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Effect of fruit flavor compounds on biogas production

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Abstract:

The purpose of the experiment was to investigate the effect of fruit flavor compounds on biogas production from fruit wastes. The flavor compounds from different fruits were selected and synthetic medium was used throughout the experiment. The experiment was carried out both in batch and continuous process using thermophilic inoculums obtained from Söbacken waste management plant. The inhibitors were added at three different concentrations 0.05g/L, 0.5g/L and 5g/L respectively and were carried out in triplicates for batch cultivation.

The result from batch cultivation showed that hexanal, (E)-2-hexanal, myrcene and octanol showed inhibitory activity. Hexanal, (E)-2-hexanal, myrcene, and octanol decreased biogas production by factor of 316.8%, 434.22%, 329.68% and 433.61% at concentration of 5g/L. Continuous experiment was carried out on Automatic Methane Potential Test System AMPTS II. Eight reactors, each with inhibitor compounds were used with a retention time of 30 days and an organic loading rate of 3g VS/day. The inhibitor concentration was increased from 0.5g/L to 5g/L and for some compounds up to 10g/L. During cultivation, several factors were measured periodically such as pH, total biogas production, biogas composition, FOS/TAC and VFA.

The inhibitory effect was clearly shown at concentration higher than 0.5g/L. Addition of 5g/L hexanal, nonanal, (E)-2-hexanal, α-pinene , car-3-ene, myrcene and octanol resulted in reduction of biogas production by 81.2%, 4.67%, 50.74%, 7.06%, 24.01%, 31.84% and 52.85% respectively. When compared to batch process, continuous process required higher concentration of flavor compounds to reduce biogas production. This might be due to adaptation of cells towards toxic compounds during continuous process.

Keywords: *Fruit flavor, Batch process, Continuous process, Inhibition.*

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1. Introduction

1.1 Background

Fruits are one of the important commodities in the world. The increase in population results in rising fruit production annually. According to FAO 746 million tons of fruits were produced globally in the year 2009. A long distribution processes were involved from production of fruits till consumption of fruits. Fruit wastes are mainly due to physiological deterioration and mechanical damage of fruits. During post-harvest chain careless harvesting, improper storage and processing facilities leads to fruit wastes. Nearly 4.4 million tons of apple wastes are produced per day, 1.6million tons of banana wastes are produced per day and 1.2 million tons of orange wastes are produced per day [1]. Fruit wastes consist of 73-91% of carbohydrate, 4-10% of protein and 3-14% of fat on dry basis.

Recently, the traditional way of disposing fruit wastes is dumping it in landfill. This type of open dumping leads to many environmental problems such as emission of greenhouse gases and water pollution. Depletion of fossil fuel and energy crisis leads to an alternative energy biogas. Production of biogas requires a sustainable raw material; fruit wastes are a promising feedstock as it has high moisture content and organic matter. Other benefits of using fruit wastes are cheap and available throughout the year. So it becomes pertinent to convert the wastes into biogas as there is huge demand for biogas at present [1].

However, there are some constraints for biogas production from fruit wastes which are due to the presence of inhibitor compound that prevent microbial attack in fruits. Previous researches showed that in the presence of 0.1% of limonene from orange inhibited biogas production, thus terming it as inhibitory compound. On the other hand researchers in food industry are interested to extract food flavor to develop natural preservative and for food flavoring [1].

Compounds such as aldehyde, alcohol, terpenoids, phenols, lactones and esters comprise fruit flavor compounds and very scarce information is known about the antimicrobial activity of these flavor compounds on anaerobic digesting bacteria other than limonene [1].

1.2 Objective

To investigate the effect of fruit flavor compounds (aldehyde, alcohol, terpenoids) on biogas production from fruit wastes.

2. Literature review

2.1 Anaerobic digestion

The anaerobic digestion refers to a natural biological process where a stable and self-regulating fermentation carried out by three independent bacterial groups namely hydrolytic bacteria, acid-forming bacteria and methanogenic bacteria to convert organic matter into methane gases and carbon dioxide [2]. The domestic sewage sludge and effluents from industries containing high concentrations of organic material waste create a big hurdle for the clean environment which can be purified by this process. Though it is well known that the final products of converting organic material would be methane and carbon dioxide, but the type of digesters, bacterial populations and the sequential reactions vary widely from each other [3].

Anaerobic digestion of fruit wastes was preferred over composting because unmatured compost disperses very bad odor, it requires a large surface area for the process to be fast or it might take a year for the compost to be ready. Higher temperature is mandatory for the decomposition to take place. In case of anaerobic compost there is more risk of contaminating the groundwater if the landfill is not properly filled with impermeable materials.

Incineration is not usually recommended as the process may be simple but the maintenance of the Incinerator is not so simple and the building cost of the equipment is very expensive. Harmful gases such as dioxins and furans are released out which are carcinogenic. This may be an instant reduction of wastes but the left over ash has to be land filled again as it contains toxic material. This process is not so advisable for organic wastes, so an easy and simple method anaerobic digestion was picked among the three processes [4].

The methane rich gas produced during anaerobic digestion is called biogas [5]. Biogas production involves four main steps hydrolysis, acidogensis, acetogensis and methanogenesis. Biogas is a mixture of gases such as methane (40-70%), carbon dioxide (30-60%) and small amounts of hydrogen and hydrogen sulfide [6].

Biogas is majorly used as vehicle fuel and to increase the energy content upgrading is done. It is a process where carbon dioxide and other gases are removed and only pure methane is obtained [7].

2.2 Stages of anaerobic digestion

2.2.1 Hydrolysis

This stage is called the polymer break down stage where the large complex chains are disintegrated to small molecules in the presence of fermentative bacteria. The microbes produce hydrolytic enzymes (exoenzymes) such as proteases, lipase and cellulase which convert proteins to amino acids, lipids to fatty acids and polysaccharides to monosaccharides. Degradation of lignin and lignocelluloses takes more time and also the degradation is incomplete [8].

2.2.2 Acidification

The monomers from the hydrolytic phase are degraded and converted to short chain organic acids and alcohols. Some examples are butyric acid, propionic acid and acetic acid. The hydrogen ion concentration affects products of fermentation [8].

2.2.3 Acetogensis

The monomers and fermented products are converted to acetic acid (CH_3COOH), hydrogen (H_2) and carbon dioxide (CO_2) [6]. There is a symbiotic relation between acetogenic and methanogenic bacteria. Acetogenic bacteria are H_2 producers and for the survival and growth of these microorganisms, low concentration of hydrogen is required whereas methanogenic bacteria require higher partial pressure of hydrogen. There is an interspecies hydrogen transfer from acetogenic bacteria to methanogenic bacteria [8].

2.2.4 Methanogenesis

The methanogens converts acetate, hydrogen and carbon dioxide to methane. This process is strictly anaerobic process. The methanogenic microorganisms are sensitive to environmental variations.

The three types of methanogenic bacteria involved are:

1. *Methanosarcina* genus
2. *Methanothrix* bacteria
3. Bacteria that catabolize furfural and sulfates

The equations below explain the formation of methane [9].

CH_3COOH (acetic acid) $\longrightarrow$ CH_4 (methane) + CO_2 (Carbon dioxide)

$2CH_3CH_2OH$ (Ethanol) + CO_2 $\longrightarrow$ CH_4 + $2CH_3COOH$ (acetic acid)

$CO_2 + 4H_2$ (Hydrogen) $\longrightarrow$ CH_4 + $2H_20$ (water)

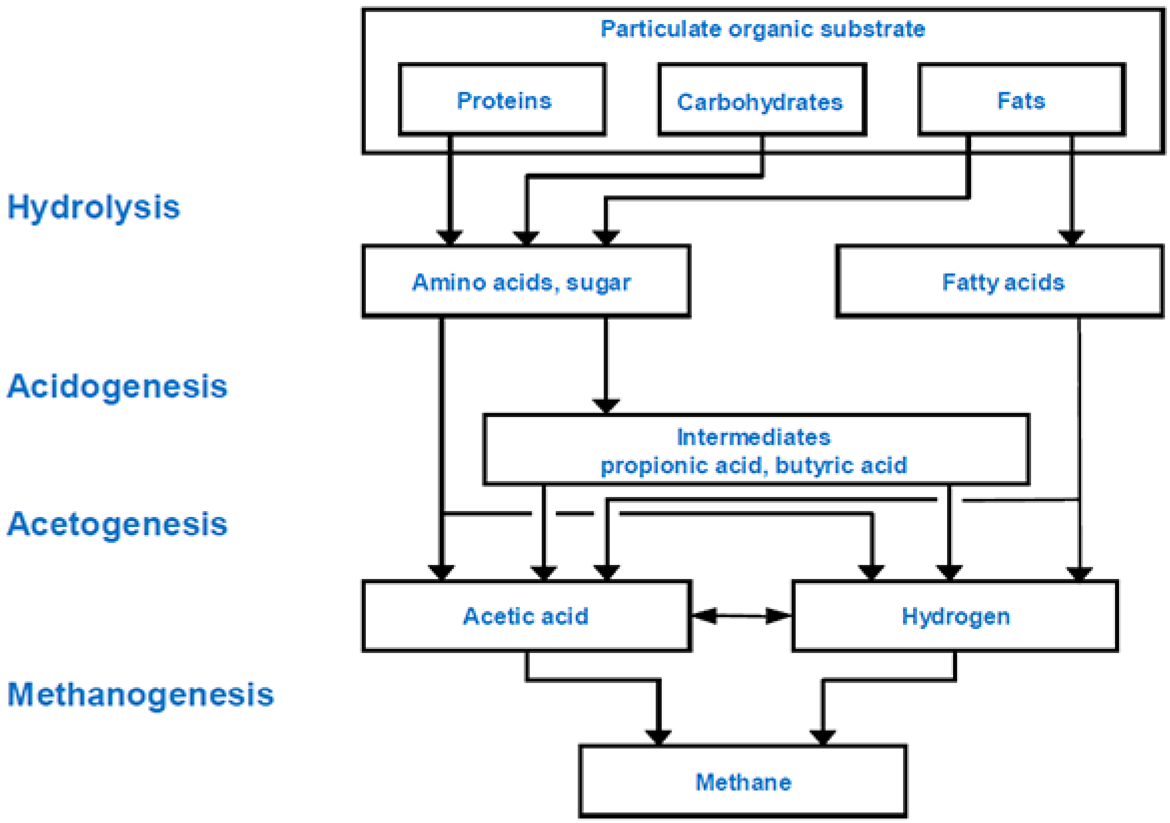


Figure 1. Anaerobic digestion process for the methane production involving hydrolysis, acidogensis, acetogensis and methanogenesis [10].

2.3 Operating conditions

There are many factors to be considered in anaerobic digestion process as these microorganisms are very sensitive to changes in temperature, pH and several other factors listed below.

2.3.1 Temperature

Anaerobic microorganisms work at three different temperatures, the psychrophilic (below 20^0C), mesophilic (20^0C -40^0C) and thermophilic (above 40^0C) [9]. The temperature must be maintained accordingly to get optimum methane production. Increasing temperature can increase biogas production but simultaneously ammonia concentration also increases thus leading to inhibitory effect. The thermophilic microorganisms can withstand change in temperature around 0.5^0C. Usually mesophilic and thermophilic microorganisms are preferred for a large scale process [10].

2.3.2 pH

Any anaerobic process should have optimum pH for better biogas production. The pH value should be around 6.8 to 7. Methanogenic bacteria decease if pH drops below 6.5. By maintaining the organic loading rate the pH can be maintained accordingly. Increased concentration of volatile acids in a digester decreases the pH, thus inhibiting the fermentation [9].

2.3.3 Nutrient

Microorganisms require energy to breakdown organic content. Besides carbon, nitrogen and oxygen other macronutrients such as potassium, magnesium, sulphur, calcium and trace amounts of compounds like molybdenum, manganese, selenium, nickel and selenium are necessary. An important factor to be concentrated is due to the presence of these compounds in a digester as agricultural and municipal solid wastes already include these compounds, the further addition of these nutrients may lead to inhibitory effect [11].

2.3.4 C/N ratio

Carbon and nitrogen are the most important nutrients in anaerobic digestion process. The energy is obtained from carbon and nitrogen is used for building the cell structure. The value of C/N ratio differs for different substrates but the optimum range is around 25-30:1 [11]. High value of carbon nitrogen ratio leads to increase in nitrogen consumption by methanogenic bacteria leading to lower biogas production. In contrast accumulation of ammonia leads to increase in pH at low values of C/N ratio.

2.3.5 Retention time

It is the amount of time the substrate remains in the digester. Process temperature and substrate type are two main factors influencing retention time. Other factors such as vessel geometry, mixing rate also has effects on retention time [11]. Too short retention time leads to inefficient extraction of methane and too long retention time means less amount of substrate being added [12].

2.3.6 Loading rate

This factor is mainly considered in continuous and semi continuous process. It is defined as the amount of raw materials fed per unit volume of the digester per day. The high amount of feeding leads to excessive ammonia accumulation and inhibits biogas production. Low feeding results such as insufficient supply of nutrients leads to lower biogas production. Hence, appropriate amount of the raw material should be added for optimal biogas production [13].

2.3.7 FOS/TAC

FOS stands for Fluchtige Organische Sauren while TAC stands for Totals Anorganisches Carbonate. The former is the measure of volatile organic acids while the latter is the measurement of total inorganic carbonate. It is the ratio of volatile organic acid to alkaline buffer capacity which helps in measuring the risk of acidification of a biogas process [14].

2.3.8 Volatile Fatty Acids (VFA)

The volatile fatty acids are measured by High Performance Liquid Chromatography (HPLC). The undissociated VFA at lowered pH had an inhibiting effect as they cannot diffuse into the cell and resulting in denaturation of proteins. It is important to estimate the amount of VFA as it as an indicator for the efficiency of a digester [15].

The amount of propionic acid is observed periodically as it is one of the best indicators in a balanced system. The decomposition of propionic acid is balanced if the concentration of propionic acid increases it is an alarm for an unstable process [16].

2.4 Fruit

2.4.1 Global fruit production

Fruit is an important part of human diet. It has a very high nutritional value with 70-85% of water content and quite a high amount of carbohydrates, low contents of fat and protein, traces of vitamins, minerals, fiber and antioxidants.

According to FAO the total global fruit production in the year 2011 was 637,864,630 tons. The total fruit production in the world from 2000 to 2010 is shown in figure 2. The steady increase in the consumption of fruit has been observed annually due to increase in population. The standard of living has been considerably changed over the years due to the effective encouragement by the health agencies promoting the benefits of consuming fruits.

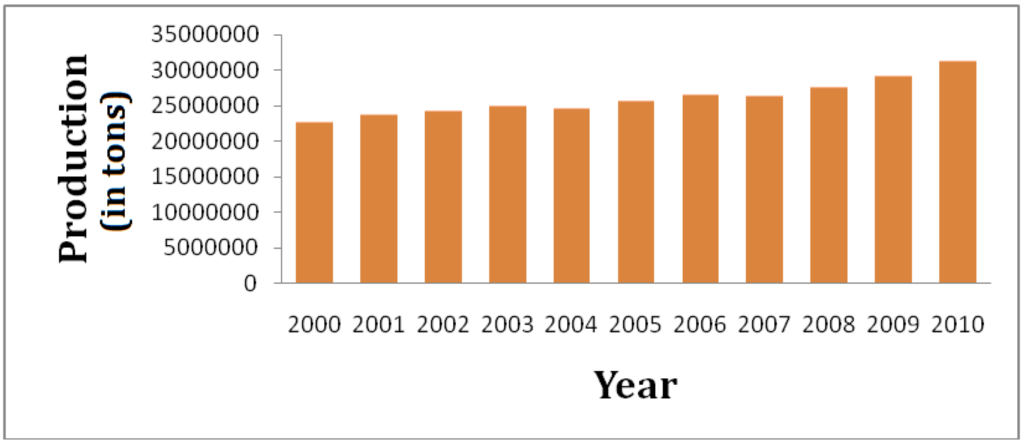


Figure 2. Graph depicting annual global fruit production in tons from 2000-2010 [17].

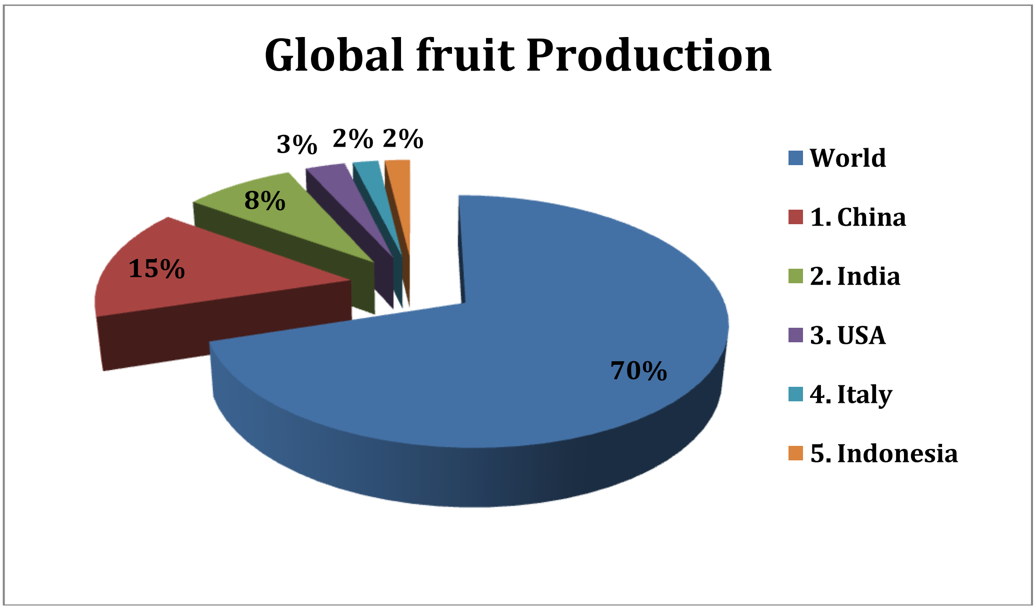


Figure 3. Pie chart description of top five fruit producing countries in the world [17].

This pie chart shows the leading producers of fruit in the world. As it is clearly visible from the figure that the major fruit producing country in the whole world is China contributing about 15% with an annual production of 134.95 million tons followed by India contributing 8% with a production of 74.83 million tons annually [17]. The reason for the large variety of fruit production in India and China is due to the favorable growing conditions like temperate climatic conditions and good arable land.

There is increase in trade volume of fruits in developing countries due to increased consumer demand in developed countries thus leading to the growth of small farms and the addition of new products, creating more job opportunities in both rural and urban sector.

2.4.2 Generation of fruit wastes

The fruit wastes are produced due to post harvest losses which includes harvesting, technological origin such as deterioration by biological or microbial agents and mechanical damage. Most post-harvest losses happen during improper handling, storage, transport and processing [18].

Physical deterioration is a natural aging process of fruits during storage period. This might be caused by chemical or biochemical agents thus producing undesirable intermediates and products resulting in loss of nutritional value of the fruit thus making it a waste [18].

Biological deterioration is the loss of fruits due to bacteria, insects, yeast and other microorganisms. Microorganisms like *Listeria monocytogenes*, *Aeromonashydrophila and E.coli O157:H7* are good examples for such a type of deterioration [19].

Mechanical damage leads to tissue wounds, breakage, abrasion and squeezing of fruits. This is mainly due to improper methods used during harvesting, packing and inappropriate transporting. This type of damage increases susceptibility to decay and growth of microorganisms [19].

There is no proper disposal for the by- products from fruits. They are merely dumped due to lack of machinery and infrastructure to process wastes in developing countries. Nearly 49-80% of the fruit production reaches consumers and the remaining is lost as fruit wastes [19]. For example in India which is the largest producer nation with an annual production of 150 million tons has nearly 30 % of fruit wastes [19]. Other factors like lack of clear policy for the production and maintaining fruit, unknown technologies associated with storage, processing and packaging of fruit. Vehicles used for transportation of fruits are not properly equipped with good refrigeration system thus leading to softening the tissue and elevated temperature enhances the growth of microorganisms thus deteriorating the fruit [19].

Below is a flow chart explaining the steps involved in transporting the fruits to the consumers from producers. The fruits are collected from the producers by traders and sold at wholesale markets from where is it sold to retails markets and then to consumers [19].

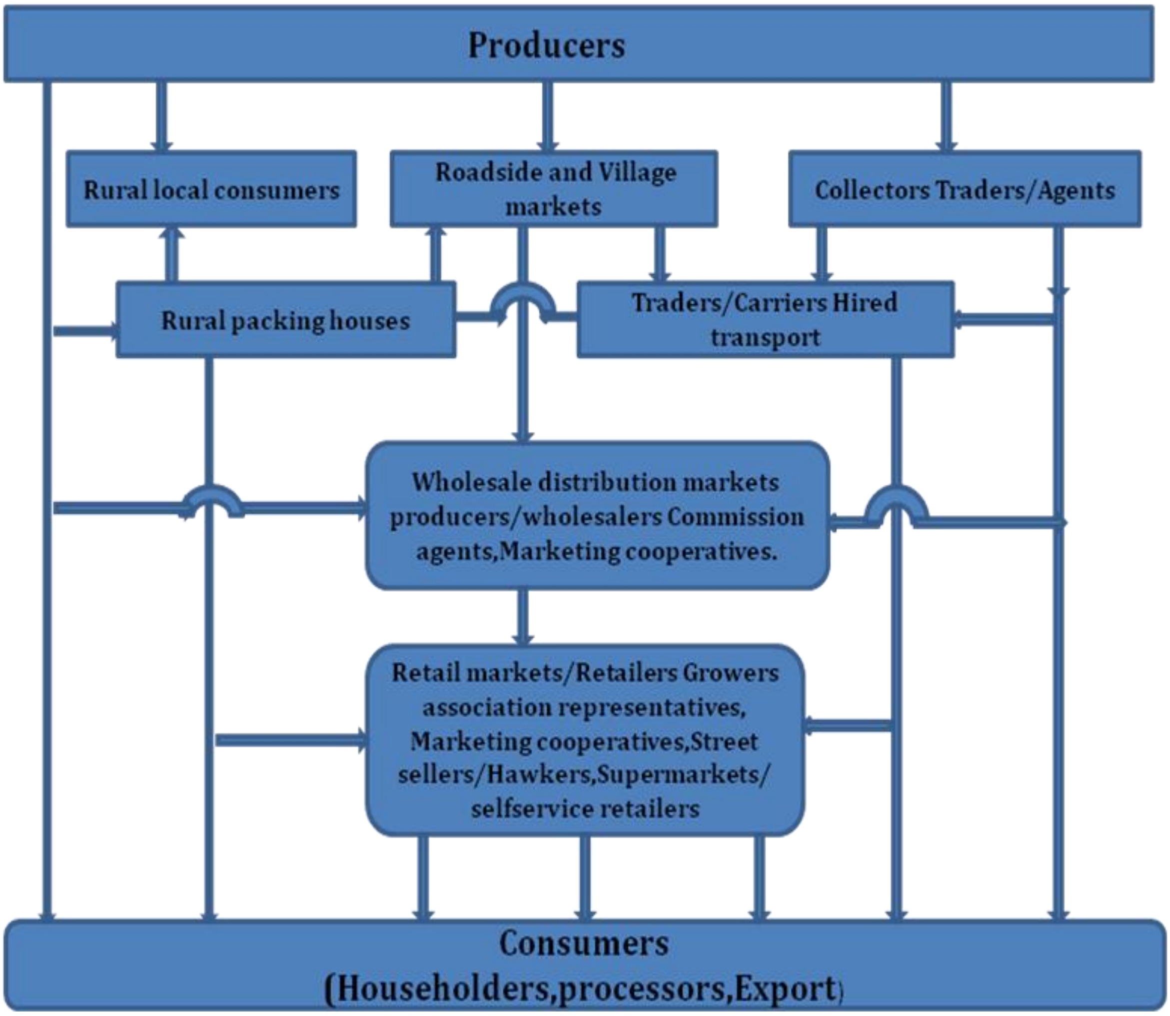


Figure 4. Flow chart of the steps involved from production of fruits to the consumption of the fruits [19].

2.4.3 Fruit Flavor

Flavor which is mainly determined by taste and smell is one important compound in all food. Flavor compounds can be mainly classified into groups like alcohols, aldehydes, lactones, phenols, esters and terpenoids.

The flavor compounds are present in all fruits at different proportions. Some examples of compounds and their presence in each fruit are given below in the table 1.

Table 1. Fruit flavor compounds.

Compounds	Fruits	Composition
Hexanal	Grape, mango, avocado, papaya, plum	NA*
(E)-2-hexanal	Grape, mango, plum, yellow passion fruit	NA*
Nonanal	Papaya, plum	NA*
Furanone	Strawberry, pineapple, raspberry, tomato	NA*
Octanol	Yellow passion fruit, orange juice	NA*
α-pinene	Chimoya, brazillainsoursop, custard apple, Venezuelan mango, orange, African atemoya, pond apple	23mg/kg; NA; 50-100mg/kg;25%; 25.6%; 28.9%; NA; 5.01 µg/kg, NA*
Myrcene	Alfonso mango, Indian mango, avocado, papaya	47%; 46%:NA;
Car-3-ene	Alfonso mango, keitt mango, Venezuelan mango, Brazilian mango	60%; NA: 15.88 µg/kg; NA*
Catechin	apple, apricot, blackberry, sweet cherry, black current, red current, mango, peach, pear, plum,	4-15: 50:7:22:7:12:17:23: 1-2:33:44 (in ppm)
Epicatechin	Apple, blackberry, bilberry: sweet cherry, black current, red current, red grape, peach, plum, raspberry, strawberry.	67-103, 61, 181, 11, 95, 5, 5, 29-37, 28

*(NA-Not Available)

Not much information is known about the quantity of these compounds present in fruits except for some compounds like myrcene, 3-carene and α-pinene.

2.4.4 Antimicrobial activity of fruit flavor

Antimicrobial activity can be defined as the ability to reduce the growth of bacteria and to suppress its reproduction [20]. Very scarce information is known on anaerobic microorganisms. The prolonged shelf life of fruit might be due to the antimicrobial activity of the fruit. One best example of fruit flavor compound showing antimicrobial activity is limonene in orange which can cease *S. cerevisiae* and digesting bacteria. Several researches are being done simultaneously to find out the antimicrobial behavior of flavor compounds and few compounds such as furanone, nonanal and hexanal were against gram positive and negative bacteria [20, 21].

All the microorganisms including gram negative bacteria exhibited visible antimicrobial activity when reacted against (E)-2-hexanal. Even by combining a sub-lethal amount of (E)-2-hexanal with *Escherichia coli* improved to a 4-fold increase in the antimicrobial activity of indole [22]. Highest activity was observed against the test bacteria by organic extracts like petroleum and methanol whereas contradictory results were observed from the aqueous extracts as they did not express major activity against the test bacteria [20]. In these two cases gram-positive organisms like *Staphylococcus aureus* were more vulnerable whereas on contrary *Salmonella paratyphi A* was more resistant [20]. When compared to *Salmonella* serotypes, *E.coli* exhibited higher sensitivity to the most of the oil extracts from fruits [21]. Extended lag phase was observed in fresh apple slices for *E.coli* and *Salmonella enteritidis* due to the effect of hexanal and (E)-2-hexanal on *Listeria monocytogenes* [23].

3. Materials and Method

3.1 Material

3.1.1 Sludge

The sludge used for our experiment was obtained from Söbacken, a wastes management plant, which is operated and owned by ‘Borås energy och miljö AB’. The inoculum in that case has thermophilic organisms which were stable at a temperature of about 55^0C.

3.1.2 Medium

The medium consisted of 20g/L glucose, 20g/L yeast extract and 20g/L nutrient broth. Except for the inoculum the nutrient medium was added to samples and standard. For continuous process the medium was prepared daily (27g of each was weighed and prepared for 900ml) and the inhibitors were added to the medium and mixed with magnetic stirrers finally adding it into the reactors.

3.1.3 Fruit flavors

Table 2. Compounds used in the experiment are the following

Groups	Compounds
Aldehyde	Hexanal
	(E)-2-hexanal
	Nonanal
Alcohol	Octanol
Terpenoid	α-pinene
	Myrcene
	Car-3-ene

These chemicals were ordered from sigma Aldrich a company producing and selling broad range of biochemicals including organic and inorganic chemicals and related products.

3.2 Method

3.2.1 Batch Cultivation

The reactors used in batch process were 119ml glass bottle with a rubber cork as shown in figure.

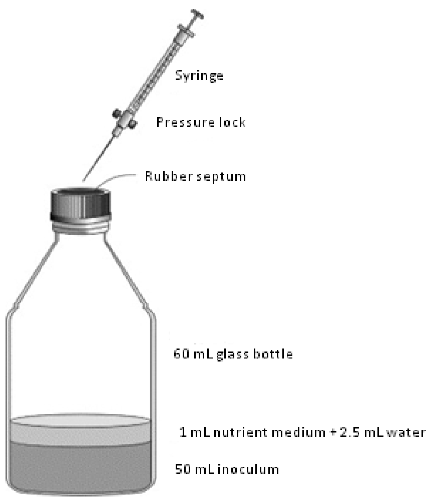


Figure 5. Reactor for the batch experiment [24].

The total working volume of the reactor was 53.5ml and a headspace of 65.5ml. The nutrient medium concentration was 20g/L. The sludge should be filtered and then added to the reactors in order to remove solid particles. The inhibitors were added in increasing concentrations from 0.05g/L,0.5g/L to 5g/L. The inhibitor compounds were diluted with water to get the specific concentration. Three replicates for each concentration was prepared. The table below shows the amount added for the sample, control and inoculum.

Table 3. Composition in each reactor

Bottle	Standard	Inoculum	Samples
Inhibitor	Not added	Not added	2,5ml
Sludge	50ml	50ml	50ml
Nutrient medium	1ml	Not added	1ml
Water	2,5ml	3,5ml	Not added

These bottles were then sealed and flushed with 80% carbon dioxide and 20% nitrogen to remove oxygen. The bottles were kept in incubator at a temperature of 55^0C as the microorganisms are thermophilic and they are stable at this temperature. The bottles were shaken in water bath two times a day for proper mixing of substrate and inoculum. At the time of shaking the temperature was maintained at 55ºC in water bath. The whole process was carried out for 30 days until the microorganisms reached stationary phase and the biogas production was stable.

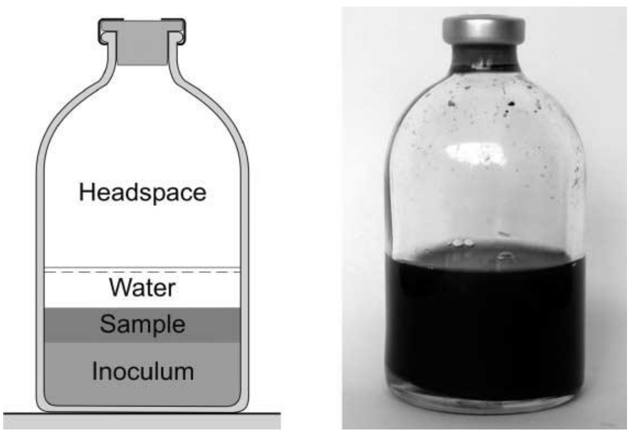


Figure 6. Reactor bottles [24].

All the flavor compounds were diluted with water depending on the concentration and added in the bottles. The amount of methane gas produced was measured using Gas Chromatography. The measurement was done every three days at the start of the experiment and then after 15 days the measurement was done 5 days once as the organism approaches stationary phase.

The gas was taken with 0.25ml pressure tight gas syringe (VICI, Precision sampling Inc., USA) from the headspace of the reactors. The samples were then immediately injected in to the gas chromatograph (Auto System, Perkin Elmer, USA). The amount of methane for each sample was measured twice i.e. one before releasing pressure and after releasing the pressure, this difference was used to determine the amount of gas produced during the period of between two measurements [24].

3.2.2 Continuous Cultivation

For this process automated methane potential test system (AMPTS II) was used. Ten reactors each of 2L in volume were used for this experiment.

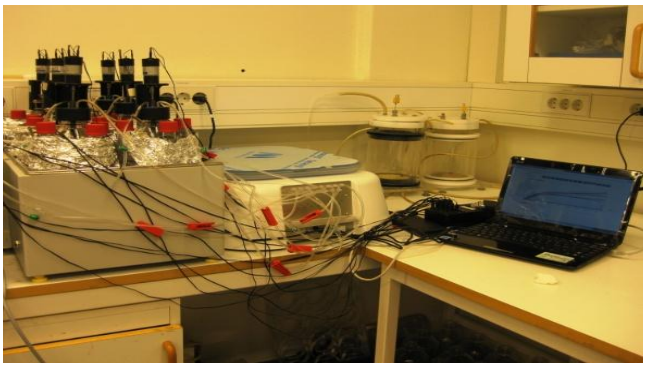


Figure 7. Reactor for Continuous process

This system has three units sample incubation unit, biomethane gas monitoring unit and data acquisition unit as shown in the figure 7.

Sample incubation unit consists of the reactors where the temperature is maintained at 55ºC. The tubes from the reactors are connected to biomethane gas unit where the paddle rises when the gas is produced and directly the data is stored in data acquisition unit. The reactors are connected with stirrers for continuous stirring.

The total working volume in the reactor 1800ml and it was equipped with the addition of inoculum and kept in incubation unit for three days and made sure that there was no leakage and the system was working properly and the amount of biogas production was automatically recorded.

The inhibitors were added in increasing concentrations starting from 0.05g/L then 0.5g/L and later 5g/L and further increased the concentration for some reactors. The hydraulic retention time of the reactor was 30 days with an organic loading rate of 3g VS/day. The inhibitor compounds were mixed with the nutrient medium first with magnetic stirrers and then added to the reactors.

3.2.2.1 Monitoring

Once the system was started certain conditions like pH, total biogas production, biogas composition, FOS/TAC and VFA should be measured periodically in order to make sure the system is running properly.

3.2.2.2 pH

The pH was measured daily with a calibrated pH meter. The pH was calibrated with two pH solutions, pH 7 neutral and pH4 acidic.

3.2.2.3 Total biogas production

For continuous process the total biogas production of each reactor is recorded in the data acquisition unit. This data was helpful to plot a graph for standard and each reactor thus finding out the inhibitory effect of individual compounds. For batch process the biogas production was measured using gas chromatography.

3.2.2.4 FOS/TAC ratio

The collected samples were centrifuged to remove coarse components. After centrifugation, 20ml of the substrate from each sample was taken and homogenized it continuously with magnetic stirrer during the titration process**.** The titration was done with 0.1N H_2SO_4 until pH 5 and the volume of acid added to achieve that respective pH is noted down and this step is continued again until pH 4.4 is observed and that particular value is noted down.

FOS/TAC ratio of 0.3 to 0.4 is optimal for any biogas plant and it was calculated by following equations:

TAC: H_2SO_4 – Volume added from start to pH 5 * 250

FOS: (H_2SO_4 – Volume added from pH 5 to pH 4.4*(1.66-0.15)*500 [14].

3.2.2.5 Biogas Composition

The biogas composition was determined using GC for both batch and continuous process. The pressure lock gas syringe (VICI, Precision Sampling Inc., USA) was used to take samples from the headspace of the reactor.0.25ml of the sample was taken from the headspace of the samples and injected in to GC (Auto System, Perkin Elmer, USA) equipped with a packed column (Perkin Elmer, 6´*1.8´´ OD, 80/100, Mesh, USA) and a thermal conductivity detector (Perkin Elmer, USA) with inject temperature of 150^{0}C where the mass of methane can be calculated. The carrier gas used in this operation was nitrogen with 25ml/min at 60^{0}C.

For reactors in batch process the methane was measured twice, before and after the release of pressure. The gas formed in the reactor was released with the needle for a minute or two and then the methane was measured again. The amount of gas released can be calculated by finding out the difference between the methane content in the headspace before and after the release. This is one important factor to release the gas during the incubation period or else it leads to building up of high pressure. The composition of methane was calculated by dividing the area of the sample with the standard.

4. Results and Discussions

4.1 Effect of different groups on methane production in batch experiment

4.1.1 Effect of fruit flavor compounds

An important advancement in the application of phytochemicals in place of antimicrobial applications is of extreme interest as they are renewable and biodegradable [22]. It is a growing interest on development of fruit flavor as a natural antimicrobial compounds since it is renewable and biodegradable. Fruit flavor can be classified into 6 groups specifically aldehydes, alcohols, terpenoids, esters, ketones and lactones. In this particular experiment the first three flavors were examined. In total seven compounds were analyzed i.e., hexanal, (E)-2-hexanol, nonanal, octanol, α-pinene, myrcene and car-3-ene anaerobic digestions were conducted with three diverse concentrations namely 0.5 g/L,0.05g/L and 0.005g/L respectively. By comparing the difference in the initial methane production and the accumulated methane production rate of the medium the evaluation of the inhibitory effects of the corresponding compounds were done.

4.1.2 Effect of aldehyde group on methane production

For the aldehyde group all the three flavour compunds namely hexanal, (E)-2-hexanal and Nonanal showed clear deviation depending on their concentrations as seen in figures 8, 9 and 10 respectively when compared to the control in the reaction. For hexanal, biogas reduction was observed at all the concentrations when compared to control. Almost no methane production was observed at 0.5 g/L and 0.05g/L concentrations in comparison to the control. Practically, no methane was produced at the presence of 0.5g/L of hexanal and (E)-2-hexanal. In the presence of nonanal at the same concentrations, 15% biogas was produced in comparative to the control. In overall, (E)-2-hexanal had higher methane inhibition when related to hexanal and nonanal. 50% more methane reduction could be observed in the presence of 0.5g/L flavor compounds. According to the trend observed in the graph of figures 8 and 9, in aldehyde group, inhibition was observed more in (E)-2-hexanal than hexanal and nonanal. At 5g/L concentration the reduction in biogas production for 30 days was found to be by factor of 329.68%, 300.74% at 0.05g/Land 283.01% at 0.05g/L respectively.

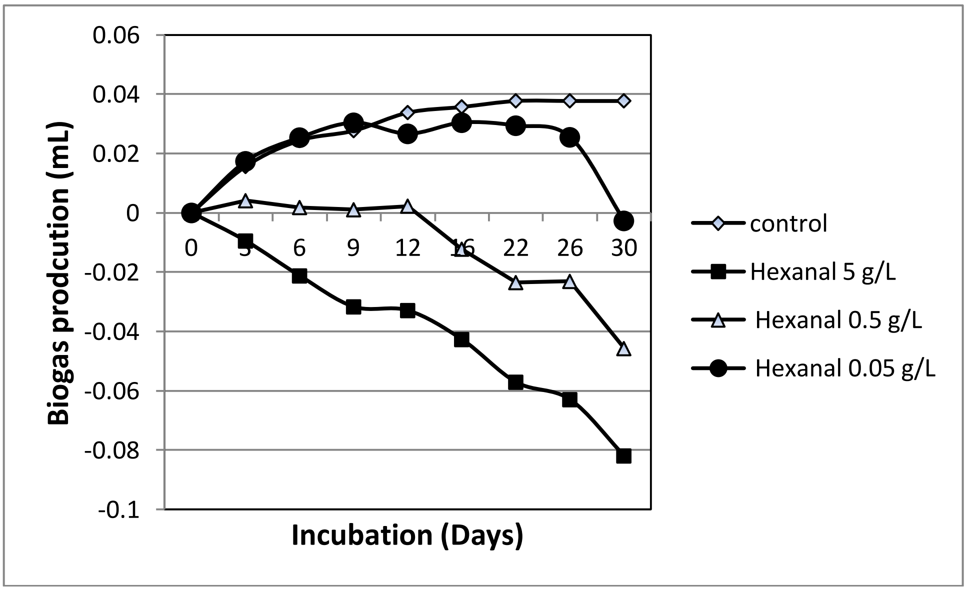


Figure 8. Effect of Hexanal on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

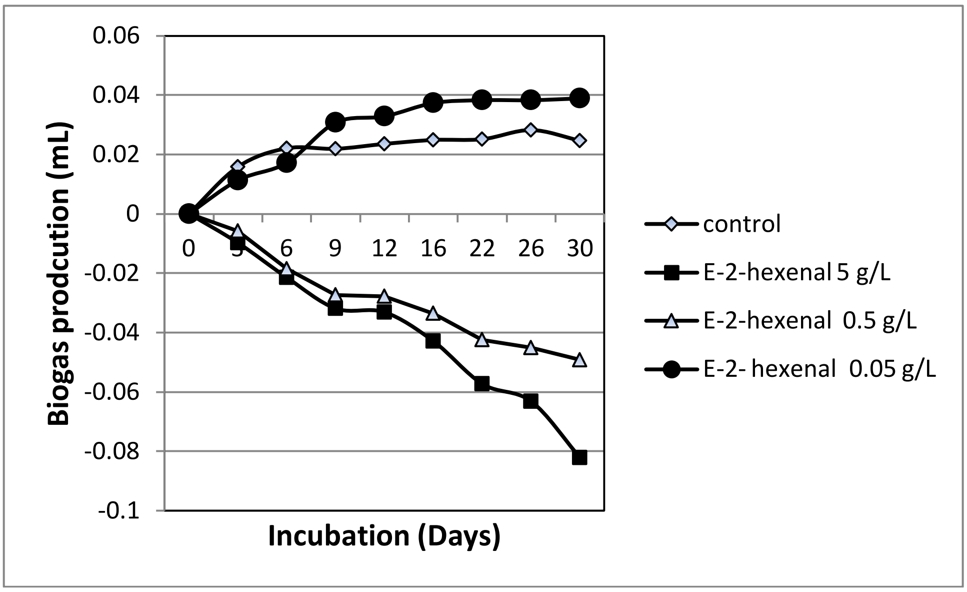


Figure 9. Effect of E-2-hexanal on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

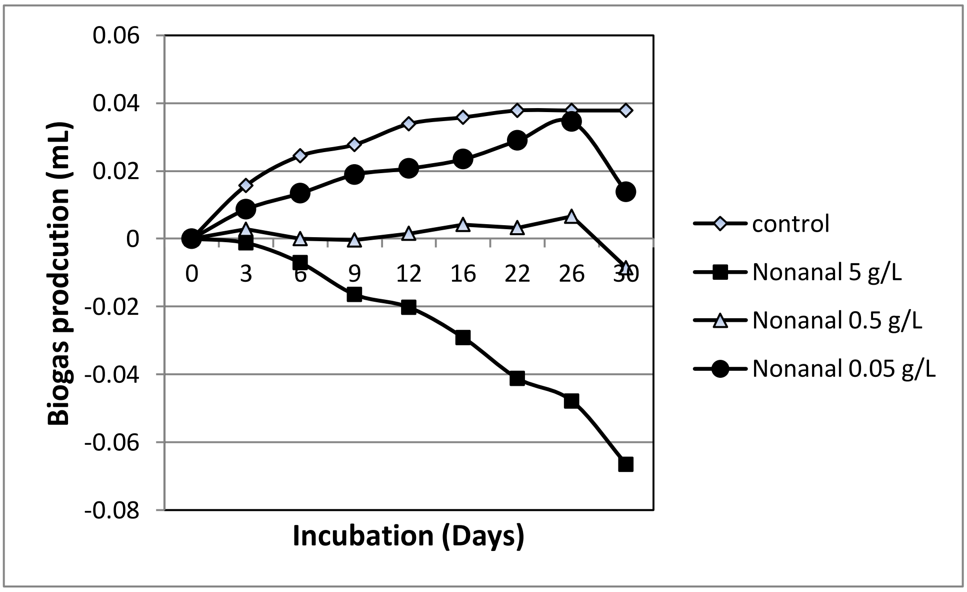


Figure 10. Effect of Nonanal on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

4.1.3 The effect of terpenoid group on methane production

Terpenoid group consists of car-3-ene, α-pinene and myrcene which were examined for the possible inhibition effects showed clear deviation depending on their concentrations as seen in figures 11, 12 and 13 respectively when compared to the control in the reaction. In case of terpenoids, myrcene was found to be the most inhibiting compound compared to α-pinene and car-3-ene. Almost three times of reduction in biogas was observed at concentration 0.05g/L and 5g/L for the compound myrcene.

Limited data was available on inhibitory effects of flavor compounds on methane production. Due to decreasing trend of methane production, assumption could be made possibly terpenoids might have inhibited it at all the examined concentrations. At the concentrations of 0.5g/L, 0.05g/L and 0.005g/L respectively, methane reduction exceeded by 50% by the presence of car-3-ene, myrcene and α-pinene. Results also indicate that the inhibitory effect of myrcene is higher when compared to car-3-ene and α-pinene. But this assumption is dubious and entails additional research. Methane production was observed for the first few days. In the presence of 0.05g/L α-pinene, highest methane production rate was achieved while at 0.5 g/L car-3-ene lowest digestion rate was attained. By the addition of 0.005g/L terpenoids low methane production rate was observed. This was around 50% production rate when compared to that of control. All tested terpenoids were inhibitory to anaerobic bacteria based on the results obtained.

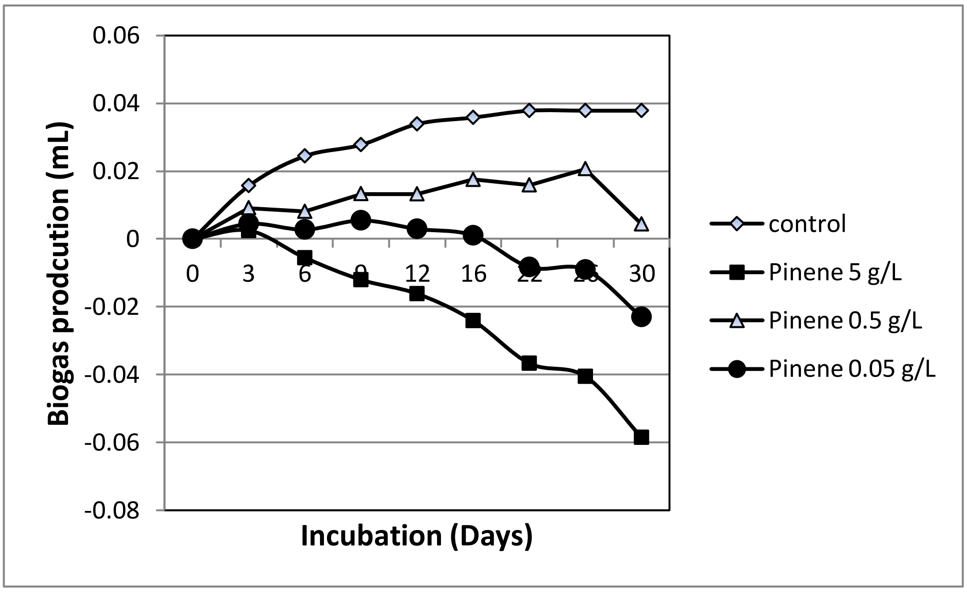


Figure 11. Effect of pinene on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

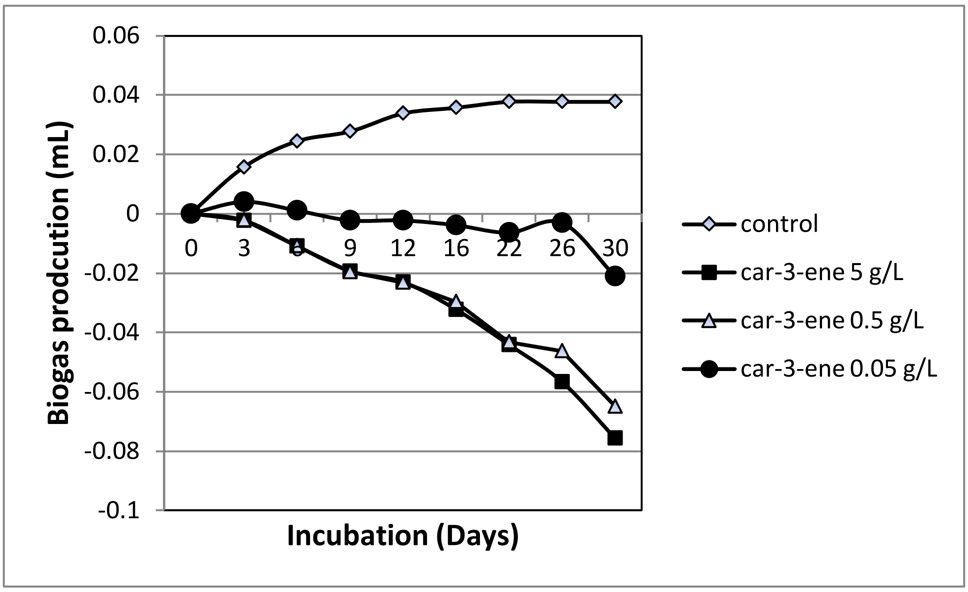


Figure 12. Effect of car-3-ene on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

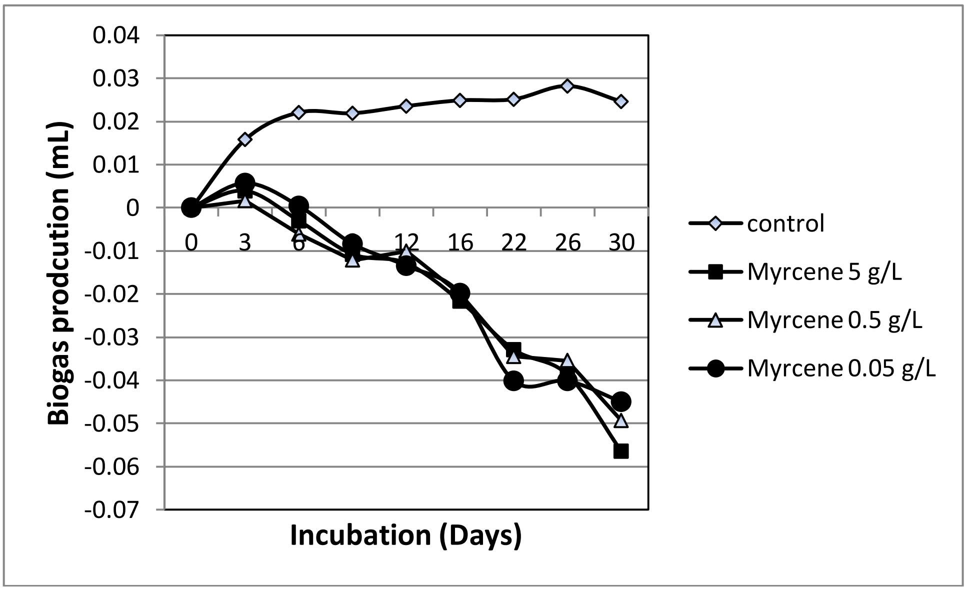


Figure 13. Effect of myrcene on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

Since terpenoids is s subclass of terpenes and Limonene belongs to terpenes family, they have similar characteristics. It is already known that limonene from citrus wastes is an inhibitor for biogas production and pretreatment is required to remove D-Limonene and open up the compact structure thus maximizing the yield of biogas. Subjecting the citrus wastes to high steam explosion at 150 C for about 20 minutes increases in production of methane. More than 94% of D-Limonene was removed under these conditions resulting in increase in methane yield by 426 %. This kind of pretreatment can be subjected to those fruits with high amount of terpenoids and then allowing them for anaerobic digestion thus increasing the methane yield [25]. Thus, probably if we do any pretreatment we can increase the methane.

4.1.4 Effect of alcohol on methane production

In this case 0.5g/L and 5g/L of octanol were found to inhibit. Initially 0.05g/L of octanol produced methane equal to the standard but after 20 days there is a drop in the production and there is no proper mechanism explained so far regarding this process. The more the concentration of octanol the less was the production of methane in the process. This also resulted in the lowering of methane production rate. 0.05 g/L of octanol was noticeably reducing the methane production rate almost to half. Similarly at 0.5 g/L of octanol, there was no evidence of gas production.

The change in the molecular chain length of any molecule depends on its toxicity which would be inversely related to the alcohol concentration. Lipid solubility plays a major role in deciding the molar effectiveness of the concerned alcohols [26].

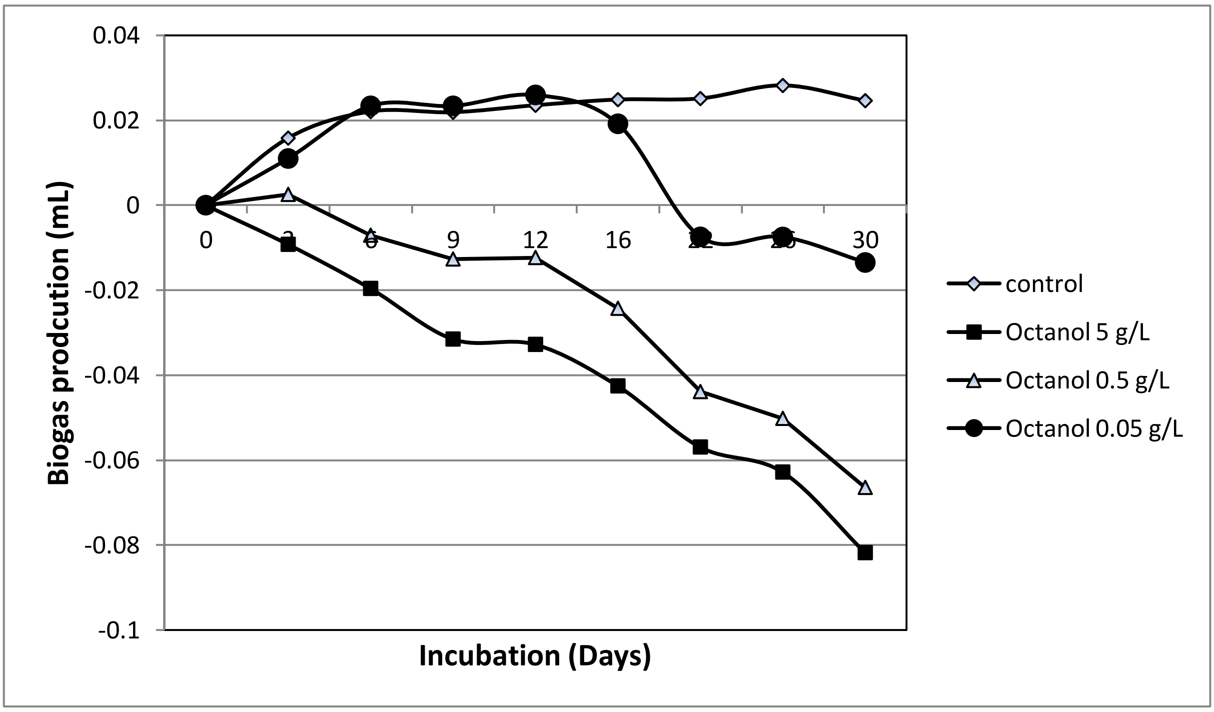


Figure 14. Effect of Octanol on methane production at different concentrations (5 g/L, 0.5 g/L and 0.05 g/L) in comparison to control.

Table 4. Batch result

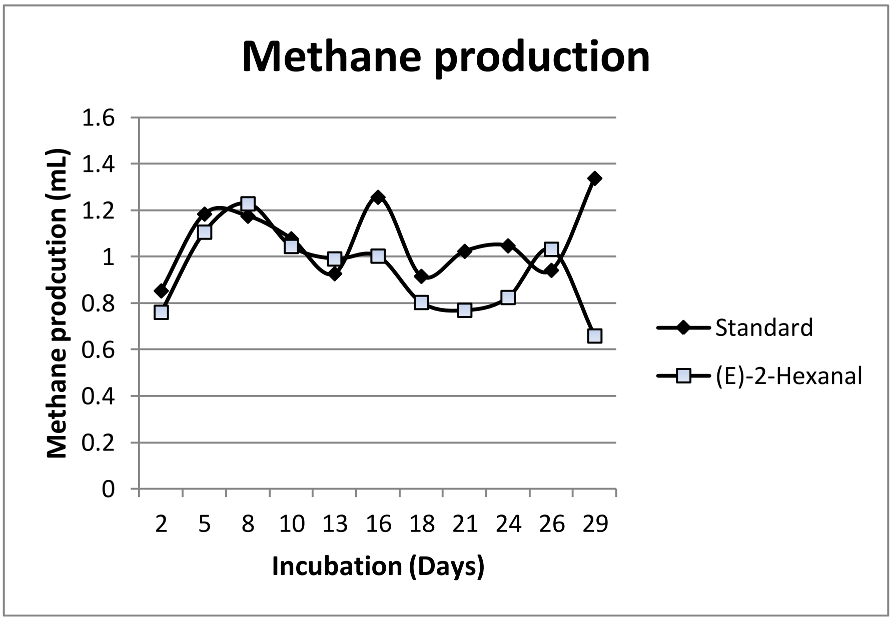
Group	Compound	Concentration (g/L)	Biogas production (ml)	Percentage of reduction of biogas (%) for 30 days.
Aldehyde	hexanal	0.05	Decrease	107.10
	nonanal		Decrease	63.43
	(E)-2-hexanal		Decrease	58.40
	hexanal	0.5	Decrease	221.02
	nonanal		Decrease	122.68
	(E)-2-hexanal		Decrease	300.74
	hexanal	5	Decrease	316.80
	nonanal		Decrease	276.01
	(E)-2-hexanal		Decrease	434.22
Terpenoids	α-pinene	0.05	Decrease	160.92
	car-3-ene		Decrease	155.73
	myrcene		Decrease	283,01
	α-pinene	0.5	Decrease	88.44
	car-3-ene		Decrease	271.78
	myrcene		Decrease	370.74
	α-pinene	5	Decrease	254.87
	car-3-ene		Decrease	300.14
	myrcene		Decrease	329.68
Alcohol	octanol	0.05	Decrease	154.88
		0.5	Decrease	370.16
		5	Decrease	433.61

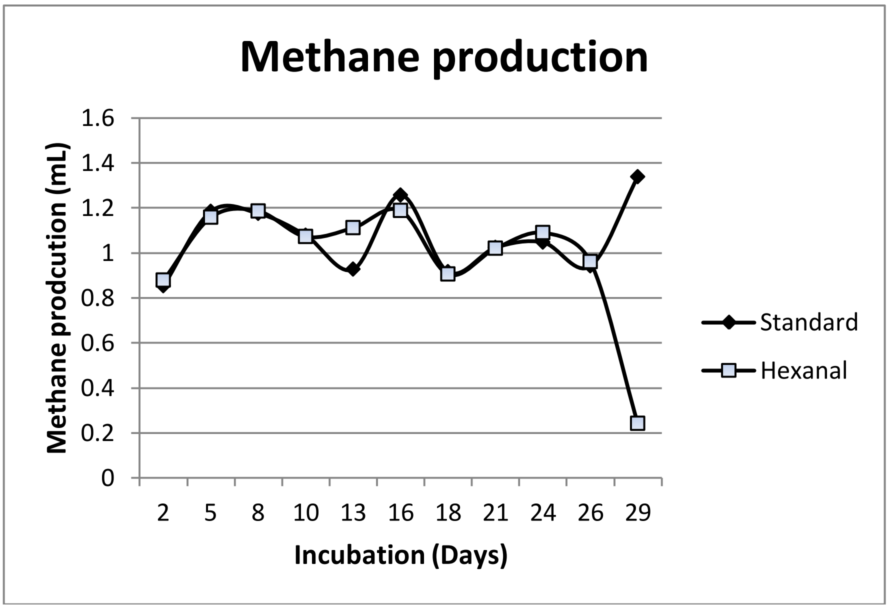
As observed from the batch results from table 4, all the flavor compounds in the reaction showed a significant reduction of methane production, i.e., almost 100% at 0.5 g/L concentrations. By assuming the above results, conclusions can be drawn as at higher concentration flavor compounds exhibited potential of reduction in the methane production whereas in the lower concentrations, the reduction was not significant. Myrcene was regarded as the most toxic flavor compound since it reduced methane production by more than half with only 0.005 g/L (lowest concentration added).

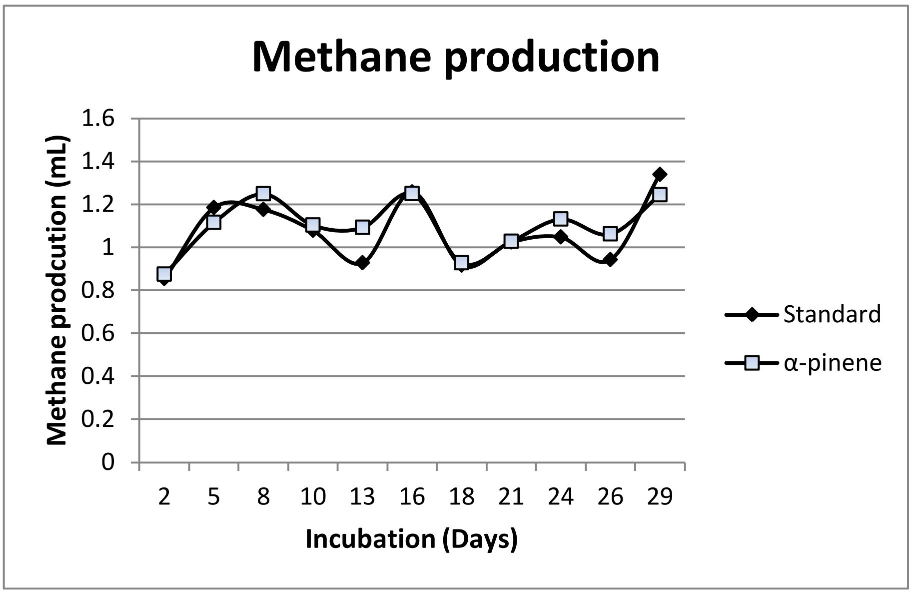
4.2 Effect of different groups on methane production in continuous experiment

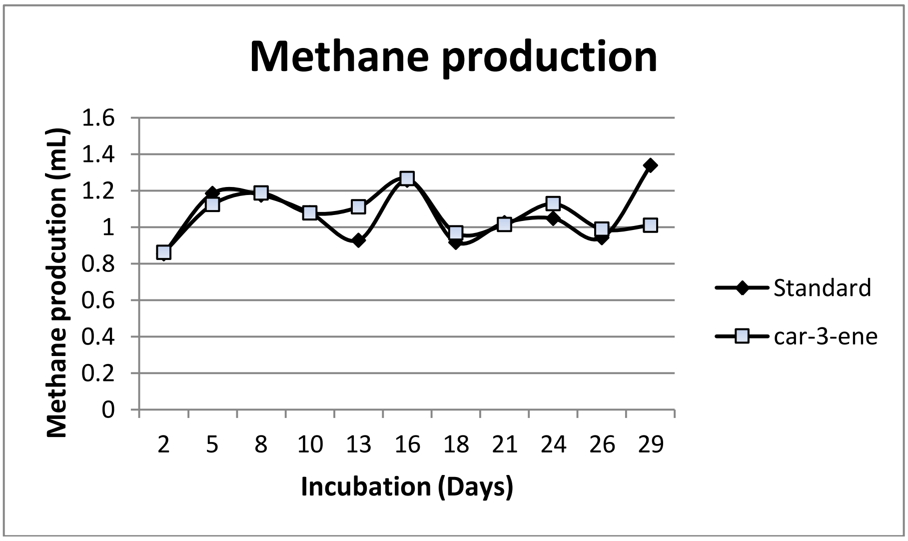
The results obtained from continuous process were different from batch process. No inhibition was observed for all the compounds at 0.05g/L concentration. The inhibition was observed with increasing concentration of the compounds. Much inhibition was observed in compounds Hexanal, (E)-2-hexanal, car-3-ene, myrcene and octanol.

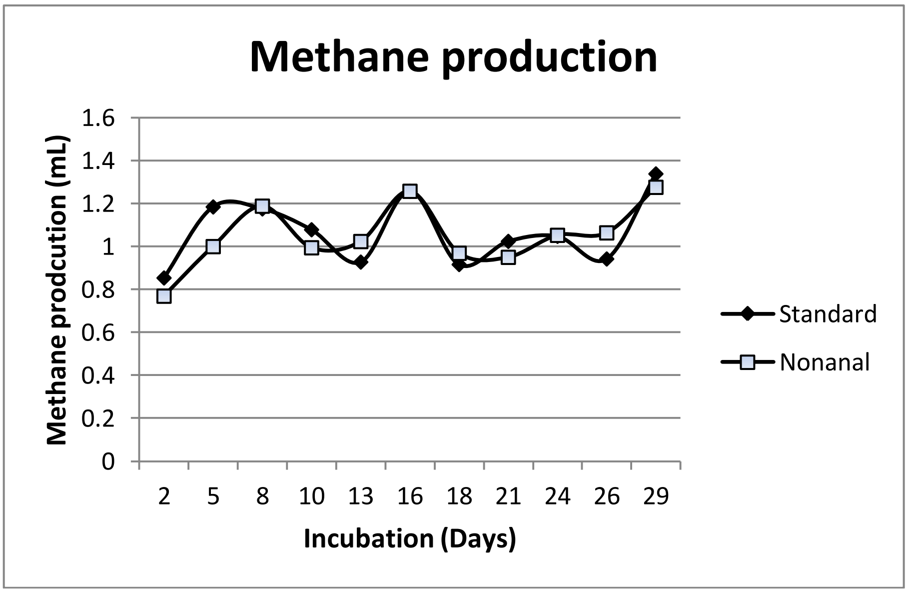
The biogas composition was measured twice a week to calculate the amount of methane produced at different concentrations which was measured using GC. Figure 15 shows the methane production of (E)-2-hexanal for 30 days.

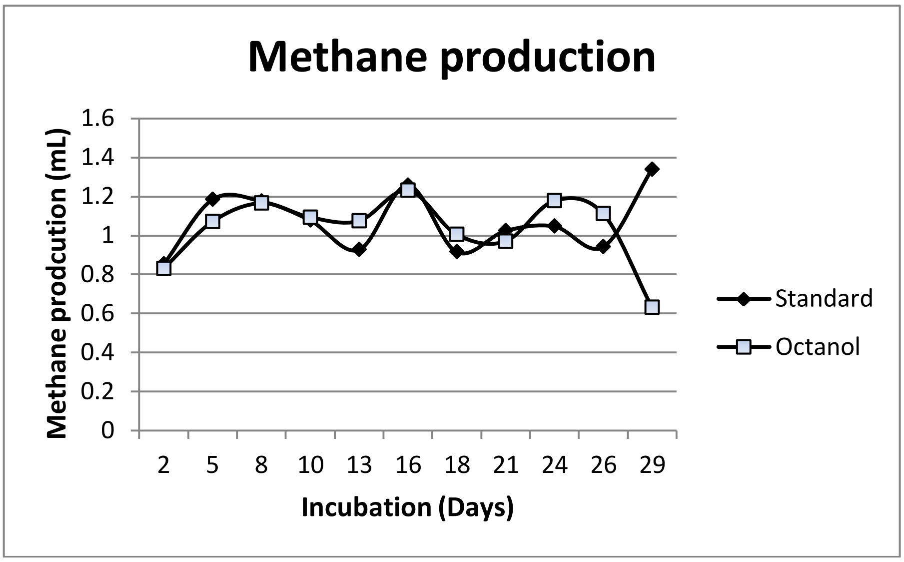





Methane production
Methane prodcution (mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
5
8
10
13
16
18
21
24
26
29
Incubation (Days)
Standard
α-pinene


Methane production
Methane prodcution (mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
5
8
10
13
16
18
21
24
26
29
Incubation (Days)
Standard
car-3-ene


Methane production
Methane prodcution (mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
5
8
10
13
16
18
21
24
26
29
Incubation (Days)
Standard
Nonanal



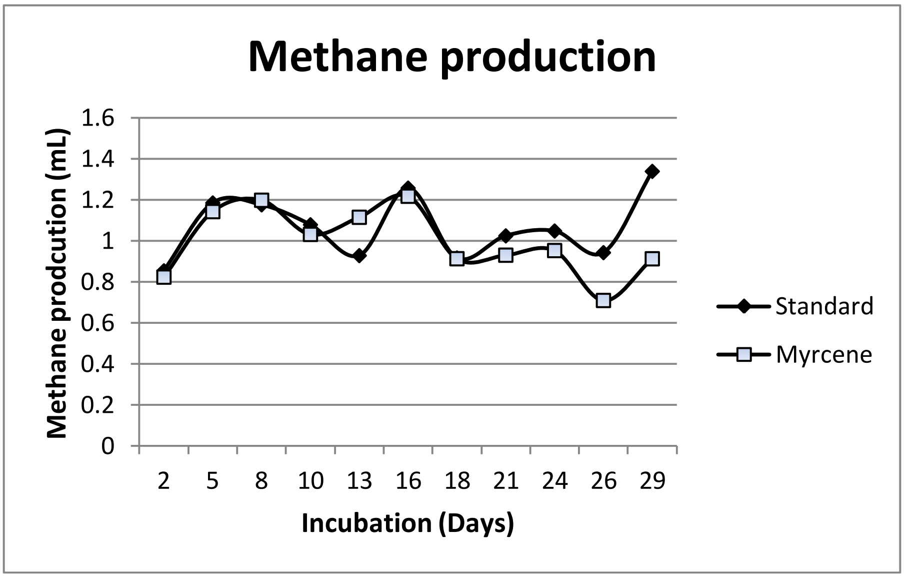


Figure 15. Effect of various flavor compounds (a) (E)-2-hexanal, (b) hexanol, (c) α-pinene, (d) car-3-ene, (e) nonanal, (f) octanol and (g) mycene on methane production at different concentrations of in comparison to standard in continuous process.

The inhibitor concentration was increased after every 15 days. As the inhibitor concentration increased the methane production was decreased as seen in the graph. This pattern was mostly observed in all other compounds.

From the table 5 below, it is clear that the reduction is mostly seen at concentration 5g/L and increasing concentration still reduces the biogas production. In case of aldehydes or terpenoids the reason might be due to increase in volatile acids which in turn decrease pH whereas for alcohol the inhibition might be due to change of permeability of the cell membrane of the bacteria which leads to leakage of the cellular component and affecting the metabolism of the bacteria [27].

In case of aldehydes, the high inhibitory compounds were hexanal and (E)-2-hexanal. (E)-2-hexanal reduced 81.92% at concentration of 5g/L. For hexanal, increasing inhibitor concentration resulted in decrease of methane production. At the lowest concentration of 0.05g/L, it reduced 5.44% of methane production. For nonanal, no inhibition was observed. In case of nonanal higher concentration of the compound is required to reduce the methane. Based on total biogas production, the high methane reduction was observed on hexanal and (E)-2-hexanal. The biogas was reduced for hexanal and (E)-2-hexanal at 5g/L by 68.85% and 98.48%, respectively which shows reduction of CO_2 for (E)-2-hexanal.

In general, terpenoids showed lower methane reduction when compared to aldehydes. The highest methane reduction was observed for myrcene. It reduced 31.84% methane and 42.87% for biogas production at concentration of 5g/L. It is followed by car-3-ene which reduced 24.51% methane and 36.98% biogas production at concentration 5g/L. The methane production of α-pinene is similar with control at all concentrations. The highest reduction was less than 10% even at the highest concentration (5 g/L).

In case of octanol there was no significant reduction in methane production at initial concentration. At 0.5g/L the percentage reduction in methane was around 12.38% which considerably increased to 52.85% at 5g/L. By comparing the results from different groups it might be interpreted that octanol and (E)-2-hexanal showed around 50 to 52% of methane reduction at 5g/L concentration.

The table 5 gives a clear depiction of the inhibitory effect of different compounds at increasing concentration. The percentage of reduction of methane around 81.92% and 50.74% was observed at 5g/L concentration for hexanal and (E)-2-hexanal. For compounds (E)-2-hexanal and myrcene, inhibitory effect was observed even at low concentration whereas for other compounds very less inhibitory effect was exhibited at 0.05 g/L. The methane reduction for (E)-2-hexanal was around 20.17 %, hexanal 5.44 % and nonanal .04% at 0.05 g/L. The difference in the methane reduction rate is clearly visible at the aldehyde group. Antimicrobial activity of car-3-ene and α-pinene was reported to inhibit organisms like *Saccharomyces cerevisiae*, *bacillus sp.* Hexanal and (E)-2-hexanal exhibited antibacterial activity against *Pseudomonas aeruginosa, Enterobacter aerogenes, Propionibacterium acnes, Staphylococus aureus and E. coli.* In the case of alcohol the inhibition might be due to change of permeability of the cell membrane of the bacteria which leads to leakage of the cellular component and affecting the metabolism of the bacteria and longer chain of alcohol causes inhibitory effect [27].

Table 5. Effect of fruit flavor compounds at different concentrations on continuous anaerobic digestion

Compound	Concentration (g/L)	Percentage reduction of methane (%)	Percentage reduction of biogas (%)
Hexanal	0.05	5.44	4.68
	0.5	4.06	4.44
	5	81.92	68.85
Nonanal	0.05	0.04	3.51
	0.5	0.44	9.70
	5	4.67	3.16
(E)-2- Hexanal	0.05	20.17	16.02
	0.5	21.35	20.72
	5	50.74	98.48
α-pinene	0.05	0.61	2.28
	0.5	7.97	5.90
	5	7.06	16.47
Car-3-ene	0.05	0.75	2.93
	0.5	7.75	11.99
	5	24.51	36.98
Myrcene	0.05	3.16	6.50
	0.5	9.08	2.89
	5	31.84	42.87
octanol	0.05	2.05	1.20
	0.5	12.38	2.22
	5	52.85	90.87

Optimum pH value around 7.2 to 7.4 was observed in the beginning of the continuous process for all the compounds as shown in figure 17. However slight drop in the pH was observed when the concentration was increased to 5 g/L. When the concentration was further increased the pH value for compounds (E)-2-hexanal and myrcene was decreased to 7 and for other compounds there was rise in the pH around 7.8. The optimum pH for a methanogenic reaction is around 6.8 to 7. Increase in acidic compounds leads to decrease in pH thus disrupting the cell function. Hence decrease in pH is a threat to anaerobic digestion. The drop in the pH might be due to accumulation of acids and thus affecting the anaerobic digestion.

Table 6. Continuous result [28]

Group	Compound	Concentration (g/L)	Percentage reduction of methane (%)	Percentage reduction of biogas (%)
Aldehyde	Hexanal	0.05	5.44	4.68
	Nonanal		0.04	3.51
	(E)-2-hexanal		20.17	16.02
	Hexanal	0.5	4.06	4.44
	Nonanal		0.44	9.70
	(E)-2-hexanal		21.35	20.72
	Hexanal	5	81.92	68.85
	Nonanal		4.67	3.16
	(E)-2-hexanal		50.74	98.48
Terpenoids	α-pinene	0.05	0.61	2.28
	Car-3-ene		0.75	2.93
	Myrcene		3.16	6.50
	α-pinene	0.5	7.97	5.90
	Car-3-ene		7.75	11.99
	Myrcene		9.08	2.89
	α-pinene	5	7.06	16.47
	Car-3-ene		24.51	36.98
	Myrcene		31.84	42.87
Alcohol	Octanol	0.05	2.05	1.20
		0.5	12.38	2.22
		5	52.85	90.87

4.2.1 FOS/TAC Value

FOS/TAC value for all compounds for the first 18 days was observed at the range of 0.3 to 0.5 (Figure 6), while the value 0.3-0.4 is optimal for an anaerobic digestion. The value was optimal during the beginning of the experiment but later after the concentration of inhibitor was increased the value was around 0.5 to 0.7 which implies acidification in the reactor. Increase in the value explains the inhibition in the reactor. As seen in the graph 16, when the concentration of the compounds is increased there is a steady growth in the FOS/TAC values. For compounds like (E)-2-hexanal and myrcene the value is around 0.6 to 1.0 at 26thday. This clearly explains the inhibition of biogas as the concentration of compound is increased in the reactor. The FOS/TAC value after 20 days was in the range of 0.5 for all the compounds. The maximum increase in the FOS/TAC value was observed for compounds (E)-2-hexanal in the range 1.28-1.90 and myrcene in the range 0.7-0.9. These high values depict the accumulation of the compound in the reactor thus inhibiting methane production.

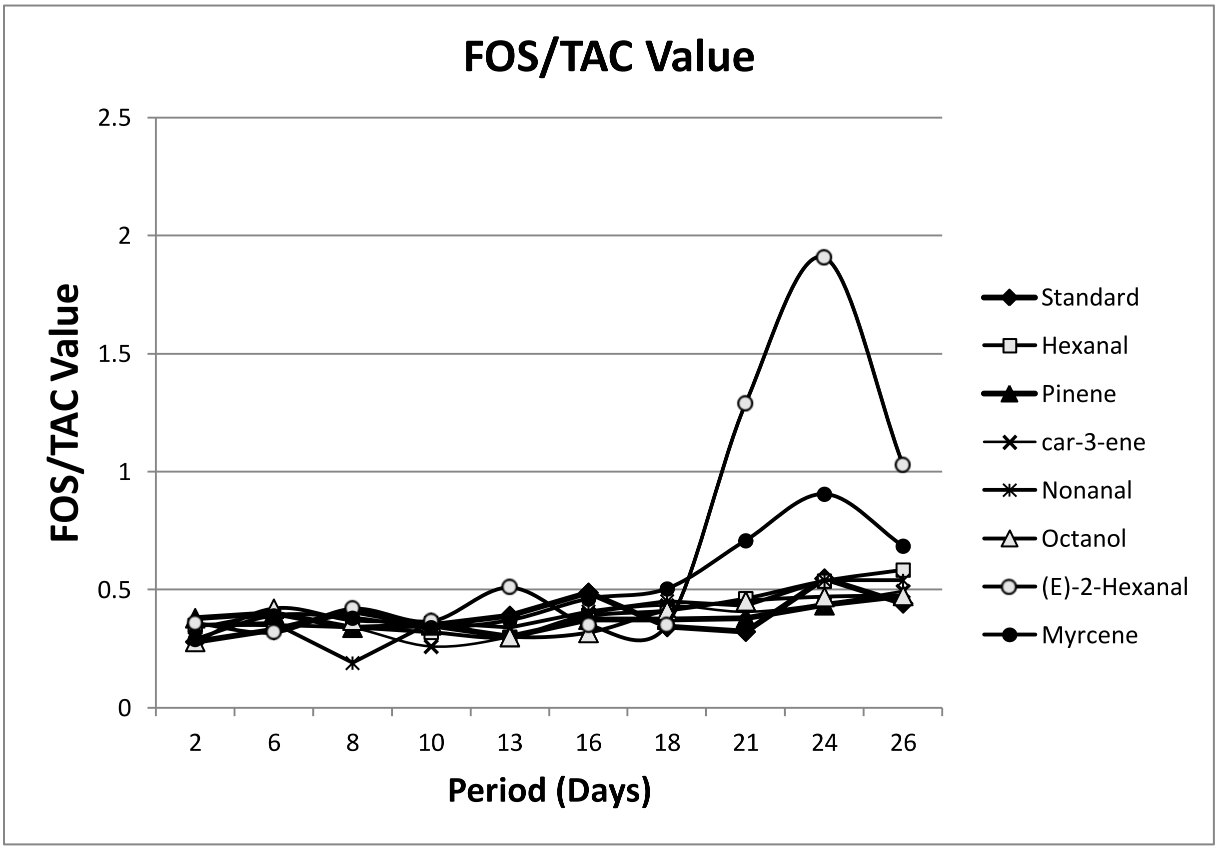


Figure 16. FOS/TAC value for a period of 30 days.

4.2.2 pH value

Optimum pH value around 7.2 to 7.4 was observed in the beginning of the continuous process for all the compounds as shown in figure 17. However slight drop in the pH was observed when the concentration was increased to 5 g/L. When the concentration was further increased the pH value for compounds (E)-2-hexanal and myrcene were decreased to 7 and for other compounds there was rise in the pH around 7.8. The optimum pH for a methanogenic reaction is around 6.8 to 7. Increase in acidic compounds leads to decrease in pH thus disrupting the cell function. Hence decrease in pH is a threat to anaerobic digestion. In the figure below (E)-2-Hexanal, myrcene and hexanal are the compounds where a slight decrease in the pH can be observed with the increasing concentration of the inhibitors. The drop in the pH might be due to accumulation of acids and thus affecting the anaerobic digestion.

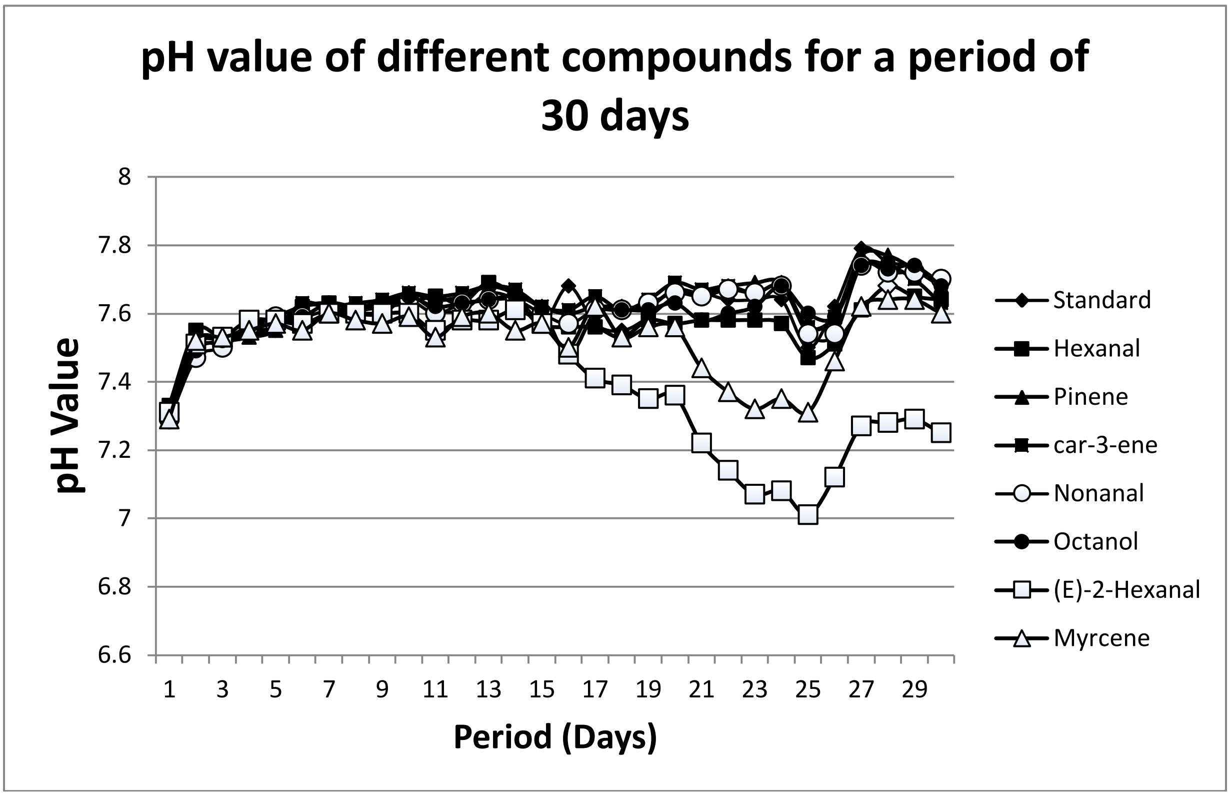


Figure 17. pH value of different compounds for a period of 30 days.

During anaerobic digestion increasing concentration of fatty acid indicates failure for biogas production . Volatile acids are produced and these have significant changes on the cell membrane composition [29].

4.3 Comparison of batch and continuous results

Similar results were obtained while interpreting the results of batch and continuous process. All the compounds were found to be inhibiting at 0.5g/l and 5g/l concentration in both processes. The most toxic compounds in both the processes were octanol, (E)-2-hexanal and myrcene but with a different percentage of reduction in biogas as shown in table 7.

As can be seen in table 7 it is clear that more inhibition of biogas is observed in batch process than in continuous process. The percentage of reduction in biogas is around 434.22%, 329.68% and 433.61% for (E)-2-hexanal, myrcene and octanol in batch process at 5g/L concentration. At the same concentration the reduction in biogas for the compounds (E)-2-hexanal, myrcene and octanol are 98.48%, 42.87% and 90.87%. The variation states that continuous process requires higher concentration to make the process failure when compared to the batch process.

Table 7. Comparison of Batch and continuous results

Compound	Concentration	Reduction in batch	Reduction in Continuous
Hexanal	0.05	107.10	5.44
	0.5	221.02	4.06
	5	316.80	81.92
Nonanal	0.05	63.43	0.04
	0.5	122.68	0.44
	5	276.01	4.67
(E)-2-hexanal	0.05	58.40	20.17
	0.5	300.74	21.35
	5	434.22	50.74
α-pinene	0.05	160.92	0.61
	0.5	88.44	7.97
	5	254.87	7.06
car-3-ene	0.05	155.73	0.75
	0.5	271.78	7.75
	5	300.14	24.51
myrcene	0.05	283,01	3.16
	0.5	370.74	9.08
	5	329.68	31.84
octanol	0.05	154.88	2.05
	0.5	370.16	12.38
	5	433.61	52.85

The target cells undergo adaptation process, when exposed to stimulus for a period of time. The cell reversibly adjusts its sensitivity to the stimulus. Adaptation enables to respond to changes in the concentration. Various ways of adaptation are slow adaptation depends on receptor down regulation; rapid adaptation involving receptor phosphorylation and some adaptation may be due to downstream changes [29]. The HRT in this case is 30 days and with continuous exposure to increasing high concentration, the number of cell surface receptors gradually decreases thus decreasing the sensitivity of the target cell to the ligand. This mechanism leads to adapting into the environment. When the concentration is kept on increasing the inhibitor gets accumulated and finally the cell dies due to the production of volatile acids thus inhibiting biogas production [30].

5. Ethical and Social aspects

According to food and agricultural organization (FAO), approximately 30% of fruit production becomes waste annually [17]. The traditional way of disposing these fruit wastes is by dumping it into the landfills. They have adverse affect on the environment like emission of greenhouse gases, water and soil pollution. A more eco-friendly method is used for the disposal of fruit waste to overcome this problem.

A smart and feasible solution to overcome this problem is anaerobic digestion. Anaerobic digestion is widely used as a source of renewable energy and is used to treat biodegradable waste and sewage sludge. This process produces biogas and reduces the emission of landfill gas into the atmosphere. The biogas can be used directly as fuel for cooking or in CHP (combined heat and power) gas engines. The nutrient rich digester effluent can be used as fertilizer [31]. On the other hand fruit waste being rich in sugars, polysaccharides and organic acids thus increasing the possibility to convert it in to biogas.

In present scenario, this type of alternative fuel is in more demand leading to intensive research and development of biogas techniques because of the depletion of the fossil fuels and oil crises. Fruit waste can be an important source for biogas production thus producing clean fuel reducing environmental problems and dependency on oil. Other feedstock's used in biogas production were kitchen waste, animal waste, agricultural and municipal sewage waste. Manure and kitchen waste were used as a source of biogas for a very long time. The primary incentive was waste management. Fruit waste as a feedstock to biogas production can be advantageous, raw material cost is cheap. Biogas yield from manure is relatively the same produced from fruit waste.

It has been a debate on the environmental harm occurred due to the monocultural production of feedstock for agrofuel production which could be overcome by fruit wastes. Female farmers have inadequate access to the inputs and intense use of resources in the large scale of fruit production, which hinders their progress. With the help of consistent monitoring system, international certification of this process could be imposed and achieved [32]. People should be aware of the intellectual property rights during the production of biogas. Constant development of the financial markets reduces the negative consequences of speculation on the prices. This technique can provide to a great deal of knowledge and benefits when compared the traditional processes.

6. Conclusion

This work was carried out to minimize the constraints during biogas production from fruit waste as world is revolving around sustainable energy. From this research work it was observed that depending on the concentration all the flavor compounds tested were inhibiting the methane production. Methane production could be reduced by the presence of the flavor compounds by 0-63%, 29-83%, and 75-100% at respective concentrations i.e. 0.005 g/L, 0.05 g/L and 0.5 g/L. In the Continuous process, inhibition was higher in myrcene when compared to that of α-pinene and car-3-ene. The percentage of reduction of methane was around 81.92% and 50.74% at 5g/L concentration for hexanal and (E)-2-hexanal. For compounds (E)-2-hexanal and myrcene, inhibitory effect was observed even at low concentration whereas for other compounds very less inhibitory effect was exhibited at 0.05 g/L. Lastly, when compared to batch process, continuous process required higher concentration of flavor compounds to reduce biogas production. This might be due to adaptation of cells towards toxic compounds during continuous process.

7. Future Work

This work would be complete if these further researches are carried out

- The whole experiment should be tried with real fruits than synthetic medium.
- The compounds can be mimicked to a fruit and further investigations can be carried out.
- Higher concentrations of the compounds can be used in continuous process to know exactly the concentration at which the inhibition takes place.

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APPENDIX

Appendix 1. Batch results of flavor compounds on biogas production for 30 days.

Appendix 2. Graphical representation of total biogas produced and methane production for 30 days.

Appendix 3. Methane data for continuous process.

Appendix 4. FOS/TAC values for the compounds in continuous process.

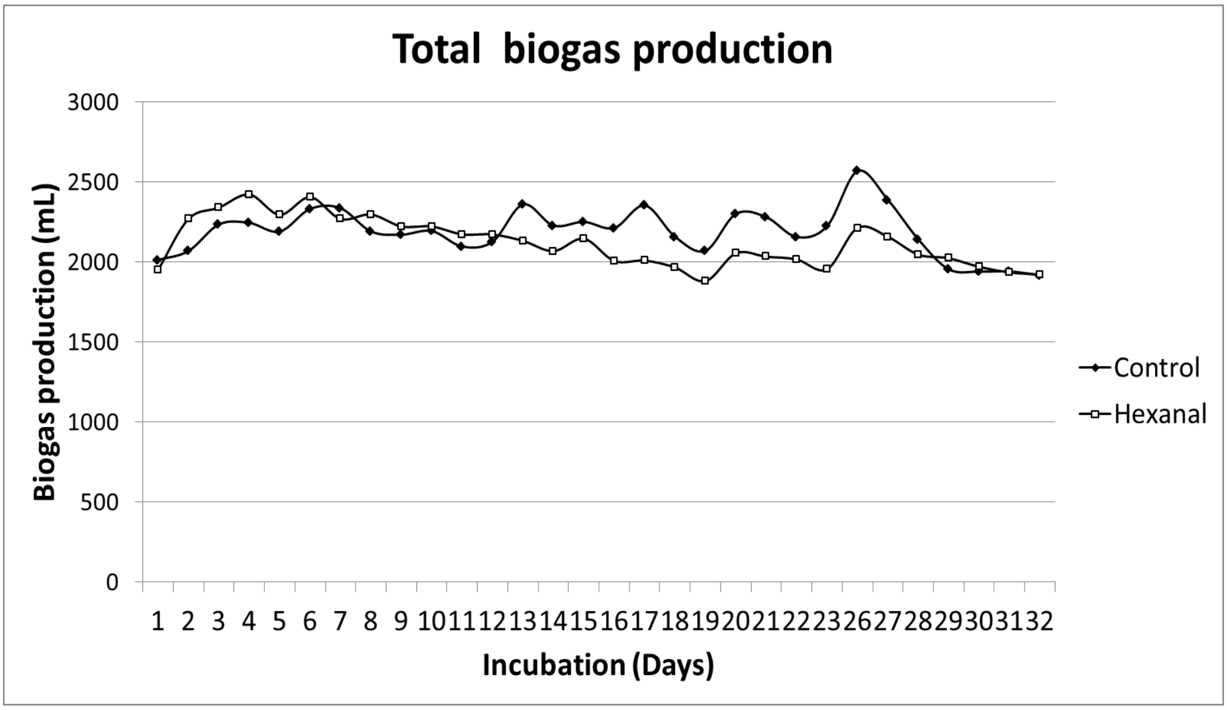
Appendix 5. pH values for each reactor in continuous process.

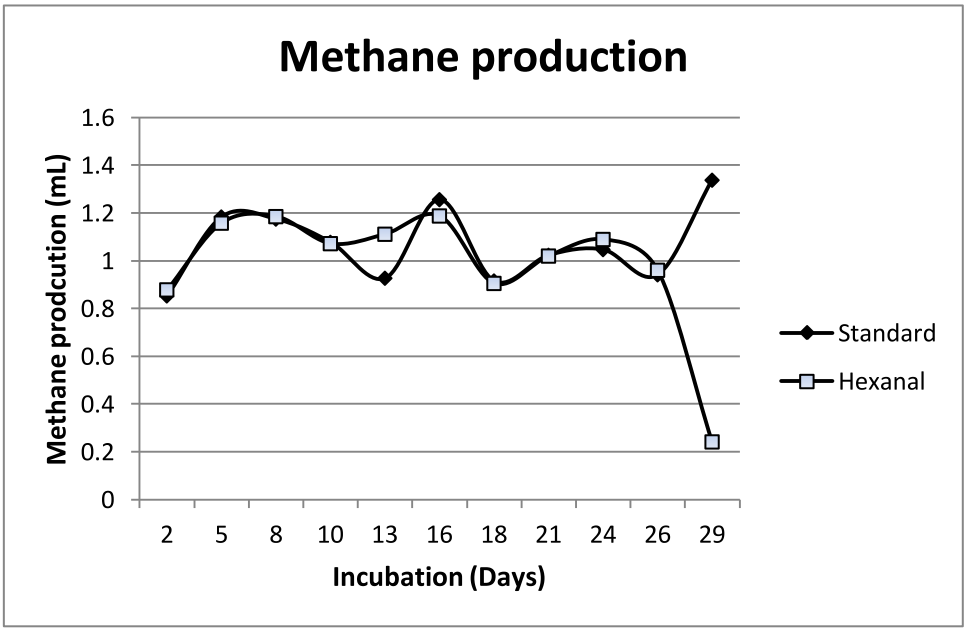
Appendix 1. Batch results of flavor compounds on biogas production for 30 days

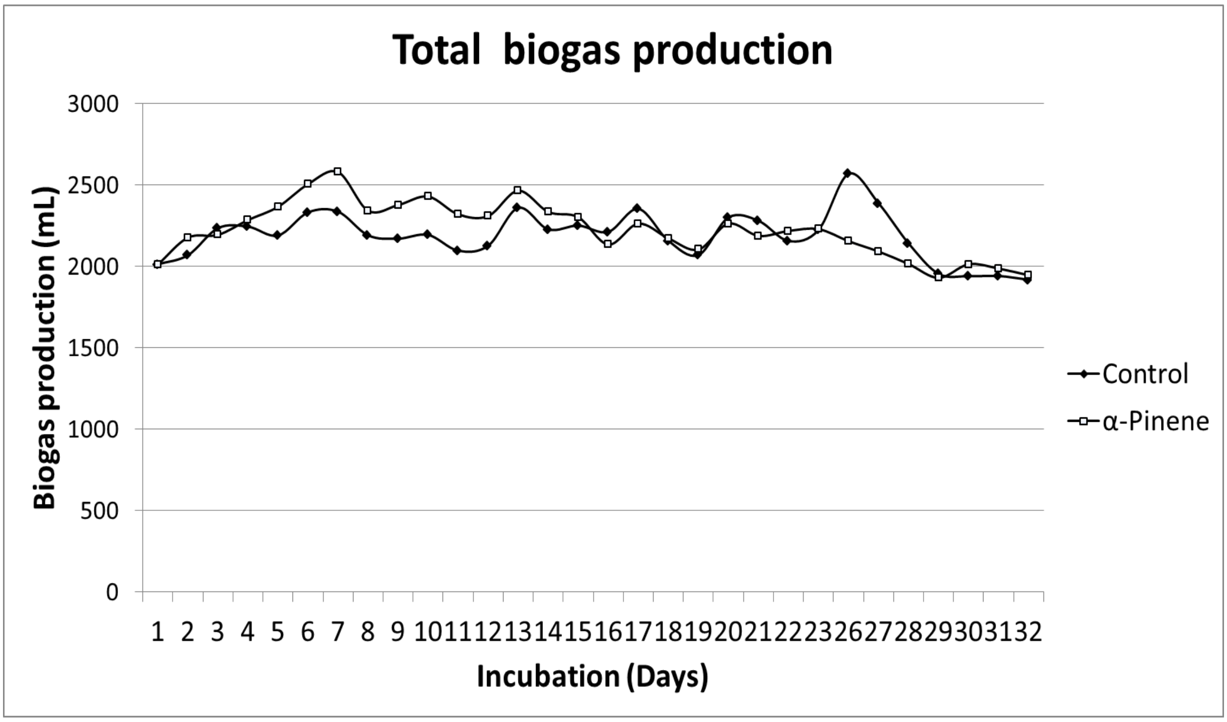
Compound	Average methane production for 30 days (ml)								
	0	3	6	9	12	16	22	26	30
Control	0	0,015674	0,024427	0,027745	0,033852	0,035775	0,037796	0,037796	0,037796
Hexanal 5 g/L	0	-0,00947	-0,02124	-0,03167	-0,03293	-0,0427	-0,05708	-0,06297	-0,08194
Hexanal 0.5 g/L	0	0,004127	0,001817	0,001078	0,002297	-0,01222	-0,02348	-0,02309	-0,04574
Hexanal 0.05 g/L	0	0,017383	0,025384	0,030377	0,026667	0,030425	0,029409	0,025489	-0,00269
α-pinene 5 g/L	0	0,00242	-0,00561	-0,01207	-0,01619	-0,02413	-0,03669	-0,04056	-0,05853
α-pinene 0.5 g/L	0	0,009142	0,008085	0,01331	0,013244	0,017495	0,01588	0,020672	0,004368
α-pinene 0.05 g/L	0	0,00445	0,002749	0,005445	0,002942	0,001022	-0,00832	-0,00907	-0,02302
Car-3-ene 5 g/L	0	-0,0023	-0,01092	-0,0194	-0,02307	-0,0323	-0,04418	-0,05668	-0,07565
Car-3-ene 0.5 g/L	0	-0,00207	-0,01081	-0,01959	-0,02313	-0,02964	-0,0432	-0,04622	-0,06493
Car-3-ene 0.05 g/L	0	0,004005	0,001101	-0,00224	-0,00224	-0,00385	-0,00636	-0,00305	-0,02107
Nonanal 5 g/L	0	-0,00123	-0,00706	-0,01644	-0,02023	-0,02917	-0,0412	-0,04788	-0,06653
Nonanal 0.5 g/L	0	0,002772	1,24E-05	-0,00038	0,001594	0,004197	0,00327	0,006605	-0,00857
Nonanal 0.05 g/L	0	0,008689	0,01342	0,018895	0,020735	0,023495	0,028999	0,034557	0,013821

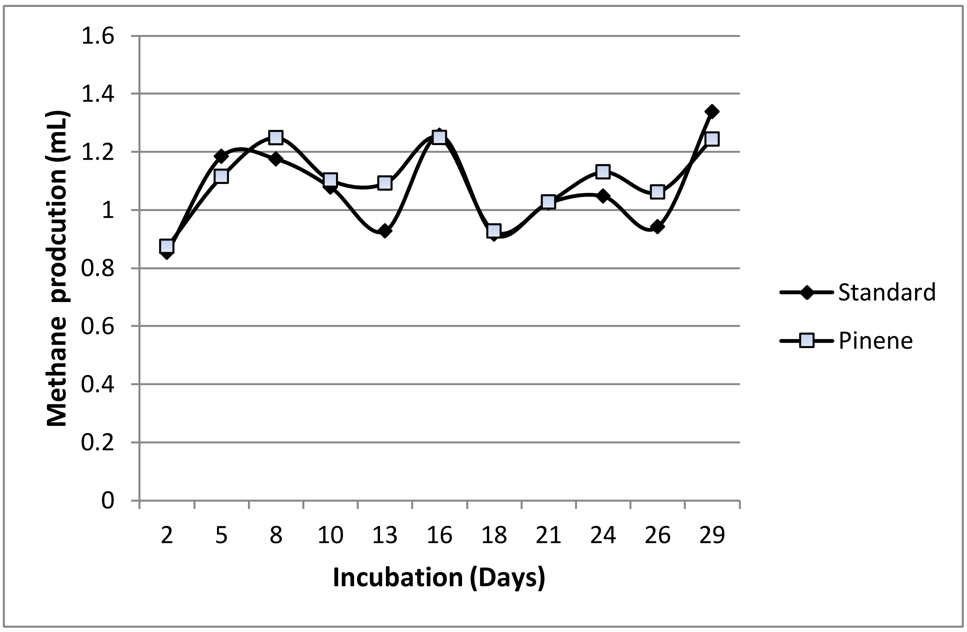
Compound	Average methane production for 30 days (ml)								
	0	3	6	9	12	16	22	26	30
Control	0	0,015789	0,022029	0,021854	0,023506	0,024835	0,025091	0,028165	0,024586
(E)-2-hexenal 5 g/L	0	-0,0098	-0,02147	-0,0319	-0,03316	-0,04293	-0,05731	-0,0632	-0,08217
(E)-2-hexenal 0.5 g/L	0	-0,00584	-0,01859	-0,02736	-0,02782	-0,03355	-0,04245	-0,04514	-0,04925
(E)-2-hexanal 0.05 g/L	0	0,011307	0,017179	0,030785	0,032886	0,037392	0,038337	0,038337	0,038943
Myrcene 5 g/L	0	0,003864	-0,00309	-0,01085	-0,01292	-0,02168	-0,03294	-0,03877	-0,05647
Myrcene 0.5 g/L	0	0,001633	-0,00613	-0,01214	-0,01009	-0,02006	-0,0345	-0,03543	-0,04935
Myrcene 0.05 g/L	0	0,005685	0,000382	-0,00841	-0,01346	-0,01982	-0,04016	-0,04016	-0,04499
Octanol 5 g/L	0	-0,00921	-0,01959	-0,03151	-0,03276	-0,04253	-0,05691	-0,06281	-0,08177
Octanol 0.5 g/L	0	0,002541	-0,00706	-0,01266	-0,01232	-0,02427	-0,04387	-0,05017	-0,06642
Octanol 0.05 g/L	0	0,010985	0,02338	0,02338	0,02595	0,019155	-0,00746	-0,00749	-0,01349

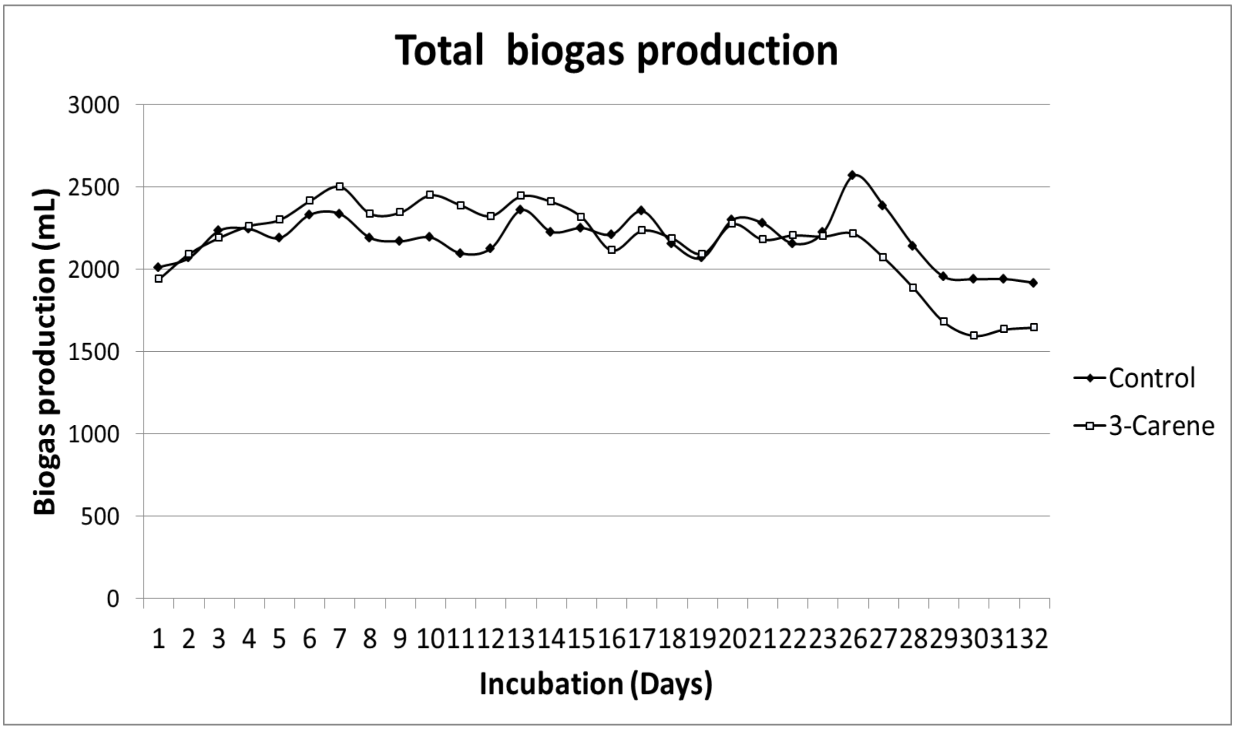
Appendix 2. Graphical representation of total biogas produced and methane production for 30 days

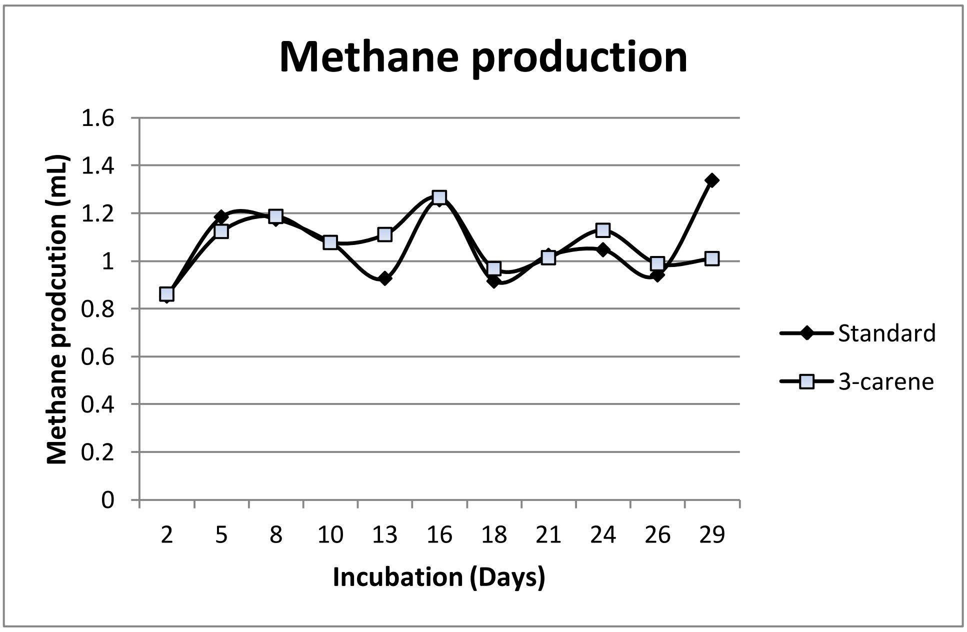


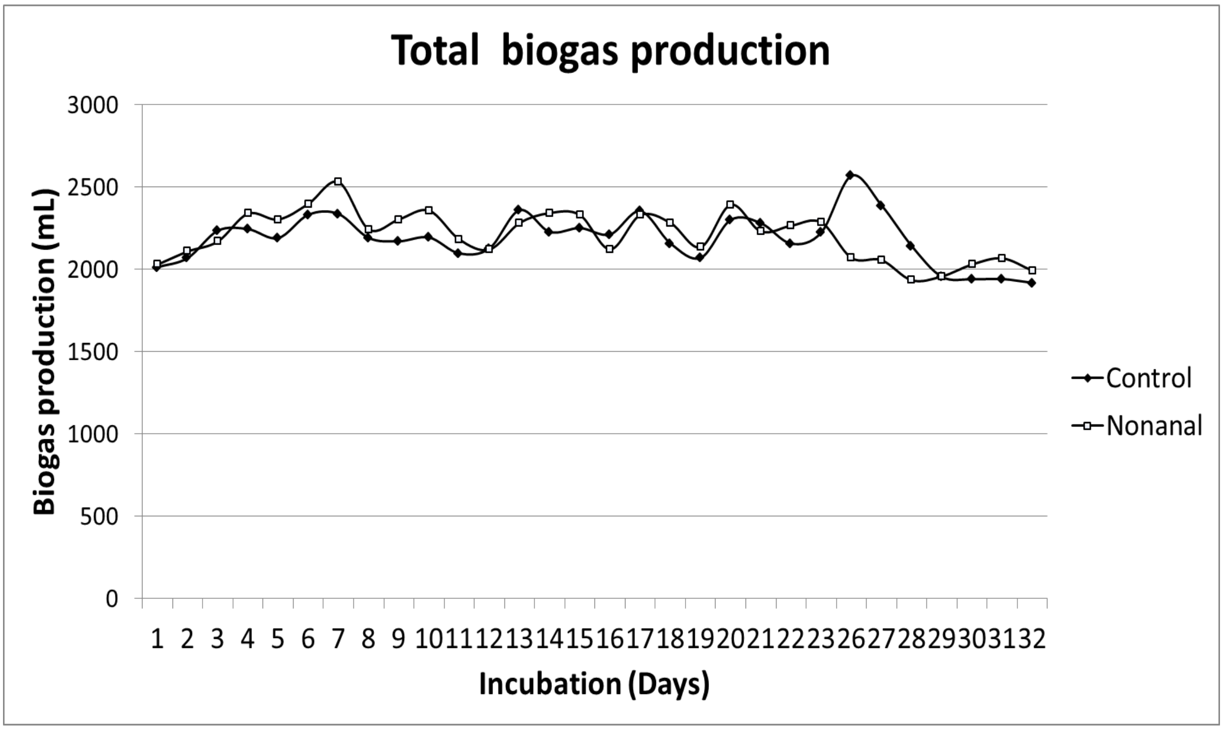


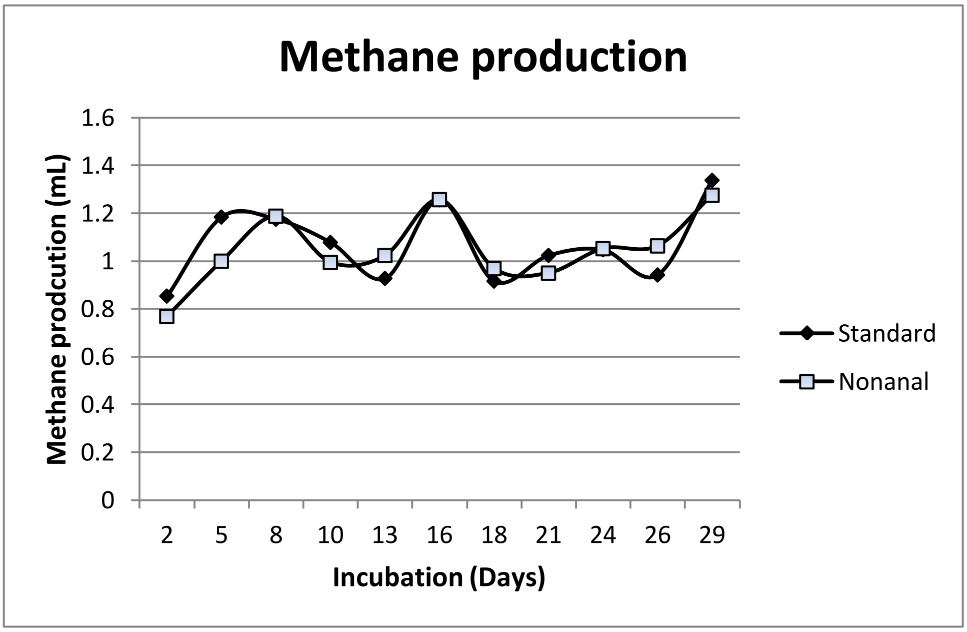

Total biogas production
Biogas production (mL)
3000
2500
2000
1500
1000
500
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 26 27 28 29 30 31 32
Incubation (Days)
Control
α-Pinene

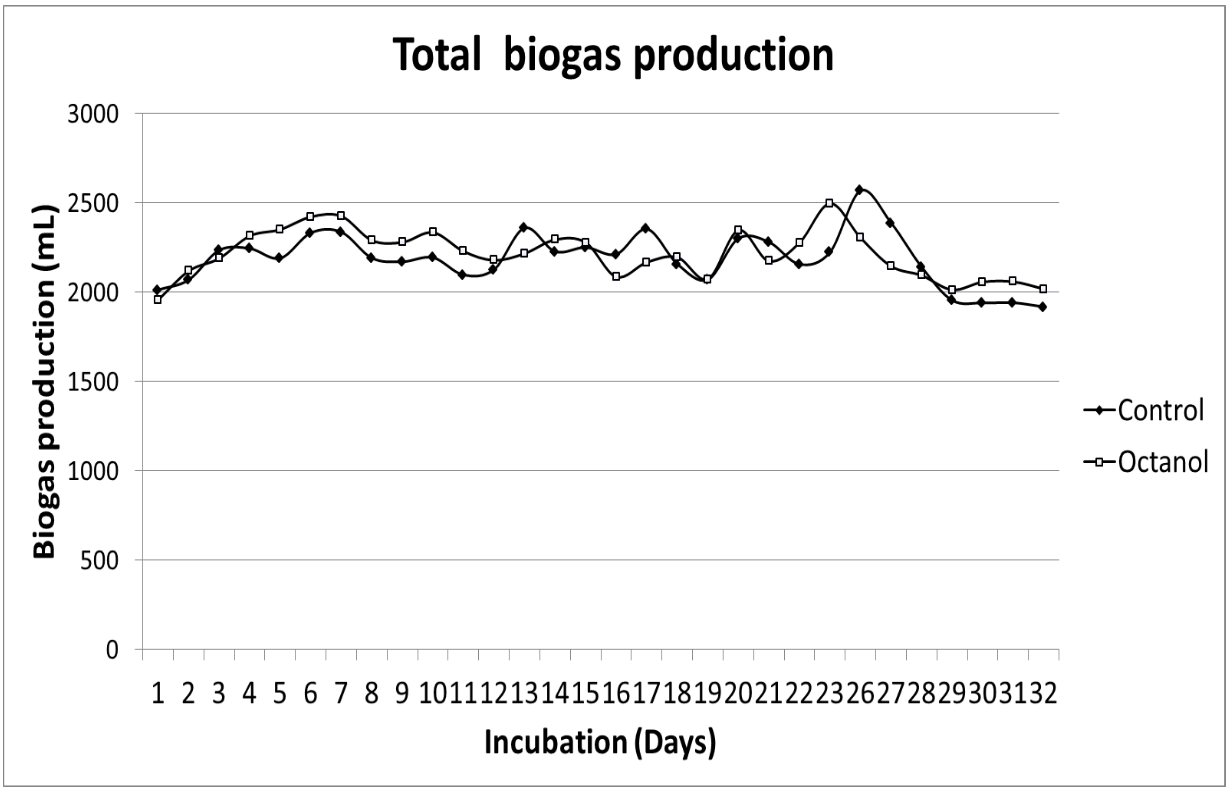

Methane prodcution (mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2 5 8 10 13 16 18 21 24 26 29
Incubation (Days)
Standard
Pinene

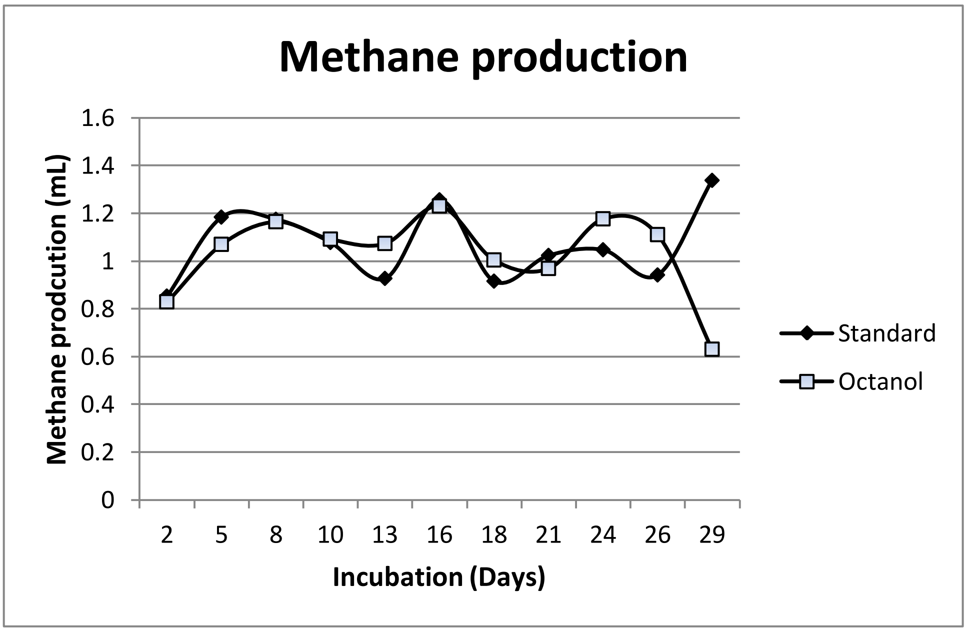

Total biogas production
Biogas production (mL)
3000
2500
2000
1500
1000
500
0
1 2 3 4 5 6 7 8 9 10111213141516171819202122232627282930313 2
Incubation (Days)
Control
3-Carene

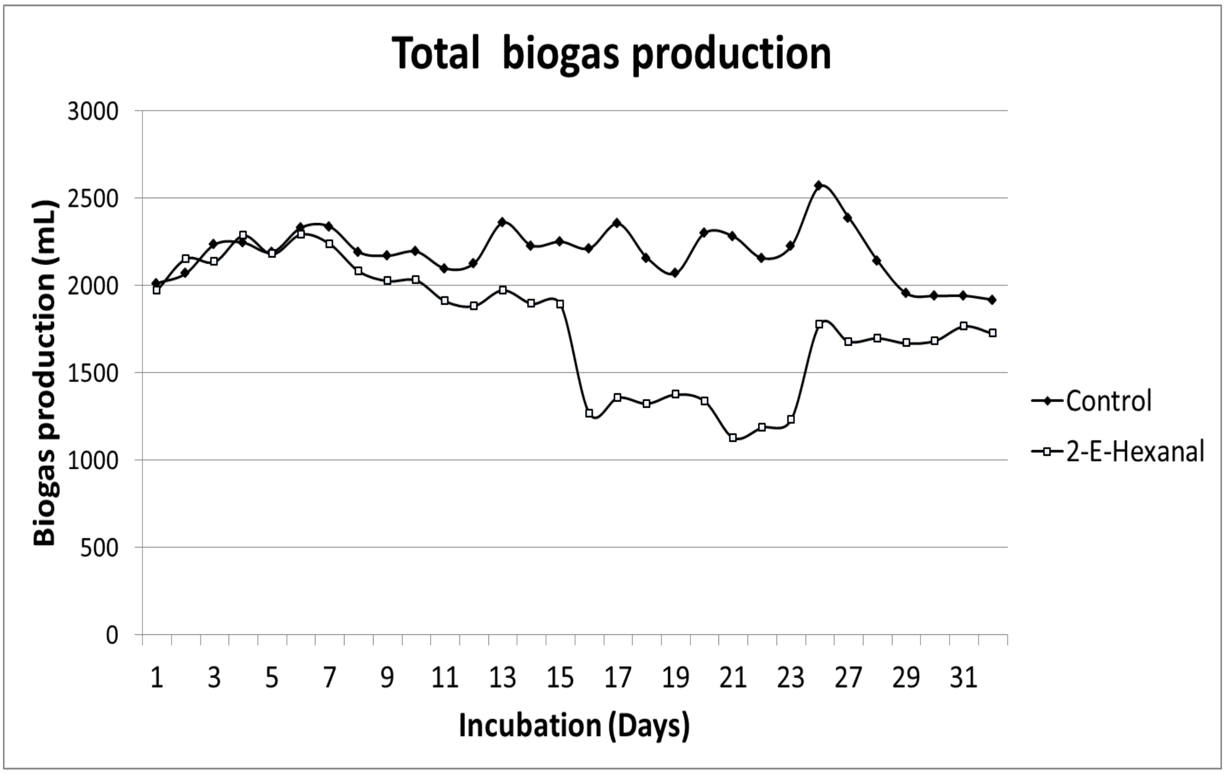

Methane production
Methane prodcution (mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2 5 8 10 13 16 18 21 24 26 29
Incubation (Days)
Standard
3-carene


Total biogas production
3000
2500
2000
1500
1000
500
0
Biogas production (mL)
1 2 3 4 5 6 7 8 9 10111213141516171819202122232627282930313132
Incubation (Days)
Control
Nonanal

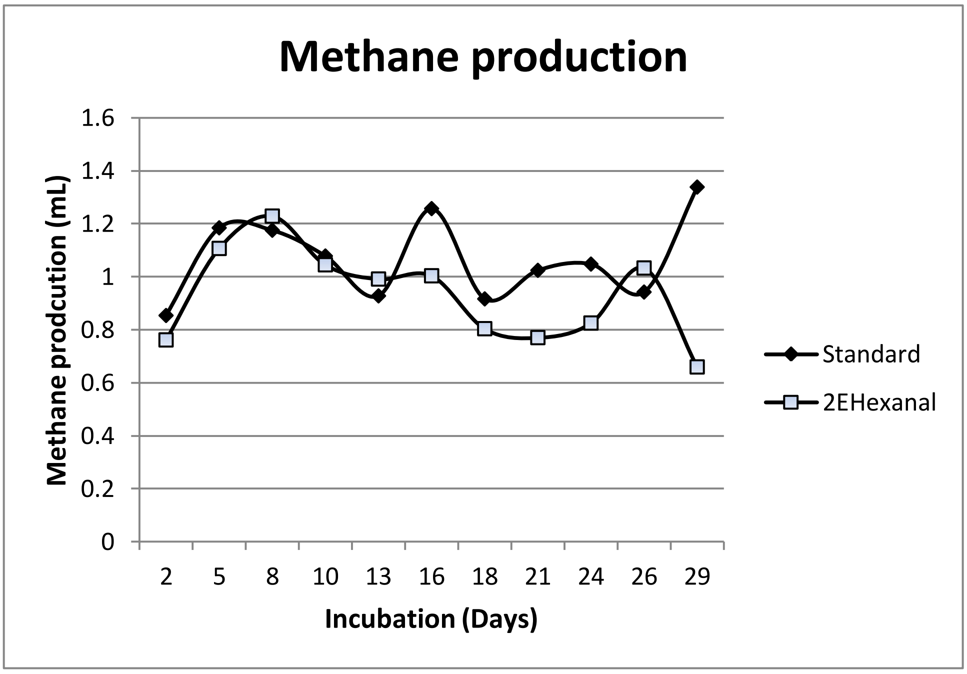

Methane production
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Methane prodcution (mL)
2 5 8 10 13 16 18 21 24 26 29
Incubation (Days)
Standard
Nonanal


Total biogas production
3000
2500
2000
1500
1000
500
0
Biogas production (mL)
1 2 3 4 5 6 7 8 9 10111213141516171819202122232627282930313 2
Incubation (Days)
Control
Octanol

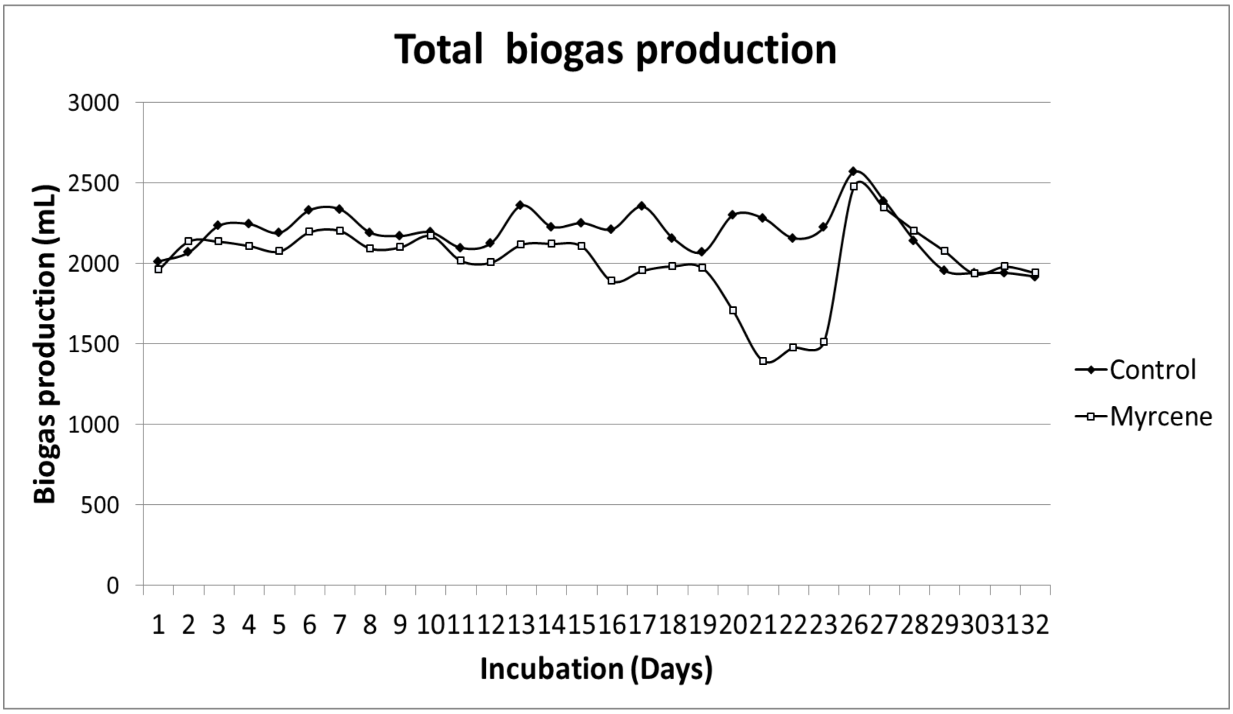

Methane production
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Methane prodcution (mL)
2 5 8 10 13 16 18 21 24 26 29
Incubation (Days)
Standard
Octanol



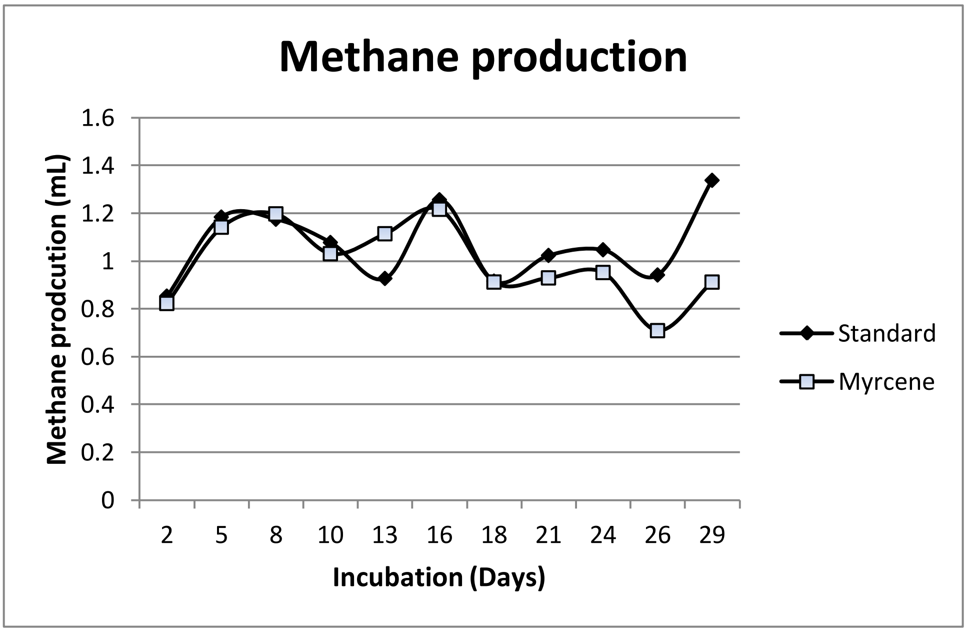
Total biogas production
3000
2500
2000
1500
1000
500
0
Biogas production (mL)
1
3
5
7
9
11
13
15
17
19
21
23
27
29
31
Incubation (Days)
Control
2-E-Hexanal



Methane production
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Methane prodcution (mL)
2
5
8
10
13
16
18
21
24
26
29
Incubation (Days)
Standard
2EHexanal



Total biogas production
Biogas production (mL)
3000
2500
2000
1500
1000
500
0
1 2 3 4 5 6 7 8 9 10111213141516171819202122232627282930313132
Incubation (Days)
Control
Myrcene



Methane production
Methane prodcution (mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2 5 8 10 13 16 18 21 24 26 29
Incubation (Days)
Standard
Myrcene

Appendix 3. Methane data for continuous process

Average methane production for 30 days (ml)											
Compd/ Day	2	5	8	10	13	16	18	21	24	26	29
Standard	0,853357	1,1841223	1,175051	1,077847	0,92735	1,256635	0,915968	1,023715	1,047276	0,941797	1,338033
Hexanal	0,878644	1,1581517	1,185526	1,072314	1,111732	1,18824	0,9057	1,020326	1,089838	0,960747	0,241901
α-pinene	0,874146	1,1151215	1,248378	1,102592	1,092114	1,249021	0,927057	1,027015	1,13072	1,061363	1,243555
Car-3-ene	0,861559	1,1240026	1,186843	1,077712	1,111008	1,266023	0,968147	1,014059	1,128539	0,988883	1,010062
Nonanal	0,768444	0,9993739	1,18774	0,993882	1,023031	1,257072	0,968377	0,949493	1,051917	1,063351	1,275532
Octanol	0,829413	1,0701478	1,165018	1,092034	1,073756	1,230801	1,00463	0,969115	1,177004	1,110851	0,630823
(E)-2-Hexanal	0,761116	1,106409	1,228401	1,044672	0,990963	1,003205	0,803483	0,769854	0,824887	1,032587	0,659141
Myrcene	0,82261	1,1419749	1,197402	1,030911	1,114352	1,216897	0,912273	0,929512	0,952119	0,708821	0,911947

Appendix 4. FOS/TAC values for the compounds in continuous process

Compd / Day	FOS/TAC values									
	2	6	8	10	13	16	18	21	24	26
Standard	0,28	0,33	0,41	0,35	0,39	0,488	0,343	0,323	0,548	0,443
Hexanal	0,35	0,35	0,34	0,32	0,3	0,388	0,412	0,463	0,537	0,584
α-pinene	0,38	0,4	0,34	0,35	0,3	0,374	0,374	0,379	0,435	0,475
Car-3-ene	0,34	0,39	0,34	0,26	0,3	0,397	0,433	0,406	0,44	0,494
Nonanal	0,35	0,36	0,19	0,361	0,34	0,408	0,452	0,433	0,54	0,54
Octanol	0,28	0,42	0,37	0,361	0,301	0,318	0,412	0,452	0,47	0,475
(E)-2-Hexanal	0,36	0,32	0,42	0,367	0,51	0,352	0,352	1,289	1,907	1,03
Myrcene	0,29	0,39	0,38	0,34	0,37	0,461	0,504	0,708	0,905	0,685

Appendix 5. pH values for each reactor in continuous process

Compd / Day	pH value for 15 days														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Standard	7,31	7,5	7,51	7,54	7,55	7,62	7,62	7,6	7,6	7,65	7,61	7,61	7,66	7,64	7,57
Hexanal	7,33	7,55	7,53	7,56	7,59	7,62	7,63	7,61	7,62	7,63	7,65	7,63	7,69	7,65	7,57
α-pinene	7,3	7,52	7,52	7,53	7,55	7,62	7,63	7,62	7,63	7,66	7,64	7,65	7,68	7,66	7,62
Car-3-ene	7,3	7,52	7,52	7,55	7,56	7,63	7,62	7,63	7,64	7,66	7,65	7,66	7,68	7,67	7,62
Nonanal	7,29	7,47	7,5	7,56	7,59	7,59	7,61	7,61	7,62	7,64	7,6	7,63	7,64	7,64	7,6
Octanol	7,29	7,49	7,52	7,56	7,57	7,59	7,63	7,62	7,63	7,65	7,62	7,63	7,64	7,65	7,61
(E)-2-Hexanal	7,31	7,51	7,53	7,58	7,58	7,57	7,6	7,6	7,6	7,6	7,55	7,58	7,58	7,61	7,57
Myrcene	7,29	7,52	7,53	7,55	7,57	7,55	7,6	7,58	7,57	7,59	7,53	7,59	7,6	7,55	7,57

Compd / Day	pH value till 30th day														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Standard	7,68	7,57	7,55	7,59	7,65	7,66	7,64	7,64	7,64	7,5	7,62	7,79	7,75	7,74	7,69
Hexanal	7,56	7,56	7,53	7,58	7,57	7,58	7,58	7,58	7,57	7,47	7,51	7,61	7,68	7,65	7,64
α-pinene	7,6	7,62	7,62	7,62	7,67	7,66	7,68	7,69	7,69	7,56	7,59	7,77	7,77	7,73	7,68
Car-3-ene	7,61	7,65	7,61	7,64	7,69	7,67	7,68	7,66	7,68	7,56	7,59	7,74	7,74	7,7	7,63
Nonanal	7,57	7,61	7,61	7,63	7,66	7,65	7,67	7,66	7,68	7,54	7,54	7,74	7,72	7,72	7,7
Octanol	7,48	7,6	7,61	7,61	7,63	7,58	7,6	7,62	7,68	7,6	7,59	7,74	7,73	7,74	7,68
(E)-2-Hexanal	7,48	7,41	7,39	7,35	7,36	7,22	7,14	7,07	7,08	7,01	7,12	7,27	7,28	7,29	7,25
Myrcene	7,5	7,62	7,53	7,56	7,56	7,44	7,37	7,32	7,35	7,31	7,46	7,62	7,64	7,64	7,6