# Methane Optimization in Multi-Stage Anaerobic Reactor (MS-AR)

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## Abstract

The biological conversion of biomass in Anaerobic Digestion (AD) into methane was studied by many researchers in recent years. In the present study, optimization of methane production during chemical oxygen demand (COD) removal was observed in a novel Multi-Stage Anaerobic Reactor (MS-AR). A synthetic glucose was used as a feed substrate and the reactor was operated at a hydraulic retention time (HRT) of 1 to 4 d. Two complementary test procedures for methane optimization were evaluated; the theoretical and experimental. The theoretical methane gas was recorded as 50.13, 50.02, 50.16, and 50.22 % for HRT of 4, 3, 2 and 1 day, respectively. The results signify well with the empirical formula at each HRTs studied in the reactor. However, the quantity of methane gas present in the real application is significantly lower than the theoretical. This is due to the microorganism activity in the reactor that may have interfere with the efficiency of the biogas production. Actual data showed a decrease in the methane gas production (35.4, 21.2, 19.8, and 18.4 %) in the reactor. Thus, theoretical formula together with the actual data provides alternative method for the evaluation of bioenergy potential in AD.

## Keywords

Multi-Stage Anaerobic Reactor (MS-AR), Anaerobic Digestion (AD), Chemical Oxygen Demand (COD), Hydraulic Retention Time (HRT), Gas Law, Methane Generation

## INTRODUCTION

The growth in wide range of wastewaters addressing two important issues at global scale: treatability and production of biogases. Recently, researchers have looked towards the development of AD process as an optional for both disposal route and energy recovery (Kim et al., 2013; Fdez-Guelfo et al., 2011). AD process biologically metabolizes organic material in the absence of oxygen and produces methane ($CH_4$). It works on either high rate or low rate system. At high rate, it comprised biomass retention (HRT≠SRT) and at low rate without the biomass retention (HRT=SRT). Methane production in AD requires a vast group of bacteria which has its own characteristics. The degradation process consists of four stages; hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Gupta and Gupta, 2014).

During early stages of the development in AD, the single stage anaerobic process was limited by the low rates of COD removal, long HRT, accumulation of waste sludge and large reactor volume (Gannoun et al., 2009). Later, the improvement in reactor design was made to overcome those issues. The multiple stage reactors were designed to distribute organic load and to increase the contact time between the medium (sludge bed) and feed particles to react. Acidogenesis and methanogenesis process in multiple stage anaerobic reactors occurs in separate compartments enhances the AD. Even though biogas recovery in AD is a reward, still there are obstacles in achieving the process efficiency (Wendy et al., 2013). Despite many studies conducted to check the feasibility of biomethane generation through AD, only several succeeded to optimize the process. However, the outcome of previous research provides resources to improve biogas generation (Mendez and Lema, 1993).

This paper intends to determine methane generation potential in two such complimentary test procedures in reactors. The stoichiometric product estimation of the empirical formula (Erickson, 1978; Buswell and Neave, 1930) was used as a basic rule to calculate the biogas potential of the reactor (MS-AR), theoretically and experimentally. In both methods, the effect of feed COD, the COD removal efficiency, and HRT were investigated.

## MATERIAL AND METHOD

### Multi-Stage Anaerobic Reactor

In this experiment, the staging concept was incorporated in the MS-AR. The MS-AR reactors contained four identical cylindrical Plexiglas, represented as R1, R2, R3 and R4 (**Figure 1**) linked in series, with an active volume of 11 L (4 stages of 2.75 L) having internal diameter of 8.5 cm and 66 cm height. Each stage of the reactor had a 3-phase separator baffle, (1 circle disks with pore size diameter 1mm) and placed 2 cm below the effluent ports , to prevent floating granules from washing out with the effluent. Next, effluent from each stage of the reactor flowed by the gravity to the next stage. The temperature controller and heater were installed to maintain the reactor temperature at 38°C. Peristaltic pumps (Masterflex L/S, Easy Load II Pump Head) were used to control the influent feed rate. The methane gas in the MS-AR was recovered in a Tedlar gas bag and measured for routine analysis by using Gas Analyzer (GA2000).

### Seeding sludge

The MS-AR was inoculated with anaerobic sewage sludge sampled from Indah Water Konsortium (IWK), Bunus Treatment Plant, Kuala Lumpur. Each stage of the reactor was added with approximately 1.4 L of sludge sieved through 2.0 mm mesh, contained 8000 mg TSS/L and 5000 mg VSS/L. The initial total suspended solid (TSS) in the reactor was reduced to 6000 mg/L, based on the settling tests performed on the sludge. The reactor then filled with tap water in order to dilute the supernatant of the seed sludge. Next, reactor was purged with nitrogen gas to remove the air. Mesophilic temperature (38ºC) was maintained in the reactor and no feeding was assigned for 5 operational days in order to make the sludge settled. Once settled, the reactor was gradually fed with the synthetic wastewater.

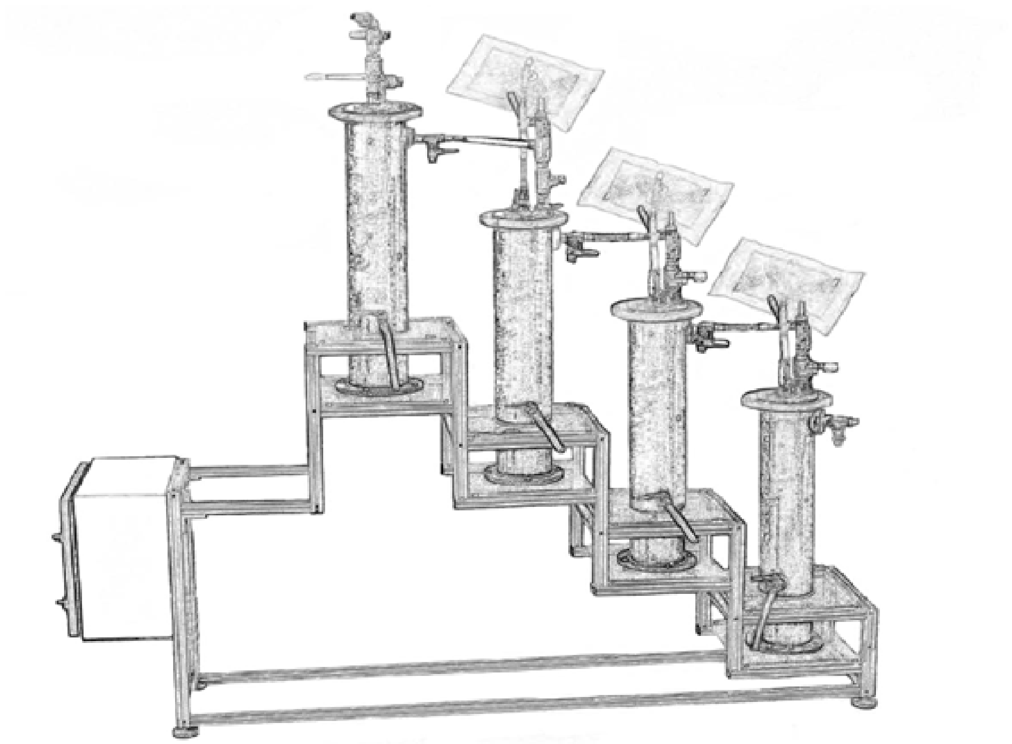


**Figure 1**: Multi-Stage Anaerobic Reactor (MS-AR)

### Feeding

The MS-AR was filled-up with synthetic wastewater, glucose as organic substance due to its degradability and does not limit the rate of anaerobic biodegradation (Noike etal., 1985). It produces readily measurable intermediary metabolites in AD and was commonly used as a carbonaceous substrate in many experimental studies (Stronach et al., 1986). The addition of nutrient and alkaline solution were prepared for the feed synthetic wastewater. Nutrient deficiency in feed was corrected using macronutrients N100 (from Bio-Systems Corporation Asia Pacific Sdn. Bhd.). It was designed to provide the essential macronutrients and micronutrients to supplement bacteria's basic metabolic needs in the anaerobic environments for enhancing the microorganisms growth rate (Speece, 1988). Inadequate nutrient will affect the anaerobic process and biogas generation. In this study, the tolerable ratio of COD: N: P in the wastewater was used at 250:5:1 for the anaerobic treatment process (Metcalf and Eddy, 1991; USEPA, 1995; Maier, 1999 a; Bashar, 2004). The analysis of parameters in this experiment was carried out using Standard Methods (APHA, 1998).

### Methane Production Potential

The potential of biogas generation in wastewater can be estimated by the amount of organic substance utilized in the treatment process. During AD process, the biodegradable of organic substance is converted to end products, specifically biogases. This process can simply described as biomethanation. It reflects complex microbial degradation of organic compound into methane and carbon dioxide by diverse group of anaerobes (Yukihiko, 2008). Adhering to the gas law, the determination of theoretical biogas product can be evaluated by the empirical formula of biomass used for stoichiometric product estimation. In this case, an organic compound ($C_nH_aO_b$) was assumed completely biodegradable and converted by the anaerobic organism (sludge yield is assumed to be zero) into biomethane. Thus, the theoretical amount of the gases produced can be calculated by stoichiometric calculation (Buswell and Neave, 1930), Eq. 1 and Van Der Waals theory, in Eq.2.

$$\mathbf{C_nH \quad {}_b \quad n - - - \frac{b}{2}\ H_2 \rightarrow \frac{n}{2} \quad - - \frac{b}{} \ CH \qquad \frac{n}{2} - - \quad \frac{b}{} \ C \ {}_2} \ldots \text{Eq. 1}$$

and;

$$\mathbf{P \qquad \frac{n}{}^{\ 2} \qquad - \ nb \qquad nRT} \ldots \text{Eq.2}$$

Where P = pressure pump
n = number of mole
V = volume of gas
a* = constant correction for the intermolecular forces
(Methane = 0.2303 Pa $m^6/mol^2$, Carbon Dioxide = 0.3658 Pa $m^6/mol^2$)
b* = constant correction for molecular size
(Methane = 0.0000431 $m^3$/mol, Carbon Dioxide = 0.0000439 $m^3$/mol)
T = temperature (Kelvin)
R = gas constant 8.31441 $m^2\ kg/s^2.K^{-1}mol^{-1}$

In previous research, the theoretical biogas yield was predicted using an ideal gas law (Fountoulakis and Manio, 2009) without considering forces and intermolecular activity inside the reactor. The implications of theoretical ideal gas law into the real gas law by Van Der Waals theory for biogas potential were studied. The degree of intermolecular attraction was represented by the constant *a* and *b* (**Table 1**) for a particular gas respectively (Reid et al., 1987).

During the experiment, synthetic wastewater (glucose) was used as organic source for generating methane biogas. This process was considered to be completely degradable.

$$\mathbf{C \ H_{12} \quad \rightarrow \quad CH \qquad C \ {}_2}$$

The degradation of carbon content was calculated based on influent COD from the beginning to the end of reactor operations. The influent COD was gradually increased from 990, 1049, 1043, and 1043 mg/L at different HRT (4, 3, 2, and 1d). Flow rate was calculated by measuring volume of inlet to the feed per unit time. For pressure, the calculations were based on Eq. 3 as the pump's horse power was 0.1 hp, Thus;

$$\mathbf{Pressure\ in\ pump\ \ psi \qquad \frac{Horsepower \quad 1 \quad 1}{flowr \quad te \quad pm}} \ldots.\text{Eq.3}$$

The converting factor in pump pressure from psi to $kg/ms^2$ was 1 psi = 6894.75729 pa or $kg/ms^2$

**Table 1**: Van Der Waals Constant for methane ($CH_4$) and carbon dioxide ($CO_2$)

| Substance | a<br>bar L $^2$/mol$^2$ | b<br>L /mol | Substance | a<br>bar L $^2$/mol$^2$ | b<br>L /mol |
|---|---|---|---|---|---|
| Acetic Acid | 17.71 | 0.1065 | Hydrogen sulfide | 4.544 | 0.043 |
| Aceton | 16.02 | 0.1124 | Isobutane | 13.22 | 0.116 |
| Acetylene | 4.516 | 0.0522 | Krypton | 5.193 | 0.01 |
| Ammonia | 4.225 | 0.0371 | Methane | 2.303 | 0.043 |
| Aniline | 29.14 | 0.1486 | **Methane** | **9.476** | **0.065** |
| Argon | 1.355 | 0.032 | Methylamine | 7.106 | 0.058 |
| Benzene | 18.82 | 0.1193 | Neon | 0.208 | 0.016 |
| Bromine | 9.75 | 0.0591 | Neopentane | 17.17 | 0.141 |
| Butane | 13.86 | 0.1164 | Nitric oxide | 1.46 | 0.028 |
| 1-Butanol | 20.94 | 0.1326 | Nitrogen | 1.37 | 0.038 |
| 2-Butatone | 19.97 | 0.1326 | Nitrogen dioxide | 5.36 | 0.044 |
| Carbon Dioxide | 3.658 | 0.0429 | Nitrogen trifluride | 3.58 | 0.054 |
| Carbon disulfide | 11.25 | 0.0726 | Nitrous oxide | 3.852 | 0.044 |
| Carbon monoxide | 1.472 | 0.0395 | Octane | 37.88 | 0.237 |
| Chlorine | 6.343 | 0.0542 | 1-Octanol | 44.71 | 0.244 |
| Chlorobenzene | 25.8 | 0.1454 | Oxygen | 1.382 | 0.031 |
| Chloroethane | 11.66 | 0.0903 | Ozone | 3.57 | 0.048 |
| Chloromethane | 7.566 | 0.0648 | Pentane | 19.09 | 0.144 |
| Cycloexane | 21.92 | 0.1411 | 1-Pentanol | 25.88 | 0.156 |
| Cyclopropane | 8.34 | 0.0747 | Phenol | 22.93 | 0.117 |
| Decane | 52.74 | 0.3043 | Propane | 9.39 | 0.09 |
| 1-Decanol | 59.51 | 0.3086 | 1-Propanol | 16.26 | 0.107 |
| Diethyl ether | 17.46 | 0.1333 | 2-Propanol | 15.82 | 0.11 |
| Dimethyl ether | 8.69 | 0.0774 | Propane | 8.442 | 0.082 |

*To convert van der Waals constants to SI units, note that 1 bar L$^2$/mol$^2$ = 0.1 Pa m$^6$/mol$^2$ and 1 L/mol = 0.001 m$^3$/mol

Meanwhile, the degradation pathway of substrate (glucose) will result in three mol of methane and three mol of carbon dioxide. The number of moles in substances can be predicted by Eq. 4.

$$\textbf{Number of moles} \quad \frac{\textbf{m} \quad \textbf{ssutilize}}{\textbf{molecul} \quad \textbf{rwei}} \; \underset{\textbf{t}}{\ldots} .\,\text{Eq. 4}$$

Molecular weight for $C_6H_{12}0_6$ was 180mg/L and mass for substance utilized was measured experimentally.

Once significant value obtained from Eq. 4, results were applied into Eq.2 to determine the volume of biogas. Subsequently, the gas potential was calculated using Eq. 5 and Eq. 6;

$$\mathbf{s\ \ iel}_{,}\ \overset{\mathbf{l}}{-}.\mathbf{C}\quad .\quad {}^{\mathbf{1}}\ \frac{\mathbf{olume}\qquad \mathbf{s}}{\mathbf{C}\qquad \mathbf{utile}\qquad \mathbf{olumere}\quad \mathbf{ctor}}\ \ \mathbf{HRT}\ \ldots.\ \mathrm{Eq.}\,5$$

and,

$$\mathbf{Percent}\qquad \mathbf{ef}\qquad \mathbf{ses}_{,}\%\quad \frac{\mathbf{s\ \ iel}}{\mathbf{Tot\quad l\qquad s\ iel}}\qquad \mathbf{1}\qquad \ldots.\ \mathrm{Eq.}\,6$$

## RESULTS AND DISCUSSION

The potential of Multi Stage Anaerobic Reactor (MS-AR) on methane gas generation was discussed in Table 2 and 3 respectively. The performance of methane generation was deliberate on the basis of COD inlet at each operational time. The organic content (COD) was found to be (990, 1049, 1043 and 1043 mg/L) for corresponding operational times of 4, 3, 2 and 1 d (HRT). The yield of methane gas was increased with the increasing of operation time. Theoretically, the highest methane yield achieved was found at 0.1202 l/g COD.d$^{-1}$ for HRT 3 day. As per observation, the maximum methane yield in real application was 0.0706 l/g COD.d$^{-1}$ at 4d HRT and constantly decreased along with HRT (0.0509, 0.0150 and 0.0082 l/g COD.d$^{-1}$ at HRT 3, 2, and 1d).

**Table 2**: Theoretic Potential of Methane Gas

| **Total Removal (%)** | **HRT (day)** | **Gas Properties** | | **Methane Yield (l/g.COD.d$^{-1}$)** | | | | | **Methane Composition (%)** | | | | | **Ratio methane: COD** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Volume (L)** | **Gas Produced (l/d)** | **R1** | **R2** | **R3** | **R4** | **Total** | **R1** | **R2** | **R3** | **R4** | **Total** | |
| 79.52 | 4 | 0.0109 | 0.00272 | 0.011 | 0.023 | 0.028 | 0.038 | 0.100 | 5.4 | 11.7 | 14.0 | 19.1 | 50.1 | 0.63 |
| 95.09 | 3 | 0.0116 | 0.00386 | 0.007 | 0.010 | 0.017 | 0.086 | 0.120 | 7.5 | 10.8 | 19.1 | 12.7 | 50.0 | 0.53 |
| 87.61 | 2 | 0.0117 | 0.00584 | 0.008 | 0.009 | 0.010 | 0.011 | 0.038 | 10.1 | 12.1 | 13.2 | 14.8 | 50.2 | 0.57 |
| 81.21 | 1 | 0.0120 | 0.01201 | 0.003 | 0.006 | 0.006 | 0.007 | 0.022 | 7.3 | 14.5 | 13.5 | 14.9 | 50.2 | 0.62 |

Results were evident from Figure 2 (a) that as the highest organic removal (95.09%) achieved at HRT 3 days, the yield of methane production become the utmost. The theoretic composition of methane gas was contradicted from its production. Result demonstrate the highest composition was (50.13% at 4 d HRT), declined to (50.02% at 3d HRT) and boost again over lower operation time (50.16 and 50.22 % at HRT 2 and 1 day).

**Table 3:** Methane gas in actual (experimental)

| Total Removal (%) | HRT (day) | Methane Composition (%) | | | | | Gas Yield (l/g.COD.d-1) | | | | | Methane Properties | | Ratio methane: COD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | R4 | Total | R1 | R2 | R3 | R4 | Total | Volume (L) | Gas Produced (l/d) | |
| 79.52 | 4 | 4.35 | 11.80 | 13.35 | 5.90 | 35.4 | 0.0087 | 0.0235 | 0.0266 | 0.0118 | 0.0706 | 0.00886 | 0.00221 | 0.45 |
| 95.09 | 3 | 3.10 | 7.55 | 7.50 | 3.05 | 21.2 | 0.0028 | 0.0069 | 0.0069 | 0.0028 | 0.0194 | 0.00478 | 0.00159 | 0.22 |
| 87.61 | 2 | 2.80 | 7.00 | 7.10 | 2.90 | 19.8 | 0.0021 | 0.0053 | 0.0054 | 0.0022 | 0.0150 | 0.00324 | 0.00016 | 0.23 |
| 81.21 | 1 | 2.60 | 6.40 | 6.60 | 2.80 | 18.4 | 0.0012 | 0.0029 | 0.0030 | 0.0013 | 0.0082 | 0.00427 | 0.00023 | 0.23 |

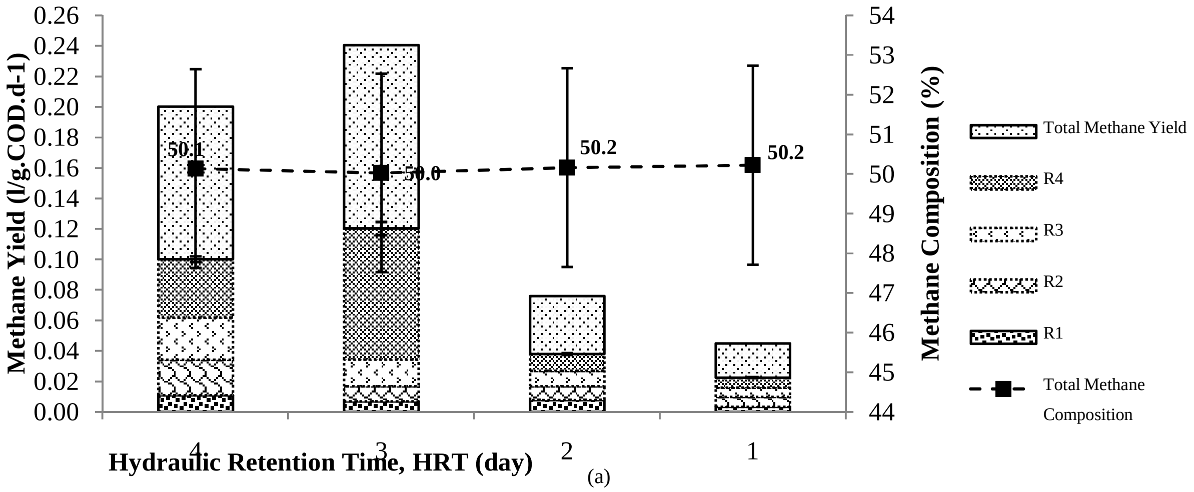
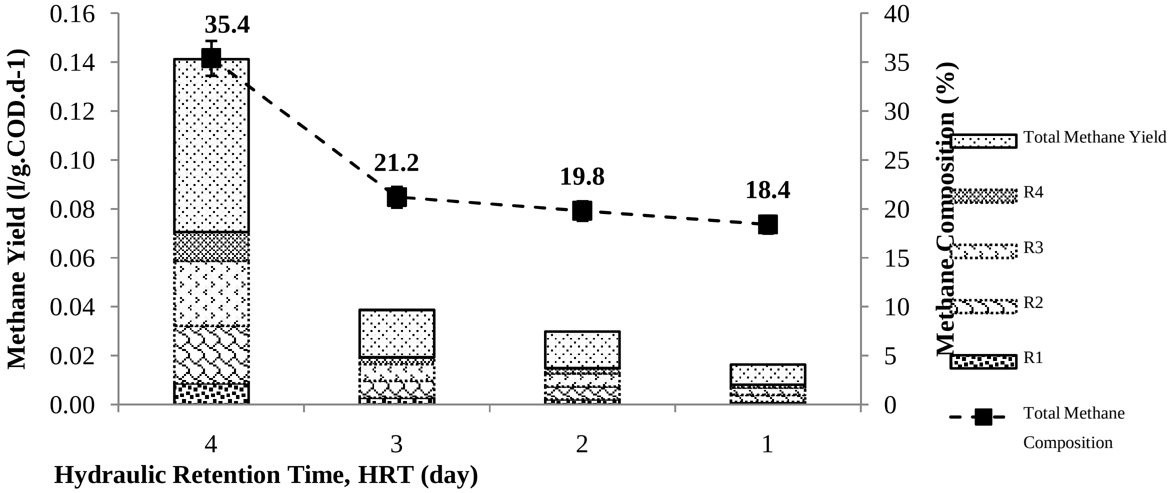


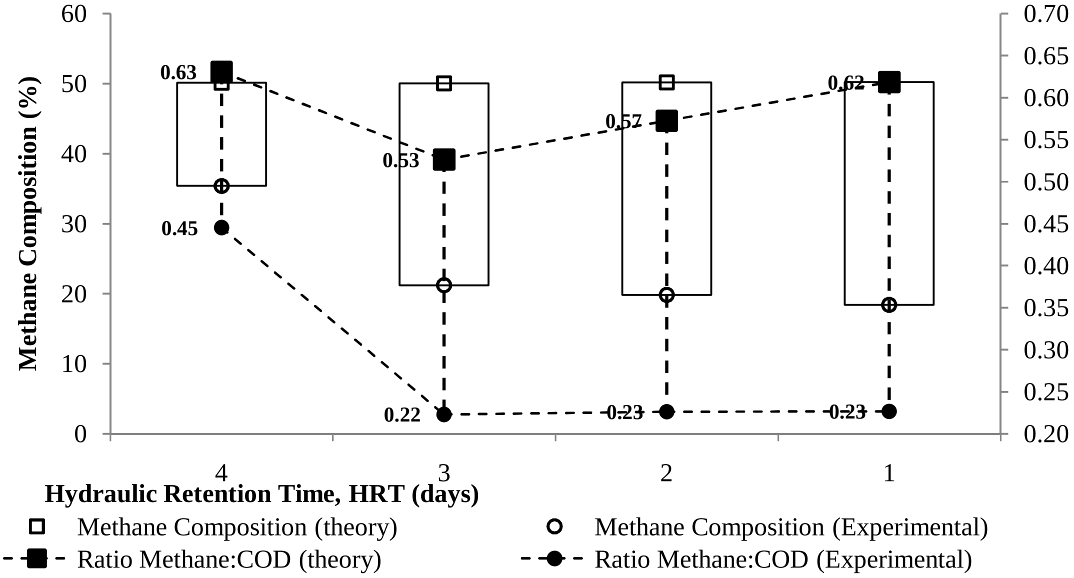
**Figure 2 (a)**: Theoretic potential in methane generation

On the other hand, the maximum experimental methane composition(Fig 2(b)) was about 35.40 % gas at HRT of 4d and decreased (21.20, 19.80 and 18.40 %) at HRT of 3, 2, 1 day which varies significantly. Result in Figure 2 (c) showed an effect of COD and HRTs ratios toward Methane yield. The ratio signified the relationship between COD degraded and converted into methane gas at each HRT. The highest ratio obtained was 0.63 and 0.45 (theory and experiment) at HRT of 4 days. Therefore, the ratio of COD and methane composition were found to be the optimization parameter in methane generation



(b)

**Figure 2 (b)**: Actual methane generation (experimental)



( c )

**Figure 2 (c)**: Relationship of methane Potential (theoretical and experimental)

Methane gas generation was optimum at long HRTs and low OLRs (MasudHossain et al., 2009). Extended time given to reactors (longer HRT), served as a sufficient platform adapt to a new environment for bacterial growth and this improved biomethanation. Unfortunately, longer HRT will weaken the main advantage of anaerobic system in term of reactor volume and operational cost (ErguderT. H. et. al, 2001). The feed (glucose) initially have high COD value and lack of alkalinity and nutrient deficiency in reactors (Franco A. et al., 2007). In higher influent COD, feed tend to acidify rapidly and the methane yield gradually decreased. The methanogensrequired optimum pH to generate methane.

Apparently, longer HRT imposed to reactors will provide sufficient time for the anaerobic microorganisms to degrade the organic substances. The outcomes will achieved high treatment efficiency associated with highest COD removal and methane generation. Comparatively, the theoretical result for defining methane potential was not similar as the real experiment. Results for those expected theory are higher than real data. On conjunction with predictable methane potential theory, the biological degradation will approximately change into around 40% of the potential methane gas (Nallathambi, 1997). The theoretical methanegases in this study are slightly higher (50. ++%) as result included actual temperature, pressure and also intermolecular activity inside reactor rather than Standard Temperature and Pressure (STP). In actual condition, the microbial activity inside reactor can affect the methane potential. The highest removal in organic content will probably turned the highest methane generation. In this experiment, highest organic degradation was achieved at 3 days of HRT (95.09% removal) corresponds well with increased methane generation Somehow, experimental results differs from the removal conception. It shows the actual for HRT at 4 days endure higher methanegeneration. Theratio for both conceptual and real dataat HRT 4 days tends to show a similar result and yet show a highest outcome. In future research, this time operation will be used as optimum value in the Multi-Stage Anaerobic Reactor (MS-AR) for optimizing other methane potential parameters.

From this experiment, the real value of methane generation was observed to be lesser than theoretical value. Result were significance with the microbial process inside reactors. Hence, by controlling microbial activity inside reactor, the methane production process can be optimized. The process depends on factors such as digestibility of the organic matter, pH condition, digestion kinetics, the retention time and the digestion temperature. Thus, optimization will lead the bacteria to have enough time for converting the substrate into methane in better ways.

## CONCLUSION

The mechanism of methane potential appears to depend on the HRTs and the COD concentration in the influent. The feed composition somehow was also initiated to inhibit the growth rate of methanogens as it turns acidify rapidly and lower the metabolic process in producing gas. Consequently, in this study, the higher methane potential adhered to the HRTs, concentration of substrate (OLR) and COD removal rate. Practically, higher carbon utilization will endeavour higher biogas yield especially methane.

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