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Identification of Anaerobic Microorganisms for Converting Kitchen Waste to Biogas

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***Abstract*—**Anaerobic digestion process is one of the alternative methods to convert organic waste into methane gas which is a fuel and energy source. Activities of various kinds of microorganisms are the main factor for anaerobic digestion which produces methane gas. Therefore, in this study a modified Anaerobic Baffled Reactor (ABR) with working volume of 50 liters was designed to identify the microorganisms through biogas production. The mixture of 75% kitchen waste and 25% sewage sludge was used as substrate. Observations on microorganisms in the ABR showed that there exists a small amount of protozoa (5%) and fungi (2%) in the system, but almost 93% of the microorganism population consists of bacteria. It is definitely clear that bacteria are responsible for anaerobic biodegradation of kitchen waste. Results show that in the acidification zone of the ABR (front compartments of reactor) fast growing bacteria capable of growth at high substrate levels and reduced pH was dominant. A shift to slower growing scavenging bacteria that grow better at higher pH was occurring towards the end of the reactor. Due to the ability of activity in acetate environment the percentages of *Methanococcus*, *Methanosarcina* and *Methanotrix* were higher than other kinds of methane former in the system.

***Keywords*—**Anaerobic microorganism identification, Kitchen waste, Biogas.

I. Introduction

THE use of oil and other fossil resources as transportation fuels and commodity chemicals is deeply engrained in today's society, but use of these resources is unsustainable. The unsustainable nature of fossil fuels stems from their finite reserves and their negative environmental impact. Combustion of fuels releases carbon dioxide and various pollutants, such as sulfur and nitrogen oxides. The promotion of waste minimization and recycling are important components of modern waste management strategies. Nevertheless, even when the minimization and recycling potentials are fully exploited, there is still a residual fraction, which has to be disposed of. The burdens resulting from landfilling can be minimized by pre-treating the waste and thus limiting its emission potential [1]. Anaerobic treatment processes could reach an average of 50–55% reduction of organic content in the treatment of residual waste. Practical tests have shown that threshold values can be achieved at the above-mentioned reduction of organic content with a post-decomposition duration of approximately 4–6 weeks [2]. The transition towards a sustainable energy supply will take considerable time. In the meantime, short-term solutions will aim to lessen the environmental impact of fossil fuels [3]. This study aims to identify most active microorganisms for converting kitchen waste to biogas in a laboratory scale Anaerobic Baffled Reactor (ABR).

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II. Anaerobic Digestion

Anaerobic digestion is the classical example of a process that combines the objectives of elimination of organic compounds from a waste stream with the generation of a valuable product in the form of methane-containing biogas. Different bioreactor configurations have been developed for the treatment of liquid and solid waste streams [1-3]. For wastewater treatment, the application potential of anaerobic digestion has been extended from medium to highly concentrated wastewaters of agro-industrial origin, to more complex applications like those generated in petrochemical industries [4, 5], paper industries [5, 6] and even sewage [7]. There are three clear advantages of anaerobic treatment over aerobic degradation of organic substrates.

- The high product and low biomass yield resulting in a limited generation of waste sludge as an unwanted side product.
- The *in situ* separation of the product as biogas, which is limiting costs for product separation.
- The use of simple technology, as mixing by the biogas produced circumvents the need for other mixing requirements.

An anaerobic baffled reactor (ABR) operates with a combination of several anaerobic process principles, the three basic steps involved are: (a) hydrolysis, (b) fermentation, and (c) methanogenesis. The ABR is a fluidized bed reactor similar to the Upflow Anaerobic Sludge Bed (UASB) process. Equal inflow distribution, and the wide spread contact between new and old substrate are important process features. It is known that a three-chamber reactor, together with physical modifications, provided a longer solid retention time

and superior performance than the reactor with only two compartments [8].

In the absence of an external electron acceptor such as oxygen, organic substrates can only be fermented; a process where the organic substrate is both the electron donor and acceptor. The final end products of the organic substrate fermentation are methane, carbon dioxide and ammonia. Methane is the organic compound with the lowest free energy content per electron upon oxidation to carbon dioxide (Fig. 1). This indicates that in a thermodynamically closed system substrates will eventually be converted to methane and carbon dioxide. Microorganisms can obtain the energy required for growth by (stepwise) catalyzing the conversion of organic substrates to methane and carbon dioxide. Ideally, the production of methane and carbon dioxide can be calculated using the following (1) [9]:

$$C_aH_bO_cN_d + (4a–b–2c+3d / 4)\ H_2O \rightarrow (4a+b–2c–3d / 8)\ CH_4 + (4a–b–2c+3d / 4)\ CO_2 + d\ NH_3 \quad (1)$$

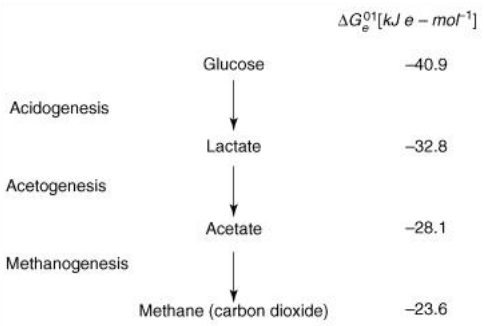


Fig. 1 Fermentation of Glucose to Methane

III. Methane Generation By Anaerobic Digestion

The two ways for methane generation are to capture the gases produced in landfills, or to pre-treat the refuse and digest it in tanks similar to those used in wastewater treatment plants through anaerobic digestion [10]. In fact, the chemical composition of kitchen waste is difficult, if not impossible to be determined. Although some attempts have been made to do so the best approximation is that the organic fraction of refuse to be described by the chemical formula, $C_{99}H_{149}O_{59}N$. In using this formula the only carbon that can participate in the production of gas is from decomposable materials such as food waste and paper. Other organics, most importantly plastics, do not decompose to produce gas.

IV. Materials And Methods

A. Reactor Design

An Anaerobic Baffled Reactor (ABR) was used to determine the biogas generation from kitchen waste. Additional vertical baffles in a plug-flow reactor constitute an ABR, which enhances solids retention to allow better substrate accessibility to methanogens. The laboratory-scale unit shown in Fig. 2 was made with a total working volume of 50 liters. Two tanks as influent tank and effluent tank were designed for feeding and removing the materials to and from the reactor. A gas collector was also provided for collection and analysis on the amount of biogas.

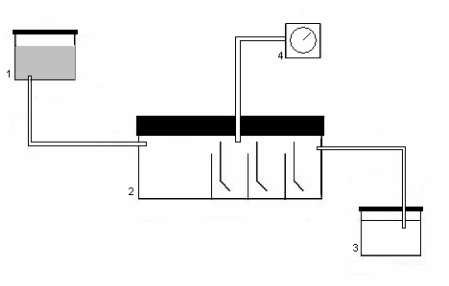


Fig. 2 Laboratory Scale Anaerobic Baffled Reactor
(1: influent tank, 2: ABR system, 3: effluent tank, 4: wet gas meter)

For the purpose of the study, this kitchen waste was brought from a university cafeteria. The wastes were collected and combined in an approximately equal proportion, then mixed thoroughly in the laboratory, shredded and grounded. Then it was mixed with sewage sludge, at the ratio of 75% kitchen waste and 25% sewage sludge. The sewage sludge was brought from a municipal wastewater treatment plant. The sewage sludge was collected from the sewage sludge return pipeline and immediately brought to the laboratory.

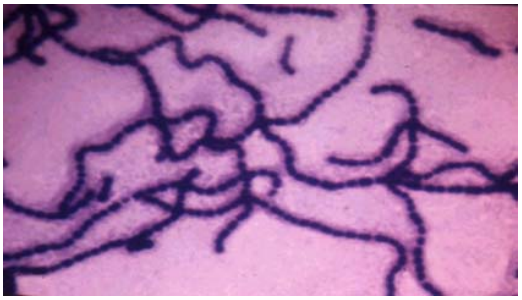
B. Microbiological Examinations

Microbiological examinations were done regularly to identify the most important and active species in each compartment of ABR as an anaerobic biological reactor. All the experiments for identifying microorganism in the anaerobic process were done according to the Standard Methods [11]. Biological water sampler was used for sample collections according to Edwards [12].

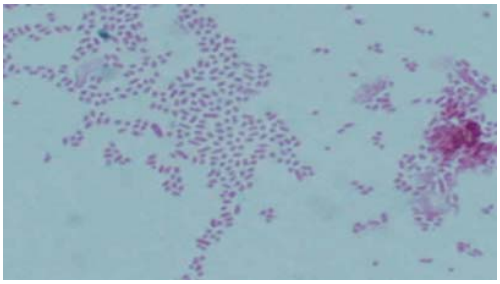
V. Results And Discussion

A. Microorganisms in the ABR System

Observations on microorganisms showed that, there exists small amount of protozoa (5%) and fungi (2%) in the system, but almost 93 % of the microorganism population consists of bacteria. Figure 3 shows hydrolytic, acetogenic and methanogenic bacteria in the ABR system.



(*a*)



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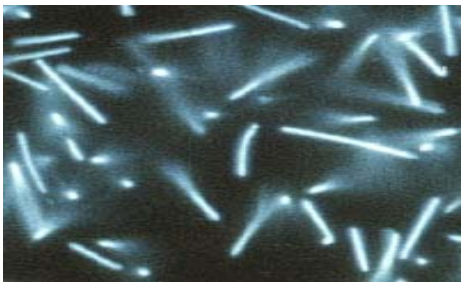

(*c*)

Fig. 3 Three Main Categories of Anaerobic Bacteria in the ABR System (*a*) Hydrolytic Bacteria (*b*) Acetogenic Bacteria and (*c*) Methanogenic Bacteria

The anaerobic digestion of organic material is a very complicated biochemical process involving hundreds of possible intermediate compounds and reactions, each of which is catalyzed by specific enzymes or catalysts. In general, anaerobic digestion is considered to occur in three stages. Many organic wastes consist of complex organic polymers such as proteins, fats, carbohydrates, cellulose, lignin, etc., some of which are in the form of insoluble solids. It was observed through microorganism examinations in the ABR that in the first stage the organic polymers of kitchen waste such as carbohydrates, fats and proteins are broken down by extracellular enzymes produced by hydrolytic bacteria (Fig. 3(a)), and dissolve in water. In the second stage monomeric compounds released by the hydrolytic break down due to bacterial action in first stage are further converted to volatile fatty acids, H2 and CO2 by the acetogenic bacteria (Fig. 3(b)). Finally, the products of second stage are converted to CH4 and other end products by a group of bacteria called methanogens (Fig. 3(c)). Methanogenic bacteria are obligate anaerobes whose growth rate is generally slower than the bacteria in first and second stages.

Results show that in the acidification zone of the ABR (front compartments of reactor) fast growing bacteria capable of growth at high substrate levels and reduced pH was dominant. A shift to slower growing scavenging bacteria that grow better at higher pH was occurring towards the end of the reactor. Table 1 shows the quantitative content of all bacteria during the anaerobic degradation in the ABR system.

The met hanogenic bacteria were composed of both gram-positive and gram-negative bacteria with a wide variety of shapes. Methanogenic microorganisms grew slowly in the reactor and their generation time ranged from 2 days at 35°C. About two thirds of methane was derived from acetate conversion by methanogens. The other third was the result of carbon dioxide reduction by hydrogen. Methanobacterium, Methanosprilium, Methanococcus, Methanosarcina and Methanotrix were observed in the biodegradation of kitchen waste. Due to the ability of activity in acetate environment, the percentages of Methanococcus, Methanosarcina and Methanotrix were higher than other kinds of methane former in the anaerobic baffled reactor (ABR).

TABLE I
QUANTITATIVE CONTENT OF BACTERIA

Bacteria Species	Percentage
Methanobacterium	4%
Methanospirilium	2%
Methanococcus	21
Methanosarcina	16
Methanotrix	15
Cintrobacteroloini	7
Cintrofermonas	5
Proteolytic Eubacterium	6
Acetobacterium	4
Biofidobacteria	3
Bacteroides	7
Streptococi	5
Entrobacteriaceoe	3

B. Fate of Pathogens in the ABR System

The pathogenic Salmonella sp. was observed at the start of the digestion process. But it was eliminated by the ninth day of digestion. Kunte et al. [13] reported that Salmonella typhi added to cattle dung-fed digesters as single dose at the start of the digestion was completely eliminated after 12 days of digestion with shorter retention period of 15 days, whereas it needed 26 days for complete elimination with 30 day HRT. Similarly, with daily dose of the pathogen too, four-fold log reduction was observed in 15 day HRT, whereas it was only two fold with 30 day HRT. This was attributed to higher production of volatile fatty acids in digesters with shorter retention period. Earlier Anupama et al. [14] observed that enteric pathogens were eliminated after two weeks of digestion.

C. Bacterial Population Development in the ABR System

With the unique construction of the ABR various profiles of microbial communities may develop within each compartment. The microbial ecology within each reactor chamber will depend on the type and amount of substrate present, as well as external parameters such as pH and temperature.

Large population of Methanosarcina was found from the sampling at the front part of the reactor while toward the end the amount for Methanosaeta was increased. The performance and bacterial population of the ABR compared with the performance and bacterial population of anaerobic filter at pilot scale was done by Yang et al. [15] and according to them firstly, the acetate loading in the first chamber of ABR was

1000 mg/l, which was close to the apparent Ks value for Methanosarcina and may have favored its growth. Secondly, lower superficial gas production rates in the anaerobic filter resulted in lower gas turbulence and therefore fewer washouts of bioflocs compared with the anaerobic baffled reactor. Hydrogen levels were also measured and the highest concentration (4 x 10-4 atm) was noted in the first chamber of the baffled reactor and this may explain the presence of Methanobacterium. The results are subsequently supported by Polprasert et al. [16] where acetate concentrations as low as 20 mg/l enabled the domination of Methanosaeta like bacteria throughout a four compartment reactor.

When methane concentrations was high, acetate concentrations were relatively high and therefore provided the best environmental conditions for the growth of Methanosarcina which can grow quickly and efficiency even at pH values as low as 6. Another source of methane would be from hydrogen scavenging bacteria such as Methanobacterium and Methanobrevibacter, which would be stimulated by the higher hydrogen concentrations.

Stable granules of 0.5 mm appeared after one month in all chambers of the reactor; microscopic studies subsequently showed that the granules were primarily of acetoclastic methanogens. Similarly, Tilche and Yang [17] found Methanosarcina coated flocs held together by fibrous bacteria resembling Methanosaeta. The flocs which were formed after one month were found to be small with diameters less than 1.5 mm. Under the same loading conditions the authors also found densely packed granules typical of a UASB (d < 3 mm) formed after 3 months in an aerobic filter. Boopathy and Tichle [18] noticed similar particles of both types described above, which grew from 0.5 mm after one month to 3.5 mm after three months in a hybrid reactor. Granules, which were made from Methanosarcina clusters, were of low density and full of gas cavities and therefore lifted to the surface of the reactor due to high gas and liquid velocities during high loading.

VI. Conclusion

The anaerobic digestion of organic material is a very complicated process, involving hundreds of possible intermediate compounds and reactions, each of which is catalyzed by specific enzymes or catalysts. With the unique construction of the ABR, various profiles of microbial communities developed within the reactor. Observations of microorganisms showed that, there exists a small amount of protozoa and fungi in the system, but almost 93% of the microorganism population consists of bacteria. About two thirds of methane was derived from acetate conversion by methanogenic bacteria. The other third was the result of carbon dioxide reduction by hydrogen. Methanobacterium, Methanosprilium, Methanococcus, Methanosarcina and Methanotrix were observed in the biodegradation of kitchen waste. Due to the ability of activity in acetate environment, the percentages of Methanococcus, Methanosarcina and Methanotrix were higher than other kinds of methane formers in the anaerobic baffled reactor (ABR).

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