

THE PRODUCTION OF BIOHYDROGEN AND BIOMETHANE FROM CASSAVA WASTEWATER UNDER MESOPHILIC ANAEROBIC FERMENTATION BY USING UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS (UASB)

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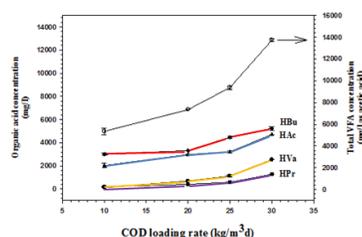
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Graphical abstract



Abstract

The main objective was to separately generate biohydrogen (H₂) and biomethane (CH₄) with the cassava wastewater via the upflow anaerobic sludge blanket reactors (UASB) under the mesophilic temperature (37 °C). For the first part, the production of H₂, the controlled system was managed on the fixed temperature (37 °C) and pH (5.5) included the varied organic concentration in term of chemical oxygen demand (COD) loading rates. As the proper COD loading rate of 25 kg/m³ d, H₂ and carbon dioxide (CO₂) were mainly generated gases which provided the highest specific H₂ production rate of 0.39 l H₂/l d and the highest H₂ yield of 39.83 l H₂/kg COD removed. For the second part, the effluent liquid that generated from the stage of H₂ production on COD loading rate of 25 kg/m³ d was fed to the UASB with the fixed temperature (37 °C) and no pH control. The highest specific CH₄ production rate of 0.91 l CH₄/l d and the highest CH₄ yield of 115.23 l CH₄/kg COD removed were shown on the proper COD loading rate of 8 kg/m³ d.

Keywords: Biohydrogen production, biomethane production, cassava wastewater, upflow anaerobic sludge blanket reactor (UASB)

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1.0 INTRODUCTION

Anaerobic digestion is a biological degradation process to convert the organic compounds like the carbohydrates, proteins, and fats into CH₄ and CO₂ that known as biogas under the absence of dissolved oxygen in which the biological wastewater treatment as an anaerobic dark fermentation process. It is an attractive process for the biogas production from the high strength wastewater and normal waste because it can reduce the post-treatment cost and the

generated biogas can be used as an alternative fuel [1]. Cassava wastewater, one of the industrial wastewaters is considered to be feasible economically for biogas production. In Thailand, the several cassava factories have already employed the process of the anaerobic digestion to generate the biogas economically.

The anaerobic digestion process comprises the four stages of the hydrolysis stage, acidogenesis stage, acetogenesis stage, and methanogenesis stage. Firstly, the complex organic compounds are

hydrolyzed by the external enzyme to form the water soluble organic compounds. For the acidogenesis stage, the organic compounds are changed into the organic acids, H_2 , CO_2 , and alcohols. For the third stage of acetogenesis, the generated organic acids are further broken down to the acetic acid (CH_3COOH), H_2 , and CO_2 . Both CH_3COOH and H_2 are changed into CH_4 and CO_2 in the final stage of methanogenesis [2]. To gain more profit and higher energy conversion efficiency, the generated H_2 in both stages of acidogenesis and acetogenesis has to be separated immediately once it is generated. This can be done via the controlled system of the two-stage anaerobic digestion. A first bioreactor is operated to generate H_2 in which is taken out from the controlled system while the effluent liquid contained mostly the organic acids are directly pumped to a second bioreactor to change the generated organic acids into CH_4 and CO_2 [3].

Cassava wastewater has been investigated for H_2 and CH_4 production that reported as our previous works [4-6]. Intanoo *et al.*, [4] studied H_2 and CH_4 production with the cassava wastewater via the two-stage UASB under the thermophilic temperature (55 °C). The highest H_2 yield of 80.25 ml H_2 /g COD removed was shown on COD loading rate of 90 kg/ m^3 d. Whereas, the highest CH_4 yield of 183.31 ml CH_4 /g COD removed was shown on COD loading rate of 90 kg/ m^3 d with the same proper COD loading rate. Wangmor *et al.*, [5] also studied H_2 and CH_4 production with the cassava wastewater and added cassava residue under the thermophilic two-stage UASB process. The highest H_2 and CH_4 yield of 15 ml H_2 /g COD removed and 259 ml CH_4 /g COD removed, respectively were found on the proper cassava residue concentration of 1,200 mg/l. Limwattanalert *et al.*, [6] indicated the production of H_2 from the ethanol wastewater via the upflow anaerobic sludge blanket (UASB) reactor under the mesophilic operation (37 °C). As the proper COD loading rate of 30 kg/ m^3 d, the controlled system provided the highest performance of the H_2 production in term of the highest H_2 yield (114.5 ml H_2 /g COD removed) and the highest COD removal efficiency.

In this study, the cassava wastewater was fermented via the upflow anaerobic sludge blanket (UASB) on the varied COD loading rates (10-30 kg COD/ m^3 d) to generate H_2 under a controlled pH (5.5) and the mesophilic temperature (37 °C). The effluent liquid from the first H_2 production stage fermented further separately the reactor to generate CH_4 with no pH control.

2.0 EXPERIMENTAL

2.1 The Seed Sludge

The bacterial sludge was collected to be used as the seed sludge on start-up the bioreactors that supported by Sahamitr Tapioca Chonburi Ltd., Part,

Thailand. The total suspended solids (TSS) and pH of it were 54 g/L and 4.5, respectively. Before being introduced into the reactor, the sludge was screened by sieving to remove the large particulates and inorganic materials. The boiling of the screened sludge was performed for 15 min at 95 °C in order to remove the H_2 consumers (or methane-producing bacteria) and it was added to the bioreactor in the first H_2 production stage [4, 6]. For the second CH_4 production stage, the screened sludge was not boiled.

The cassava wastewater Sahamitr Tapioca Chonburi Ltd., Part, Thailand supported the cassava wastewater that it was screened by sieving to remove the large particulates and inorganic materials. The cassava wastewater on the H_2 production stage had the COD values of 19,000-22,000 mg/l included the ratio of COD:Nitrogen:Phosphorous (100:0.7:2.3) that indicated the cassava wastewater had the insufficient nitrogen on the bacterial growth and the anaerobic degradation (the theoretical ratio of COD:N:P of 100:1:0.4 on the anaerobic decomposition as biogas production [4-5]). Therefore, NH_4HCO_3 was only added to the cassava wastewater. The cassava wastewater with nitrogen supplement was fed to both bioreactors for the H_2 production rate UASB operation.

For the first H_2 production stage, two identical reactor units of UASB were built with the borosilicate glass of 24 L working volume. The fixed temperature (37 °C) inside the bioreactor was controlled by circulating water through the system of circulating/heating bath as the water jacket in order to prevent the inhibition of biological activity of anaerobic bacteria from the operating temperature variation. The cassava wastewater was continuously fed into the bottom of reactor on the varied flow rate via using the peristaltic pump resulted in the varied COD loading rates (10, 20, 25, and 30 kg/ m^3 d). The effluent liquid was maintained at pH (5.5) by using a pH controller and the effluent liquid was fixed at the recycle ratio of 1:1. At any given COD loading rate, the UASB system was operated to reach steady state before taking experimental data. The steady state was justified when both of the gas production rate and the effluent COD were invariant with time. The experimental data after the steady state were averaged and the average data were used to access the process performance.

For the second CH_4 production stage, the effluent from hydrogen UASB unit operated at an proper COD loading rate was used as a feed to both methane UASB units at different COD loading rate under 37 °C without pH control. The same two UASB units were with the seed sludge without heat treatment. At any given COD loading rate (2, 4, 6, 8, and 10 kg/ m^3 d), the studied UASB system was operated to reach steady state before being taken effluent and generated gas samples for analysis and measurement. Steady state conditions were attained

when both effluent COD and gas production rate were invariant with time.

2.2 Measurements and Analytical Methods

The organic contents in the cassava wastewater and the effluent samples of both UASB units were quantified by using the chemical oxygen demand method (COD). The microbial concentration in the UASB bioreactor was measured by taking the whole sludge in the bioreactor at the end of operation for each COD loading rate. The sludge sample was filtered, and the filtered solids were burnt at 550 °C to obtain MLVSS (mixed liquor volatile suspended solids) to represent the microbial growth in the controlled system. The analytical methods of COD and effluent VSS were followed the standard method [7]. The gas production rate was measured by using the water replacement method. The gas composition was analyzed by a gas chromatograph (AutoSystem GC, Perkin-Elmer) equipped with a thermal conductivity detector (TCD) and a packed column (stainless-steel 10'x 1/8' x .085" HayeSep D 100/120 mesh, Altech). Injector, column, and detector temperatures were kept at 60, 35, and 150 °C, respectively. Argon was used as the carrier gas. The total volatile fatty acids (VFA) of the effluent samples were measured by the steam distillation and titration method [7]. The samples obtained from the steam distillation were taken for the determination of organic acid compositions by using a gas chromatograph (PR2100, Perichrom) equipped with a flame ionization detector (FID) and a capillary column (50 m x 0.32 ID, 0.25 µm film thickness DB-WAXetr, J & W scientific) in the splitless mode with helium as a carrier gas, hydrogen as a combustion gas, and air zero as a combustion-supporting gas. The column temperature program was started at 60 °C, heated to 125 °C at a ramping rate of 10 °C/min, held for 2 min, then heated to 180 °C at a ramping rate of 15 °C/min, and held for 15 min. The temperatures of both injector and conductor were kept constant 250 °C.

3.0 RESULTS AND DISCUSSION

3.1 Biohydrogen Production Results

The effect of COD loading rate on COD removal efficiency and gas production rate of the hydrogen UASB units is shown in Figure 1a. The COD removal efficiency increased with increasing COD loading rate from 10 to 25 kg/m³d and then decreased with further increasing COD loading rate from 25 to 30 kg/m³d. The highest COD removal was 43.8 % at a COD loading rate of 25 kg/m³d. The increase in COD loading rate resulted in an increase in organic compounds available for microbial degradation, leading to increasing COD removal. However, at a very high COD loading rate greater than 25 kg/m³d, the concentration of generated volatile fatty acids exceeded their inhibitory level to the hydrogen-

producing bacteria, causing both reductions in both COD removal and the microbial concentration which will be further discussed later. The gas production rate rapidly increased with increasing COD loading rate from 10 kg/m³d to 25 kg/m³d. However, the gas production rate slightly increased when the COD loading rate increased from 25 to 30 kg/m³d. The results can be explained in that an increase in COD loading rate provided higher substrates available for microbes to generate higher quantities of gaseous products.

Figure 1b shows the composition of the generated gas and biohydrogen production rate at different COD loading rates. Both of the biohydrogen production rate and biohydrogen content in the generated gas had similar trends to that of the gas production rate from the hydrogen UASB unit whereas the CO₂ content had an opposite trend. The same explanation from the effect of COD loading rate on COD removal and gas production rate can be used for the results of biohydrogen production rate and the composition the generated gas. Interestingly, the biomethane content decreased from 10 % to zero when the COD loading rate increased from 10 to 25 kg/m³d. The results of the reduction of methanogenic activity with increasing COD loading rate can be explained by the fact that the growth of methanogens require both organic acids and hydrogen generated from both stages of acidogenesis and acetogenesis and the inhibitory level by the accumulated organic acids (400 mg/l as acetic acid) to methanogens is much lower than that (10,000 mg/l as acetic acid) to hydrogen producing bacteria [4].

Specific biohydrogen production rate (SHPR) is defined as the biohydrogen production rate per unit weight of the microbial cells in the controlled system or per volume of the controlled system. Figure 1c shows that the specific biohydrogen production rate increases from 1.96 l H₂/kg MLVSS d (0.03 l H₂/l d) at a COD loading rate of 10 kg/m³d to 10.92 l H₂/kg MLVSS d (0.39 l H₂/l d) at a COD loading rate of 25 kg/m³d. After that, it slightly declined to 8.67 l H₂/kg MLVSS d (0.27 l H₂/l d) with increasing COD loading rate from 25 to 30 kg/m³d. The profile of SHPR showed a similar trend to those of COD removal, biohydrogen production rate and biohydrogen content in the generated gas. The results will be further discussed later.

Figure 1d shows that the yields of biohydrogen increased from 15.82 l H₂/kg COD removed (or 3.15 l H₂/kg COD applied) at a COD loading rate of 10 kg/m³d to 39.83 l H₂/kg COD removed (or 15.84 l H₂/kg COD applied) at a COD loading rate of 25 kg/m³d which was the highest yield of biohydrogen production in this study. However, at a COD loading rate of 30 kg/m³d, the yield of biohydrogen adversely decreased to 36.38 l H₂/kg COD removed (or 8.91 l H₂/kg COD applied).

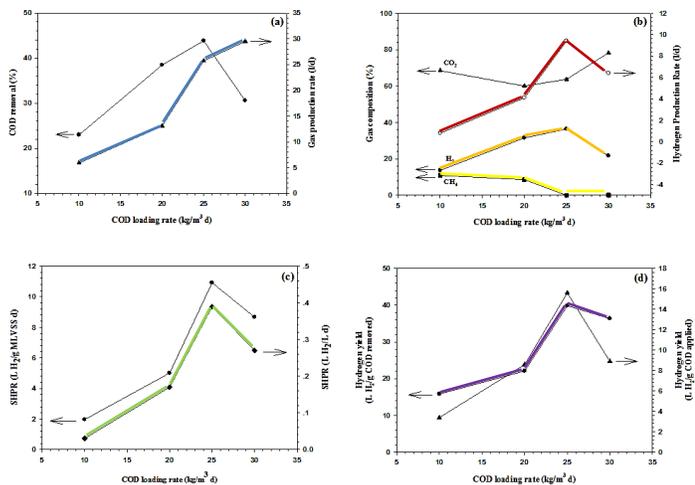


Figure 1 Effects of COD loading rate based on biohydrogen production process on (a) COD removal and gas production rate, (b) gas composition and biohydrogen production rate, (c) Specific biohydrogen production rates and (d) biohydrogen yield at pH 5.5 and 37 °C in hydrogen UASB unit

Figure 2 shows the total VFA (mg/l as acetic acid) and its composition in the biohydrogen reactors as a function of COD loading rate. The total VFA concentration increased at any given COD loading rate and reached a highest value of 13,740 mg/l as acetic acid at the highest COD loading rate of 30 kg/m³d. However, at this the highest COD loading rate of 30 kg/m³d, biohydrogen production performance decreased due to the toxicity of VFA accumulation [4]. It can be concluded that a highest VFA tolerance level to the mesophiles for biohydrogen production from this cassava wastewater may be around 650 mg/l as acetic acid. The major components of VFA in the liquid effluent are acetic acid (HAc), propionic acid (HPr), butyric acid (HBu), and valeric acid (HVa). Under proper COD loading rate of 25 kg/m³d, the concentrations of all organic acid were 48 % HBu, 34 % HAc, 12 % HVa, and 6 % HPr. Moreover, the highest HBu-to-HAc ratio of 1.41:1 was obtained at this proper condition causing the highest biohydrogen production. In general, the production of HAc and HBu is favorable when biohydrogen production is dominant, according to Equations 1 and 2 [8].



In addition, the production of HPr was also important in affecting the consumption of hydrogen initially generated, according to Equations 3 [8].

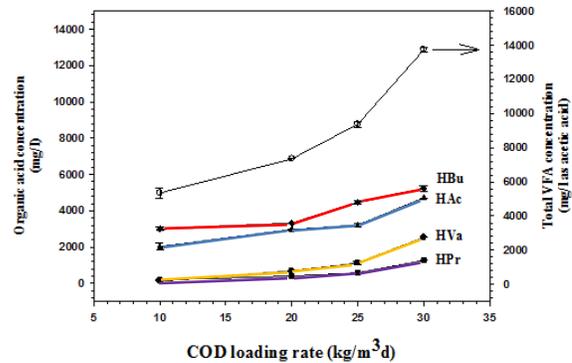


Figure 2 Total VFA, and VFA composition versus COD loading rate based on biohydrogen production process at 37°C and pH 5.5 in hydrogen UASB unit

3.2 Biomethane Production Results

The liquid effluent from both hydrogen UASB units at the proper condition, which was operated at a COD loading rate of 25 kg/m³d, was further fed into both biomethane reactors. The biomethane production was operated without pH control and at the mesophilic temperature (37 °C) to obtain the highest biomethane production. The effect of COD loading rate on COD removal efficiency and gas production rate is shown in Figure 3a. The COD removal efficiency increased with increasing COD loading rate from 2 to 8 kg/m³d and then decreased with further increasing COD loading rate from 8 to 10 kg/m³d. The highest COD removal was 93.2 % at a COD loading rate of 8 kg/m³d. At the too high COD loading rate, the controlled system has a very short hydraulic retention time (HRT), at which microorganism have too short time for digesting the organic compounds. This indicated that at a COD loading rate of 8 kg/m³d was the highest cassava wastewater utilization by microbes. Moreover, the COD removal of biomethane production process was much higher than that of biohydrogen production process, suggesting the most of liquid effluent from the both biohydrogen reactors was mostly short chain molecule of organic compounds which is easily digested [10].

The composition of the generated gas and biomethane production rate as a function of COD loading rate are shown in Figure 3b. Under the studied conditions, the generated gas contained biomethane and carbon dioxide without the production of biohydrogen. Both biomethane content and biomethane production rate had a similar trend to COD removal efficiency. The highest value of biomethane content (83 %) and biomethane production rate (23 l/d) were found at a COD loading rate of 8 kg/m³d. For carbon dioxide content, it had an opposite trend to the biomethane content. A large amount of biomethane content

resulted from biohydrogen, and carbon dioxide were utilized by methanogens and converted to biomethane [11]. In addition, there is longer HRT when compared with biohydrogen production process; therefore the methanogenic bacteria have enough time to digest the organic waste and converted to biomethane gas [12].

Specific biomethane production rate (SMPR) and biomethane yield were calculated in accordance with biohydrogen production process. SMPR and biomethane yield also showed a similar trend to biomethane content and biomethane production rate, as discussed before (Figure 3c-d). The decrease in both SMPR and biomethane yield at a COD loading rate of 10 kg/m³d can be explained by the fact that the decrease in pH in both biomethane reactors due to more organic acid generated [8]. The decrease in pH below 6, in this work the pH at a COD loading rate of 10 kg/m³d was around 5.83, caused the inhibition of methane-forming bacteria growth [13-16], as shown in Figure 5, which will be discussed later.

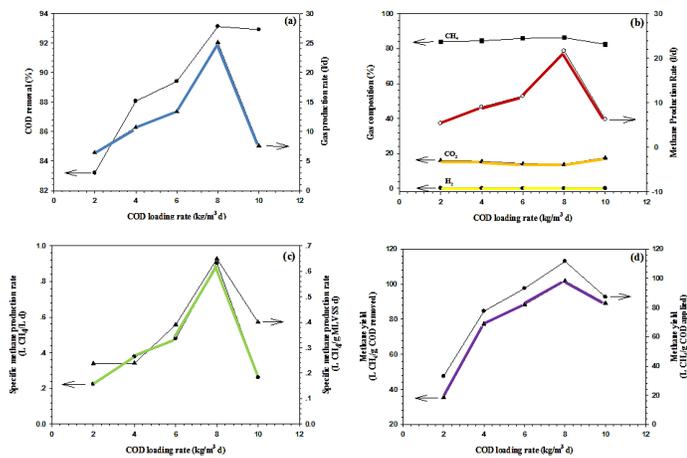
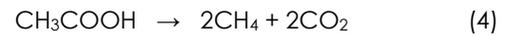


Figure 3 Effects of COD loading rate based on biomethane production process on (a) COD removal and gas production rate, (b) gas composition and biomethane production rate, (c) Specific biomethane production rates and (d) biomethane yield at 37°C without pH control in methane UASB unit

The effect of COD loading rate on the total VFA concentration (mg/l as acetic acid) and composition in the biomethane UASB system is shown in Figure 4. From the total VFA concentration result of both biohydrogen and biomethane production process (Figure 2) was higher than that of biomethane production process [8]. It indicated that the substrate feeding to biomethane reactors was mostly small molecule of organic compound [8]. The components of VFA were HAc, HPr, HBU, and HVa. It was found that HAc concentration was the highest, followed by the concentration of HPr, HBU and HVa, respectively

[8]. The highest HAc concentration, the controlled system gave the highest biomethane production performance in term of the highest biomethane yield and SMPR (Equation 4). The concentration of HAc decreased at any given COD loading rate. Under proper COD loading rate of 8 kg/m³d, the VFA concentrations were 32 % HAc, 28 % HPr, 22 % HBU, and 18 % HVa. Interestingly the concentration of HAc was the lowest at proper COD loading rate suggesting that HAc is the most utilizable at this proper COD loading rate and the highest biomethane production performance was also obtained [17-19]. Mohan S.V. *et al.*, [8] also found the similar result that the highest HAc concentration under methanogenic process contributed to the highest biomethane production. In general, the high amount of HAc formation from biohydrogen production process (Figure 3) at proper COD loading rate of 25 kg/m³d also contributed to the highest biomethane production [19-20].



Moreover, another organic acid that can affect to the biomethane production performance is HPr concentration, according to Equation 5 [21], it decreased with increasing COD loading rate and continuously increased with further increasing COD loading rate from 8 to 10 kg/m³d. At a very high COD loading rate of 10 kg/m³d, both HAc and HPr concentration are maximizing. This result showed that the negative effect to biomethane production performance (Figure 3a-d) resulted from the toxicity from VFA accumulation to the controlled system and inhibition the growth of microbes [22-23] (Figure 5).

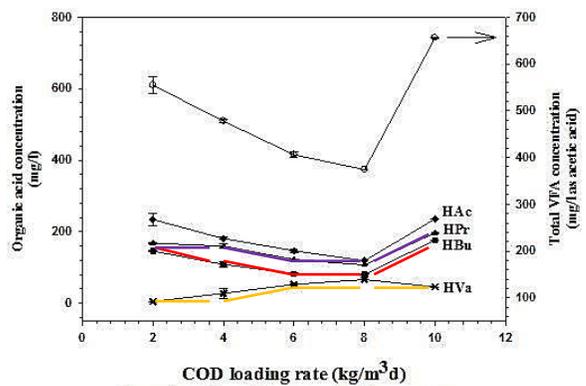
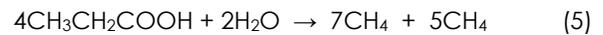


Figure 4 Total VFA, and VFA composition versus COD loading rate based on biomethane production process at 37°C without pH control in biomethane reactor

3.2 Microbial Concentration Results

The microbial growth is a parameter used for determining the capacity of the microorganism of growing and degrading the organic compounds present in the reactors. In this study, the microbial growth in terms of mixed liquid volatile suspended solids (MLVSS) and the wash-out of microorganism from the reactors in terms of volatile suspended solids (Effluent VSS) were also examined. Figure 5a-b shows the effect of COD loading rate on both MLVSS and the effluent VSS. The results showed that with increasing COD loading rate from 10 to 25 kg/m³d based on biohydrogen production process (or 2 to 8 kg/m³d based on biomethane production process), the MLVSS increased to reach a highest, but the effluent VSS decreased to reach a minimum. Beyond COD loading rate of 25 kg/m³d based on biohydrogen production process (or 8 kg/m³d based on biomethane production process), it was found that the MLVSS decreased and the effluent VSS increased. This point out that at the proper COD loading rate, the microbial growth in the reactors was the highest, accompanying with the least loss of the microbes from the reactors. The important parameter that affects to the anaerobic bacteria growth is pH, the acceptable pH for the acidogens and methanogens growth is around 5.5 and 7.0, respectively [24].

4.0 CONCLUSION

This study focused on the production of both biohydrogen and biomethane from cassava wastewater using upflow anaerobic sludge blanket reactors (UASB). For the first biohydrogen production, the results showed the highest biohydrogen production was achieved at a COD loading rate of 25 kg/m³d under a controlled pH of 5.5 and a mesophilic temperature (37 °C). The generated gas contained mainly hydrogen and carbon dioxide. Under proper conditions, the highest biohydrogen yield (39.8 l/kg COD removed), the highest biohydrogen production rate (0.4 l/l d), the highest specific biohydrogen production rate (10.92 l H₂/kg MLVSS d), the highest COD removal (43.8%), the highest butyric acid concentration (25 %), and the lowest propionic acid concentration (10 %) were obtained. Moreover, the lowest butyric acid-to-acetic acid ratio of 1.41:1 was also obtained at this proper condition. For the second biomethane production, the studied UASB was fed by the effluent from the biohydrogen production process, which was operated at a proper COD loading rate of 25 kg/m³d. The controlled system was operated at a constant temperature of 37 °C without pH control to obtain the highest biomethane production. The highest biomethane production was found at an proper COD loading rate of 8 kg/m³d which correspond to the highest biomethane production

rate (23 l/d), the highest COD removal (93.2 %), the highest biomethane yield (100 l/kg COD removed), the highest specific biomethane production rate (0.65 l CH₄/kg MLVSS d), and the lowest acetic acid concentration (33 %). Therefore, it is interesting for studying the biomethane and biohydrogen production from other organic waste such as the food waste by using UASB reactor under the ambient condition due to the characteristic of the food waste mostly contain the carbohydrate-rich organic solid waste.

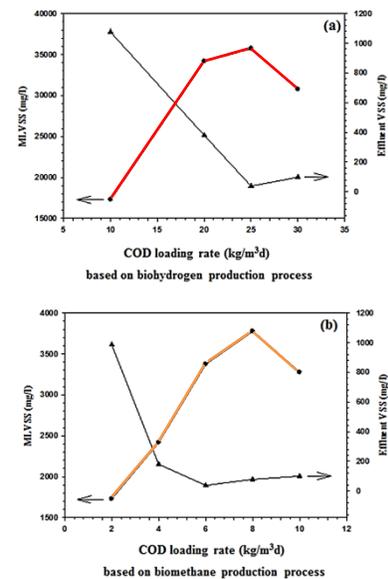


Figure 5 Effect of COD loading rate on MLVSS and effluent VSS unUASB unit reactor at pH 5.5 and (b) methane UASB unit without control pH

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