

## Effect of Specific Methanogenic Activity (SMA) of Anaerobic Sludge under High Salinity

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### ABSTRACT

Anaerobic wastewater treatment technology has become one of the most popular technologies due to its economic and environmental benefits. Therefore, a reliable method to study the specific methanogenic activity (SMA) of anaerobic sludge is presented using SMA test. This test helps to determine suitable organic loading rate (OLR) to the anaerobic process. Meanwhile, SMA act as a benchmark to the anaerobic system performance where a slight change in SMA stipulate the accumulation of inhibitory substances from influent wastewater. One of the possible substances that inhibit the anaerobic digestion process is the presence of excessive light metal ions such as sodium (Na<sup>+</sup>) from the wastewater. The objective of this research was to investigate the specific methanogenic activity of anaerobic sludge in anaerobic filter and anaerobic digester as well as to compare and analyze anaerobic methanogenic bacteria under the effect of potentially inhibitory compound in both anaerobic digester and anaerobic filter. Two sources of inoculums (one from the anaerobic digester and one from the anaerobic filter) were adapted with sodium with varied range of concentrations which was 0 to 5 g Na<sup>+</sup> /l, before conducting SMA test by means of pressure differences. For the anaerobic digester, the SMA varied from 0.25 to 0.31 g COD/g VS.day meanwhile for the anaerobic filter, the SMA varied from 0.40 to 0.51 g COD/g VS.day. The result obtained confirmed that sludge from POME have better tolerance towards sodium than influents from petrochemical wastewater.

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## 1. Introduction

Anaerobic process is an example of biological treatment in which the organic matter in wastewater is degraded in the absence of oxygen and producing carbon dioxide and methane biogas as by- product. Examples of anaerobic process are anaerobic digester and anaerobic filter. This technique has been widely used since the 19th century in treating wastewater where it is very cost-effective and less biomass yield. In addition, this treatment produce energy in the form of methane

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gas instead of consumes energy such as aerobic treatment [1] and the biogas produces has been widely used as an alternative source of energy. This process is achieved via enzymatic and bacterial activities [2].

In this context, the activity of methanogenic bacteria is the key factor for the production of the methane gas as well as a criterion for the effectiveness of anaerobic treatment since these bacteria works to degrade organic matter in the wastewater to carbon dioxide and methane gas. These methanogenic bacteria are very sensitive where changed operational circumstance will influence its growth and behavior activity [3]. Sodium is an alkali metal group where at concentration of 8 g/L, sodium became the strongest inhibitor on a molar basis compared to other metal cations as reported by [4] in their research. High sodium concentration (saline) in wastewater will possibly constrain the methanogenic activity and decline the effluent quality of wastewater treatment.

Anaerobic activity test which is Specific Methanogenic Activity (SMA) test is one of the key tools to assessing the performance and stability of anaerobic digester. Specific methanogenic activity represents the rate at which the methanogens (microbe) utilize the substrate (food) to yield methane and carbon dioxide. The SMA test is done to determine the maximum activity of the inoculum which can be produced per day per gram of bacteria present in unit of g COD/ g VSS.d [5]. A methanogenic test is designated based on pressure difference analysis of the headspace of closed serum bottle in anaerobic condition that containing sample with acetate as substrate and filled with macronutrient and micronutrients at various sodium concentrations. In Malaysia, there are limited studies of methanogenic activity of high sodium wastewater. In order to enhance anaerobic treatment performance as well as to improve sludge properties, knowledge on degree of sodium inhibition on anaerobic bacteria is prerequisite.

For better understanding of anaerobic treatment under high salinity, the characteristics of the sludge need to be analysed. In addition, the purpose of this study is to determine to what extend high salt level ( $\text{Na}^+$ ) in wastewater affects the activity of methanogen in anaerobic digester at different concentration of sodium. The objectives of this study are to determine the specific methanogenic activity of anaerobic sludge by means of pressure differences using pressure meter as well as to compare and analyse anaerobic methanogenic bacteria under the effect of potentially inhibitory compound ( $\text{Na}^+$ ) in both anaerobic digester and anaerobic filter. In this paper, the acclimatization of methanogenic bacteria in both anaerobic digester of Palm Oil Mill factory of Felda Chalok, Terengganu and anaerobic filter of Optimistic Organic Sdn Bhd were examined under high salinity.

## **2. Methodology**

### **2.1 Batch Experiment**

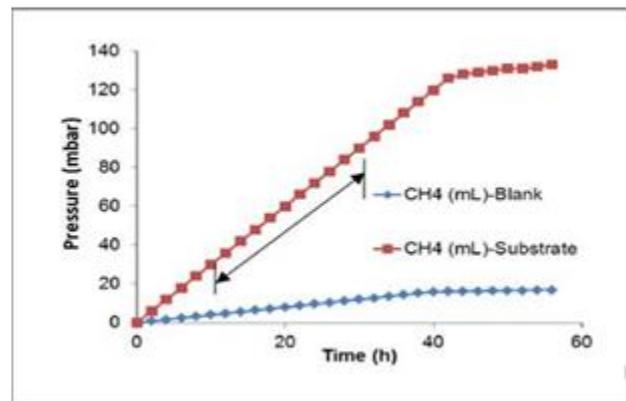
Anaerobic sludge was incubated in a batch system for 60 days according to the operating conditions as stated in Table 1. This batch experiment was conducted to acclimatize the sludge to sodium. 1000 mL Scott bottle was used as reactors and was filled with anaerobic sludge. Sodium chloride was used as a source of sodium and diluted with water to obtain 0.05, 0.5 and 5 g  $\text{Na}^+$ /L. For the R4, the sodium was gradually injected to prevent the microbe shock. The Scott bottle was then sealed tightly prior to submerge in water bath. Mechanical shaker was used to mix the substrate and the anaerobic sludge. This incubation process was carried out under optimal pH and temperature. The reactor was submerged in the water bath so that the temperature can keep constant at 30°C.

**Table 1**  
 Characteristics for batch experiment set-up

Parameter	Reactor			
	R1	R2	R3	R4
Liquid Volume, mL	800	800	800	800
[Na <sup>+</sup> ], g/L	0	0.05	0.5	5
COD, g/L	1	1	1	1

## 2.2 Feed Composition

A medium consisting of macronutrient, trace elements and acetate was used as a substrate. The acetate was used because approximately 72 percent of methane formed during anaerobic degradation of complex substrate results from acetic acid. The macronutrients were added containing (mg/L): NH<sub>4</sub>Cl (170), CaCl<sub>2</sub>.2H<sub>2</sub>O (8.0), MgSO<sub>4</sub>.7H<sub>2</sub>O (9.00). The trace elements (mg/L) were used as described in [6] which contained FeCl<sub>3</sub>.4H<sub>2</sub>O (2.00), CoCl<sub>2</sub>.6H<sub>2</sub>O (2.00), MnCl<sub>2</sub>.4H<sub>2</sub>O (0.50), CuCl<sub>2</sub>.2H<sub>2</sub>O (0.03), ZnCl<sub>2</sub> (0.05), HBO<sub>3</sub> (0.05), (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>2</sub>.4H<sub>2</sub>O (0.09), Na<sub>2</sub>SeO<sub>3</sub>.5H<sub>2</sub>O (0.10), NiCl<sub>2</sub>.6H<sub>2</sub>O (0.05), EDTA (1.00), Resazurin (0.50) and HCL 36% (1 ml/L). The macronutrient and micronutrient was essential for the stimulation of microorganism which enhance methane production [7] while the Sorensen buffer was added for maintaining its pH. The feed was characterized as a soluble and highly biodegradable substrate, with the essential of macro and micro-nutrients for optimal bacterial growth [8].

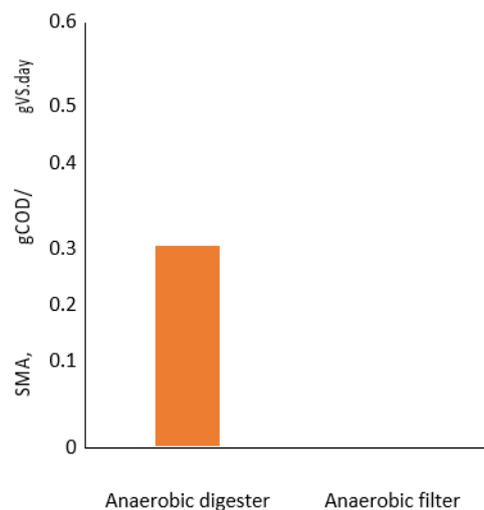


**Fig. 1,** Example of pressure increment graph for collected methane

## 3. Results and Discussion

According to Colleran and Pender [11], there is no international accepted test protocols to determine specific activity of anaerobic biomass. There are 3 types of technique usually used to measure the specific methanogenic activity which is volumetric method, manometric method and gas chromatography method [12]. This method which included in volumetric method was chosen due to its simplicity and inexpensive compared to other techniques. In this paper, two sources of inoculum were used which were from anaerobic digester and anaerobic filter. The SMA test was conducted on these samples by using pressure difference method and the results are shown below. The reactor R1 has only contained inoculum and other medium without addition of sodium.

From Figure 2, it shows that the SMA value of reactor R1 for anaerobic filter is 40% higher than anaerobic digester which is 0.51 g COD/ g VS .day. According to Aenaiz *et al.*, [13] Chen *et al.*, [14] and Bodkhe [15] anaerobic filter used the porous media packed that formed biofilm made by sludge adherence which produce higher activity than suspended sludge such as anaerobic digester. Study conducted by Checinel *et al.*, [16] shows that the sodium concentration in petrochemical wastewater ranged from 154 to 241 mg Na<sup>+</sup>/L. As reported by Kugelman and Chin [17], the optimum sodium concentration in wastewater treatment were 230 mg Na<sup>+</sup>/ L or equals to 10 mM. At low sodium concentration within the ranged of 100-200 mg Na<sup>+</sup>/L, the sodium enhances the growth of microbial activity by supplying energy to the methanogens [18]. However, excessive salt in the synthetic wastewater will reduce the methanisation process by increasing osmotic pressure in bacteria cell and causing plasmolysis and eventually the bacteria will die [19]. Lying on that, highly saline wastewater will reduce anaerobic activity as well as affect the biomass decay [20].

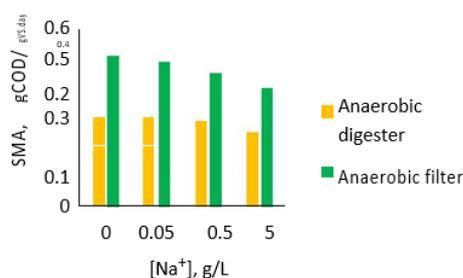


**Fig. 2.** SMA value for reactor R1

After the inoculum has come in contact with sodium, the SMA values for both anaerobic filter and anaerobic digester were decreased. The results for a reactor that contained sodium were presented in Figure 3. For the reactor 2 (R2), the SMA value for anaerobic digester and anaerobic filter sample at concentration 0.05 g Na<sup>+</sup>/ L were 0.30 and 0.49 g COD/ g VS .day respectively. Meanwhile, for the reactor R3 with sodium concentration 0.5 g Na<sup>+</sup>/l, the SMA value of anaerobic filter reduced 8.16% from the value of R2 which was 0.45 g COD/ g VS .day. Comparing with reactor R3 of anaerobic digester, the SMA value reduced only 3.3% from reactor R2 value which was 0.29 g COD/ g VS .day. The reactor R4 which contained 0.5 g Na<sup>+</sup>/ l has SMA value of 0.25 and 0.40 g COD/g VS .day with respect to anaerobic digester and anaerobic filter. The reduction of SMA value from reactor R1 to R4 for anaerobic digester was 16.1% which was from 0.31 to 0.25 g COD/ g VS .day while anaerobic filter reduce about 21.6% from 0.51 to 0.40 g COD/ g VS .day.

The results show that the inoculum derived from anaerobic filter cannot tolerate with the sodium whereby the SMA values are lower than anaerobic digester. Furthermore, as stated by Lefebvre [21], selection of sludge become a major factor to determine the effectiveness of biological treatment of saline wastewater. As stated before, petrochemical wastewater contains a favourable concentration of sodium before acclimatization process. Notably, after adaptation period, sludge from the anaerobic filter unable to tolerate with addition of sodium. This shows that the sludge already reached the maximum adaptation towards salinity while sludge from POME also slowly affected by

the increment of sodium. As reported by Lakaniemi *et al.*, [22], the adaptability of the methanogenic bacteria varies at difference salt levels. Hence, increasing  $\text{Na}^+$  concentrations to the 5 g/L have a slight impact on the sludge from POME and greatly affect the sludge from petrochemical wastewater.



**Fig. 3** SMA value for reactor R1- R4 with its sodium concentration for anaerobic filter and anaerobic digester

#### 4. Conclusion

From the research, it can be concluded that the SMA value for inoculum varies with types of wastewater treatment where anaerobic filter have higher SMA value than anaerobic digester which is 0.51 and 0.31 g COD/ g VS .day respectively. Other than that, when sodium is added, sodium affect the SMA in anaerobic sludge where the higher sodium concentrations, the lower its value and the degree of sodium inhibitory on SMA for anaerobic digester is lower than anaerobic filter. Thus, this study proved that POME wastewater can tolerate better with high concentration of sodium than petrochemical wastewater.

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