IB DIPLOMA PROGRAMME

TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

BIOLOGY EXTENDED ESSAY

‘Comparison of Biomethane Yields of Two Different Methanogenic Bacteria Cultures at Different Temperature’

“How does the biogas production and biomethane content that are produced by the *Methanosarcina thermophila* and *Methanobrevibacter ruminantium* change at 37℃ (mesophilic temperature conditions); 55℃ (thermophilic temperature conditions) by using the same amount of 100.0 mL stock solutions of each methanogenic bacteria cultures by feeding with %8 dry matter content substrate every 16 hours until reaching twentieth day at constant conditions.’

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Contents

ABSTRACT

The Research Question of the study is "How does the biogas production and biomethane content that are produced by the *Methanosarcina thermophila* and *Methanobrevibacter ruminantium* change at 37℃ (mesophilic temperature conditions); 55 ℃ (thermophilic temperature conditions) by using the same amount of 100.0 mL stock solutions of each methanogenic bacteria cultures by feeding with %8 dry matter content substrate every 16 hours until reaching twentieth day at constant conditions.'

In this study, I aimed to compare the biomethane yields of two different methanogenic bacteria in thermophilic (55℃) and mesophilic (37℃) temperature conditions using pasteurized cow manure as substrate. Pasteurization via autoclaving was necessary to avoid contamination by native bacteria that is abundant in the digestive tract of the animal. The experiment was conducted with biodigesters with a working volumes of 1 liters, with stock cultures of bacteria kindly provided by the institution that this experiment took place. The substrate solution which were given every sixteen hours until reaching twentieth day and recorded the volume of biogas in liters. Then by the help of the results (volume of biogas) that were taken every sixteen hours, the cumulative biogas production was calculated of each different types of bacteria *Methanosarcina thermophila* and *Methanobrevibacter ruminantium*, were compared statistically and the hypothesis was supported.

The result of the experiment showed that, improvement of biogas production and biomethane content at thermophilic conditions, *Methanosarcina thermophila* produced the highest amount

of biogas in liters. At mesophilic conditions, *Methanobrevibacter ruminantium* produced the highest amount of biogas in liters. Overall biomethane content per given volume of biogas did not changed significantly according to T-test by considering the results of experiment. The

p value for Mesophilic Conditions (37℃) is 7.04239×10^{-15}. *p value* of the investigation were calculated as 2.46299×10^{-8} for Thermophilic Condition. As a result of the *P value* those two bacteria *Methanosarcina thermophila* and *Methanobrevibacter ruminantium* are able to produce highest volume of biogas at their optimum conditions.

Keywords: biomethane, biogas, methanogenic bacteria

Word Count: 317

INTRODUCTION

When I was taking biology class, our teacher started to talk about how can people put account the environmental agile. Then I remember the enormous garbage in Mamak, Turkey. Since I know there is also organic matters in this garbage, I started to search how those organic matters can be transformed to beneficial products for the environment. While I was searching I found that there is a huge biogas production center in Mamak. Biogas production is fast becoming a trend topic in Turkish energy production. With vast bio-resources such as agricultural residues, municipal solid wastes of organic content of fast growing urban population, Turkey has the potential to produce one tenth of its energy directly from renewable bio resources[1] [2]. I was really surprised and then I decided to create my own station with designing my experiment. Furthermore, according to my searching I need to determine the kind of bacteria because it varies in order to their workout mechanisms. Each of them are able to produce high content of biogas in different conditions.

In this study, I focused on methanogenic bacteria, which has crucial importance on biogas production and overall biomethane content in biogas. Biogas production generally happens via the process of anaerobic fermentation, the valuable content in biogas is the biomethane part, which can be easily combusted in generators in order to produce electricity.

[1] http://www.biyogaz.web.tr/tr/dokumanlar/egitim-dokumanlari

[2] www.uteg.org/makaleler/biyokutle_enerjisi_turkiye.pdf

Biogas typically refers to a gas produced by the biological breakdown organic material, usually by a wide variety of micro-organisms in the absence of oxygen (anaerobic fermentation)[3] . Release of biogas via anaerobic fermentation is actually a natural process, takes place more than frequently especially in hot and humid parts of the world like swamps. Even on dump sites or landfill sites, biogas accumulation happens frequently and inadequately designed sites can even explode. Mankind observed this phenomena for a long time, even the ancient Persians have records of flammable gases observed in swamp areas. But it took until late 20th century for mankind to truly understand the nature of biogas and learned to harness its potential by providing controlled environments in the form of biodigesters for methane producing bacteria to create a sustainable and clean energy source.

As being a pure bacteriological process, unlike any other means of renewable energy production, the biogas production requires not as much engineering like the other alternatives. The real know-how of the biogas industry is biological, hundreds of research groups around the world are concentrating their efforts for identifying methane generating bacteria (the methanogens), obtaining further information on anaerobic fermentation and means to optimize the process in order to get the best biomethane yield per unit of substrate used.

During the literature search, one thing that surprised me was the wide variety of defined methanogenic bacteria and even much more undefined. Although there was considerable information and very established understanding about the anaerobic process, biochemical pathways for methane conversion and design elements to enhance the biogas production and its

[3] en.wikipedia.org/wiki/Biogas

biomethane content [4], the main research interest seemed to be focused on the identification of methanogenic bacteria on molecular level, and the determination of the biogas potential of the bacteria in different types of environment and identifying their biomethane conversion efficiency for different types of substrates[5] [6] [7]. One of the factor that influences the biogas production is temperature.

In the light of the information I obtained from the literature, I decided to study the net biomethane yields of different methanogenic bacteria in different temperature conditions between thermophilic and mesophilic operating environments, using the same substrate in order to determine the best performing bacteria in terms of biomethane conversion. The bacteria I selected were *Methanosarcina thermophila* and Methanobrevibacter *ruminantium,* which are known as common bacteria found in ruminant digestive tract and found dominant in most of the samples obtained from biodigesters worldwide working on animal manure. Another reason for me to select these organisms they both have different optimum temperature conditions according to literature mentioned as 'The optimum temperature for activity was between 48℃ and 55 ℃ consistent with the optimum growth temperature for M. thermophila.' [8] For *Methanobrevibacter ruminantium; '*Optimum temperature is 37℃ .'[9] Also the availability of

[4] en.wikipedia.org/wiki/Anaerobic_Digestion
[5] Chaper one: an introduction to microbiology, Microbiology: An introduction Fortora, *Funke, Case; 2001*

[6] http://ocw.mit.edu/courses/civil-and-environmental-engineering/1-89-environmental-microbiology-fall-2004/lecture-notes/, lecture 17
[7] Anaerobic hydrolysis and fermentation of fats and proteins. Biology of Anaerobic Microorganisms *(Zehnder. J.B. ed) John Wiley and Sons, Inc. McInerney, M.J. (1988). (USA): 373-415.*

[8] http://www.ncbi.nlm.nih.gov/pubmed/10613861
[9] http://ijs.sgmjournals.org/content/52/3/819

Methanosarcina thermophila and Methanobrevibacter *ruminantium* as isolation of single bacterial lines from heterogeneous cultures is a difficult process requiring high expertise, and cultures of the bacteria mentioned above were kindly provided to me by Onkosel Biyoteknoloji laboratory at Hacettepe Technopark.

My research question which is developed from the research I did is that; "How does the biogas production and biomethane content that are produced by the *Methanosarcina thermophila* and *Methanobrevibacter ruminantium* change at *37*℃ (mesophilic temperature conditions); 55℃ (thermophilic temperature conditions) by using the same amount of 100.0 mL stock solutions of each methanogenic bacteria cultures by feeding with %8 dry matter content substrate every 16 hours until reaching twentieth day at constant conditions.'

HYPOTHESIS

Two types of digester operating conditions apply to biogas production being thermophilic and mesophilic conditions, thermophilic operation usually preferred when plant based substrates which have cellulosic content have been used. However, animal manure also contains partially digested plant residues along with a diverse population of bacteria. The thermophilic conditions also provide a level of enhanced hydrolysis of the substrate, therefore making it easier for the methanogens to convert readily available substrate to biogas. But the disadvantage is, most bacteria cannot operate efficiently at thermophilic conditions. According to the literature information the research question is supported. 'The process of organic material anaerobic digestion takes place in two main temperature ranges from 30℃-37℃(mesophilic conditions) and from 48℃ − 55℃ **(**thermophilic conditions). The majority of methanogens (the microorganisms that form methane from organic matter) belong to the mesophilic. They grow quickly in this temperature range and exhibit high degrees of conversion. A smaller proportion of methanogenic organisms are thermophilic, meaning that are attached perfectly to higher temperatures. Generally, at these temperatures all bacteria consume the organic substrate with higher rates and grow faster. Because of this, the digesters operated at thermophilic conditions may be constructed in smaller dimensions (which means lower manufacturing costs) while maintaining very high levels of biogas.'[10]

[10] http://www.biomassenergy.gr/en

Clearly, temperature is a determining factor on net biomethane yield per unit of substrate used which directly effects the bacteria involved in the process. As it is the effectiveness of bacteria that determines the net output and the temperature being one of the most effective parameters in biogas production.

I predict that; in a controlled environment using a single type of standard substrate at mesophilic (37℃)and thermophilic (55℃) operating conditions, *Methanosarcina thermophila* will produce the highest amount of biogas in liters because of its optimum conditions at 55℃. At *37*℃ *Methanobrevibacter ruminantium* will produce the highest amount of biogas in liters because of its optimum conditions.

METHODOLOGY

Method and Material Development

In order to create an experimental setup to find the answer to the research question "How does the biogas production and biomethane content that are produced by the *Methanosarcina thermophila* and *Methanobrevibacter ruminantium* change at 37℃ (mesophilic temperature conditions); 55℃ (thermophilic temperature conditions) by using the same amount of 100.0 mL stock solutions of each methanogenic bacteria cultures by feeding with %8 dry matter content substrate every 16 hours until reaching twentieth day at constant conditions.' I have started with creating the desired environment for methanogens. As temperature is a determining factor in the process, a biodigester that can provide complete control over all process variables, especially sensitive temperature control to the extent of 0.1ºC-100ºC and total impermeability which is necessary for providing anaerobic conditions to bacteria involved. In order to elicit the temperature in the biodigester; heating plate is used.

pH is also an important variable for the experiment because it affects the workout of the bacteria while biogas production. This variable is controlled by the sensitive detectors in the biodigester mechanism. If there would be a high deviation in the pH values, this situation can be altered by buffer solution. However, this would be an unexpected situation.

I tried to use minimum amount of materials during the experiment to less harm the environment and also keep the cost low.

To compare the biogas yields of bacteria, a gas flow meter is used that can volumetrically in liters determine the amount of biogas produced, and for measuring the methane content in a

given volume of biogas produced, an infrared methane sensor array has been utilized. IR methane sensor is used to measure the yield of the biomethane. I preferred this gas sensor because it is a well-developed measurement. By this way the errors are minimized. Those sensors are very durable to high and low temperature. The other measurements such as gathering the biomethane in a syringe would cause high deviations in the results that's why I didn't choose that way. Those well- developed equipment was procured by Hacettepe University Department of Environmental Engineering.

In order to compare the methanogens, with each other, a control group was required, as control group, a standard heterogeneous mixture of bacteria taken from an operational laboratory scale biodigester kindly provided by Hacettepe University Department of Environmental Engineering. The hydraulic retention time is an important parameter for biogas production, as it is the time needed for complete conversion of volatile solids to biogas. According to the literature[11], general retention time for cow manure is between 20-30 days, 20 days of hydraulic retention time has been determined for the experiment according to the literature data and after my counsel with Alper K. Doğan MSc. Phd., head of Onkosel Biyoteknoloji. Since it is too hard to stabilize the amount of bacteria, I prefer to stabilize the amount of cow manure which I used as stock substrate while feeding and stock solutions of each methanogenic bacteria cultures at the beginning by this way I am able to control the

[11] McInerney, M.J. (1988). *Anaerobic hydrolysis and fermentation of fats and proteins.* Biology of Anaerobic Microorganisms (Zehnder. J.B. ed) John Wiley and Sons, Inc. (USA): 373-415.

amount of bacteria. The reason why I couldn't stabilize the amount of bacteria is they can multiply by theirselves in the biodigester while the experiment.

Process conditions have been set after determining the retention time, the biodigester have been operated in a continuous manner, by daily feeds of substrate and removal of the same amount of digestate. Because it maintains the volumetric amount of substrate and solution in order to not affect the workout mechanism of bacteria during the experiment. If we did not stabilize the volume of substrate which is obtained from cow manure by dilution and stock solution of bacteria cultures then we would obtain unreliable data. Substrate material which was cow manure cannot be fed to the digester as a whole, it has to be homogenized and diluted, in order to provide enzymatic reaction. Also, for the sake of avoiding any bacterial contamination, substrate material has to be autoclaved in order to be sure that no native bacteria originating from the cow digestive tract interfere with the experimental outcome. The volume of biogas measured each sixteen hours until reaching the twentieth day and recorded then the mean values were taken in order to make comparison between those two bacteria known as *Methanosarcina thermophila* and *Methanobrevibacter ruminantium.*

General sterility of the work environment is mandatory when conducting biogas experimentation, as the substrates used are usually waste products. Precautions have been made in this experiment as using gloves during experiment, although the methanogens used are generally considered non-pathogenic, the substrate, which is cow manure, may contain pathogens that can be dangerous. Utmost care has been taken especially during the preparation of stock substrate solution. Acting according to the advice of Onkosel personnel, adequate amount of stock solution has been prepared in the beginning of the experiment and stored in refrigerator until the end of experimental phase.

The dilution ratio while preparing stock solution from cow manure is decided by the help of literature ; %8 dry matter content substrate is required for the optimum results for the workout mechanism of bacteria. If the dry matter in the cow manure will be more than that value than the bacteria will be over-feeded and infertile. This experiment is repeated for three times for *Methanosarcina thermophila*, *Methanobrevibacter ruminantium*, Heterogeneous control at mesophilic and thermophilic conditions.

Materials and experimental apparatus

1- Laboratory scale biodigesters (Sartorius Biostat control unit) set with 1 L working volume.
2- Beakers of 500 mL, 250 mL and 100 mL for solutions and dilution.
3- Automatic pipettes of varied volumes for dilution
4- Homogenizer (Daihan Scientific) for the preparation of substrate
5- Gas bags of 30.0 L volume for as sampling
6- Mettler gas flow meter
7- IR methane sensors (Draeger)
8- Graduated cylinders of 1000 mL volume for substrate transport
9- Custom made graduated cylinder for volumetric gas measurement and sampling.
10- Custom made 50 mL injector for inoculation.
11- %90 Ethanol (Sigma) for general sterilization purposes
12- Nuve OT100 vertical autoclave for sterilization of the substrate.
13- 100.0 mL stock solutions of each methanogenic bacteria cultures used in experiment. Delivered as bacterial viability verified with Vericon VIT methanogen kit. (Appendix I).
14- Magnetic Agitation and Heating Plate
15- Gloves

Procedure

1- Preparation and equipment setup

- Biodigesters to be used have been thoroughly washed and sterilized with 90% ethanol.
- 5.0 kilograms of dairy cow manure have been diluted in 4.0 L of water each to prepare the standard (required for the optimum results) %8 dry matter content substrate. The diluted stock substrate had been homogenized for 2 hours. After homogenization, the stock substrate have been partitioned into 50.0 mL aliquots and refrigerated for feeding the stock solutions of methanogens every 16 hours.

2- Inoculation

- 100.0 mL stock solutions of each methanogen was diluted to 20% concentration with autoclaved stock substrate, in order to achieve the optimum conditions for the bacteria to multiply in the biodigester. This is required for their enzymatic reaction while producing biogas. The stock solution has been inoculated to their respective biodigester. General layout of the experiment has been figuratively summarized in Diagram 1.
- As control group, two digesters have been fed with 1.0 L volumes of stock solution containing heterogeneous mixture of bacteria and substrate taken directly from a working digester.
- Average retention time has been set to 20 days, digester agitation set at 120 RPM, this is the velocity of mixture blend in order to avoid downfall of the bacteria and

not influence stock solution of methanogens and stock solution while the experiment. This action is achieved by the help of Magnetic Agitation. Average pH was stabilized between 7-7.5 at all times on all biodigesters.

- Temperatures have been set to 37.0^{o}C (mesophilic), and 55.0 ^{o}C (thermophilic) conditions for each methanogen involved, along with two control groups.

3- Observation of experimental progress and biogas formation

- Start of biogas formation of each biodigester have been noted in terms of any differences or lags, which will help as an additional data on the interpretation of results.
- All produced biogas has been stored in gas bags connected to each biodigester during the course of experiment.
- 30 samples per group has been considered adequate as for statistical analysis and experimental resolution, gas flow measurements have been conducted in order to keep track of the biogas production rate.
- Experiment has been finished at the end of 20 days, as the hydraulic retention time dictates and observation of biogas production has been established and started being produced on a steady rate.

4- Finalizing the experiment and data collection

- Cumulative volumetric biogas production took place for every biodigester has been measured every 16 hours at the end of 20 days time and the cumulative biogas volume values were taken.

- Gas samples taken from each gas bag have been analyzed with Draeger IR methane sensor array and volumetric amount of cumulative biomethane content of biogas produced from every biodigester have been quantified and documented.

5- *Data evaluation and comparison*

- Documented data collected from the experiment have been evaluated and compared for statistical significance using T-test and all the calculations and graphs were done by taking in the consideration cumulative biogas production and the amount of biomethane per volume of biogas for selected methanogens in mesophilic and thermophilic conditions.

The Experiment Setup

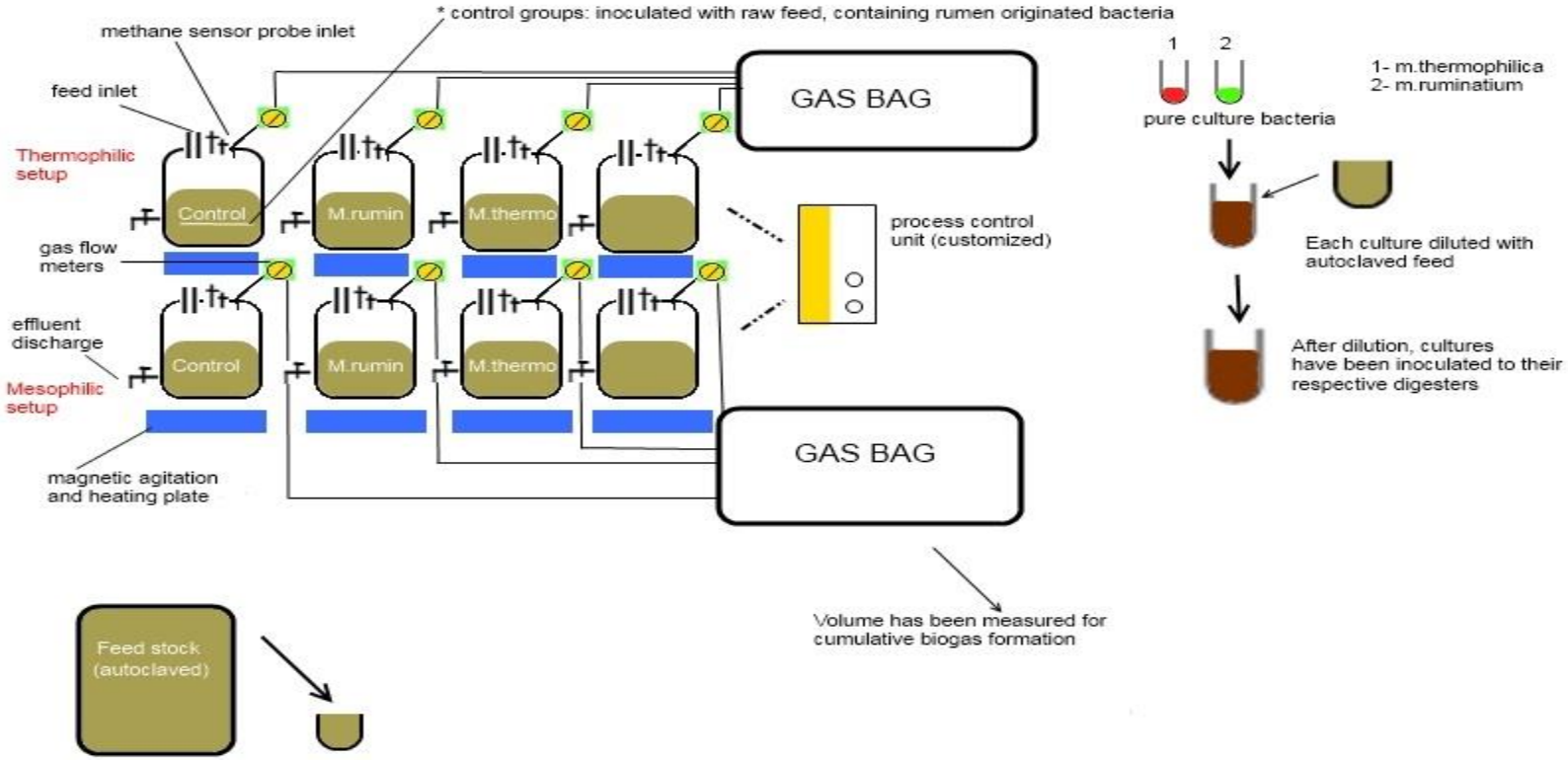


Diagram 1: Summarizes the materials of the experiment visually.

DATA COLLECTION AND PROCESSING

Biogas production

Cumulative biogas production and the amount of biomethane per volume of biogas for selected methanogens in mesophilic and thermophilic conditions at the end of 20 days are as follows:

Table 1: Cumulative Biogas production of 100.0 mL Heterogeneous Control, 100.0 mL *Methanobrevibacter ruminantium*, 100.0 mL *Methanosarcina thermophile* at Mesophilic conditions (37℃)

	Cumulative Volume of Biogas At the End of the Experiment								
Trial	Heterogeneous control $(\pm 0.1L)$	*Methanobrevibacter ruminantium* $(\pm 0.1L)$	*Methanosarcina thermophile* $(\pm 0.1L)$	Temperature $(\pm 0.1℃)$	pH (± 0.5)	Amount of Stock Substrate injected every 16 hours $(\pm 0.1mL)$	Amount of Stock Solution of Methanogenic Bacteria $(\pm 0.1mL)$	Time $(\pm 0.1hour)$	Velocity of Blender Mixture in the Biodigester $(\pm 0.1\text{RPM})$
1	110.7	162.4	72.5	37.0	7.0	50.0	100.0	480.0	120.0
2	111.8	162.4	72.2	37.0	7.0	50.0	100.0	480.0	120.0
3	112.5	163.1	72.9	37.0	7.0	50.0	100.0	480.0	120.0

Table 2: Cumulative Biogas production of 100.0 mL Heterogeneous Control, 100.0 mL *Methanobrevibacter ruminantium*, 100.0 mL *Methanosarcina thermophile* at Thermophilic conditions (55℃)

	Cumulative Volume of Biogas At the End of the Experiment								
Trial	Heterogeneous control $(\pm 0.1L)$	*Methanobrevibacter ruminantium* $(\pm 0.1L)$	*Methanosarcina thermophile* $(\pm 0.1L)$	Temperature $(\pm 0.1℃)$	pH (± 0.5)	Amount of Stock Substrate injected every 16 hours $(\pm 0.1mL)$	Amount of Stock Solution of Methanogenic Bacteria $(\pm 0.1mL)$	Time $(\pm 0.1hour)$	Velocity of Blender Mixture in the Biodigester $(\pm 0.1\text{RPM})$
1	145.8	65.1	227.1	55.0	7.0	50.0	100.0	480.0	120.0
2	146.9	65.1	228.6	55.0	7.0	50.0	100.0	480.0	120.0
3	147.3	65.2	229.3	55.0	7.0	50.0	100.0	480.0	120.0

According to the mean values of Cumulative biogas production of *Methanobrevibacter ruminantium* at Mesophilic Condition (37℃) and *Methanosarcina thermophile* at Thermophilic Condition (55℃),which was shown in Table 1 for Mesophilic Condition and Table 2 for Thermophilic Condition, T- Test was done to each different conditions. By this way I am able to find if there is a significant difference in the volume of biogas or not. T-Test values are shown as below for both conditions.

Table 3: T-Test values for *Methanobrevibacter ruminantium, Methanosarcina thermophile* at Mesophilic Conditions (37℃).

	Methanobrevibacter ruminantium	*Methanosarcina thermophile*
Mean	162.6	72.6
Variance	0.1	0.1
Observations	4	4
Pooled Variance	0.09625	
df	6	
t Stat	410.599957	
P(T<=t) one-tail	**7.04239E-15**	
t Critical one-tail	1.943180274	
P(T<=t) two-tail	1.40848E-14	
t Critical two-tail	2.446911846	

P value of the investigation were calculated as $\mathbf{7.04239 \times 10^{-15}}$ for Mesophilic Condition.

Table 4: T-Test values for *Methanobrevibacter ruminantium, Methanosarcina thermophile* at Thermophilic Conditions (55℃).

	Methanobrevibacter ruminantium	*Methanosarcina thermophile*
Mean	65.1	228.3
Variance	0.0	0.8
Observations	4	4
df	3	
t Stat	-355.0763898	
P(T<=t) one-tail	**2.46299E-08**	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	4.92598E-08	
t Critical two-tail	3.182446305	

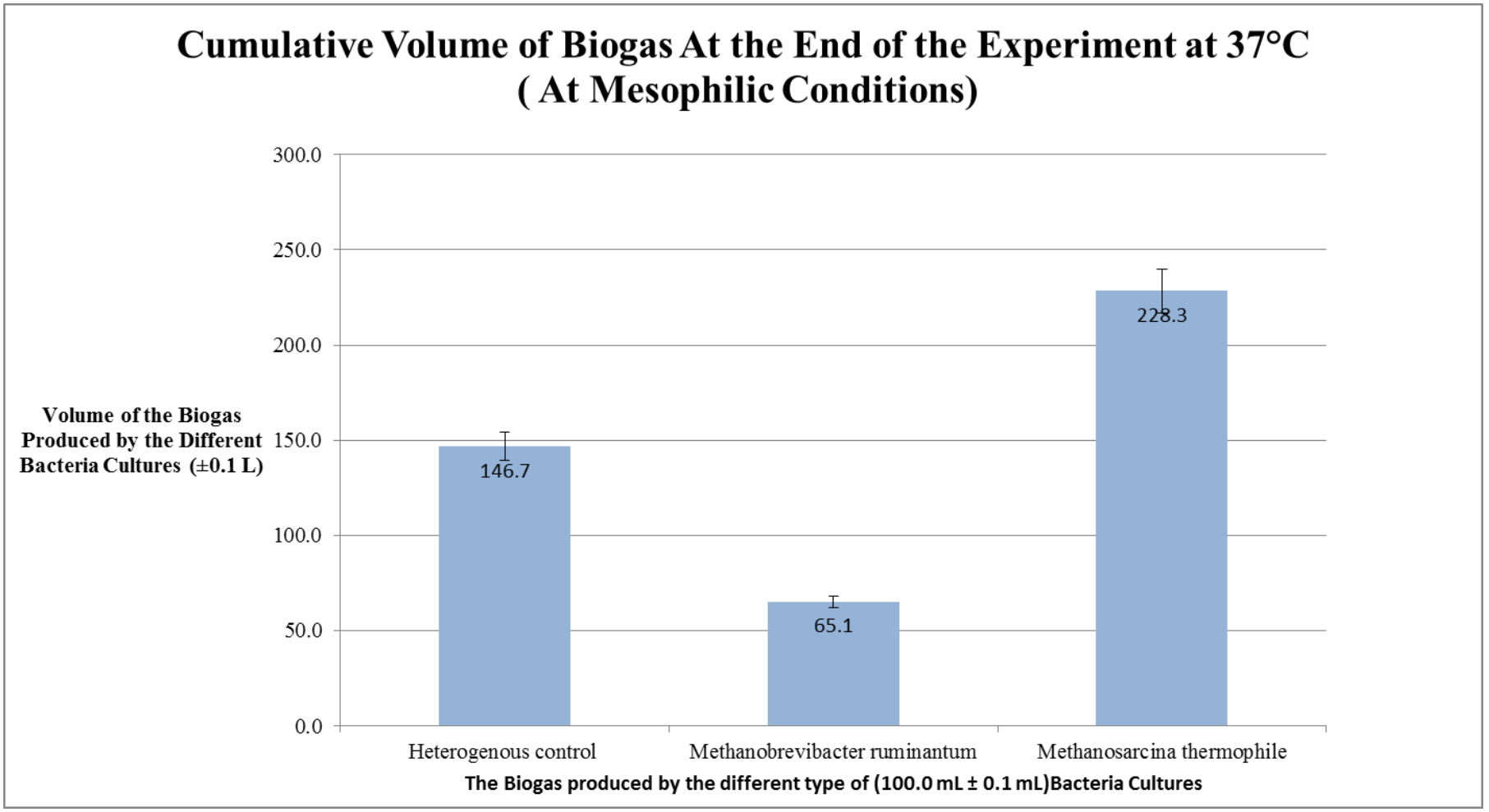
P value of the investigation were calculated as $\mathbf{2.46299 \times 10^{-8}}$ for Thermophilic Condition.

Table 5: The Mean values of the Heterogeneous Control, *Methanobrevibacter ruminantium*, *Methanosarcina thermophile* according to their Cumulative Volume of Biogas at the end of the experiment at Mesophilic Conditions (37℃).

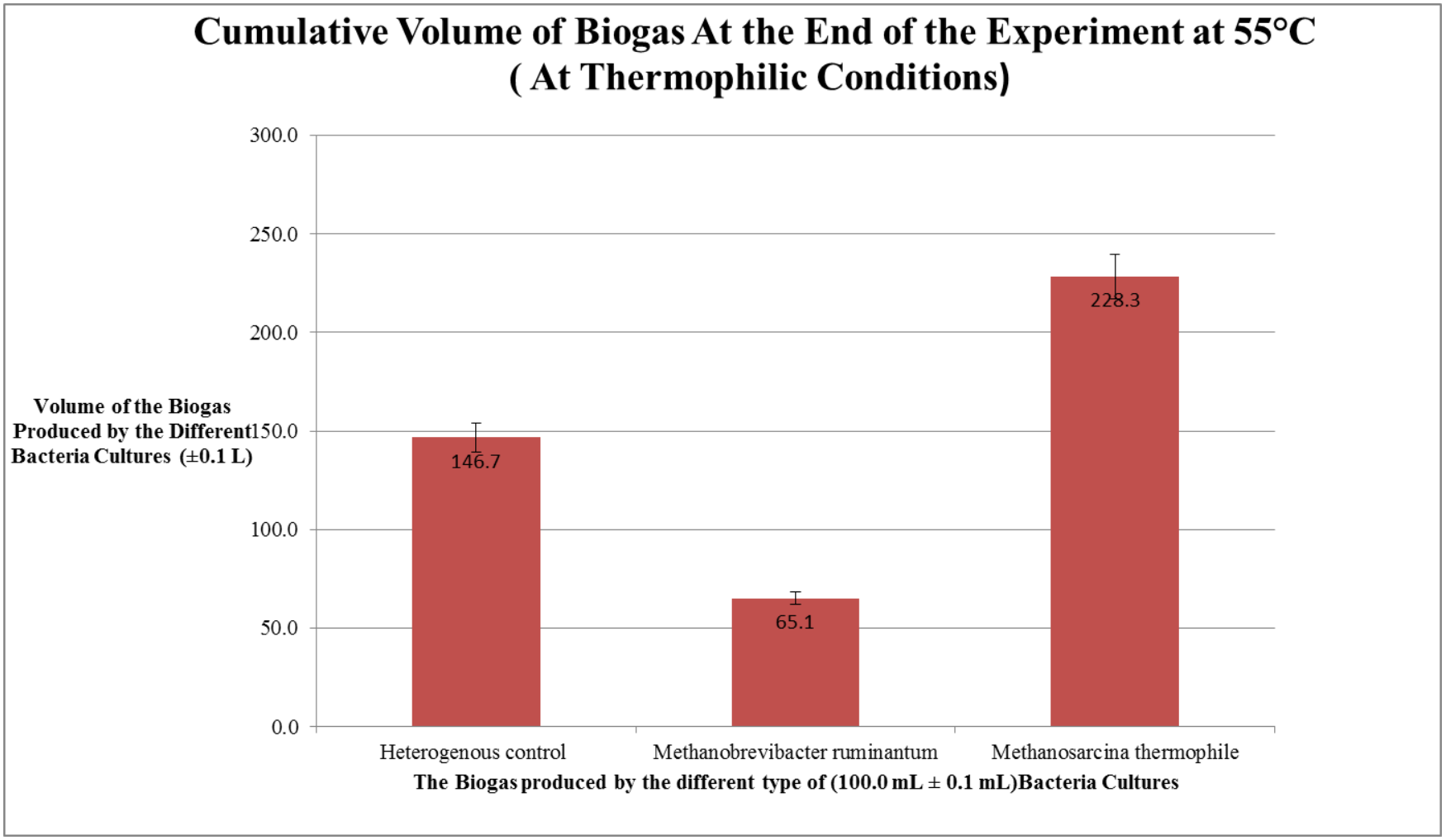
	Cumulative Volume of Biogas At the End of the Experiment		
Trial	Heterogeneous Control $(\pm 0.1L)$	*Methanobrevibacter ruminantium* $(\pm 0.1L)$	*Methanosarcina thermophile* $(\pm 0.1L)$
1	110.7	162.4	72.5
2	111.8	162.4	72.2
3	112.5	163.1	72.9
Mean	111.7	162.6	72.6

Table 6: The Mean values of the Heterogeneous Control, *Methanobrevibacter ruminantium*, *Methanosarcina thermophile* according to their Cumulative Volume of Biogas at the end of the experiment at Thermophilic Conditions (55℃)

	Cumulative Volume of Biogas At the End of the Experiment		
Trial	Heterogeneous Control $(\pm 0.1L)$	*Methanobrevibacter ruminantium* $(\pm 0.1L)$	*Methanosarcina thermophile* $(\pm 0.1L)$
1	110.7	162.4	72.5
2	111.8	162.4	72.2
3	112.5	163.1	72.9
Mean	111.7	162.6	72.6



Graph 1: Cumulative Volume of Biogas at end of twentieth day at Mesophilic Conditions (37°C)



Graph 2: Cumulative Volume of Biogas at the end of twentieth day at Thermophilic Conditions (55℃)

Amount of biomethane produced:

As being an important indicator for a methanogens effectiveness, net methane as volumetric percentage (v/v %) of cumulative biogas produced has been measured by IR methane Sensor and documented for mesophilic and thermophilic conditions respectively.

Table 7: Biometahane content at mesophilic conditions

Heterogeneous control	*Methanobrevibacter ruminantium*	*Methanosarcina thermophila*
49.0 %	**58.0 %**	46.0 %

Table 8: Biomethane content at thermophilic conditions

Heterogeneous control	*Methanobrevibacter ruminantium*	*Methanosarcina thermophila*
52.0 %	39.0 %	**62.0 %**

EVALUATION

The aim of the investigation was to compare the biomethane yields of two different methanogenic bacteria cultures of *Methanosarcina thermophila, Methanobrevibacter ruminantium* in thermophilic (55℃) and mesophilic (37℃) conditions using pasteurized cow manure as substrate. Experimentation phase has been concluded after 20 days. It is observed that rate of biogas production has been stabilized for all experimental digesters. There had been no significant changes in terms of biogas production in last five samples measured.

When obtained results are evaluated, it can be seen that my hypothesis has proved correct at thermophilic conditions. Although statistical significance is only slight at thermophilic conditions and there seems to be no statistical significance between groups at mesophilic conditions. Conducting post-hoc tests for further significance proved inessential as experimental group population is low and supplementing evidence arising from data collected output biomethane content and cumulative biogas production gave us enough data proved to be supporting indicators for the comparison of methanogens. By the help of the raw data, T-Test was done and the *p value* is calculated for both conditions. At mesophilic condition the *p value* is observed as $\mathbf{7.04239 \times 10^{-15}}$. At thermophilic condition the *p value* is observed as $\mathbf{2.46299 \times 10^{-8}}$. Since both *p values* are smaller than 0.05, the hypothesis is authenticated. As a result, *Methanosarcina thermophila* is the best performer at thermophilic condition and produced the highest volume of biogas. On the other hand, *Methanobrevibacter ruminantium* is the best performer *at* mesophilic condition and produced the highest volume of biogas. Those results are due to their optimum conditions and workout mechanisms.

When I look at the cumulative amount of biogas produced along with average biomethane content measurements of the Heterogeneous Control, *Methanobrevibacter ruminantium*, *Methanosarcina thermophile*; 52.0%, 39.0%, 62.0% respectively for thermophilic condition and it is clearly indicate that *Methanosarcina thermophila* has produced more biogas with significantly higher methane content especially in thermophilic conditions. At mesophilic conditions average biomethane content measurements of the Heterogeneous Control, *Methanobrevibacter ruminantium, Methanosarcina thermophile, Methanobrevibacter ruminantium;* 49.0%, 58.0%, 46.0% respectively and it excels among other groups in terms of cumulative amount along with biomethane content, but the bacteria performed inefficiently at thermophilic conditions.

At all conditions, rate of biogas production seemed to come to a steady state at day after day 16.

One interesting result I have noticed is the slight difference between the cumulative biogas production and the results obtained from samples. Although very close but not exactly the same. This may be originating from gas bag permeability, especially in the joints that connects the pipes to the bag itself. In order to avoid that limitation; an insulation to the joints which connects the pipes of the bag can be made for air proof.

If there would be a change in pH, a certain amount of buffer solution could be used, however in the experiment I didn't come across as a situation like this.

I chose to use IR Methane Sensor because it is a well-developed type of measurement nonetheless, I minimized the errors of scaling the volume of biomethane content.

I stabilized the initial amount of bacteria in volume by taking the cultures 100.0 mL, however I couldn't stabilize the number of bacteria in the digester while the experiment. Because they are able to multiply by theirselves and this could affect the cumulative biogas at the end of the experiment.

Since the biogas production is an exothermic reaction. The temperature while the experiment could undergo a momentary change because of the bacterial metabolism and affect the biogas production. At this point I was hard pressed. Because a detector in the biodigester controlled the temperature and it might not be a prevention for the momentary change in temperature. 'the operators of anaerobic digesters are forced to either cool down their digesters further on or to let them heat up and thus risk a maybe significant temporary reduction of the biogas yield caused by thermal inhibition of the microbial community.'[12] Also my statement according to my results are supported by literally.

The reason that I did three trials for each condition is; the bacteria cultures need to be finely processed and this event is very expensive and hard so I used minimum amount of bacteria culture to not waste. Also, I really take care the sterility of materials to avoid contamination and fallacy in the data. Overall results indicate that there is no contamination arising from substrate content, proving that autoclave and general sterilization procedures have been implemented correctly.

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[12] *Self-heating of anaerobic digesters using energy crops H. Lindorfer *, R. Kirchmayr, R. Braun* BOKU - University of Natural Resources and Applied Life Sciences, Vienna Department for Agrobiotechnology, IFA-Tulln, *Institute for Environmental Biotechnology, Konrad Lorenz Str. 20, 3430 Tulln, Austria*

CONCLUSION

According to the literature, biomethane content of biogas obtained from dairy cow manure with heterogeneous cultures of bacteria is within the range of %49-69[9]. Results of this experiment is generally in harmony with the literature data in that aspect.

Literature suggests that certain methanogenic bacteria have better adaptation ability to changes in temperature. In my study, it can be clearly seen that, when all other parameters such as agitation, bacterial load and substrate load has been constant, biogas production and content nearly stable at either mesophilic or thermophilic conditions. *Methanosarcina thermophila* excelling at thermophilic conditions as its name implies, signifying an evolutionary adaptation for operating more effectively at higher temperatures. *Methanobrevibacter ruminantium* performed best on mesophilic conditions, as this organism is known to be the most abundant in ruminant digestive tract, therefore its conversion efficiency is at best when operating in environments which temperature is close to body temperature[13] [14].

I have expected a significantly higher rate of biogas production and biomethane content in all thermophilic experiment groups because of autoclaving the substrate and increased heat in the environment causing further hydrolysis on the substrate but the results proved otherwise. Further literature research about the subject proved that although heat has positive effect especially on breaking the ligno-cellulosic bonds, most of the hydrolytic bacteria cannot survive

[13] *Schink, B. (2002). Synergistic interactions in the microbial world.* Antonie van Leeuwenhoek. 81: 257-261.

[14] *Chaban, B., Ng, S.Y.S. and Jarell, K.F. (2006) Archael habitats – from the extreme to the ordinary.* Canadian Journal of Microbiology. 52:,73-116.

at thermophilic conditions[15], along with the fact that my experiment groups lacked hydrolytic bacteria as the bacterial load purely consisting of selected culture of methanogen.

Also, in anaerobic digestion of organic material by methanogens, metabolic pathway is a determining factor on biogas production and quality (methane content). There is more than one pathway for producing methane gas, from acetic acid, from hydrogen and even from CO_2. Generally literature states that hydrogen pathway is the most efficient in terms of biogas amount, acetic acid pathway is more efficient in terms of biogas quality[16]. *Methanosarcina thermophila* is a known hydronegotrophic, this fact explains that how it produced so much especially in high temperature conditions, *and Methanobrevibacter ruminantium* is an acetotrophic bacteria, making effective conversion via acetic acid pathway, which explains its mediocre biogas production performance but increased methane content per volume of biogas.

Literature also states that hydrogen pathway is generally favored in thermophilic conditions and biogas yield of both quantity and quality from industrial facilities are

[15] *Ahring B, Ibrahim AA, Mladenovska Z (2001).* Effect of temperature increase from 55 to 65; on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. Water Resour. 35: 2446-2452

[16] *Demirel B, Scherer P (2008).* The roles of acetotrophic and hydrogenotrophic methanogens Turing anaerobic conversion of biomass to methane: a review. Rev. Environ. Sci. Biotechnol. 7: 173-190.

significantly higher than the ones operated at mesophilic conditions, especially for plant derived substrates[17] [18]. My findings also support that statement.

In general, both my study and literature data suggests that industrial biodigesters should be operated in thermophilic conditions for getting the most out of the substrate used. But in real-world conditions, maintaining a constant 55^{o}C temperature will be quite hard and will consume a lot of energy. Especially in cold climatic zones this fact alone is a major setback on thermophilic operation on an industrial scale. The amount of biogas must be used to heat the facility will be more than the extra production. But in my opinion, further research efforts especially focused on identifying new bacteria and even genetically modifying them will lead to very bright future on efficient production of biogas, which I see as a truly sustainable renewable energy resource.

[17] Microbial handbook for biogas plants: Swedish Waste Management U2009/03; ana Schührer, Asa jarvis, 2010 Edition

[18] Zieminski and Frac, Methane fermentation process as anaerobic digestion of biomass; African Journal of Biotechnology Vol. 11(18), pp. 4127-4139, 1 March, 2012 DOI: 10.5897/AJBX11.054 ISSN 1684–5315 © 2012 Academic Journals

APPENDIX I

Materials and experimental apparatus

Delivered as bacterial viability verified with Vericon VIT methanogen kit.

Our test kit VIT-Methanogenic bacteria enables the identification of living methanogenic Achaea (or: methanogenic bacteria) in samples of the anaerobic sludge treatment, biogas reactors and pellet sludges.

With VIT-Methanogenic bacteria the microbiology of methane producing plants reasonably can be monitored.

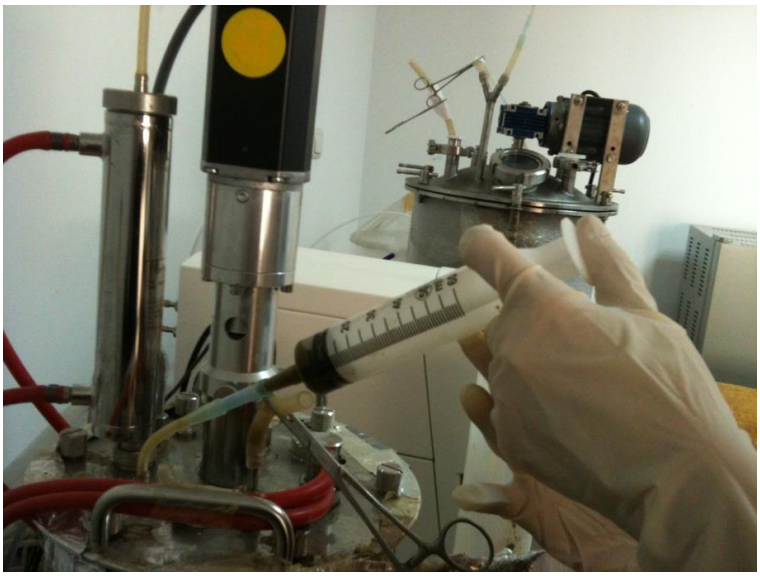
Advantages of this kit:

- Application as continuous monitoring system
- Detection is based upon the reliable VIT® gene probe technology[19]

[19] http://www.vermicon.com/en/en/products/VIT-Methanogenic_bacteria-462

APPENDIX II

Pictures of biodigester system and process controlling equipment



Picture1: Substrate loading

Picture 2: Me preparing Stock Solution for feeding

Table 9: Rate of biogas production at mesophilic conditions (37℃). Those results were taken every 16 hours until reaching the twentieth day and the volume of the biogas in the gas bags measured by the mettler gas flow meter every sixteen hour. In order to calculate cumulative biogas the results at each 16 hour were summed and remarked in Table 1. Here the results were shown in each tables and emphasizes the values at each trials while the experiment.

Hours (±0.1 hour)	Heterogeneous control (L). (± 0.1)			Methanobrevibacter ruminantium (L). (± 0.1)			Methanosarcina thermophila (L). (± 0.1)		
	Trials			Trials			Trials		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.0	0.0	0.0	0.0	0.2	0.2	0.1	0.0	0.0	0.0
48.0	0.1	0.2	0.1	0.8	0.8	0.7	0.1	0.1	0.1
64.0	0.9	1.0	1.1	1.1	1.2	1.3	0.4	0.3	0.4
80.0	1.1	1.2	1.3	1.4	1.4	1.4	0.6	0.6	0.7
96.0	1.3	1.5	1.6	1.6	1.6	1.7	0.9	0.9	0.9
112.0	1.6	1.7	1.7	1.9	1.9	1.9	1.1	1.1	1.1
128.0	2.0	2.0	2.2	2.6	2.4	2.6	1.2	1.2	1.2
144.0	2.4	2.4	2.4	2.9	2.8	2.8	1.5	1.5	1.4
160.0	2.6	2.6	2.5	3.6	3.7	3.8	1.8	1.7	1.8
176.0	2.9	2.9	2.9	3.9	3.9	3.9	2.1	2.1	2.1
192.0	3.2	3.4	3.3	4.3	4.3	4.3	2.2	2.4	2.4
208.0	3.8	3.8	3.8	4.9	5.0	5.0	2.6	2.6	2.6
224.0	3.9	3.9	4.0	5.0	5.2	5.3	2.8	2.8	2.8
240.0	4.3	4.5	4.5	5.4	5.4	5.5	3.1	3.1	3.2
256.0	4.5	4.7	4.8	5.6	5.6	5.6	3.2	3.2	3.4
272.0	4.8	4.8	4.8	6.7	6.7	6.7	3.5	3.5	3.5
288.0	4.9	4.9	4.9	7.3	7.3	7.3	3.5	3.6	3.6
304.0	5.2	5.2	5.3	7.9	8.0	8.1	3.2	3.2	3.6
320.0	5.1	5.1	5.1	8.1	8.2	8.2	3.4	3.4	3.4
336.0	5.3	5.3	5.3	8.4	8.4	8.4	3.5	3.5	3.5
352.0	5.6	5.6	5.5	8.6	8.6	8.7	3.6	3.4	3.4
368.0	5.5	5.6	5.6	8.7	8.7	8.7	3.4	3.4	3.5
384.0	5.7	5.7	5.8	8.6	8.5	8.5	3.6	3.5	3.5
400.0	5.6	5.6	5.6	8.7	8.6	8.6	3.4	3.4	3.5
416.0	5.6	5.6	5.6	8.8	8.8	8.8	3.6	3.6	3.6
432.0	5.5	5.5	5.5	8.9	8.9	8.9	3.5	3.5	3.5
448.0	5.7	5.7	5.8	8.8	8.8	8.9	3.5	3.4	3.4
464.0	5.8	5.8	5.8	8.9	8.7	8.7	3.6	3.6	3.5
480.0	5.8	5.6	5.7	8.8	8.8	8.7	3.6	3.6	3.3

Table 10: Rate of biogas production at thermophilic conditions (55℃) Those results were taken every 16 hours until reaching the twentieth day and the volume of the biogas in the gas bags measured by the mettler gas flow meter every sixteen hour. In order to calculate cumulative biogas the results at each 16 hour were summed and remarked in Table 2. Here the results were shown in each tables and emphasizes values at each the trials while the experiment.

Hours (±0.1 hour)	Heterogeneous control (L). (± 0.1)			Methanobrevibacter ruminantium (L). (± 0.1)			Methanosarcina thermophila (L). (± 0.1)		
	Trials			Trials			Trials		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.8	0.9
48.0	0.3	0.5	0.5	0.4	0.4	0.5	1.2	1.3	1.2
64.0	0.7	0.8	0.7	0.5	0.5	0.5	1.5	1.6	1.5
80.0	2.9	2.9	3.0	0.6	0.6	0.6	2.4	2.5	2.5
96.0	3.2	3.4	3.2	0.7	0.7	0.7	2.9	2.9	2.9
112.0	3.6	3.6	3.5	0.7	0.8	0.7	3.5	3.5	3.6
128.0	4.1	4.1	4.2	0.9	1.0	0.9	3.8	3.8	3.9
144.0	4.1	4.2	4.2	1.1	1.1	1.1	4.4	4.4	4.6
160.0	4.5	4.5	4.5	1.2	1.2	1.2	4.9	4.9	4.9
176.0	4.8	4.8	4.8	1.3	1.3	1.3	5.3	5.3	5.7
192.0	4.9	4.9	4.9	1.5	1.5	1.6	6.5	6.5	6.5
208.0	5.0	5.0	5.2	1.9	1.8	1.9	7.1	7.2	7.5
224.0	5.3	5.3	5.3	1.8	1.9	1.8	7.8	7.8	7.8
240.0	5.2	5.2	5.2	1.9	1.9	1.9	8.6	8.7	8.6
256.0	5.5	5.4	5.6	2.2	2.1	2.1	9.3	9.4	9.6
272.0	5.9	5.9	5.9	2.6	2.5	2.5	9.9	9.9	9.9
288.0	6.1	6.1	6.3	2.8	2.8	2.7	10.1	10.1	10.1
304.0	6.4	6.5	6.4	2.9	2.9	2.9	10.5	10.6	10.5
320.0	6.6	6.6	6.6	3.2	3.2	3.2	10.8	10.8	10.8
336.0	6.5	6.7	6.6	3.3	3.3	3.3	10.9	10.9	10.9
352.0	6.4	6.4	6.5	3.5	3.4	3.6	11.4	11.7	11.6
368.0	6.3	6.5	6.4	3.7	3.7	3.7	11.6	11.6	11.6
384.0	6.8	6.8	6.8	3.8	3.8	3.8	11.5	11.7	11.6
400.0	6.9	6.9	6.9	3.7	3.8	3.7	11.6	11.7	11.7
416.0	6.7	6.7	6.8	3.9	3.8	3.9	11.8	11.8	11.8
432.0	6.9	6.9	6.9	3.7	3.7	3.7	11.7	11.7	11.8
448.0	6.8	6.9	6.8	3.8	3.9	3.8	11.8	11.9	11.8
464.0	6.7	6.7	6.8	3.7	3.7	3.8	11.8	11.9	11.8
480.0	6.7	6.7	6.8	3.8	3.8	3.8	11.7	11.7	11.7

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