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RESEARCH ARTICLE

The Biogas Production from mixture of Agar and Rumen Wastes

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Abstract

The effect of Agar waste on biogas production from the co-digestion of Rumen waste was studied at room temperature and prevailing atmosphere pressure conditions. The slurries were used in the fermentor in 5 litre of glass bottles working volume on a batch reactor for over 40 days. The preparation of slurry in different ratio of mixture of Agar waste and Rumen waste were 1:1, 1:3, 3:1 and control. The experiments was done in triplicate. Biogas production was measured indirectly by water displacement method. The result indicated that the mixture of 1:1 ratio of substrate provide the highest average volume of biogas production in 2445ml than followed by 3:1, 1:3 and control of agar and rumen waste gave average yield of 2272, 1836, 837 and 407.36ml. The result showed fastest onset of gas flammability from 1:1 and 3:1 ratio after the 4th day than followed by 1:3, control of agar and rumen waste started producing flammable biogas on the 7^{th}, 8^{th} and 20th day. The pH of the 1:1 ratio substrates was increased in before and after the biogas production experiments provided a reading of pH 5.4 and 6.4. In this present the investigation the utilization of 1:1 ratio substrates for biogas production could eliminate its disposal problems and create another abundant source of sustainable energy. The biogas process has established to be cheap and practically feasible.

INTRODUCTION

Biogas can provide a clean, easily controlled source of renewable energy from organic waste materials for a small labour input, replacing firewood or fossil fuels which are becoming more expensive as supply falls behind demand. During the conversion process pathogen levels are reduced and plant nutrients made more readily available, so better crops can be grown while existing resources are conserved. Since small scale units can be relatively simple to build and operate, biogas should be used directly if possible for cooking, heating, lighting and absorption refrigeration, since both electricity generation and compression of gas for storage or use in vehicles use large amounts of energy for a small output of useful energy (Corral *et al,* 2007).

Biogas generation is a chemical process whereby organic matter is decomposed. Slurry of cow dung and other similar feedstock is retained in the biogas plant for a period of time called the hydraulic retention time (HRT) of the plant. When organic matter like animal dung, human excreta, leafy plant materials, etc. are digested an aerobically (in the absence of oxygen), a highly combustible mixture of gases comprising 60% methane (CH_4) and 37% carbon dioxide (CO_2) with traces of sulphur dioxide and 3% Hydrogen (H_2) is produced (Babel *et al,* 2009).

A number of studies have been conducted to increase biogas yield in the anaerobic digestion. An attempt to improve the biomass conversion efficiency and biogas yield are abundant which include; pre-treatment of manure by separating solids from digested materials(Moller *et al,* 2008). Improving substrate composition by co-digesting with other substrate (Gelegenis *et al,* 2007).

The seas contain prominent diversity of seaweed. Nevertheless, so far only five types from the genus of *Gelidium, Gelidiella, Hypnea, Eucheuma*, and *Gracilaria* that are valuable for export-trade purposes. Two of them, i.e. *Eucheuma* and *Gracilaria* have been cultured by community (Soegiarto, 1968)

Seaweed is the most widely distributed organisms in the ocean. It is a lower cryptogam growing in the ocean, rich in protein, amino acid, inorganic salt, vitamin, alginate, a small amount of enzyme, plant hormones, polyphenols and polysaccharides (Gao *et al*, 2004). Usually, the seaweed waste is treated as chemical solid waste after extraction of alginate, iodine and mannitol. There are many problems in existing disposal ways, such as the small utilization scale and the high utilization cost. Thus, it has caused environmental pollution and waste of resources. Results show that seaweed waste contains about 20% crude protein, 50% crude fiber and 3% ash content respectively (Gan *et al,* 1999).

Seaweed from the genus of *Gracilaria* has been used as a raw material for jelly powder production (Chapman, 1949; Soegiarto and Sulistijo, 1981). During the process of jelly powder making, both solid and liquid wastes are generated. The solid waste represent the biomass of the seaweed. One of the big companies, PT Agarindo Bogatama, that is located at Pasar Kemis, Tangerang, produces jelly powder from the seaweed. The amount of solid wastes generated is 60 tonnes per day with the water content of 70%. Examination on a field, which is devoted for purging the solid wastes revealed that several farmers have attempted to utilize them as media for growing vegetables and demonstrated good results.

Seaweeds are used for various applications, as food as well as in the textile, pharmaceutical, cosmetic, and biotechnological industry. High sulfur content can affect the digestion process, if the substrate which is digested contains too much sulfur (as sulfate ions) it may sulphate the activated bacteria. These bacteria compete for methanogenesis (Zehnder, 1988). The result of this competition leads to the formation of hydrogen sulfide (H_2S) instead of methane. Besides giving reduced methane production, it is not desirable because hydrogen sulfide is very corrosive and toxic.

Rumen is one of slaughterhouse wastes that is frequently disposed into drainage system. This waste disposal system may cause environmental nuisance, particularly pose health hazard to human due its content of millions microorganisms. However, rumen may be useful to be used as an activator in producing biogas through anaerobic fermentation. Since some of rumen microorganisms are cellulolitic and methanogenic bacteria. Rumen is part of digestion system in ruminant where the microbial fermentation occurs. This fermentation process is similar to that in biogas digester (Haryati *et al,* 2006). So that, microorganism in rumen will have significant role in producing biogas by accelerating degradation process of organic matter in fermentation substrate to produce methane. However, sometimes this degradation process results in too low pH that may kill most microorganisms in the digester. Therefore, this process needs more acidophilic microorganisms. One of the microorganisms that can be used for this purpose is *Saccharomyces cerevisiae*. The addition of this yeast may increase degradation rate of cellulose and stimulate the growth of cellulolitic bacteria and fungi (Williams *et al,*1991). The increase of the two microorganism’s population is important. Both of them will cooperate in increasing cellulose degradation. Besides, *S.cerevisiae* will decrease propionate acid and increase acetic acid proportions in Volatile Fatty Acid (VFA) (Kumar *et al,* 1994; Newbold *et al,* 1998), and increase acetogenesis after VFA formation, and the resulted acetic acid will be increased accordingly (Chaucheyras *et al,* 1995). Acetic acid is a main precursor of methane. Therefore, the more acetic acid produced the higher the methane generated.

In 2010, world’s ruminant population was about 3.6 billion, of which 5.38%, 39.59%, 25.19%, and 29.84% were for buffaloes, cattle, goats and sheep, respectively. The relative distribution of the number of ruminant animals in different parts of the world according to (FAOSTAT, 2013).

1) Hydrolysis, in which enzymes secreted by hydrolytic bacteria break down organic polymers (proteins, carbohydrates) into their monomer components (amino acids, sugars)

2) Acidogenesis, in which acidogenic bacteria break down the amino acids and sugars into volatile fatty acids (VFAs) and alcohols

3) Acetogenesis, in which acetogenic bacteria convert the VFAs into acetic (and propionic) acid and some CO_2 is liberated and

4) Methanogenesis, in which the acetic acids are converted to methane and CO_2 by methanogenic bacteria.

The aim of this research is to study the influence of mixture of waste content for biogas production. The problem caused by solid waste can be significantly mitigated through the adoption of environment- friendly waste - to - energy technologies that will allow the treatment and the processing of wastes before their disposal. These measures would reduce the quantity of wastes, generate a substantial quantity of energy from them, and greatly reduce the air and water pollution.

MATERIALS AND METHODS

Sea weed industry waste

There is a cluster of more than 100 industries in and around kappalur industrial estates of Madurai, Tamil Nadu. These industrial estates are processing sea weed and extracts phycolloids from them and accumulates a large quantity of sea weed residues after extraction. The residues are collectively called seaweed waste. If it is collected from agar industry, it is called agar waste. In this present investigation, agar waste is collected from Omega agar industries near Tirumangalam.

Rumen wastes

Goat rumen wastes were collected from Madurai goat slaughter house, Nellupattai in Madurai city.

Solid waste management through anaerobic digestion

In this present investigation solid wastes like slaughter house waste like rumen waste and agar waste were treated anaerobically for biogas production from them.

Anaerobic digestion for biogas generation

Anaerobic digestion experiments were carried out with laboratory scale batch digesters of 2.5 litre working capacity. To produce slurry each sample was mixed separately with tap water, as per the table given below. Totally eight types of slurries were prepared and were designated as T_0,$T_{1,}T_{2,}T_{3,}$ and T_4 respectively. One litre of each such slurry was taken separately in different sets of digesters for anaerobic digestion (A set consists three digesters). After loading the slurry, the digesters were perfectly sealed and kept at room temperature for 40 days. The digesters were shaken well periodically. The gas produced in the digesters were measured once in a day by water displacement method. The measured gas was subjected to burning test. The dry weight of the slurry was measured before and after digestion. Total amount of dry weight reduced during the digestion was calculated and correlated with the amount of biogas produced.

Digester setup

A 2.5 litre narrow mouthed reagent bottle was used to setup laboratory scale digester. The bottles were cleaned and dried. The mouth of the bottle was closed with one hole rubber cork (No.3); then a known length of saline tube was taken and its one end was inserted through the hole of rubber cork. The other end of the saline tube was closed with a metal pinch cork.

Measurement of gas by water displacement method

In order to measure the quantity of gas produced in the digester, a measuring cylinder was filled with water. Then a glass plate was placed over the mouth of the cylinder ; then the cylinder filled with water was inverted carefully and placed in a rectangular tray with some water. The other free end of the saline tube of the digester was inserted in such a way into the inverted measuring cylinder. The pinch cock of the tube was loosened to allow the biogas produced inside the digester to pass through and was collected at the top of the cylinder by displacing water. The amount of water displaced from the cylinder was equal to the amount of gas collected. The level of water displaced in cylinder was noted by observing the graduation marked on the cylinder. Biogas formed was measured by using 'liquid displacement method' as described previously by (Budiyono *et al,* 2010).

Burning Test

After collection, the measuring cylinder filled with gas was carefully turned up and lighted match sticks were placed near the mouth of cylinder. If blue flame appeared the gas produced in the digester is the burnable gas (contain more amount of methane) if no flame is formed the gas produced in the digester containing is non-burnable gas which containing more amount of CO_2 or H_2S then it is methane.

Measurement of dry weight and pH

50ml of slurries was taken before and after digestion in different china dishes. It was kept at 100^oC overnight in a hot air oven. Then the dry weight of the slurry was measured. The differences between the initial and final weight of the slurry can also be recorded as moisture content (Trivedy and Goel,1984). pH of the slurry was measured before and after digestion by standard method.

Results and Discussion

Table 1 Biogas production from various concentration of Agar waste and Rumen

SL.No	Slurry type	Rumen (g)	Agar waste (g)	Water (ml)	Taken slurry(lit)
1	T0	500	-	500	1
2	T1	-	500	500	1
3	T2	750	250	1000	1
4	T3	500	500	1000	1
5	T4	250	750	1000	1

Photo 1 Production of biogas for anaerobic digestion

Photo 2 Measurement of gas by water displacement method

Photo 3 Burning Test of biogas

A range of pH values suitable for anaerobic digestion has been reported by various researchers. (Liu *et al*, 2008) showed that the most favourable range of pH to attain maximal biogas yield in anaerobic digestion is 6.5-7.5. The pH of the mixture slurry before and after the biogas production experiments was recorded. The rate of pH measured the after digestion in maximum increased to 1.0 in T_3 treatment and was 0.5, 0.3, 0.0,&-0.2 in respectively T_4,T_0,T_2 & T_1. When the rate of pH was increased the biogas production also increased (Table 2).

Table 2 Biogas generation from Agar waste mixed with Rumen waste

SL.No	Treatments (g)	Designation	Total dry weight of slurry(g)			pH of the slurry			Total biogas production
			Before Digestion	After Digestion	Reduction during the Digestion	Before Digestion	After Digestion	Reduction during the Digestion	
1	Rumen	T_0	60	59.82	0.18	4.6	4.9	0.3	407.36
2	Agar waste	T_1	100	99.50	0.50	6.4	6.2	-0.2	837
3	AW250+RU750	T_2	80	78.20	1.80	6.5	6.5	0.0	2272
4	AW500+RU500	T_3	80	77.80	2.20	5.4	6.4	1.0	2445
5	AW750+RU250	T_4	90	88.80	1.20	5.8	6.3	0.5	1836

RU= Rumen; AW= Agar Waste

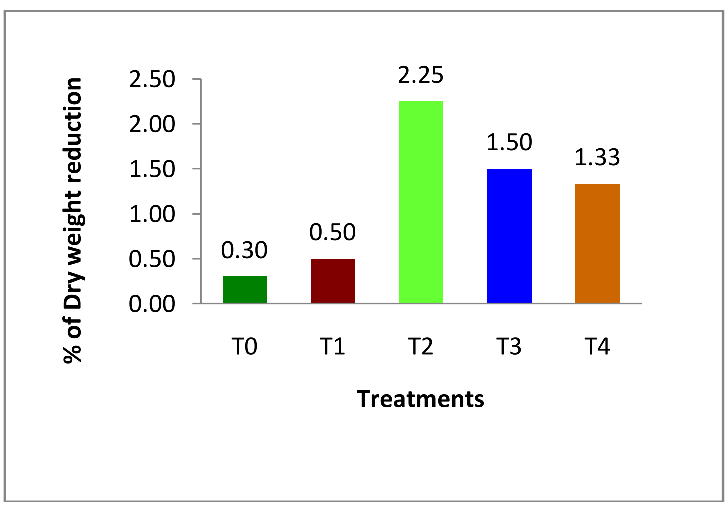

Figure 1 % of Dry weight reduction of substrates

The reduction of the dry weight substrate occurred the after digestion. The maximum degradation of T_2 treatment was in 2.25% and 1.50, 1.33, 0.50 & 0.30% in T_3, T_4, T_1 &T_0 respectively (Fig 1). The maximum degradation of substrate generated high amount of biogas production. The minimum degradation of substrate resulted in less amount of biogas generation. Another factor that enhanced higher volume of gas was the lower moisture content of the substrate (10%) compared to (20%) for (Bagudo *et al*, 2010), since the higher the moisture content the lower the potential of the substrate to produce biogas (Maishanu *et al*,1991). This is because high moisture content resulted in low total solid. Marine algae consist of polysaccharides (agar, alginate, carrageenan, laminaran and manitol), which zero lignin and low cellulose content, which make them an easy material to convert to methane by anaerobic digestion process (Alberto *et al,* 2008). High concentration of anaerobic bacteria content in liquid rumen works effectively to degrade organic substrate from manure. Rumen of the ruminant animals contains the highly anaerobic bacteria dominated by cellulolytic bacteria able to biodegrade cellulose material from manure (Aurora,1983).

Table 3 Biogas generation from Agar waste & Rumen mixture of various phase of digestion

SL.No	Treatments(g)	Designation	Total biogas production	Gas production in various phase			
				Ist Phase	IInd Phase	IIIrd Phase	IVth Phase
1	Rumen	T_0	407.36	204	69.53	14.50	119.33
2	Agar waste	T_1	837	293	272.50	164.00	107.87
3	AW250+RU750	T_2	2272	544	826.00	404.00	497.5
4	AW500+RU500	T_3	2445	847.5	933.50	441.40	222.5
5	AW750+RU250	T_4	1836	852.5	573.50	243.00	166.5

RU= Rumen; AW= Agar Waste

The (Table 3) shows that the highest biogas produced was recorded in 2445ml of T_3 treatments than followed by 2272ml, 1836 and 837 in respectively T_2, T_4 and T_1. In this treatment group the least biogas produced was recorded on T_0 treatment of 407.36ml. These result suggest that the optimum rumen content for giving the best performance of biogas production. Similar to this results, (Sunarso *et al*, 2012) reported that samples with 50 % rumen waste exhibit still there is the tendency to increase biogas production after 90 days observation. This is suggest that, in case of very abundance of rumen fluid such as occur in slaughterhouse, the rumen fluid content of 50 % (Manure : Rumen fluid ratio 1:1) will give the best performance for biogas production. The biogas produced in all of the treatment groups increased as observation days increased which corroborated with the findings of (Nopharatana *et al,* 2007) that observed a very slow rate of biogas being produce at the beginning of the experiment. In this study biogas production gradually increased until 10 days of T_2 and T_3 treatments. This gradually decreased until 40 days of T_1 and T_4. T_0 treatment was biogas gradually decrease until 30 days and slightly increase the biogas throughout experiments periods

The optimum dose of rumen liquid that can be supplemented in biogas production of fresh market garbage is 5 %/kg of garbage in combination with *Saccharomyces* cerevisiae. The addition of *S. cerevisiae* culture to ruminant diets has improved the digestibility of dry matter, crude protein, and hemicelluloses; has increased ruminal bacterial numbers and activity, which in turn leads to increase degradability of forages and flow of microbial N postruminally; and has decreased ruminal lactate concentrations (Wiedmeier *et al,* 1987).

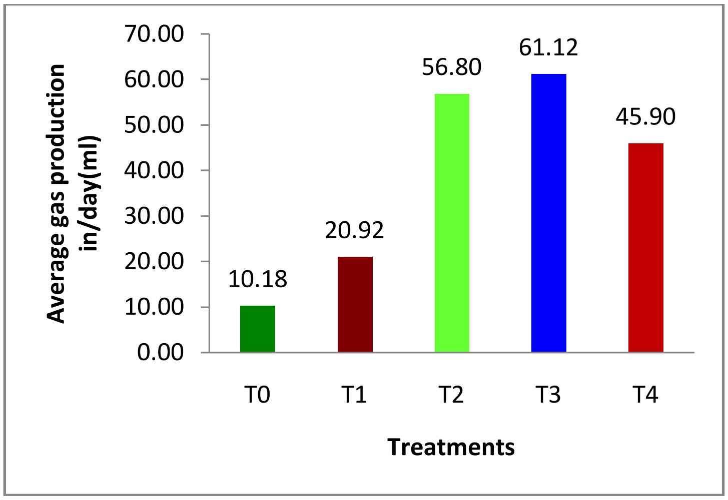

Figure 2 Average gas production in/day(ml)

In this study the maximum biogas production in T_3 treatment of 61.12ml/day than followed by 56.80, 45.90 and 20.92ml/day in respectively T_2,T_4 and T_1, whereas was less the amount of biogas produced from T_0 treatment of 10.18ml/day (Fig 2). (Aurora, 1983) The reported high concentration of anaerobic bacteria content in liquid rumen works effectively to degrade organic substrate from manure. Rumen of the ruminant animals contains the highly anaerobic bacteria dominated by cellulolytic bacteria able to biodegrade cellulose material from manure.

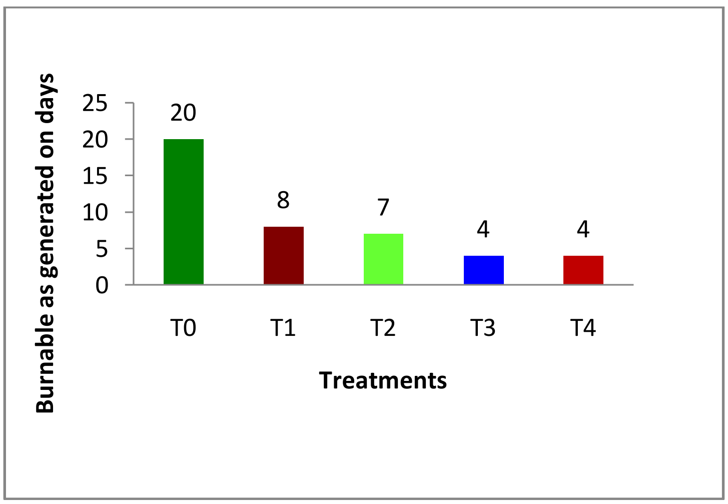

Figure 3 Burnable as generated on days

Burning test of biogas revealed that burnable gas was recorded on 4^{th} day of fermentation in T_3 & T_4 treatments, whereas, It was on 7^{th}, 8^{th} and above 20 days in T_2, T_1 and T_0 respectively (Fig 3). In the anaerobic environment, the predominance of CO_2 over CH_4 in gas production at the initial stage indicated the strong activity of hydrolysis. In contrast, higher CH_4 content over CO_2 in the gas production implied strong activity of the methanogens (Borhan *et al,* 2012).

CONCLUSION

The result of the investigation shows that the mixture of equal amount of substrate (T_3 treatment) provided the highest biogas production. Overall results indicate that the very shortly flammable biogas production happen in T_3 and T_4 treatments. The utilization of these substrates (T_3) for biogas production could eliminate its disposal problems and create another abundant source of sustainable energy. The result of study also indicates that the biogas production process is economically feasible.

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