



Laboratory Preparation of Simulated Sludge for Anaerobic Digestion Experimentation

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ABSTRACT

Health and environmental factors as well as operational difficulties are major challenges facing the development of an anaerobic digestion process. Some of these problems relate to the use of sludge collected from primary and secondary clarifier units in wastewater treatment plants for laboratory purposes.

The present study addresses the preparation of sludge for laboratory purposes by using a mixture that consists of the digested sludge, which is less pathogenic, compared to the collected sludge from the primary or secondary clarifier, and food wastes. The sludge has been tested experimentally for 19 and 32 days under mesophilic conditions. The results show a steady methane production rate from the anaerobic digester which used sludge with a rate of 1.5 l/day and concentration around 60%, with comparatively low H₂S gas content (10 ppm). The methane produced from the digester that used only digested sludge decreases during the experimental period.

Key words: Anaerobic digestion, Digested sludge, Food waste, Biogas.

التحضير المختبري للوحل الافتراضي لاختبار الهضم اللاهوائي

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الملخص

العوامل الصحية والبيئية بالإضافة الى الصعوبات التشغيلية هي تحديات رئيسية لا زالت تواجه تطوير عملية الهضم اللاهوائي. بعض هذه المشاكل تتعلق باستخدام الوحل الذي يؤخذ من وحدات الترسيب (الكلايفير) الابتدائية والثانوية في وحدات معالجة المياه الثقيلة للاغراض المختبرية. البحث الحالي يتناول تحضير الوحل للاغراض المختبرية مستخدماً خليطاً من الوحل المعالج بايولوجيا في الهضم اللاهوائي، والذي يكون اقل تسبباً لأمراض بالمقارنة مع المأخوذ من وحدات الترسيب الابتدائية والثانوية، مع مخلفات الطعام. الوحل المقترح اختبر تجريبياً لمدة 19 يوماً و 32 يوماً تحت ظروف حرارية متوسطة. النتائج بينت استقرارية معدل انتاج الميثان من الهضم اللاهوائي المستخدم الوحل مع معدل 1.5 لتر/يوم وبتركيز 60% تقريباً، مع محتوى قليل من غاز كبريتيد الهيدروجين (10 جزء بالمليون). بينما معدل انتاج الميثان المنتج من الهاضم الذي يستخدم الوحل المعالج بيولوجيا فقط يتناقص خلال الفترة التجريبية.

الكلمات الرئيسية: الهضم اللاهوائي، الوحل المعالج بايولوجيا، مخلفات الطعام، الغاز الحيوي.



1. INTRODUCTION

Anaerobic digestion is an important economic and biological process. It includes four stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) in the absence of oxygen. The main benefits of this process are: sludge stabilisation; reduction of pathogens; reduction of odour and solids content in the sludge; conversion of organic matter into energy (biogas) for use as a renewable energy source; and production of fertilizer for agriculture, **Montgomery and Bochmann, 2014. ,Castellucci et al., 2013. ,Chelliapan et al., 2012. ,Batstone et al., 2002. ,Angenent et al., 2004.**

Collection of the sludge from either primary or secondary clarifier units for laboratory purposes has become unacceptable for health and environmental reasons, which have become a major concern for many researchers and being an additional burden when they are conducting their experimental work.

Even though several attempts have been made to resolve these problems through the use of simulated sludge, the results obtained from these experiments have been compromised by the use of simulated sludge, and the question of how closely it conforms to real sludge. In most cases, the physical, chemical and biological properties of fresh sludge, collected from wastewater treatment plants, are unknown; in addition they continually change. Such changes can be influenced by, for instance, the type of wastewater, sampling and storage duration, handlings and transfers from wastewater treatment plant to the laboratory, weather conditions and seasonal changes, variation of water treatment equipment design and operating conditions. Biologically, many types of anaerobic bacteria exist in wastewater, **Baudez et al., 2007.**

The activity and type of these bacteria mainly depend on the characteristics of wastewater and weather conditions at the time of sampling, as well as on the collection method used. These parameters, for instance, would strongly affect the biogas production rate and the efficiency of biodegradation of the organic matter in the sludge. Chemical and physical properties of sewage sludge vary with time; this makes it difficult to link the results obtained from experiments carried out using different sludge batches (e.g. starter inoculums).

Therefore, in an attempt to prevent this problem occurring, the authors of the present study considered using an identical sampling procedure for the sludge by taking sludge samples from one pre-identified source. The sludge inoculums which been simultaneously taken from a wastewater treatment plant would have been introduced into the two reactors under similar operating conditions, in an attempt to produce the most accurate results possible; as is reported in this study. However, the collection of sludge samples from primary and/or secondary clarifiers for lab-tests has become unacceptable due to numerous health and environmental restrictions.

Hence, a process for the simulation of sludge samples has been adopted in an attempt to overcome these restrictions.

In earlier studies, a synthesised sludge consisting of organic and inorganic synthetic components has been used, **Baudez et al., 2007. ,Dursun et al., 2004.** However, the suggested methods still need to be further clarified through simpler procedures for preparation and use.

This study suggests using a mixture of digested real sludge (which pose less danger than sludge collected from primary and secondary clarifiers) and simulated sludge formed from food waste.

2. MATERIALS AND METHODS

2.1 Synthetic the Suggested Sludge

Achieving secure discharge of the food waste collected from households, restaurants, and residues from the food industry, which makes up about 70% of the total municipal solid waste in Malaysia, **Hassan et al., 2001.** and three billion tons in Europe in 2003, **Pavan et al., 2007.** has become a major challenge for the environment. Several methods have been used for the treatment of food waste. Although landfill dumping of food waste has been the most common method of reducing the volume, pathogens and odour of such waste, the incurred costs and comparatively large areas taken up by landfill sites are serious drawbacks of this method. Utilisation of food waste as a source of energy generation has become the best practice both, environmentally and economically, through the use of biological processes such as anaerobic digestion. Such treatments have the combined benefits of reducing the effects of food wastes as well as producing biogas and digested sludge (compost) which can be used as soil fertiliser. The different types of organic matter which make up food waste are presented in **Table. 1** ,which shows the substrates that are required for the anaerobic digestion process.

The simulated sludge, which was suggested in this study, consists of a mixture of anaerobic digested sludge mixed with food wastes. The components and quantities of the food wastes constituents used in the present study are presented in **Table 2.**

Fresh meat, red beans, peas, lentils, white beans, chickpeas, carrots, and rice are the principle materials used in the sludge in the present study, since these materials have the organic material (lipids, polysaccharides, proteins and nucleic acids) necessary for anaerobic bacteria. The first six materials were initially boiled at 100 °C for 1 hour, before being added to the rice, which has been soaked in water for 24 hours. Then, the product from the previous stage was thoroughly mixed for 30 min to make a simulated food waste with more slurry after adding the water.

According to the recommendations provided by earlier studies, feeding the digester with nutrients or trace metals was not necessary, **Perez-elvira et al., 2011.** **Chamy and Ramos, 2011.** **Braguglia et al., 2011.** **Kim et al., 2011.** **Siggins et al., 2011.** The studies reported that sludge taken from wastewater treatment plant did not require the addition of any supplementary nutrients or trace metals, as the used sludge already contained lipids, polysaccharides, proteins and nucleic acids that are required for the digestion process.

The digested sludge, used in these experiments, was collected from an outlet stream of a full-scale mesophilic digester at “Woodhouse wastewater treatment plant” in the UK.

2.2 The Experimental Setup

Two main setup procedures were applied in this study. The first procedure was experimental testing of the suggested mixture, which was used in the first setup trial. This mixture consisted of a portion of the digested sludge collected from our earlier experiments and a portion of the digested sludge collected from the anaerobic digester from wastewater treatment, in addition to the food waste which was simulated and prepared in the present study. The aim of the second setup practice was to analyse the prepared simulated sludge in order to create a semi-continues process for long operation periods.

In this experiment, two identical bench-scale anaerobic digesters were setup. The digester has an overall volume of 15 litres, with a working volume of 9 litres. The working days of this



experimental work were 19 and 32 days. The digesters were operated under mesophilic conditions (36°C to 38°C). Continuous measurements of biogas produced from both digesters were achieved by downward displacement of acidic aqueous solution (pH <4). All volumes of biogas given in this study have been corrected to 1atm pressure and 20°C. Thus, the total volume of biogas equals to volume of the collector (i.e volume of cylindrical tube). Continuous measurement of methane, carbon dioxide and hydrogen sulphide concentrations in the biogas mixture was carried out daily by biogas analyser (Data gas analyser, Model 0518) at 1atm pressure. A schematic diagram and photograph of the experimental apparatus is shown in **Fig.1** and **Fig.2** respectively. The digested sludge collected from Woodhouse Wastewater Treatment Plant (WWTP) in the United Kingdom, was used with the same preparation procedure that was used in first stage.

A PID controller was used in the present study to maintain the temperature in the reactor with mesophilic conditions. The digester was fitted with a pH controller, type ON/OFF controller (model BL931700 pH minicontroller) to monitor pH values in the digester. 0.2M sodium bicarbonate (NaHCO₃) was used to adjust the digester pH to the optimum pH value, (6.8 - 7.4, which provides a suitable environment for growth of the anaerobic bacteria).

Volatile fatty acids (VFAs) content was measured according to Hach Lange for Water Quality procedure, **Esterification method, 1962**. in which the sample was filtered by centrifugal device (Eppendorf centrifuge 5810) at 2000 rpm for 10 min. Then, an aliquot of 0.5 ml of centrifuged sample was pipetted into a dry 25 ml sample cell. While the second dry sample cell has 0.5 of deionised (DI) which was prepared to calibrate the spectrometer device. Ethylene glycol (1.5 ml) and sulphuric acid (0.2 ml and 19.6 N) were also introduced into each sample cell. The hydroxylamine hydrochloride solution (0.5 ml), sodium hydroxide (2.0 ml and 4.5 N) and ferric chloride sulphuric acid solution (10 ml) are used in the evolution of VFAs.

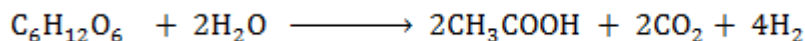
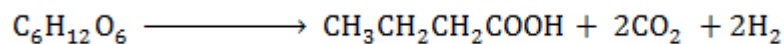
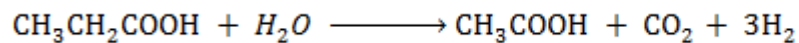
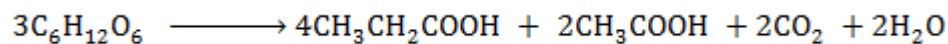
3. RESULTS AND DISCUSSION

Many experiments with different conditions and methods were conducted in order to achieve the best simulation process. The experiments were carried out starting with raw sludge collected from WWTP, followed by the direct use of the food waste and digested sludge. Although many experiments gave negative results, some of the experiments which showed positive results have encountered environmental problems (e.g. producing huge amounts of H₂S gas). For instance, when the food waste was used as a sole feed stock with no added digested sludge; huge amounts of biogas were produced with comparatively high H₂S content, which was out of the range of the biogas analyser used in this experiment. This increase was result of high organic loading rate that led to inhibit the methanogenesis bacteria, **Babae and Shayegan, 2011**. Thus, hydrogen and acetate produced from the early stage of this process can be consumed by sulfate-reducing bacteria, which considers thermodynamically favourable more than methanogenesis bacteria in consumption of hydrogen and acetate, to produce hydrogen sulphide, **Isa et al., 1985**. Therefore, concentration of hydrogen sulphide in the biogas is an indicator of the success or otherwise of the anaerobic digestion process, **Karhadkar et al., 1986**. This problem, consequently led to a full shut down of the digestion process.

In further experiments, the efficiency of the simulated sludge prepared for use in this study was evaluated by measuring the biogas production rate from anaerobic digestion as methane gas. The latter is produced by methanogenic bacteria through the anaerobic digestion process, which, when encounters any problem, prevents or slows biogas production (methane and carbon dioxide). Methane, carbon dioxide and hydrogen sulphide, as well as oxygen, were continuously monitored during the experimental operation period.

The effect of the addition of the food waste supplement to the feed substrate on biogas production has been investigated in this section. **Fig.3** and **Fig.4** show yield of biogas from two anaerobic digesters fed with and without supplementary food waste substrate, respectively. It can be clearly seen that the amount of biogas produced from the first digester, which was fed with supplementary food waste, is more than that produced from the second digester (e.g. fed with only digested sludge).

Fig.5 shows the biogas concentration produced from the digester that was fed with 15 ml of food waste. During the first six days of the experiment, the carbon dioxide produced from the digester was more than the methane. The main reason for this is that high production of carbon dioxide takes place in the second stage of the anaerobic digestion process via converting the propionate and butyrate to acetate, hydrogen and carbon dioxide as shown in the following equations. Moreover, the acidogenesis bacteria are faster growing than methanogenesis bacteria. However, it depends on the activity of anaerobic bacteria, sampling and operational conditions.



As some of the produced carbon dioxide remains as a dissolved gas in the sludge, another portion of CO₂ gas strips up of the digester to biogas collector.

In addition, in this stage, the methanogenic bacteria have a very slow growth and they need time to complete the fermentation process. These bacteria convert a part of the produced carbon dioxide to methane by reducing the partial pressure of hydrogen produced from the second phase to around 10⁻⁴ atmosphere, **Schink, 1997.** **Stams et al., 2005.** Reduction of hydrogen concentration to this level ensures the success the whole process by reducing the accumulation of volatile fatty acids, as well as increasing the production of methane. The amount of methane produced from the reaction of hydrogen with carbon dioxide is estimated by 30%, **Appels, et al., 2008.** **Sahlstrom, 2003.** **Ahring, 2003.** **Metcalf, and Eddy, 2003.** Thus, the methane production usually increases day per day until reaches to a known-value (e.g. ≈60%). During the operation of the anaerobic digestion process, 15 ml of food waste slurry was fed into the digester every day. Equally, similar quantities of sludge were removed daily from the digester in order to maintain a constant working volume.

In order to evaluate the efficiency of the degradation process of organic material (food waste) at different stages, the concentration of volatile fatty acid (VFAs) was measured at different intervals during the operating period, considering that the VFAs are raw materials to produce the acetates molecules by acetogenic bacteria. **Fig.6** below indicates that the concentration of VFAs in the digester fed with sludge and food waste show higher values than that in the digester with sludge only. Moreover, there were no significant variations in pH values throughout the experiments, and the pH values were kept at optimal conditions as shown in **Fig.7**. Thus, this finding evidently supports the fact that the increase in VFAs was not because of an accumulation



process, but these values would be converted into acetate by acetogenic bacteria and then to the methane by methanogenic bacteria.

Although the experiment in this stage has shown encouraging results with both digested sludge and food waste, this experiment was repeated but for a longer operation period in an attempt to confirm the obtained results and the behaviour of the process. **Fig.8** and **Fig.9** show the methane and carbon dioxide produced from the anaerobic digester, respectively, over 32 days of operation. The trend of methane production has been stable during the operation period.

Fig.10 below shows percentages of biogas components (e.g. CH₄ and CO₂) produced from the anaerobic digester fed with a simulated sludge. It can be seen that the percentages of main gases (methane and carbon dioxide) were around 60-70% and around 20%, respectively. The data obtained from the experiments shows that the use of suggested simulated sludge keeps operation of the anaerobic digestion with desired results, providing that H₂S values are kept low as shown in **Fig.11**.

It would, therefore, be expected that any accumulation of dissolved CO₂ in the digesters without pH control would lead to lowering of pH, however, as shown in **Fig.12**, the results showed the capability of the used pH control system to maintain the pH within an ideal range in both digesters. It should also be noted that there was also a natural buffering effect whereby acids produced can immediately react with ammonia produced from biodegradation of proteins.

4. CONCLUSION

Collecting sludge from either primary or secondary clarifier units in wastewater treatment plants, for laboratory purpose, has been unacceptable due to several health and environmental reasons. The present study suggested mixture, which consists of digested sludge fed with food waste as semi-continuous process, for laboratory purposes. The results obtained from the experiments shows that the use of the suggested simulated sludge through 19 and 32 day, keeps operation of the anaerobic digestion with desired results (60% methane), while the hydrogen sulphide values are kept low no more than 10 ppm. According to these results, the use of such a preparation for the purposes of laboratory when dealing with anaerobic digestion, provides the stability of the process with less environmental and health hazard.

5. ACKNOWLEDGMENT

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**Table 1.** Type of organic matters in food waste.

Organic materials	Source
Lipids	<i>Butter, Cheese, Whole Milk, Ice Cream, Cream And Fatty Meats</i>
Polysaccharides	<i>Potatoes, Wheat, Corn, Rice, and Cassava</i>
Protein	Lamb, Egg, Beef, Marmite
Nucleic acids	Plant and animal foods like meat, Certain vegetables and alcohol

Table 2. Details of materials which was used in the synthetic sludge.

	Name	Organic content	Quantity (g)
1	Rise	<i>Carbohydrates (80%), Fat, Protein, Vitamin B1,2,3,5,6,9,C, Calcium, Phosphorus, Potassium, Iron</i>	1250
2	Meat	Protien	400
3	Red bean	Protien, Carbohydrates, Fat	600
4	Peas	Carbohydrates, Fat, Protein, Vitamin A, B1,2,3,5,6,9,C, Iron	
5	Lentils	Protien, Sugars, Carbohydrates, Fat, Vitamen B1,9, Cadium, Iron, Phosphorous, Potassium, Sodium	
6	White bean	Protien, Carbohydrates, Fat	
7	Chickpea	Carbohydrates, Fat, Sugar, Protein, Vitamin A, B1,2,3,5,6,9,12,C,E, K Iron, Phosphorus, Potassium, Sodium	
8	Carrot	Carbohydrates, Fat, Sugar, Protein, Vitamin , Iron, Phosphorus, Potassium, Sodium	

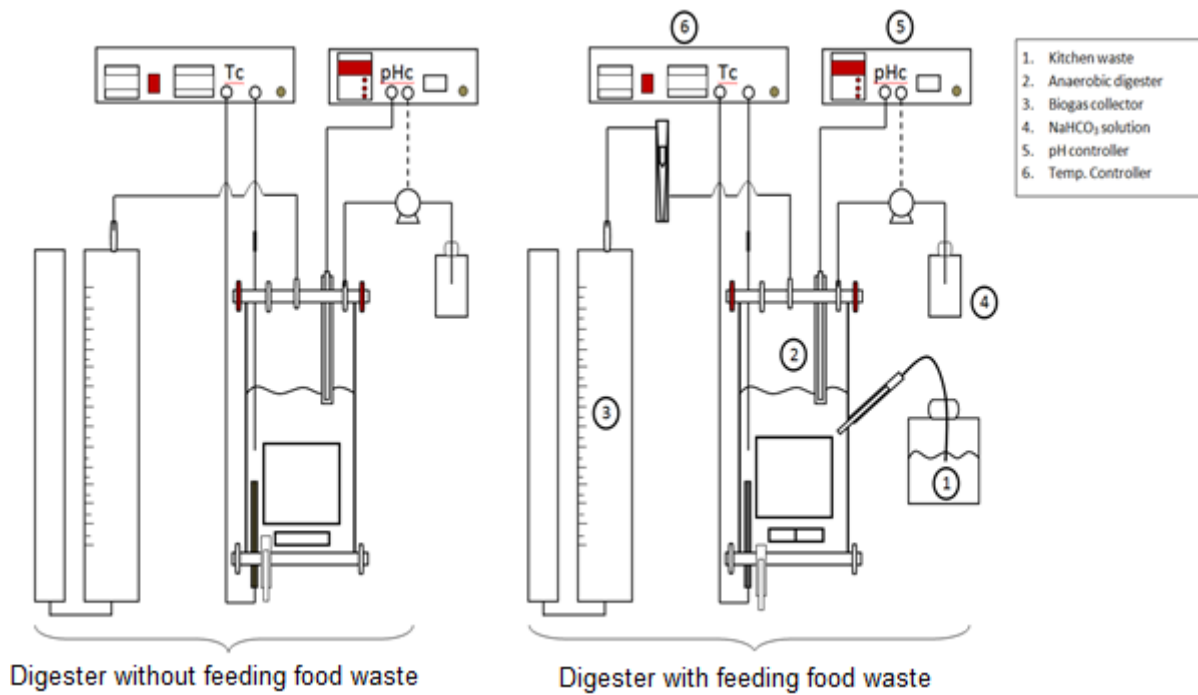


Figure 1. A schematic diagram of the experimental apparatus.

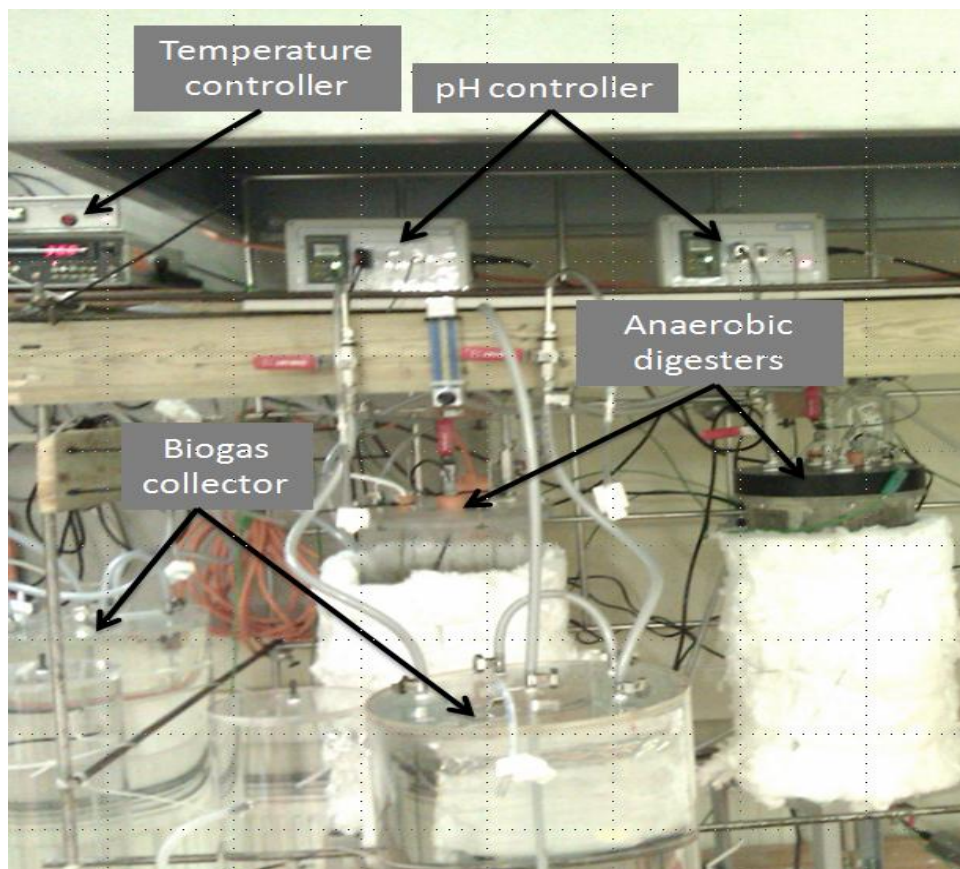


Figure 2. Photograph of the experimental apparatus.

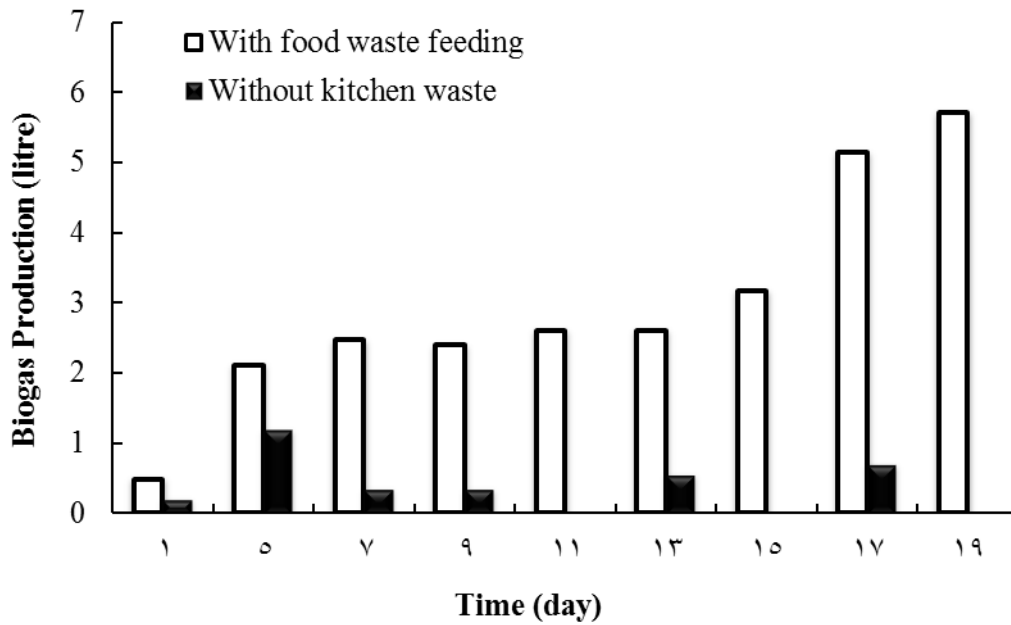


Figure 3. Effect of food waste feeding on biogas production.

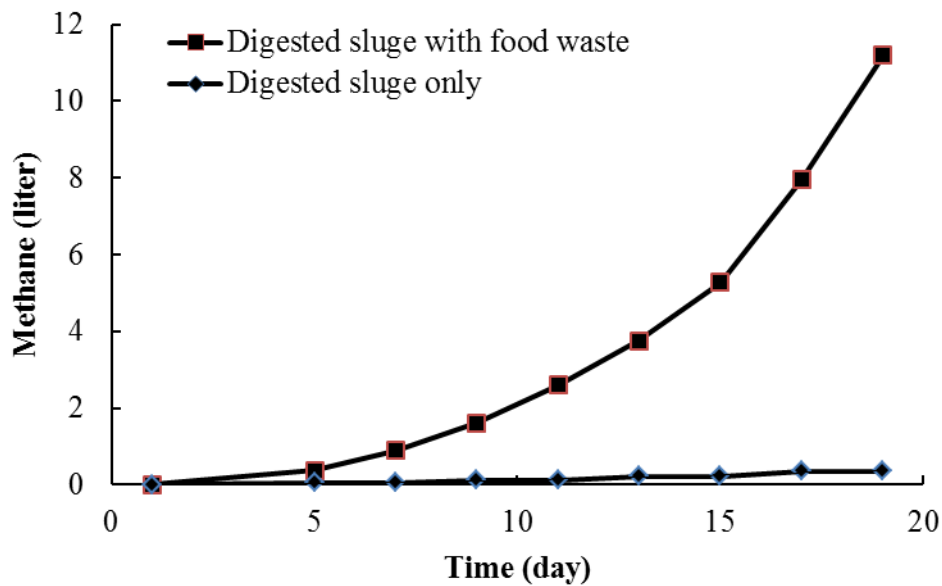


Figure 4. Cumulative methane production from anaerobic digester containing digested sludge only compared to that also fed by food waste.

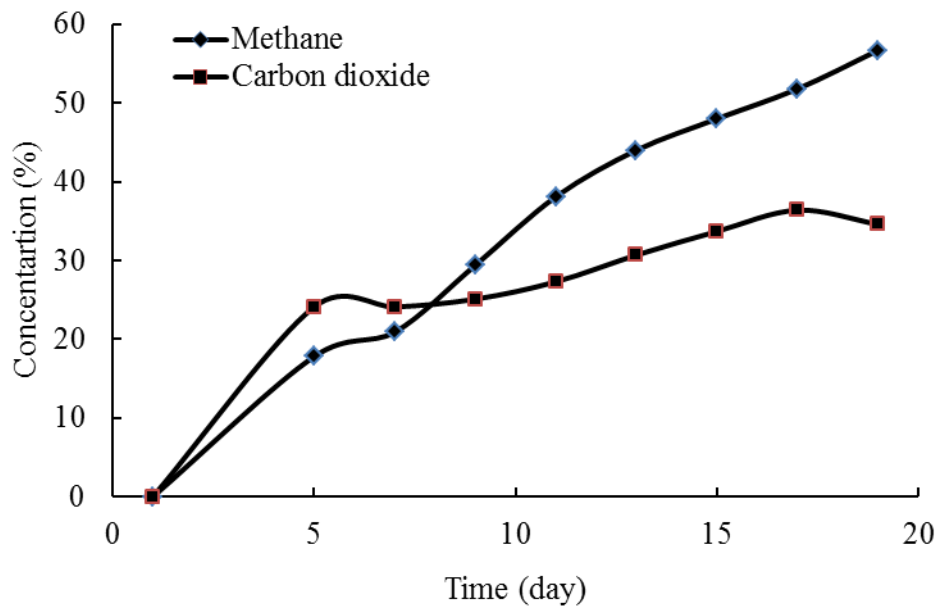


Figure 5. Concentration of methane (CH_4) and Carbon dioxide (CO_2) in biogas produced from the digester containing digested sludge and fed by food waste.

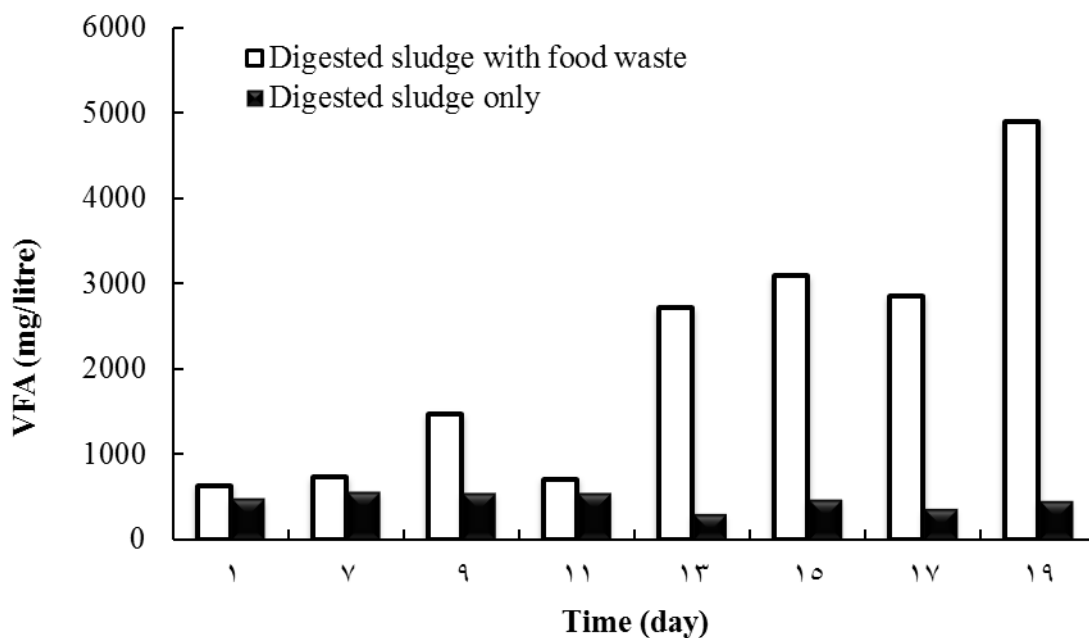


Figure 6. Variation in volatile fatty acids concentration in the digester fed with food waste and the digester fed only with digested sludge.

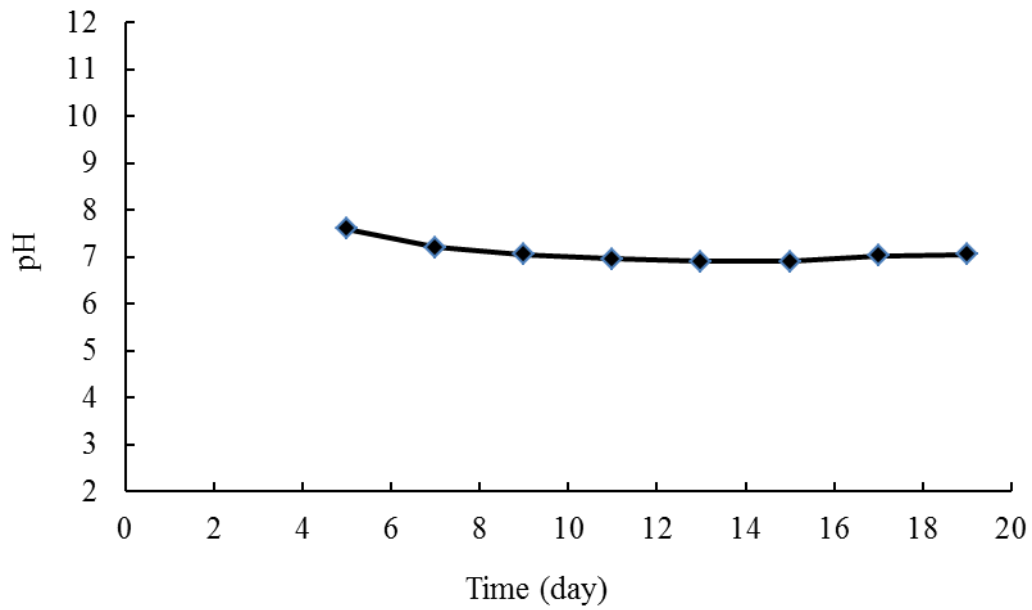


Figure 7. pH values in the anaerobic digester that contain simulated sludge. The pH value remained stable at optimum conditions.

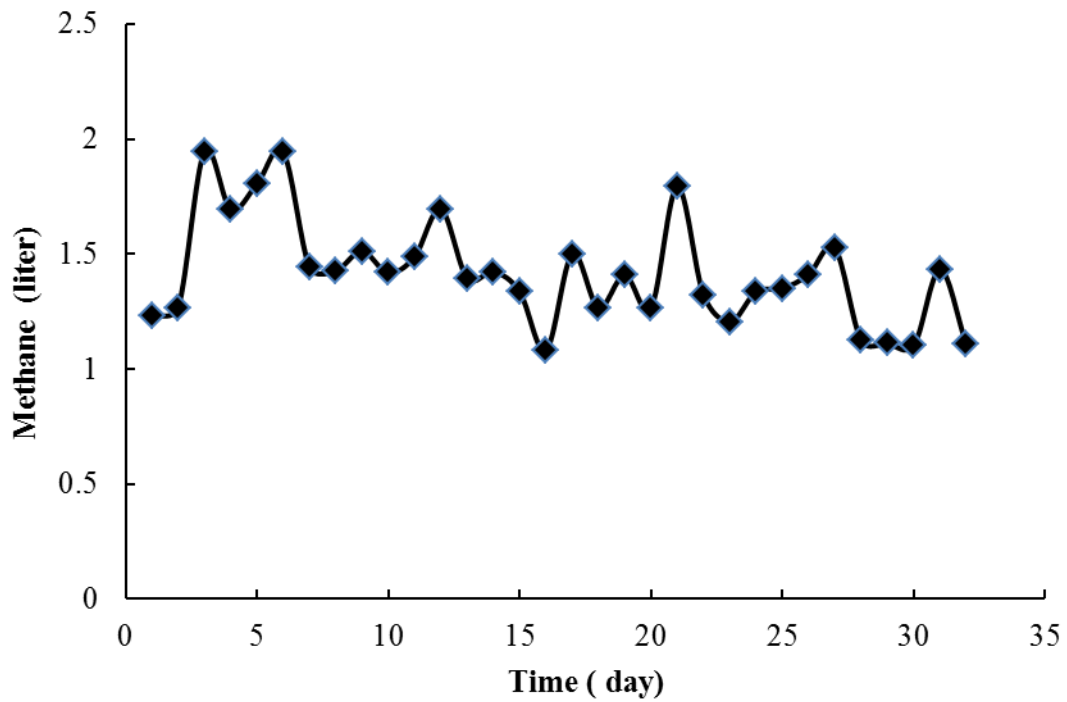


Figure 8. Methane production from the anaerobic digester fed with digested sludge and food waste.

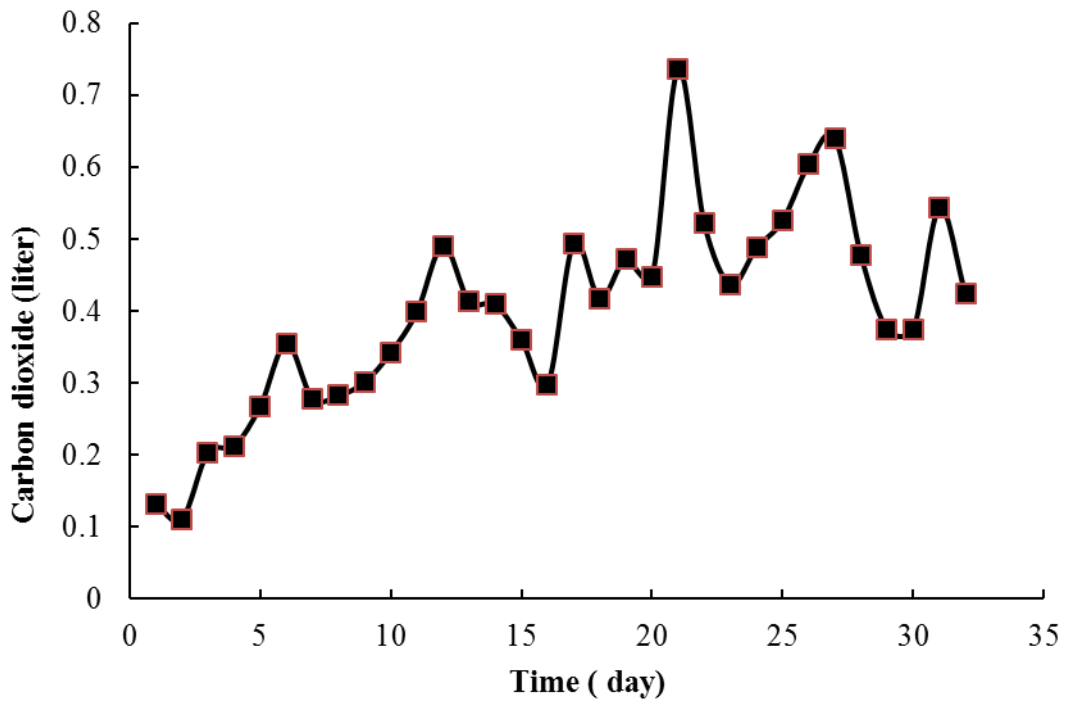


Figure 9. Values of carbon dioxide produced from anaerobic digester fed with digested sludge and food waste.

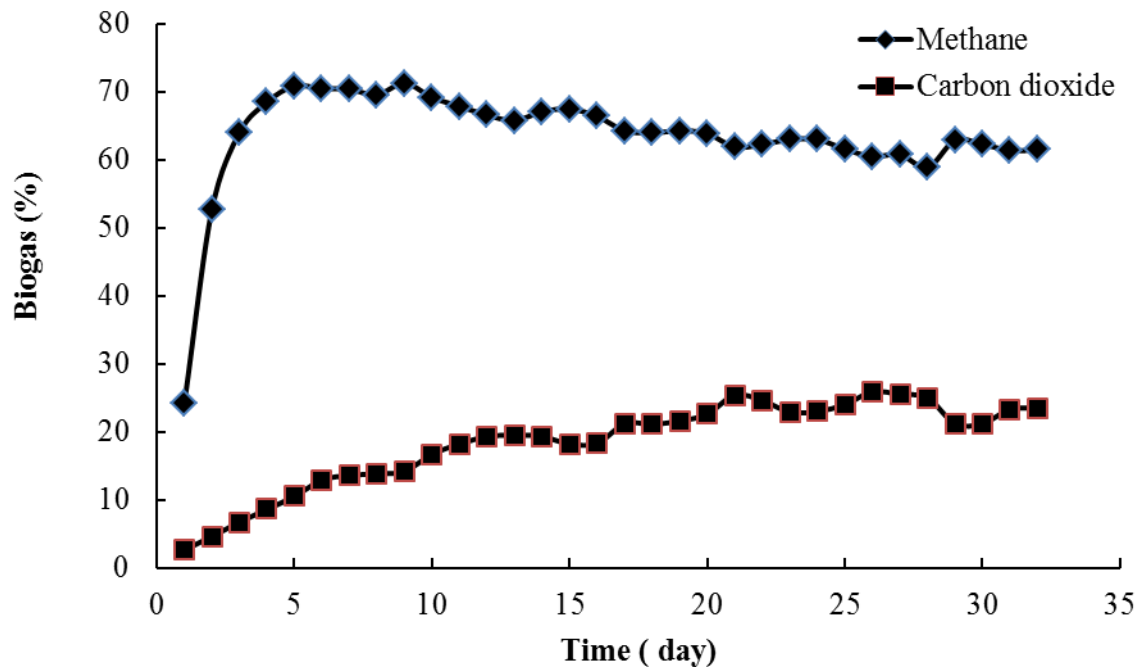


Figure 10. Concentration of methane and carbon dioxide in the biogas produced from the anaerobic digester fed with simulated sludge.

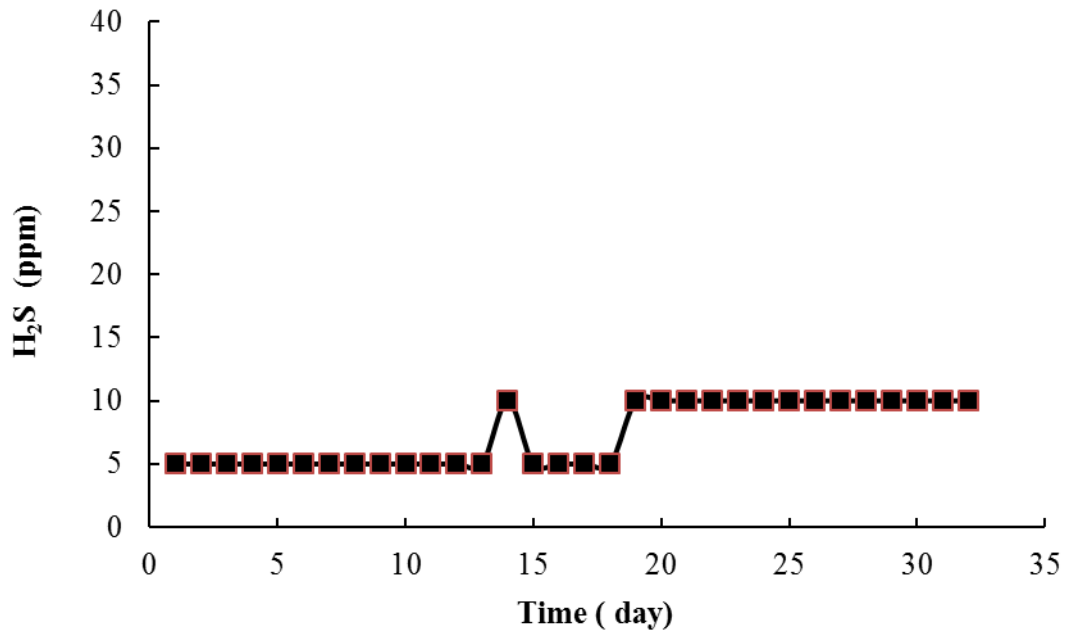


Figure 11. Hydrogen sulphide production by the anaerobic digester fed with simulated sludge.

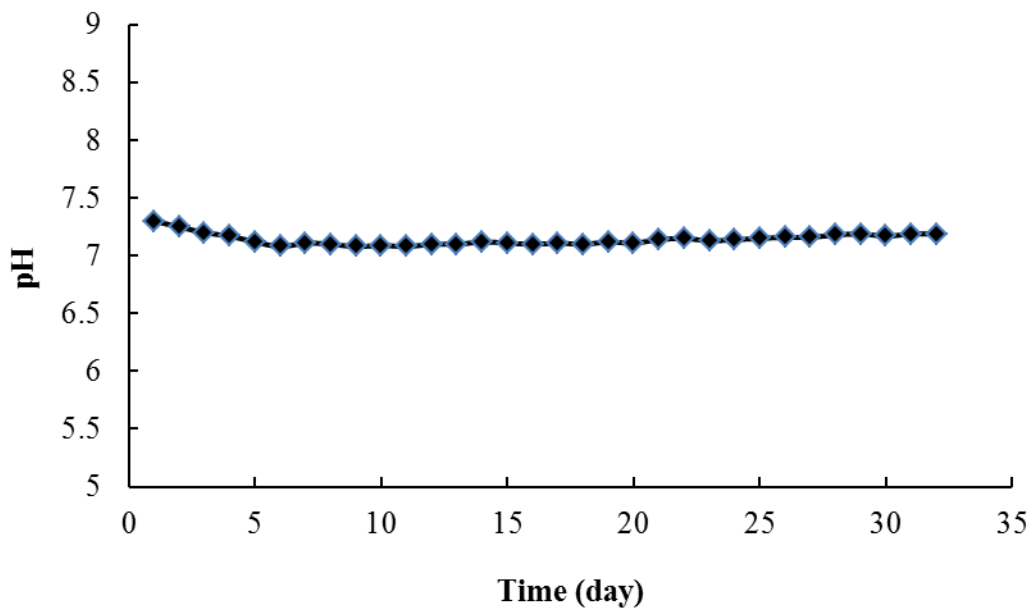


Figure 12. pH value in the anaerobic digester containing simulated sludge.