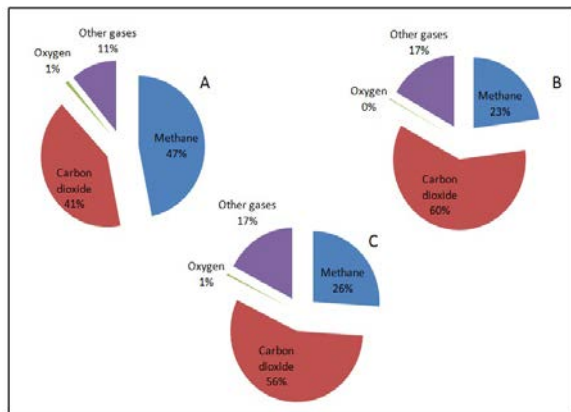


Figure 1. Composition of biogas produced from cow manure incubated at different temperatures: 40°C (A), Room temperature (B) and 30°C (C) for 35 days. The highest methane volume percent (47%) was achieved at 40°C. All experiments were done in triplicates.

Different experiments were also carried out at different initial pH values (4, 5 and 6), and the effect of initial pH on the methane production was evaluated (Figure 2). It was found that the highest volume of methane (42 %) was

produced from cow manure at pH 4, and the lowest volume of methane reached (16 %) was obtained at pH 6. This indicates that the optimal initial pH value was four.

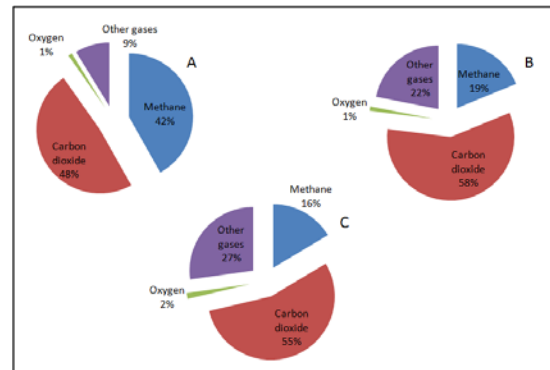


Figure 2. Composition of biogas produced from cow manure at different pH values: pH 4 (A), pH 5 (B) and pH 6 (C). All experiments were done in triplicates. Incubation was done for 35 days. The highest methane level (42%) was produced at pH 4.

Trace-element supplementation is an important factor that affects methane production. In these experiments, some elements (nickel, iron, zinc, copper, cobalt, manganese and boron) were used to support microbial growth. Figure 3 shows the enhancement of trace-element supplementation on the production of methane. The results showed that the methane production was three times higher with the use of trace elements in comparison with methane production without trace elements.

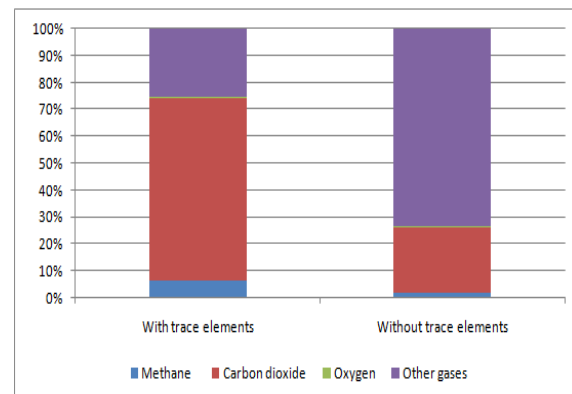


Figure 3. Composition of biogas produced from cow manure supplemented with trace element supplementation (right) and without trace-element supplementation (left). A higher percent of methane volume was obtained with trace-element supplementation. All experiments were done in triplicates. Incubation was done for 35 days.

3.3. Methane Production under Optimized Conditions

In this study, the production of methane was carried out under optimized conditions. Optimal conditions were achieved at pH4, 40°C and addition of trace element. Results showed that the methane level was 68.5 %. This is higher than any other experiment carried out before (Figure 4).

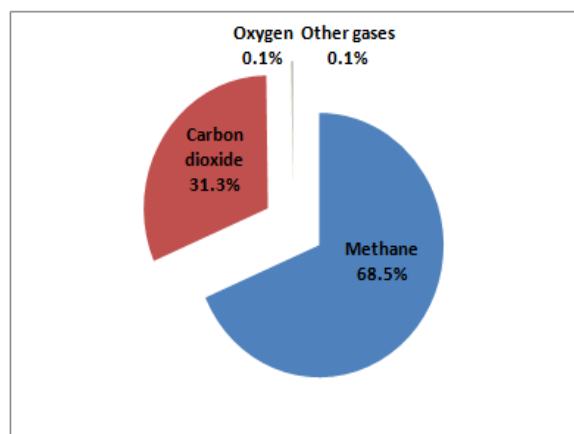


Figure 4. Composition of biogas produced from cow manure after incubation under optimized conditions (40°C, pH 4, and trace element supplementation). Incubation was done for 35 days. The methane level was the highest compared to other experiments in this study.

4. Discussion

In this study, different types of animal manure (cow, sheep, and chicken manures) were collected from animal farms in the northern part of Jordan to be used in the methane production. The selected sampling site is well-known by high livestock numbers. According to the Jordanian Ministry of Agriculture reports of 2014, there are about 3537989 sheep and 69408 cows in Jordan, and most of these animals are concentrated in the northern parts of Jordan (Jordanian Ministry of Agriculture, 2014).

According to the results of this study, cow manure as a methane bio-source gives the highest methane production, whereas chicken manure does not support methane production. This conclusion can be explained based on several factors including the number and activity of microorganisms in the manure, manure pH, and manure minerals. In more details, the cow manure may possess a higher number and activity of microorganisms in their rumen and ultimately in the manure itself than either the sheep or chicken manure. In respect to manure pH, chicken manure is more acidic than the cow manure, due to the secretion of uric acid in the chicken waste (Abu-Ashour *et al.*, 2010). Accordingly, the lower acidity in the chicken manure seems to negatively affect the number and activity of microbiota including the methanogens. Finally, in respect to the manure minerals, it was reported that the concentration of some nutrients such as (potassium and magnesium) was found to be higher in the cow manure than in the chicken manure (Abu-Ashour *et al.*, 2010). Higher nutrients in the cow manure are expected to positively affect the number and activity of methanogens. The results in this study came in agreement with similar studies that reported the advantage of using cow manure as a source of methane compared to chicken manure. For instance, Recebli *et al.* (2015) reported that the biogas production from cow manure is higher than poultry manure, whereas Song *et al.* (2013) reported that the energy-production rates (kcal/h/animal) were higher when using cow manure compared to poultry manure.

In respect to the effect of incubation conditions on methane production, three incubation conditions were

tested (temperature, initial pH, and trace-element supplementation). Temperature is considered as one of the important physical factors that affect methanogenesis. The literature review indicates that large scale anaerobic digesters are usually run at mesophilic conditions (the optimum growth temperature ranges between 25 to 40 °C) or thermophilic conditions (between 50 to 65°C) [Boe and Angelidaki, 2010; Mussoline *et al.*, 2013]. This study showed that when temperature was increased from room temperature to 40°C, methane production was also increased. In the temperature experiments, the highest methane production was observed at 40°C (46.8%). One possible explanation of these findings is that metabolism and methanogenesis increase as temperature increases. More importantly, it must be noted that the methanogens responsible for methane liberation originate in the cow rumen which is characterized by a temperature higher than the body temperature. The rumen is usually characterized by constant and warm temperatures reaching 39°C (Madigan *et al.*, 2012).

In the pH experiments, the percent of methane volume was highest at initial pH4 compared to other higher initial pH values (pH 5 and 6). Methanogenesis usually require acidic conditions to proceed (Bergey and Holt, 1994). In nature, the rumen environment is characterized by narrow pH range (5.5–7); however, this range may change depending on when the animal was last fed (Madigan *et al.*, 2012). This pH range is optimal to acidogenic and acetogenic bacteria as well as methanogenic *Archaea* that which can grow and function normally at low pH range (Mussoline *et al.*, 2013; Boe and Angelidaki, 2010). High pH value becomes more inhibiting to rumen bacteria and *Archaea* and sometimes toxic because ammonia is converted to ammonia at higher pH conditions (Jorgensen, 2009; Chen *et al.*, 2008).

Microorganisms typically require certain nutrients for the growth and maintenance of metabolic functions. In this study, biogas production using cow manure with trace elements was compared to biogas production using cow manure without trace elements. The results showed that the manure with the additional supplementation of trace elements positively affected the methane production. Microorganisms require several trace elements for growth. Chief among these is iron (Fe), which plays a major role in the cellular respiration (Madigan *et al.*, 2012). Iron is a key component of cytochromes and of iron-sulfur proteins involved in the electron transport reactions (Madigan *et al.*, 2012). The cell uses magnesium (Mg) for the stabilization of ribosome, nucleic acid and membrane, and it is important for the activity of many enzymes (Madigan *et al.*, 2012). Manganese (Mn) is important as an activator of many enzymes and as a component of certain superoxide dismutase. Nickel (Ni) is a cofactor in methanogens (Pramanik and Kim, 2013), whereas Cobalt (Co) is an essential trace element and an important part of the active site of vitamin B12 (Madigan *et al.*, 2012). Copper (Cu) plays a role in respiration (Madigan *et al.*, 2012). Our results came in agreement with Glass and Orphan (2012) who reported that methanogenic *Archaea* need a large amount of Ni, Fe and Co, and that the limited concentration of these metals can negatively affect methanogenesis. Moreover, trace-element supplementation can enhance the growth of methanogenic

Archaea, improve process stability, and increase methanogenesis (Mussoline *et al.*, 2013; Kayhanian and Rich, 1995; Jorgensen, 2009).

Biogas production from animal waste can solve significant environmental microbial pollution problems by lowering the levels of contamination in the air, fresh water, and groundwater. Moreover, the utilization of the energy potential contained in the waste and the highly enriched organic fertilizer produced from this waste are important economic benefits (Girguis *et al.*, 2005).

Renewable energy has become an alternative to fossil fuels with time. Generating energy from animal waste is not common in Jordan; therefore, new findings related to biogas production will increase the public awareness about the benefits and the efficiency of biogas at the national level.

5. Conclusion

Cow manure gives the highest methane production compared to chicken and sheep manure. After optimization (40°C, pH 4, with trace-element supplementation), the methane volume percent from cow manure was increased from 10 % to 68.5 %.

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