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Anaerobic digestion of food waste using artificially cultured and natural anaerobes under Mesophilic conditions

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Abstract
Municipal Solid waste management is becoming a critical problem in most of the megacities of the world. Anaerobic digestion is one of the technologies to convert that waste into useful form of energy. But countries like Singapore having limited resources, cannot fulfil the cow dung or other animal manure requirements in anaerobic digestion. Therefore in the present paper a comparative study has been presented for natural and artificial inocula used for anaerobic digestion of Singaporean food waste. Laboratory-scale batch type digesters were operated to study the effect of natural and artificial inoculums prepared from different animals. Effect of different parameters e.g. COD, PH, TS on digestion has been studies. Results showed that the artificial inocula are less effective as they are converting the COD into CO_2. However, natural inocula have shown good results for digestion of food waste. CN ratio and PH value has also found to have significant effect on digestion process.

Keywords: Biogas, natural inoculum, artificial inoculum, COD, Anaerobic digestion.

1. Introduction
Municipal solid waste (MSW) amount from societies have increased over the last few years, out of which, food waste contributes a considerable fraction. The food waste generated annually in Singapore was 542,700 tons in 2006 and reached about 570,000 tons in the year 2008 and 680,000 tons in 2011 [1]. Food waste management is a challenge faced by any developing nation as untreated and unmanaged food waste creates odour, hygiene concerns and cause adverse environmental impacts [1-15].
Singapore is a highly populated, industrialized city with limited land area. Semakau Landfill is Singapore's only landfill for waste disposal which is also going to be filled completely in next few years. Singapore's offshore landfill accepts only inert wastes that are inorganic. Therefore, no food waste is sent to the landfill and the majority of food waste is directed to incineration plants [2]. A remaining 10–15% is sent for recycling via anaerobic digestion (AD), followed by composting of the digestate material. But incineration also produces high amount of ash which again has to go for landfilling. Also lots of energy and cost is incurred in transporting the waste to incineration plants therefore an idea is came into picture to digest the food waste anaerobically in decentralized manner so that on one side we can reduce the amount of Ash generated through incinerator and on the other side the heavy investment in transportation can be reduced.
Anaerobic digestion (AD) is considered as the most effective method for the treatment of municipal solid waste (MSW) [3]. The AD process can be divided into low solid (⩽10% TS), semi-solid (10–20% TS), and high solid (⩾20% TS) digestion processes depending on the amount of total solid (TS) contents [4]. Compared to low solid digestion, high solid digestion is effective in terms of a low energy requirement for heating and pumping, and less production of digester effluent [5].
Thermophilic anaerobic digestion predominate over mesophilic condition, as a higher hydrolysis, gas production rate, and pathogen destruction rate can be achieved at temperature 50–60 °C [6]. However, mesophilic operation (30–40 °C) is advantageous in terms of a smaller energy requirement and less sensitivity to toxic substances [7].
Nowadays, anaerobic co-digestion has attracted more attentions [8-10]. Generally, animal manures like cow dung, piggery wastewater are considered to be excellent co-substrates because of its capacity of providing anaerobes, high buffering capacity, high nitrogen content and the wide range of nutrients needed by the anaerobes [11, 12]. Co-digestion of animal manure with a biodegradable waste appears as a robust process technology that can increase the biogas production by 80–400% in biogas plants [12, 13]. But at the same time,

the countries like Singapore, having limited resources, cannot fulfil the cow dung or other animal manure requirements of such processes. Therefore, an idea to culture anaerobes artificially came into picture. If such kind of technology will be successful then one can store the cultured anaerobes at freezing temperature and can use at the time of requirement. In the current paper, the anaerobes from 5 different animal manures were cultures in artificial medium and those cultured anaerobes were used to digest the food waste under Mesophilic batch conditions for 10 days. Also to compare the results, the food waste is also digested with 5 same animal manures (natural) under same conditions.

2. Materials

2.1 Feedstock

Food waste is collected from Chinese food stall of Engineering Canteen of National University of Singapore, Singapore. Food is collected in bulk and then blended using a blender to make it homogeneous.

2.2 Inoculum

2.2.1 Natural Inoculum

Animal manure is used as natural inoculum for anaerobic digestion of food waste. The experiments were started using fecal samples collected from Singapore Zoo as microbial inocula. 5 different animal species were chosen according to their preferred nutrition and sixth one is made by mixing all together in equal proportions. Particular attention was given to herbivourus species because their digestion was assumed to have adapted to cellulose rich substrate. Only fresh samples were collected and immediately stored in air tight plastic bags to avoid prolonged exposure to oxygen. 20 g of manure were blended with 200 ml of autoclaved water to make them homogeneous in air tight anaerobic chamber.

2.2.2 Artificial Inoculum

Samples (10 g) were used within 3 hours to inoculate 200 ml of sterile basic anaerobic medium [8] in 250 ml flasks. After anaerobic conditions had been established by flushing with pure Nitrogen in anaerobic chamber for several minutes cultures were kept in a Typ 1092 water bath (GFL) at mesophilic conditions (35°C). Every three weeks volumes of 20 ml were used to inoculate flasks containing 180 ml of fresh basic anaerobic medium. Proper cultivation conditions and existence of anaerobic environment were monitored by measuring pH, oxidation reduction potential and dissolved oxygen using an electrochemistry meter 3200M and appropriate probes according to manufacturer's instructions [Agilent Technologies]. Table 1 is showing the general nomenclature for different samples used in the present paper.

Table 1: Different inoculum samples with their names and nomenclatures

S. No.	Sample	Nomenclature
1	Rhino control	R Cont
2	Kangaroo Control	K Cont
3	Tortoise Control	T Cont
4	Giraffe Control	G Cont
5	Otter Control	O Cont
6	All sample Mixture control	ACM Cont
7	Rhino+ food waste	R
8	Kangaroo + food waste	K
9	Tortoise + food waste	T
10	Giraffe + Food waste	G
11	Otter + Food waste	O
12	All sample mixture + Food waste	ACM

3. Methods

3.1 Sample preparation for digestion

To ensure anaerobic conditions, the samples were prepared in anaerobic chamber. 20 g food waste was added in 200 ml of inoculum in Erlenmeyer flask and sealed with the help of rubber stopper with a plastic pipe going through for gas collection. In the same manner, different samples were prepared for different animal natural and artificial inocula. All the samples were added with 20 ml (0.025M) phosphate buffer to ensure the buffer conditions during the digestion process. For a better analysis of digestion process, control was prepared for each sample. All samples with 2 replicates were placed in shaking water bath at 37°C. Gas is allowed to collect in glass columns filled with water.

3.2 Analytical methods

Chemical oxygen demand (COD), total solid (TS), and volatile solid (VS) were measured according to standard methods [14], and elemental analysis was done with a CHNS Analyser. The produced gas was collected by a gas collector and

sampled using a 1-mL syringe to analyse CH_4 and CO_2 content. The CH_4 gas content was analysed using a gas chromatograph (GC, Gow Mac Series 580) equipped with a thermal conductivity detector (TCD) and a 2 m × 2 mm stainless-steel column packed with a Porapak Q mesh (80/100) with helium as carrier gas. The temperatures of the injector, detector, and column were kept at 80, 90, and 50 °C, respectively.

4. Results and discussion

4.1 Composition of samples

The results of the analysis of different samples are shown in fig 1(a, b, c). Fig 1a shows the TS of all samples. It is clear from the fig that the TS of natural inocula samples (1.9 to 2.3%) are higher as compared to artificial inoculums samples (approximately 1%). The TS of food waste was 4.67% and therefore when food waste was added to inoculums for digestion, the TS was increased for all the samples in proportionate amount. Now the TS for natural inocula with food samples were varying from 4.1 to 4.5 % and for artificial inocula with food samples, it was approximately 3%.

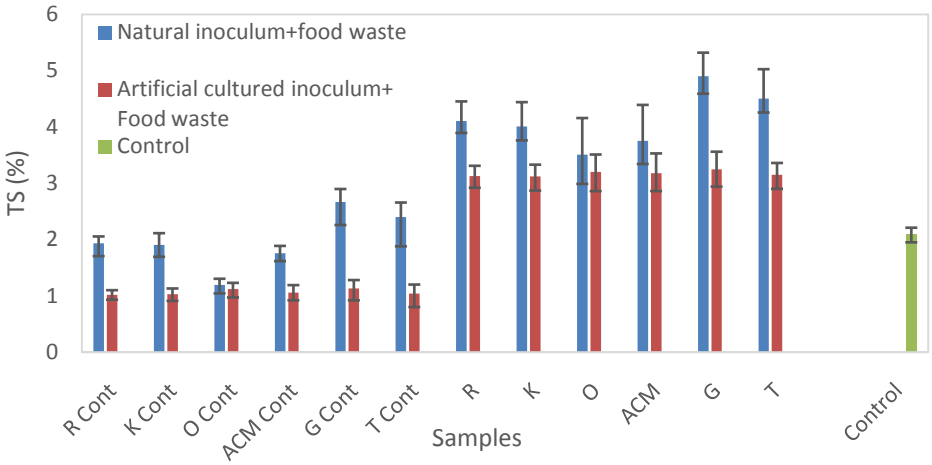

Fig 1a: Total solids (TS) of different samples before digestion

The CN ratio of all the samples is given in fig 1b. Carbon to nitrogen ratio is an important parameter while characterising the waste for digestion. The elements of carbon and nitrogen are the food of anaerobic bacteria. Carbon is used for energy and nitrogen for building the cell structure. Most of the literature recommends an operating C/N ratio range of 20 to 30 with an optimal ratio of 25/1 for anaerobic bacterial growth in an AD system. Improper C/N ratios could result in high total ammonia nitrogen (TAN) released and/or high VFA accumulation in the digester. Both TAN and VFAs are important intermediates and potential inhibitors in the AD process. High concentrations of TAN and VFAs in the digester would inhibit the methanogens activity and cause possible failure of the AD process.

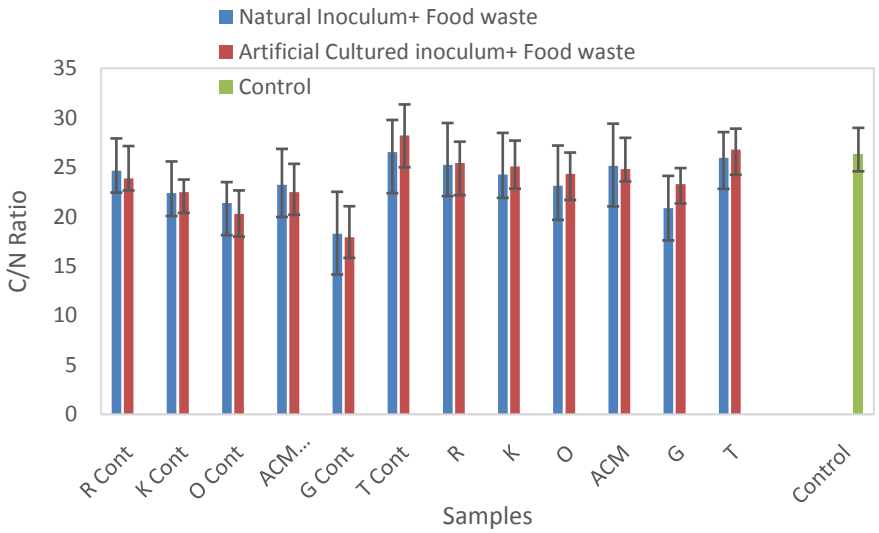

Fig 1b: C/N of samples before digestion

From the fig it is clear that the CN ratio for almost all the samples including natural and artificial inoculums samples are varying from 20 to 30 excluding Giraffe for which the CN ratio is 18. This conclude that the digestion of giraffe inoculums shall be minimum due to fast depletion of carbon as compared to nitrogen which will result in access nitrogen resulting in the production of ammonia and resulting high amount of ammonia will inhibit the methanogens. But only this reason is not sole responsible for quantitative analysis of methane but other factors are also responsible for that and one of the main factor is amount of organic matter present in the samples. The amount of organic matter present in the samples is measured in terms of COD value and is shown in fig 1c.

COD of food waste is 4.62 g meaning thereby, theoretically 1.617L of CH_4 can be produced from the selected amount of food waste. As the food waste is added to inocula, it will increase the COD of the total sample. The COD of natural inocula is high (1.4g to 1.8g) as compared to COD of artificial inocula samples (0.7g-0.8g). The reason for the fact is that artificial inocula contain less organic matter as compared to natural inocula as can be found from the TS of both the samples. This is due to the fact that the natural inoculum is the direct animal manure samples of different animals which contain plant waste, leaf waste etc

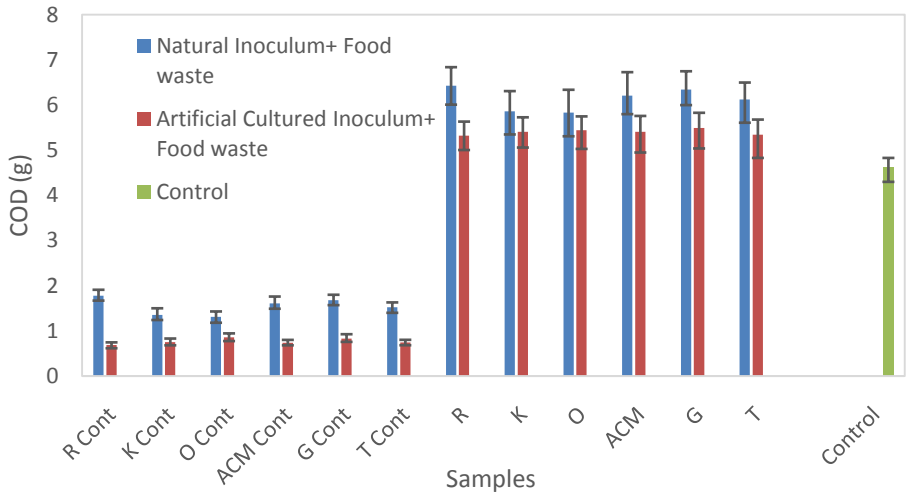

Fig 1c: COD of samples before digestion

4.2 Biogas characteristics

Biogas produced from artificial inoculum samples are shown in fig 2a. From the fig it is clear that gas volume produced is proportional to COD conversion of the samples. The COD conversion during digestion process is less for artificial inocula control samples as they contain less organic matter as discussed above resulted in less gas production however the COD conversion of food samples with artificial inocula is higher. COD conversion of Rhino and Giraffe is maximum and almost same and that is 0.4g which resulted in maximum gas production in both samples however for Kangaroo control it is minimum which is 0.05g and due to this reason it produced less gas. However the converted gas was found CO_2 instead of CH_4 in all the artificial inocula samples. The possible reason is shown in fig 3a. From the fig 3a it is clear that for all the samples the PH value was very less (varying from 3 to 4) and this may tend to create acidic conditions inside the digester which is responsible for deterioration of methanogens. The second possible reason was low organic matter which is measured in terms of COD value and the third reason was low CN ratio (between 20 to 25) which is also important while studying anaerobic digestion.

Fig 2b gives the relation between the COD conversion of natural inoculum samples with gas production during anaerobic digestion. Food sample with otter natural inoculum has given the maximum gas production. This may be supported by the fact that the COD conversion is maximum for otter sample and that is 0.85g. But at the same time the whole gas produced was CO2. the possible reason for this may be due to low PH (around 4) and low C/N ratio which inhibit all the methanogens due to intense acidic conditions inside the digester. For tortoise sample the COD conversion is 0.08g and this low COD conversion is responsible for less gas production for tortoise sample. If concentrate on Kangaroo sample then it is found that food waste with kangaroo natural inoculum is producing maximum methane. The possible reason is high amount of CN ratio which helped the methanogens to survive.

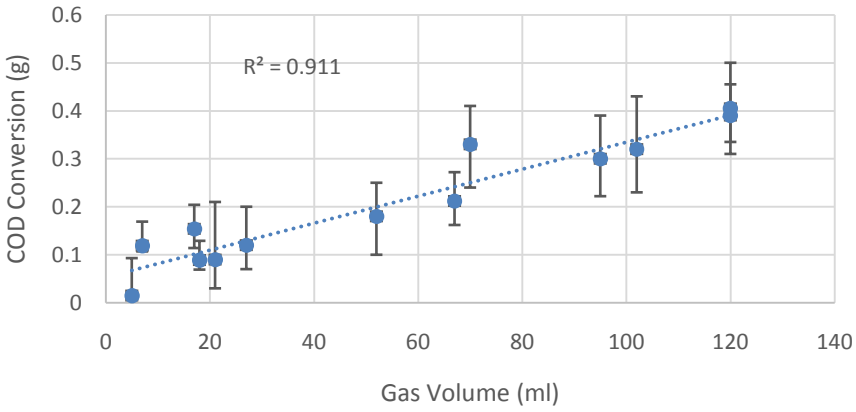

Fig 2a: Variation of gas volume with COD conversion for artificially cultured inoculum

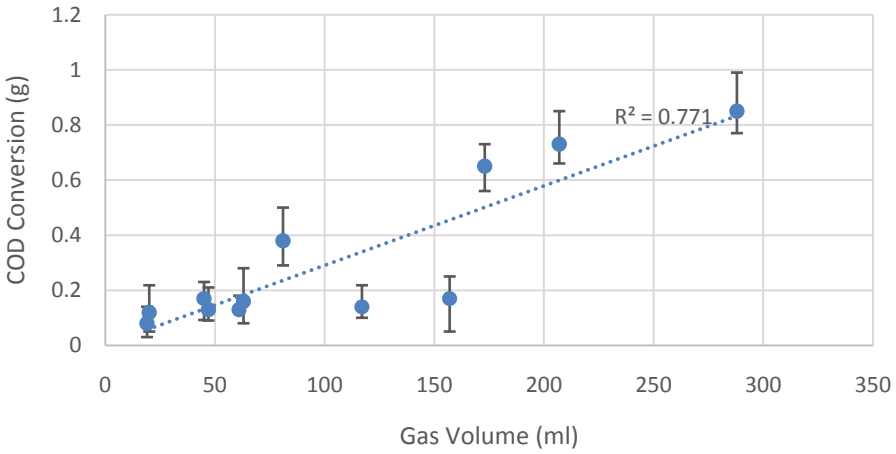

Fig 2b: Variation of gas volume with COD conversion for natural inoculum

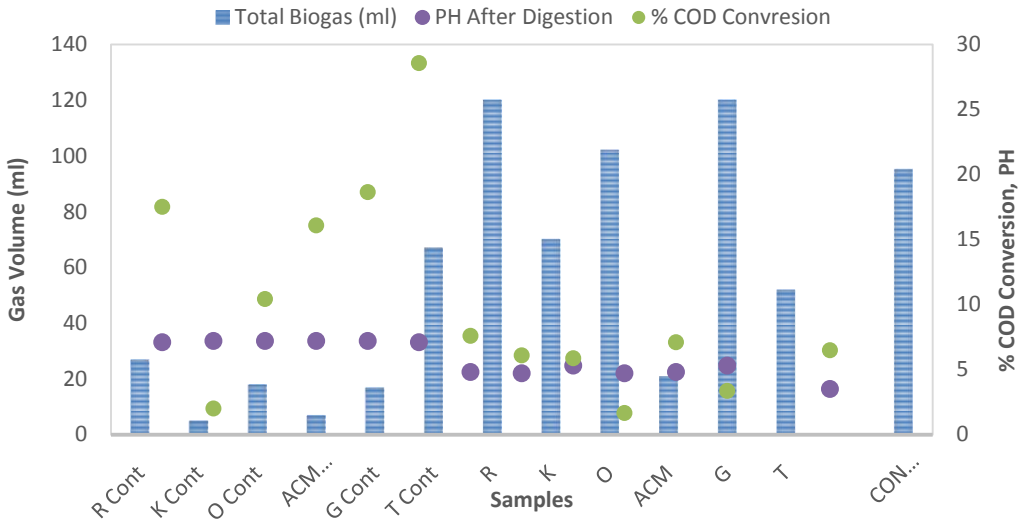

Fig 3a: Biogas volume and characteristics of different food samples with artificial inoculums

From the above analysis it is found that artificial inocula are able to convert the COD but at the same time they are converting the COD to CO_2 instead of methane gas. The possible reason is the low PH and CN ratio which are important reason of methanogens survival. At the same time natural inocula shown good results for digestion of food waste. The reason for this is high PH, high CN ratio and high TS of the samples as shown in fig 3b.

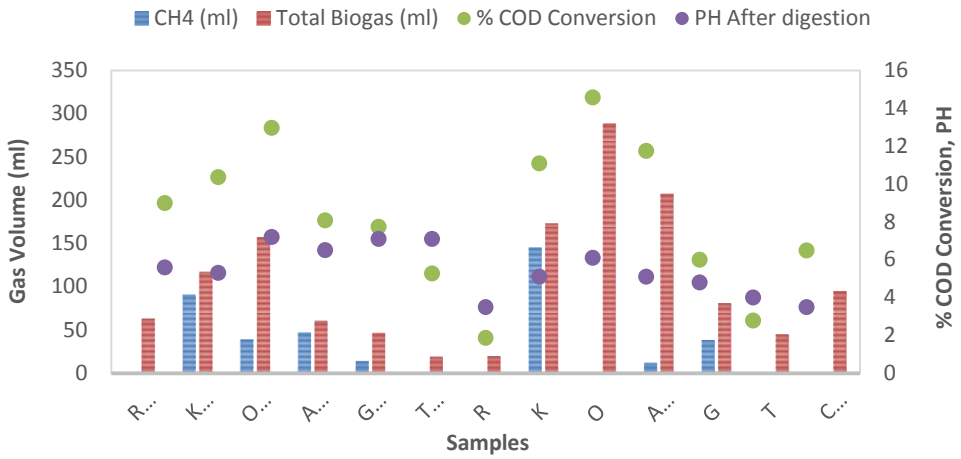

Fig 3b: Biogas volume and characteristics of different food samples with natural inoculum

Conclusion

It is observed that there is a potential of biogas production from the food waste varying differently with varying quality of inoculum. The natural inocula are giving better performance as compared to artificially cultured inocula for all the animal fecal samples. Low PH during digestion is possible reason which is responsible for acidic conditions inside the digester. Low C/N ratio is the second possible reason due to which access ammonia was generated which inhibit the methanogens. Based on the results, it is also suggested to study the behaviour of microbial community over the digestion time span using real time PCR to understand the digestion process.

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