Optimization of abiotic factors for Biogas production from vegetable Agronomic Wastes

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Abstract

Vegetable wastes, fruit wastes and cow dung was collected and brought to the laboratory. 1kg of each vegetable wastes and fruit wastes were partially sterilized and homogenized before fermentation. Then the wastes were mixed with equal volume of distilled water with 1:1 ratio. The mixture is maintained at the pH of 6.8-7.2. The biogas was collected in a balloon which was connected to the inlet slit of the reactor. From the result, clearly revealed that the biogas production efficiency completely depends upon the temperature effect of the particular types or ingredients present on the experimental sample wastes. Vegetable wastes over maximum (90%) production noted at 25-30^{0}C. Hence, the current result conformed that this optimum temperature is susceptible or support for the production of biogas from the vegetable wastes. Moreover, other parallel higher production (80%) noted on 30-40^{0}C and 40-50^{0}C from Fruit and Cow dung wastes respectively. From the result showed the vegetable wastes produces high gas production on the 2nd day resulting at 90% of biogas, followed by fruit wastes on 3rd day resulting at 50% of biogas production with lowest acidity. Hence, the results evidenced that the vegetable wastes with enhancer showed the higher production of biogas in less retention time.

Key words: Agronomic wastes, anaerobic digestion, Biogas, Abioticfactors

Introduction

Biogas is a mixture of methane and carbon dioxide, produced by the breakdown of organic waste by bacteria without oxygen (anaerobic digestion) also it was one of the excellent sources of energy (Dhanalakshmi and Ramanujam, 2012). It is produced when bacteria decompose organic material such as pineapple peel, garbage and sewage, especially in the absence of oxygen. Biogas is a mixture of about 60 percent methane and 40 percent carbon dioxide. Methane is the main component of natural gas. It is relatively clean burning, colorless and odourless. This gas can be captured and burned for cooking and heating. This practice is being done on a large scale in some countries of the world. Biogas production from fruit wastes is an efficient method of waste treatment, resulting in a highly stabilized effluent which is odourless and almost neutral in pH (Viswanath *et al*., 1992). In the recent years global energy crisis increased at a fast pace. Demand for the use of fossil fuels for cooking and other commercial activities increased along with the increasing population of India. Use of renewable sources of energy viz. biogas for cooking etc can somewhat is an alternative for the excessive demand of fossil fuels like LPG. (Das *et al.,* 2013). The rate of bio gas production varies with different conditions and parameters like temperature, stirring speed, feed concentration, catalyst concentration, etc. It has been found that the catalyst mainly increases the production rate of biogas from water hyacinth (Pöschl *et al*., 2010; Antony and Lindon, 2012). It was also found that methane production was affected by the ratio of waste to water (w/v). Wastes dilution ratio of 1:2 showed comparatively higher methane content than the wastes dilution ratio of 1:1 (Dhadse *et al.,* 2012). Performance of the reactors was

evaluated by estimating destruction of Total and Volatile Solids and by monitoring daily gas production. The performance evaluation in terms of specific gas production based on amount of total solids added and volatile solids added has indicated that the mixture of vegetable wastes chosen for the study are amenable to anaerobic digestion (Forster *et al*., 2008).

Flammable biogas production of brewery spent grain could be enhanced significantly in the presence of cow liquor waste. Cassava waste water which could not produce biogas could be made to be a cheap source of biogas by inoculating it with cow liquor waste (Giovanni *et al*., 2012) Fossil energy sources are the most used energy supply in the world today, however the increased prices of oil and increased awareness of climate change will trigger the increasing use of renewable energy, such as biogas (Mattsson *et al.,* 2011)**.** Vegetable wastes were an-aerobically digested in a fed-batch laboratory scale reactor at mesophilic conditions (35^{o}C) Dhanya *et al*., 2009. The physicochemical parameters of the wastes were determined including microbial analysis. It also indicates that blending paper waste with cow dung or any other animal waste will give sustained gas flammability throughout the digestion period of the waste since animal wastes are good starters for poor biogas producing wastes (Ofoefule et al**.,** 2010). The aim of this study was to investigate the effect of abiotic factors biogas production potentials of cow dung, vegetable waste, fruit waste and enhance the production using,.

MATERIALS AND METHODS

Sample collection and processing:

The cow dung were collected in a sterile polythene bag from houses .The collected samples were grinded and sterilized before fermentation. The sterilized substance should be mixed with distilled water in 1:1 ratio.

Design of invitro anaerobic digester:

The invitro anaerobic digester, 2.25 litre reactor was filled with grinded substrate .An inlet slit was made on the top of the reactor and connected with a balloon were the generated gas were collected. The collected gases were used for further analysis.

Isolation and Identification of *E. coli* and *Lactobacillus* as enhancers

The *E.coli* and *Lactobacillus* were isolated and identified of through Bergey's manual. The isolated organisms were purified and used as biological enhancers for biogas production.

Small scale production of bio-methanation

1kg of each vegetable waste, fruit waste and cow dung were taken and homogenized and mixed with 1 litre of distilled water in the ratio 1:1. Then the mixture was inoculated with 20 ml of starter culture (Methanogenic bacteria) as control and addition of 5 ml enhancers (*Lactobacillus* and *E. coli* culture) were added as test and the digester was allowed to incubate at various temperatures in anaerobic condition.

Biochemical process of anaerobic digestion:

Anaerobic digestion (AD) is a microbiological process of decomposition of organic matter in the absence of oxygen. Specific groups of micro-organisms are involved in each individual step. These organisms successively decompose the products of the previous steps. There are four steps namely hydrolysis, Acidogenesis, acetogenesis and methanogenesis.

Hydrolysis:

Hydrolysis is theoretically the first step of AD, during which the complex organic matter (polymers) is decomposed into smaller units (mono and oligomers). During hydrolysis, polymers like carbohydrates, lipids, nucleic acids and proteins are converted into glucose, glycerol, purines and pyrimidines.

Acidogenesis:

During acidogenesis, the products of hydrolysis are converted by acidogeneic (fermentative) bacteria into methanogeneic substrates. Simple sugars, amino acids and fatty acids are degraded into acetate, carbon dioxide and hydrogen (70%) as well as into volatile fatty acids (VFA) and alcohols (30%).

Acetogenesis:

Products from acidogenesis, which cannot be directly converted to methane by methanogenic bacteria, are converted into methanogenic substrates during acetogenesis.

Methanogenesis:

The production of methane and carbon dioxide from intermediate products is carried out by methanogeneic bacteria.

ABIOTIC FACTORS OF ANAEROBIC DIGESTION:

pH :

pH of the digester was kept within a desired range of 6.8-7.2, by feeding it at an optimum loading rate. Various pH was maintained 6.8,7. 0.7.2, 7.4 with the addition of HCl and NAOH.

TEMPERATURE:

There are different temperature ranges during anaerobic fermentation was carried out: Psychrophilic (<30^0

C), Mesophilic (30-40^0C) and Thermophilic (50-60^0C) conditions were maintained and observed the effect of temperature.

WATER:

1000 ml of slurry was diluted with equal volume of distilled water in the ratio 1:1.Hot water (40^0C) and cold water (15^0C) were used for slurry preparation.

FERMENTATION TIME:

The fermentation was started up by providing the mixture in the reactor, and allowed to ferment for 12 days in an anaerobic condition. The gas production was checked daily.

AGITATION:

Stirring of digester contents needs to be done to ensure intimate contact between microorganisms and substrate which ultimately results in improved digestion process. Agitation of digester contents can be carried out in a number of ways. Physical shaking was done for proper mixing.

COLLECTION OF BIO GAS:

The gas was collected in the balloon. The enlargement of balloon showed the production of biogas. Based on the pH, temperature, dilution, the production of biogas was varying. The biomethanation production was calculated by the weight of air

TEST FOR METHANE:

Flame test:

The balloon was removed from the bottle and was connected to the sterile pipette and allow to light. Lightening of the blue colour flame indicates the presence of methane.

Calcium Carbonate test:

The balloon was removed from the bottle and the gas was passed into the calcium carbonate solution and no colour change indicates the presence of methane.

ORGANIC ASPECTS OF SUBSTRATE:

The samples were collected before and after fermentation. The following tests were done for the biochemical analysis of substrate.

Test for Carbohydrate:

0.1gm of extract was taken in a test tube and mixed with 1ml of water and add two drops of α- naphthol reagent and 1ml of conc. H_2SO_4. A deep violet color at the junction indicates the positive result.

Test for Terpenoids:

0.5 gm of extract was taken in a test tube and add 2 ml of Chloroform and 1 ml of conc. H_2SO_4. Reddish brown color indicates the positive result.

Test for Reducing Sugar:

2 ml of extract was taken in a test tube and mixed with 5 ml of distilled water and filtered. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 mins. Orange red color indicates the positive result.

Test for Saponins:

0.2 gm of extract was taken in a test tube and add 5 ml of distilled water then heat to boil. Appearance of creamy mass of small bubbles indicates the positive result.

Test for Tannis:

2 ml of extract was mixed with 5 ml of distilled water in a test tube and heated on water bath and filtered. Then to the filterate 1 ml of Ferric chloride was added.A blue or green color indicates the positive result.

Test for Carbonyl:

2 ml of extract was taken in a test tube and add few drops of α, 4- dinitrophenyl hydrazine solution and shakes.The presence of yellow crystals immediately of an aldehyde indicates the positive result.

Test for Flavanoids:

0.5 ml of extract was taken in test tube and add few drops of NaOH and silver nitrate solution. Black precipitate indicates the positive result.

Test for Glycoxide:

2 ml of extract was taken in a test tube and add 3-4 drops of Fehling's solution and boiled for 2 mins .Black red color indicates the positive result.

Test for Protein

2 ml of extract was taken in a test tube and add 2 ml of NaOH and 2 drops of copper sulphate. Violet color indicates the positive result.

Test for Amino acid:

2 ml of extract was taken in test tube and add 5 drops of Ninhydrin solution and boiled for 2 mins. Blue color indicates the positive result.

RESULT

EFFECT OF pH ON BIOMETHANATION:

Various pH was maintained 6.8, 7.0, 7.2, 7.4 with the addition of HCl and NaOH and the production of methane was observed. The optimization of pH in the biomethanation was given in the Table-1.

EFFECT OF TEMPERATURE ON BIOMETHANATION:

Different temperature ranges such as Psychrophilic ($<30^0$C), Mesophilic (30-40^0C) and Thermophilic (50-60^0C) conditions was carried out during anaerobic fermentation and observed the effect of temperature. The optimization of temperature in the biomethanation was given in the Table-2.

EFFECT OF WATER:

Water plays a major role in the production of biomethanation. Hot water showed higher production when compared to cold water. The optimization of water in the biomethanation was given in the Table-3.

EFFECT OF ENHANCERS:

Microbial cultures (*Lactobacillus* and *E.coli* culture) were added in the slurry as enhancers. The addition of *Lactobacillus* showed higher production when compared to *E.coli*. The optimization of enhancers in the biomethanation was given in the Table-4.

FERMENTATION TIME:

The mixtures were allowed to ferment for 12 days in an anaerobic condition. The results showed the volume of biogas production from the three wastes. The close observation showed, that cow dung started production on the second day, reaching peak on the 10^{th} day and yielding 45% of biogas. Vegetable gas production started production on the second day, reaching peak on the second day itself, and the gas production ranges from 85-90%. Fruit sample gas productions were the lowest in terms of gas production because of its high acidity and started gas production on the third day and the biogas produced was 40%. The results were given in the Fig.1.

AGITATION:

Agitation helps to intimate contact between the microorganisms and the substrate. This helps increase in the production of biogas.

PRODUCTION OF BIOMETHANATION:

The gas production was observed for the first twelve days in the six digesters. It was also observed that the vegetable digester had a peak production on the second day amounting to 90%, the fruit digester had a peak production on the third day amounting to 50% and the cow dung digester had a peak production on the 10^{th} day amounting to 45% respectively. The cumulative biogas production during the study period is shown in Table-4. It was observed that biogas production was actually slow at starting and the end of observation. Various factors such as pH, temperature, water, fermentation time and enhancers are affecting the production of biogas.

The biogas production was varied from substrate to substrate and by day to days. The optimum pH was 6.8 and the temperature was 30^0 C for vegetable sample followed by fruit waste, the pH was 7.2 and the temperature was 32^0C and cow dung with pH of 6.9 and the temperature was 40^0C. The addition of enhancers (*Lactobacillus* and *E. coli*), *Lactobacillus* showed high production when compared to *E. coli*.

RESULTS AND DISCUSSIONS

Table- 1: Effect of pH for the production of Biogas from the experimental Agronomic wastes

pH	Vegetable waste (100%)	Fruit waste (100%)	Cow dung (100%)
6.8	90	45	30
7.0	75	60	75

7.2	30	80	60
7.4	5	15	10

From the table -1 shows that the present study expressed the whenever the abiotic stress of pH level is increased on the three experimental samples biogas production was dramatically decreased the range between 90, 75 30 and very low level of 5% at the remarkable level of following pH 6.8, 7.0, 7.2 and 7.4 respectively in Vegetable wastes. Similarly, according to the pH biogas production denoted maximum production (80%) takes place 7.2 level of pH for fruit wastes also in cowdung wastes maximum 75% observed on the 7.0 pH. The overall result shows, among the three samples main biogas production occurred on vegetable wastes with low pH range compared with other two experimental samples.

Furthermore another important abiotic factor of temperature also affects the production of Biogas result depicted on the table-2. From the result, clearly revealed that the biogas production efficiency completely depends upon the temperature effect of the particular types or ingredients present on the experimental sample wastes. In vegetable wastes over maximum (90%) production noted at 25-30^{0}C. Hence, the current result conformed that this optimum temperature is susceptible or support for the production of biogas from the vegetable wastes. Moreover, other parallel higher production (80%) noted on 30-40^{0}C and 40-50^{0}C from Fruit and Cow dung wastes respectively. Subsequently, water also been affected the biogas production efficiency on the three wastes results presented Table-3.

Table - 2: Effect of temperature on production of Biogas from the experimental Agronomic wastes

Temperature (^{0}C)	**Vegetable waste (100%)**	**Fruit waste (100%)**	**Cow dung (100%)**
25-30	90	60	**30**
30-40	80	80	**45**
40-50	40	30	**80**
50-60	**15**	**10**	**65**

Table - 3: Influence of water on production of Biogas from the experimental Agronomic wastes

Name of the Wastes	**Mass of waste (kg)**	**Mass of water (kg)**	**Mixed ratio**	**Result (100%)**
Vegetable waste	1	1	1:1	100
Fruit waste	1	1	1:1	100
Cow dung	1	1	1:1	100

Fermentation duration for biogas production on three wastes result presented on the table-4. It explained that the vegetable wastes produces maximum gas production takes

place on the 2nd day resulting at 90% of biogas, followed by fruit wastes produces gas production on the 3rd day resulting at 50% of biogas and the production of biogas. The cow dung sample produces biogas on the 10th day resulting at 45%. From this result clearly observed that biogas production from the normal cowdung sample took for prolonged days up to 10 days to 1month. But interestingly, when the vegetable wastes fermented for biogas production, on second day itself maximum amount 90% of biogas production takes place. It takes short term period of time for production than the cowdung sample (normal sample) (Figure-1).

Table - 4: Duration of the Fermentation period (in days) for biogas production on with three different experimental waste(s)

Number of days	Vegetable waste (100%)	Fruit waste (100%)	Cow dung (100%)
1	10	5	3
2	**90**	40	5
3	55	**50**	8
4	30	35	11
5	10	20	15
6	5	15	17
7	3	5	20
8	1	2	25
9	0	0	30

10	0	0	**45**
11	0	0	39
12	0	0	30

Figure: 1- Biogas Production within Cow Dung Sample on 2nd Day

One of the burning problems faced by the world today is management of all types of wastes and energy crisis. Rapid growth of population and uncontrolled and unmonitored urbanization has created serious problems of energy requirement and solid waste disposal. Previously studied by Dhanalakshmi and Ramanujam, 2012 vegetable market wastes contribute to a great amount of pollution hence there has been a strong need for appropriate vegetable waste management systems. Fruit and vegetable wastes (FVW) are produced in large quantities in markets, and constitute a source of nuisance in municipal landfills because of their high biodegradability (Chanchal and Biswas, 2012). In the present study, the result showed that the vegetable waste produce high amount of

gas when compared to fruit and cow dung. Futhermore, Ojolo *et al.* (2008) reported on comparative analysis of utilization with poultry, cow and kitchen wastes for biogas production and the analytical approach for predicting biogas generation in a municipal solid waste anaerobic digester respectively Adeyosoye *et al.,* (2010) The present result also confirmed by Dhanalakshmi and Ramanujam, (2012) with the other type of source estimated the proximate composition and biogas production from invitro gas fermentation of sweet potato and wild cocoa yam peels. This shows that carbohydrates have been broken down much faster than the proteins and fats present in the wastes and produced the gas (Nitin *et al.,* 2012). Waste degradation which was advantageous to the environment was also achieved in the process, thus disposal problems of wastes can be solved alongside energy generation (Angelidaki and Ahring, 1994). Similar result also been published by Chanchal Biswas, (2012) on another type of source by estimated the comparative study of biogas production from cow dung, cow pea and cassava peeling using 45 liters biogas digester. The result showed that cow pea produced the highest methane content of 76.2%, followed by cow dung with 67.9 % methane content and cassava peeling has the least methane content of 51.4%. Cow pea was favored in terms of volume of flammable biogas production of biogas and flamed on the 7^{th} day. The present work also agreed by several researchers with the production of biogas from the different types of source materials with kitchen wastes (Gunaseelan, 1987) vegetable wastes (Bouallagui *et al*., 2003); cowdung wastes (Cheerawit *et al*., 2012; Carrère *et al*., 2010; Baba and Nasir, 2012; Chanchal and Biswas, 2012).

CONCLUSION

Present study provides comparative information regarding fruitful utilization of vegetable wastes also with other two wastes of fruit and cow dung sample by anaerobic digestion process for the production of biogas. The mixture is maintained at the pH of 6.8-7.2. The biogas was collected in a balloon which was connected to the inlet slit of the reactor. The biogas production was checked daily and recorded. The result showed that , the vegetable wastes produces high gas production on the 2nd day resulting at 90% of biogas, followed by fruit wastes produces gas production on the 3rd day resulting at 50% of biogas and the production of biogas was low because of acidity. The cow dung sample produces biogas on the 10th day resulting at 45%. It takes more retention time to produce biogas. Hence, the results evidenced that the vegetable wastes with enhancer showed the higher production of biogas in less retention time.

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