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The Effects of Temperature-pH on Biochemical Degradation at Leachate Treatment in Anaerobic Bioreactor

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Abstract : Leachate is liquid waste resulting from physical, biological, and chemical decomposition of waste landfill. Leachate containing biodegradable and non-biodegradable substrates, in the form of complex dissolved organic and inorganic.Anaerobic treatment in principle is using anaerobic bacteria to degrade soluble organic material into biogas. Anaerobic treatment is highly sensitive to waste water, temperature and pH compositions. This study used anaerobic bioreactor with volume of 160 L, the ratio of leachate:biogaswas 70: 30. Seeding, acclimatization and leachate treatment was performed at temperature of 35°C and 45°CwithpH ambient, 7.2 and 8.0. Microorganism used came from cow rumen, with ratio of rumen: leachate was 1:3. Analysis and test of pH, biogas pressure, COD, BOD, and VFA were performed every two days. Decrease in COD and BOD was affected by temperature and pH. VFA concentration was affected by temperature dan pH. The higher the temperature-pH the higher VFA concentration obtained.
Keywords : anaerobic bioreactor, leachate, pH, temperature.

Introduction

Leachate

Leachate is liquid waste emerges due to the influx of external water into waste landfill, dissolving and flushing soluble and suspended organic and inorganic material in the garbage, including complex organic material resulting from physical, biological, and chemical decomposition process[1, 2]. Therefore, leachate is complex mixture consisting of soluble organic material and inorganic contaminants. Leachate contains: VFA, LCFA, fulvic and humic compound, ammoniac-nitrogen, phosphate, sulfate, heavy metal, xenobiotic organic (XOCs); aromatic hydrocarbons, phenols and chlorinated aliphatic, inorganic salts and microorganism [3, 4, 5, 6]; as well as *biorefractory contaminants*[7]. So leachate contains complex dissolved organic and inorganic substrate, which are biodegradable and non-biodegradable [3, 8].

The characteristic cause leachate is highly hazardous for environment with contamination potential higher than several industrial wastes[5, 6]. Characteristic and quantity of leachate affected by: characteristic and composition of waste, the type of landfill cover soil, weather, pH and humidity; as well as landfill age [1, 5, 6].

The objective of this study is to investigate the effect of temperature-pH combination against COD, BOD, VFA and biogas pressure on leachate treatment in anaerobic bioreactor.

Anaerobic Bioreactor

Anaerobic treatment in principle uses anaerobic bacteria to degrade soluble organic materials or soluble chemical oxygen demand (SCOD) into biogas [9]. Anaerobic degradation process degrades natural polymer, like polysaccharide, protein, nucleic acid, and lipid into methane and carbon dioxide, takes place in gradual and parallel reaction. Efficiency of anaerobic bioreactor treatment is sensitive to waste water, temperature and pH compositions [10, 11].

Organic material anaerobic treatment is complex and specific biochemical reaction. Soluble organic material biodegradation is through reaction phase; hydrolysis, acidogenesis, acetogenesis and methanogens occurs simultaneously, both serial and parallel [9, 12, 13, 14, 15, 16]. Bacteria that play role in the four phase work specifically and are interdependent [17].The final phase of organic substrate specific biochemical reaction produces CO_2andCH_4, which constitute the major product of anaerobic process [18, 19, 20].

Hydrolysis is liquefaction of organic materials using extra-cellular enzyme produced by hydrolytic bacteria [12, 13, 15, 16]. Hydrolysis from organic materials polymer like; protein, carbohydrate and lipid will create amino acid, simple sugar, fatty acid [14, 21, 22], alcohol [23, 24] and lipid into long chained fatty acid (LCFA) [14].

Acidogenesis is a reorganizing phase of organic materials resulting from hydrolysis into amino acid, simple sugar and volatile fatty acid (VFA), involving format acid, acetate, propionate, butyrate, lactate, succinate, ethanol, and CO_2, H_2, NH_3, H_2S gases by acid-forming bacteria[14, 20, 25].Acetogenesis is construction phase of acetate, carbon dioxide and hydrogen compounds [20, 26]. Intermediate compound anaerobic oxidation (intermediate acid product) like VFA (especially propionate and butyrate acid) into acetate acid and hydrogen by asetogenik bacteria is called as asetogenesis [14, 26, 27]. Glucose and ethanol are converted into acetate in asetogenesis phase as well [9].

Final phase in anaerobic biodegradation is methanogens. Methanogens is anaerobic archaea which can be divided into two groups: 1. Hydrogenophilic or hydrogenotrophic species and 2. Acetoclastic or acetotropic methanogens, which produce methane by acetate decarboxylation [28]. About 70% of the methane is produced during anaerobic biodegradation through acetoclastic phase [14, 29, 30, 31]. Most methanogens bacteria are mesophilic with temperature ranging from 28-42°C and on thermophilic temperature ranging from 55-72°C [20].

Experimental

The study was pilot scale experiment with batch system. The leachate used was from Sambutan Landfill, Samarinda, East Kalimantan, Indonesia. This study used anaerobic bioreactor with volume of 160 L. In which the ratio of leachate:biogas volume was 70:30. After leachate quality characterization and analysis were performed and thenanaerobic bioreactor establishment with design according to the necessity of the study was carried out. Furthermore, leaking test and calibration from anaerobic bioreactor system were performed.

Seeding and acclimatization phases were carried out, for 10 days respectively; while leachate treatment was performed for 21 days. Seeding, acclimatization and leachate treatment were performed in anaerobic bioreactor at 35°C and 45°Cwith ambient pH, 7.2 and 8.0. Microorganism used coming from cow rumen fluid as inoculums and leachate with the ratio of 1:3 and were filtered to obtain the extract. Analysis and parameter test were performed every two days. The parameters tested were pH, biogas pressure, COD, BOD and VFA. Leachate treatment process was terminated if COD($COD_{removal}$) decrease percentage had reached 60-80 %.

Results and Discussion

Results

pH

Seeding and acclimatization conditions at 35°C, ambient pH. In seeding, pH increases from 6.8-7.3. Meanwhile in acclimatization, pH is up and down between 7.2-7.5. In seeding at 45°C, ambient pH, pH is up and down between 7.3-7.5. Meanwhile in acclimatization, pH is up and down between 7.4-7.6 and biogas pressure increase from 0-25 mm H_2O. At 35°C, pH is 7.2, pH in seeding is up and down from 7.8-6.4 to 6.4-6.9. Meanwhile in acclimatization, pH is constant at 7.2. At 45°C, pH is 8.0, pH in seeding decreases from 7.7-6.3

and increases from 6.3-7.4. Meanwhile in acclimatization, pH increases from 7.8-8.0 and then it is constant at 8.0.

In anaerobic treatment at 35°C, ambient pH is obtained; pH tends to decrease at 7.9-7.3. Anaerobic treatment at 45°C, ambient pH is obtained; pH tends to decrease at 7.3-7.9. At 35°C, pH is 7.2, pH in anaerobic treatment is constant at 7.2. And at 45°C, pH is 8.0, pH is constant at 8.0.

Biogas Pressure

Seeding and acclimatization conditions at 35°C, biogas pressure begins to increase at day 4, with up and down fluctuation between 1 up to 4 mm H_2O. Meanwhile in acclimatization, biogas pressure increase from 7-22 mm H_2O. In seeding at 45°C, ambient pH, pH is up and down from 7.3-7.5 and biogas pressure begins to increase at day 2, with up and down fluctuation between 1 up to 4 mm H_2O. Meanwhile in acclimatization, pH is up and down between 7.4-7.6 and biogas pressure increase from 0-25 mm H_2O. At 35°C, pH is 7.2, biogas pressure begins to increase at day 4, with up and down fluctuation between 1 up to 4 mm H_2O.Meanwhile in acclimatization, biogas pressure increase from 6-23 mm H_2O. And at 45°C, pH is 8.0; biogas pressure begins to increase at day 7, with up and down fluctuation between 1 up to 3 mm H_2O.Meanwhile in acclimatization, biogas pressure increases from 4-22 mm H_2O.

And anaerobic treatment at 35°C, ambient pH, and biogas pressure increase at day 21 up to day 27, which is from 25 mm H_2O up to 199 mm H_2O, then decrease at day 28 up to day 41, from 163 mm H_2O up to 8 mm H_2O. Anaerobic treatment at 45°C, ambient pH is obtained, biogas pressure increases at day 21 up to day 26, which is 21 mm H_2O up to 152 mm H_2O, then decrease at day 27 up to day 41, from 146 mm H_2O up to 5 mm H_2O. At 35°C, pH is 7.2, biogas pressure increase at day 21 up to day 27, which is from 27 mm H_2O up to 201 mm H_2O, then decrease at day 28 up to day 41, from 185 mm H_2O up to 12 mm H_2O. Meanwhile at 45°C, pH is 8.0, biogas pressure increase at day 21 up to day 27, which is from 25 mm H_2O up to 155 mm H_2O, then decrease at day 28 up to day 41, from 140 mm H_2O up to 9 mm H_2O.

COD, BOD and VFA

Anaerobic treatment at 35°C, ambient pH is obtained; BOD experiences decrease starting from3850.32-693.50 mg/L, COD experiences decrease starting from6520-1327.45 mg/L. Meanwhile VFA concentration is up and down, at day 21 which is 166.5 mg/L, then going up with the highest concentration at day 25 by1698.97 mg/L, and next going up and down up to day 41 becoming 331.85 mg/L.

And aerobic treatment at 45°C, ambient pH is obtained; BOD experiences decrease starting from4104.18-902.88 mg/L, COD experiences decrease starting from7445.11-1563.45 mg/L. Meanwhile VFA concentration is up and down, at day 21 which is 166.5 mg/L, then going up with the highest concentration atday27 by1232.1 mg/L, and next going up and down up to day 41 becoming80.78 mg/L.

In anaerobic treatment at 35°C, pH is 7.2; BOD experiences decrease from3925.50-689.50 mg/L, COD experiences decrease from6155.9-1124.5 mg/L.Meanwhile VFA concentration is up and down, at day 21 which is 234.8 mg/L, then going up with the highest concentration at day 27 by1678.5 mg/L, and next going up and down up to day 41 becoming338.5 mg/L.

Meanwhile anaerobic treatment at 45°C, pH is 8.0; BOD experiences decrease from3904.20-702.68 mg/L, COD experiences decrease from6531.1-1306.35 mg/L. Meanwhile VFA concentration is up and down, at day 21 which is 266.5 mg/L, then going up with the highest concentration atday29 by1566.5 mg/Land next going up and down up to day 41 becoming 168.7 mg/L.

Discussion

pH

pH is one of major parameters in anaerobic treatment because methanogens bacteria are highly sensitive to pH change. Methane-forming bacteria live well in neutral to slightly base condition. pH in bioreactor directly depends on retention time [32].In Figure 1, it is seen that pH in both phases, seeding acclimatization and anaerobic treatment phases, there is difference in pH range. In which pH of seeding-acclimatization phase is at slightly

acid pH while pH in anaerobic treatment is at slightly base pH. This occurs because this biodegradation phase involves hydrolysis bacteria that produce extracellular enzyme [14, 21, 22], and cellulose [9].

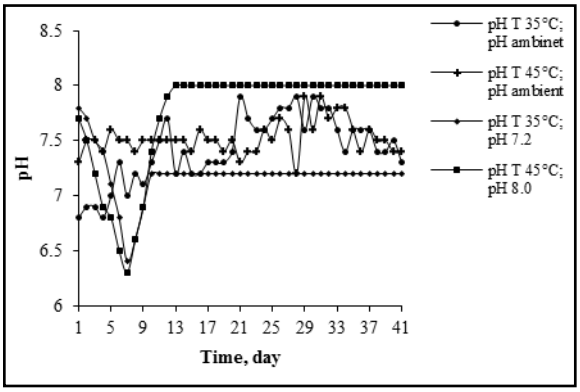

Figure1. pH in Temerature-pH variation

Biogas Pressure

Overall, biogas pressure in seeding process (day 1 up to day 10), although increase in pressure occurs but it does not experience significant increase. While in acclimatization process phase (day 11 up to day 20), biogas pressure experiences significant increase by >10%. Biogas pressure in seeding phase, at day 1 up to day 10, although pressure increase occurs, but it does not experience significant increase, which is >10%.

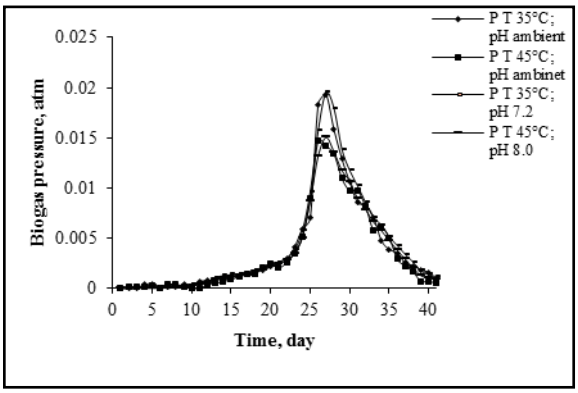

Figure2. Biogas pressure in variation of Temerature-pH

In figure 2, acclimatization in pH, Q and T variations, increase in biogas pressure, at day 10 up to day 20, seems to be sloping. In which increase in biogas pressure is >10% in variation of pH, leachate recirculation rate; Q and temperature of leachate; T, occurs at day 12–15. However until the final phase of acclimatization at day 20, increase in biogas pressure remains sloping and not exponential. And biogas pressure is at T 35ºC, pH 7.2 > T 35ºC > T 45ºC > T 45ºC, pH 8.0.

In variation of leachate temperature, it is seen that ambient T biogas pressure is lower than T 45°C and T 35°C, but biogas pressure at T 45°C is lower than T 35°C. This occurs because T 35°C is optimum temperature form esophilic bacteria growth. Meanwhile T 45ºC is minimum temperature for thermophilic bacteria growth. This is according to the statement that, most methanogen bacteria are mesophilic ranging from 28-42°C and thermophilic ranging from 55-72°C. Optimum temperature form esophilic bacteria growth is 35°C[20]. Optimum temperature required in anaerobic treatment by microorganism is ranging from 25-37°C [33].

COD and BOD

In general, all kinds of biomass can be said to be substrate, as long as they contain carbohydrate, protein, fat, cellulose, and hemicellulose as the main component [15]. And concentration of substrate is expressed as COD [3, 6]. The difference of soluble substrate concentration (organic and inorganic) in leachate is driving force from mass transfer process in leachate treatment. The soluble organic and inorganic material substrate

biodegradation will affect character, parameter and characteristic of leachate in bioreactor during anaerobic treatment process[41].

pH, COD, BOD, and VFA is chemical parameter, while biogas pressure is physical parameter. In which pH is control parameter indicating leachate acidity level in bioreactor. COD and BOD provide description of soluble substrate concentration in leachate. VFA indicates organic acids production in hydrolysis, acidogenesis and acetogenesis phases. Meanwhile biogas pressure indicates biogas production which is based on biogas molar concentration in certain unit of volume. Anaerobic treatment process is quite effective for leachate with high ratio of BOD: COD produced at the initial phase of landfill[34]. Leachate treatment depends on the characteristic. Meanwhile leachate characteristic highly depends on how the leachate is formed and accumulated [35].

Successful indicator of microorganism in biodegradation is the decrease of substrate concentration in leachate. Substrate (COD and BOD) concentration in acclimatization, day 11 up to day 21 decreases, as seen in Figure 3 and Figure 4. Minimal decrease of COD is 40.42% and maximum is 75.07%. Decrease of COD and BOD in acclimatization phase, is caused by soluble substrate concentration used by microorganism to grow, develop and adapt in leachate. So in this phase, substrate of dissoluble complex organic molecules is hydrolyzed into simple molecule which is soluble in leachate. This indicates that bacteria growth runs well. It is also seen in value of BOD/COD ratio biodegradability which ranges between 0.43–0.70.

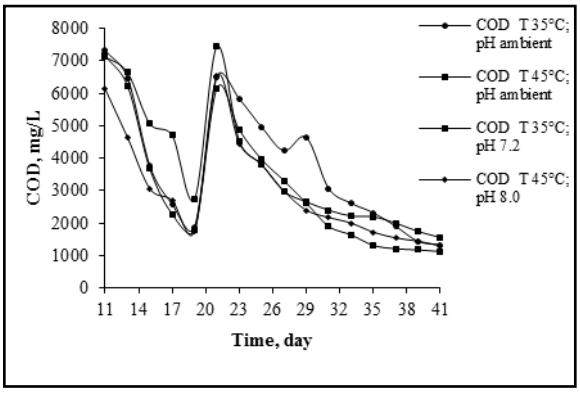

Figure 3. CODin variation of Temperature-pH

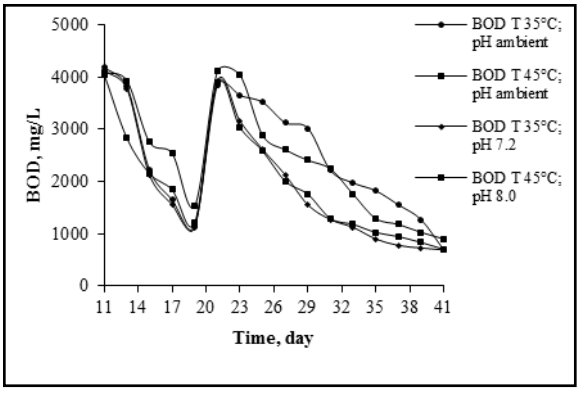

Figure 4. BODin variation of Temperature-pH

The percentage of decrease of COD at 35°C, at 45°C, at 35°C and pH 7.2 and temperature of 45°C and pH 8.0 are71.84%, 79.64%, 79.00%, 81.73% and 80.00%, respectively. So COD removal isat T 35°C; pH 7.2 > T 35°C> T 45°C; pH 8.0 > T 45°C > T ambient, as seen in Figure 3. Decrease of BOD at ambient T, T 35°C, T 45°C, T 35°C, pH 7.2; and T 45°C, pH 8.0 are68.91%, 81.99%, 78.00%, 82.44% and 82.00%, respectively. So, BOD removal is at T 35°C; pH 7.2 > T 45°C, pH 8.0> T 35°C > T 45°C, as seen in Figure 4.

In anaerobic treatment phase, at day ke-21 up to ke-41,overall, COD removal ranges between 71.84 % up to 85.31 %. Meanwhile BOD removal is obtained ranging between 68.91 up to 84.15 %. With the average of

COD and BOD removal by 80.74 % and 80.44%, respectively. The highest decrease of COD and BOD occurs at day 21–29.

In this study, it is also seen that the higher the treatment temperature condition, the bigger the organic material. This is according to the statement that in thermophilic condition, organic material biodegradation, biomass, and biogas production are high [36, 37].

VFA

VFA concentration is determined as parameter to find out how far acidogenesis and acetogenesis phases take place. In which VFA concentration is one of good parameters to monitor in determining anaerobic bioreactor stability. VFA is analyzed as acetate acid, because it is dominant volatile organic acid that contributes ±85% of total VFA [38, 39, 40].VFA is *intermediate product*[16], that will be converted into acetate acid that will be converted into acetate acid in acetogenesis phase[38, 39, 40], $CO_2$and H_2 [26, 20].

VFA concentration is obtained by80.78–1698.97 mg/L.VFA concentration is affected by temperature. The higher the temperature the higher VFA concentration obtained, but remains consistent at optimum temperature of microorganism plays the role in it, in which VFA is at T 35°C > T 45°C > T ambient. As seen in Figure 5.

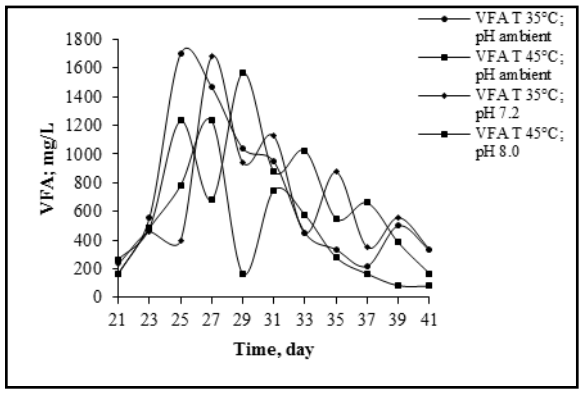

Figure 5. VFA in temperature-pH variation

In the initial treatment, increase in VFA is followed by decrease in substrate concentration. As time goes by, the bigger the decrease of COD removal the lower VFA concentration obtained. The inevitable fact is exponential increase of VFA then decrease again indicates that VFA depends on the limit of substrate concentration in leachate [41]. It is also seen that the highest VFA concentration in all operation condition is at day 25–31, as seen in Figure 6 and Figure 7.

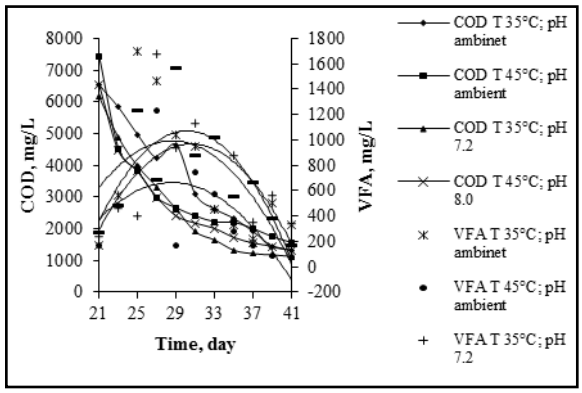

Figure 6. COD vs VFA in variation of Temperature-pH

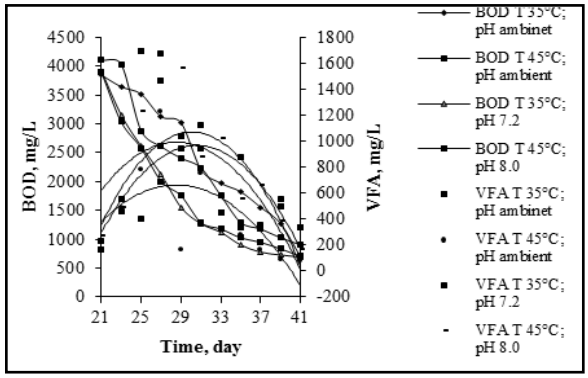

Figure 7. BOD vs VFA in variation of Temperature-pH

The growth of hydrolytic bacteria is faster than methanogen bacteria, this frequently cause the occurrence of VFA accumulation [42]. VFA accumulation in anaerobic bioreactor reflects imbalance between acid produced and acid consumed by bacteria. If bioreactor is overload and VFA concentration is high, higher than methane-producing bacteria (slow-growers) can consume, then biogas production will increase. This increase potentially increases foaming in anaerobic bioreactor [43]. VFA accumulation may cause progressive pH decrease from 7 into 5 that may disrupt decomposition process especially for methane-producing bacteria which is prone to pH.

In this study, it is obtained that the higher COD biodegraded, the higher VFA concentration. The less substrate concentration the less VFA concentration is. This is according to the statement that the higher substrate concentration reduced the higher biodegraded soluble organic material into organic acids. This organic acid is then converted into methane gas. Therefore, the higher the decrease of COD then the higher the rate of methane gas production. It is only that one thing to be considered in all conditions of leachate treatment operation in anaerobic bioreactor is before and after of the highest VFA concentration peak. In which before the highest VFA, soluble organic substrate concentration is still high. Meanwhile after the highest peak of VFA, soluble organic substrate concentration decreases. The comparison of relevant studies have been performed which is shown in Table 1.

Table1. The comparison of relevant studies that have been conducted

Bioreactor	**Wastewater**	**HRT (day)**	**Temp. (ºC)**	**$COD_{Inf.}$ (mg/L)**	**$COD_{Rem.}$ (%)**	**Biogas Production (Nm^3/kg $COD_{rem.}$)**	**Reference**
UASB	RPH	24	30	4.175	90	0.34	Manjunath *et al*., 2000.
Two-stage Anaerobic Filter	Brewery wastewater	0.5-6 d	35	1.500-2.500 (OLR 0.5-20 g SCOD/L.d	98.2	0.04 mg VSS/mg COD	Cho and Young, 2001
UASB	RPH	2-7	30-33	6.037	75	0.3	Torkian *et al*., 2003.
AFBR	Leachate	1	35	10.000-50.000 (OLR 2.5-37 kg COD/m^3.d)	80-90	0.50-0.52 L/g COD_{rem}	Gulsen and Turan, 2004.
UASB	Leachate	5.1 – 6.6	37 (pH 7.1-8.5)	15.700 (OLR 1-2.4 g COD/l d)	66-90	0.053 g VSS/g $COD_{removed}$	Fang *et al*., 2005
Anaerobic	Food Waste	10-	40-55	9.800	83	119-223	Kim *et al.*,

Bioreactor	**Wastewater**	**HRT (day)**	**Temp. (ºC)**	**$COD_{Inf.}$ (mg/L)**	**$COD_{Rem.}$ (%)**	**Biogas Production (Nm^3/kg $COD_{rem.}$)**	**Reference**
Digestion		12				L CH_4/kg $sCOD_{degraded}$	2006.
UASB	Domestic	5-10	33	522	80	0.40	Sunny *et al.*, 2010.
Anaerobic	Leachate	40	35	24.840	94	-	Safari *et al.*, 2011.
Two-Stage Anaerobic	Paper Industry	1-20	55	31.700	52.21	4.07-15.82 L/dy	Soetopo *et al.*, 2011.
Anaerobic Bioreactor	POME	14-6,5	35-45	15.000 – 66.000	70-65	0.35 m^3 CH_4/kgCOD	Chotwattanasak and Puetpaiboon, 2011.
Anaerobic Digestion (Batch)	Cow Dung	10	53	2.200	48,5	0.15 L/kgVS	Abubakar and Ismail, 2012.
ABR	Algae-Laden Water	10	30	3.000-7.000	80	99-293 mL/(L.d)	Yu *et al.*, 2014.
Anaerobic Bioreactor (Vr = 160 L)	Leachate	1.0 d	35-45	6155.9-7445.11	79.00-81.73	0.057-2.372 mol CH_4/g COD	This research

Conclusion

As for the conclusion of this study, decrease in COD is affected by temperature and pH. Decrease in COD at T 35°C, T 45°C, T 35°C; pH 7.2 and T 45°C; pH 8.0 are79.64%, 79.00%, 81.73% and 80.00%, respectively. The decrease in BOD is affected by temperature and pH. Decrease in BOD at T 35°C, T 45°C, T 35°C, pH 7.2; and T 45°C, pH 8.0 are81.99%, 78.00%, 82.44% and 82.00%, respectively. VFA concentration is affected by temperature and pH. The higher the temperature the higher the VFA concentration obtained. VFA concentration obtained is 80.78-1698.97 mg/L. And biogas production ranges between 0.057-2.372 mol CH_4/g COD.

Acknowledgement

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Abbreviation:

UASB : Upflow Anaerobic Sludge Blanket
RPH : Slaughterhouse
MABR : Modified Anaerobic Baffled Reactor
POME : Palm Oil Mill Effluent
MAS : Membrane Anaerobic System
ABR : Anaerobic Baffled Reactor
AFBR : Anaerobic Fluidized Bed Reactor

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