Microscopic Observation of Anaerobic Microorganism in a Modified Anaerobic Hybrid Baffled (MAHB) Reactor in Treating Recycled Paper Mill Effluent (RPME)

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Abstract— activities of various kinds of microorganisms are the key factor for anaerobic digestion which produces methane gas. Therefore, anaerobic digestion process is known as an alternative methods to convert waste into methane. In this present study, a modified anaerobic hybrid baffled (MAHB) reactor with a working volume of 58 L were used to treat recycled paper mill effluent (RPME) wastewater. The mixture of 90% RPME wastewater and 10% anaerobic sludge was used as substrate. The morphologies of anaerobic microorganism involved in anaerobic digestion of RPME in MAHB reactor was observed using Scanning Electron Microscopy (SEM) and Fluorescence Microscope. Only small amount of protozoa and fungi were observed in the MAHB reactor system. It was found that bacteria responsible in biodegradation of biomass inside the MAHB reactor were dominant. It was also demonstrated that fast growing bacteria which were capable to growth under high substrate levels and reduced pH was dominant at the front compartments (acidification zone). Towards the end of the reactor, a slower growing scavenging bacteria that grow better at higher pH was found. In addition, the population of *Methanococcus*, *Methanosaeta* and *Methanosarcina* were higher compare to other species of methane former bacteria.

Index Terms— Anaerobic microorganism identification, Recycled paper mill effluent, Biogas

I INTRODUCTION

Anaerobic digestion is the most common process for dealing with wastewater containing sludge. It is widely used as a source of renewable energy. The process produces a biogas, consisting of methane, carbon dioxide and traces of other 'contaminant' gases [1]. This biogas can be used directly as fuel, in combined heat and power gas engines [2] or upgraded to natural gas-quality bio methane.

From literature surveys, it is suggested that an anaerobic baffled reactor (ABR) act as an effective option for onsite sanitation in low income, waterborne and peri-urban communities [3]. ABR were consists of vertical baffles, in which the wastewater is forced through and over the vertical baffle as it moves from inlet to outlet [4]. Although ABR were extensively used to treat different types of industrial waste, literature survey shows that there is lack on the anaerobic treatment of recycled paper mill effluent (RPME) wastewater by a novel modified anaerobic hybrid baffled (MAHB) reactor.

Spatial nutrient concentration gradients resulted from compartmentalised design of MAHB reactor contributes to microbial consortia that optimally adapted to the specific conditions in each compartment [3, 5]. Previous researches demonstrates that fast growing bacteria which is capable to grow at reduced pH and high substrate levels should dominating the first two compartments whereas slower growing bacteria dominating near the end of the reactor at higher pH [5]. Phase separation provides greater stability to environmental parameters, increased conversion of suspended solids and enhanced efficiency [6].

Fluorescent microscope and scanning electron microscopy (SEM) were used to observed specific microorganism types that responsible in converting the biomass of RPME

wastewater and provide insight into the mechanisms of anaerobic digestion in the MAHB reactor [7]. Previous study indicates that hydrolytic and acidogenic bacteria existed in all compartments of the reactor [8]. Using SEM, hydrogenotrophic, methanogen-like archae were observed at the front part of reactor which includes morphotypes resembling *Methanobacterium sp, Methanococcus sp* and *Methanospirilium sp.*

This present study were done to observe microorganism which are responsible to convert biomass of recycled paper mill effluent to biogas in a laboratory scale MAHB reactor using Scanning Electron Microscopy (SEM) and Fluorescence Microscope.

II Materials and Methods

A Reactor design

A modified anaerobic hybrid baffled (MAHB) reactor was used to determine the generation of biogas from recycled paper mill effluent (RPME). Vertical baffles in each of compartments inside the MAHB reactor enhances the solids retention for better substrate accessibility to methanogens. The laboratory-scale unit as shown in Fig 1 with a total working volume of 58L. Feeding and effluent tank were designed for influent and removing the wastewater to and from the reactor. A gas collection system was for collection and analysis on the amount of biogas produced.

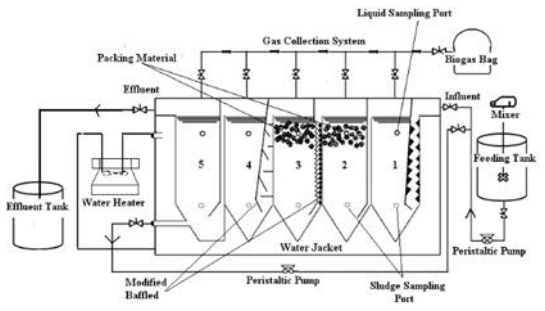

Figure 1: Laboratory Scale Modified Anaerobic Hybrid Baffled (MAHB) Reactor

For the purpose of this present study, recycled paper mill effluent (RPME) were collected from Muda Paper Mill Bhd, Bandar Tasek Mutiara, Penang, Malaysia and refrigerated at 4℃. Prior to analysis, the samples were warmed to room temperature (25 ± 2℃). The seeding and start up process of MAHB reactor have been reported previously [9]. The MAHB reactor was started at hydraulic retention time (HRT) of 4 days and organic loading rate (OLR) of 0.14 g/L/dy

B Microbiological Examinations

During steady state, microbial examinations were done to observe the most active and important species in each compartment of MAHB reactor as an anaerobic biological reactor. Scanning electron Microscope (SEM) and Fluorescence Microscopy were used to examine the microbial population involved. Sludge sample from each compartment were taken and examined in SEM according to standard protocols [10] and were produced at magnifications between 10x to 20x.

III Result and Discussion

Observation using fluorescence microscope shows existence of protozoa and fungi in the system. However from observation, most of the system were consists of microorganism population which are bacteria comprising hydrolytic, acetogenic and methanogenic bacteria. Figure 2 below illustrated the hydrolytic, acetogenic and methanogenic bac-teria found in the MAHB reactor system. Micro-scopic image of hydrolytic, acetogenic and meth-anogenic bacteria observed were

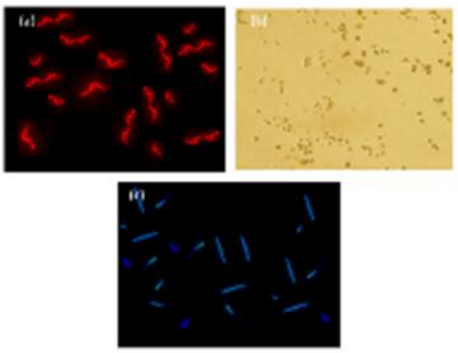

Figure 2: Three Main Categories of Anaerobic Bacteria in the ABR System (a) Hydrolytic Bacteria (b) Acetogenic Bacteria and (c) Methanogenic Bacteria

in similarities with the image previously indicates by Malakahmad et al. [11].

Generally, anaerobic digestion occurred in three stages which are hydrolysis, acetogenesis and methanogenesis. From observation through fluorescence microscope, it is thought that organic polymers of RPME wastewater such as carbohydrates are broken down by extracellular enzymes produced by hydrolytic bacteria (Fig 2a). Then, during second stage of acetogenesis process, monomeric compounds are further converted by acetogenic bacteria (Fig 2b) into volatile fatty acids, H_2 and CO_2. Lastly, methanogenic bacteria (Fig 2c), an obligate anaerobes bacteria whose growth rate is slower than bacteria in first and second stages converted products of second stage into methane and other end products.

Results indicates that in front compartments of MAHB reactor (acidification zone), a fast growing bacteria which capable to grow at reduced pH and high substrate levels was dominant. However, towards the end of the reactor, a shifted to a slower growing scavenging bacteria which grow better at higher pH was found. Similar results were reported by Malakahmad *et al* [11].

Methanogenic bacteria were consisted of both gram negative and gram positive with a diversity of shapes. They grew slowly inside the reactor with generation time ranged from 2 days under room temperature [11]. Methane were derived by two ways. One third of methane was derived by carbon dioxide reduction by hydrogen and another two third was by acetate conversion by methanogens [12]. Figure 3 shows image of methanogens observed in the biodegradation of RPME. The image of observed methanogen were compared with previous finding by Macario *et al* [13, 14] by comparing the shape of the microbes.

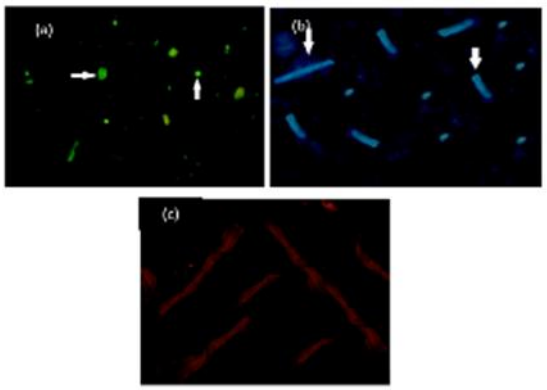

Figure 3: Fluorescence image of (a) *Methanococcus* (small cocci), (b) *Methanobacterium* (chain forming rod) and, (c) *Methanosprilium* (filamentous)

IV Bacterial Population Development in the ABR System

Regarding to the unique construction of MAHB reactor, each compartment may comprise of varieties of microbial communities within. The microbial ecology within each compartments were influenced by external parameters such as temperature and pH as well as amount and type of substrate present.

Figure 4 shows images of two microorganism, *Methanosaeta* and *Methanosarcina*, which used same substrate and can coexist in MAHB reactor treating RPME wastewater. The image of *Methanosarcina* and *Methanosaeta* were denoted using previous finding by Pillay *et al* and Lima *et al* for fluorescent and SEM image respectively by comparing the shape of both microbes in which *Methanosarcina* has coccus shape while *Methanosaeta* has a bamboo-shaped rods [8, 15]. *Methanosarcina* like bacteria was found in the wastewater sample at the front compartment while *Methanosaeta* was mainly found towards the rear end of compartment. Bacterial population of the MAHB reactor were compared to previous work by Yang et al [16] which indi-cates that acetate loading in front compartment favoured growth of Methanosarcina. As the methane concentration was high, the concentrations of acetate were also relatively high. Therefore it provides the best environmental conditions for Methanosarcina which can grow well at pH as low as 6. Subsequent compartments shows domination of *Methanosaeta* as the acetate concentration as low as 6.5 mg/l. Similar results were also recorded by Polprasert *et al.* [17] supported that acetate concentrations as low as 20 mg/l enabled the domination of *Methanosaeta* like bacteria throughout a four compartment reactor. In addition, with high k and Ks value, *Methanosarcina* will dominate when acetate concentrations are high, while *Methanosaeta* will dominating at lower acetate concentration as the k and Ks value lower [18]. For hydrogen scavenging bacteria, such as *Methanobrevibacter* and *Methanobacterium*, higher hydrogen concentration will stimulates the methane formation.

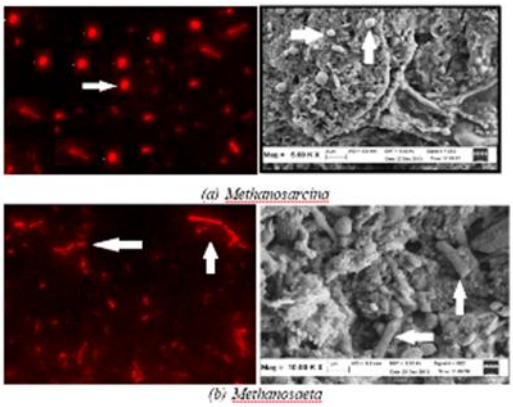

Figure 4: Fluorescent image and SEM micrograph showing: a) *Methanosarcina* (small coccus microorganism) and b) *Methanosaeta* (bamboo shape microorganism)

V CONCLUSION

It is thought that the uniqueness of MAHB reactor design influence the predominance of either acetoclastic methanogenic bacteria. Observation showed that bacteria were dominant compared to other microbes. The configuration of the ABR causes partial delinking of acidogenesis and methanogenesis. Theoretically, about two thirds of the methane was derived from conversion of acetate by methanogenic bacteria. *Methanosprilium*, *Methanobacterium*, *Methanococcus*, *Methanosarcina* and *Methanosaeta* were observed in the biodegradation of RPME wastewater. Owing to the capability of activity in acetate environment, *Methanosarcina* and *Methanosaeta* were dominant than other kinds of methane formers in the modified anaerobic hybrid baffled (MAHB) reactor.

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