Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1189705

57 (2016) 29168–29177
December

Taylor & Francis
Taylor & Francis Group

Optimization of methane production process from synthetic glucose feed in a multi-stage anaerobic bioreactor

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Received 2 September 2015; Accepted 3 May 2016

ABSTRACT

The biological conversion of biomass into methane during anaerobic digestion has been studied by many researchers in recent years. In this study, optimization of methane composition during chemical oxygen demand removal was observed in a multi-stage Anaerobic Bioreactor. Synthetic glucose was used as a feed substrate, and the reactor was operated at a hydraulic retention time (HRT) of 1–4 d. Complementary experimental and theoretical test procedures were evaluated for methane optimization. The theoretical methane was recorded as 50.13, 50.02, 50.16, and 50.22% for an HRT of 4, 3, 2, and 1 d, respectively. However, the quantity of methane determined experimentally was significantly lower than the theoretical predictions; this was likely due to the microorganism activity in the reactor that may have interfered with the efficiency of the biogas generation. Experimental data showed a decrease in the methane composition (35.4, 21.2, 19.8, and 18.4% for HRT of 4, 3, 2, and 1 d, respectively) in the reactor system. Thus, the theoretical formula and experimental data together provide an alternative method for the evaluation of bioenergy potential in anaerobic digestion.

Keywords: Multi-stage anaerobic bioreactor; Glucose; Methane production

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Presented at The 2nd IWA Malaysia Young Water Professionals Conference 2015 (YWP15)
March 17–20, 2015, Kuala Lumpur, Malaysia

1. Introduction

The number and range of wastewater treatment facilities have grown on a global scale and are faced with two critical issues: treatability and production of biogases. Recently, researchers have looked toward the development of the anaerobic digestion process as an option for both disposal route and energy recovery [1]. The anaerobic digestion process biologically metabolizes organic material (disposal) in the absence of oxygen and produces methane (CH_4, for energy recovery). Anaerobic digestion operates on either a high-rate or low-rate system; for the high-rate system, anaerobic digestion occurs under the conditions of biomass retention (HRT ≠ SRT), and for the low-rate system, it occurs under conditions without biomass retention (HRT = SRT). Methane production in anaerobic digestion requires a diversity of bacteria capable of participating in the degradation process, which consists of four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [2].

During the early periods of anaerobic digestion development, a single-stage anaerobic process was limited by the low rates of chemical oxygen demand (COD) removal, long hydraulic retention time (HRT), accumulation of waste sludge, and the requirement of a large reactor volume [3]. Later, reactor design was improved by implementing multi-stages and those issues were overcome. Multiple-stage reactors were designed to distribute organic load and to increase the contact/reaction time between the medium (sludge bed) and the feed particles. Acidogenic and methanogenic processes occur in separate compartments in multiple-stage anaerobic reactors, improving reactor performance. Although biogas recovery in anaerobic digestion is a lucrative reward, there are obstacles in achieving the process efficiency to optimize biogas output [4]. Despite many studies conducted to determine the feasibility of biomethane generation through anaerobic digestion, few have succeeded in optimizing the process; however, the results of previous research provide some resources to improve biogas generation [5].

There have been several studies on methane generation using anaerobic digestion. Tartakovsky et al. [6] investigated biomethane production using a microalgae biomass as the substrate in an up-flow anaerobic sludge bed (UASB) and reported 80% methane at an HRT of 3.5 d. Lu et al. [7] demonstrated that 60% methane can be generated within 6 h in a UASB treating starch wastewater. Khan et al. [8] reported that a UASB treating sewage was able to produce 70% methane in 8 h. An investigation by Kongjan et al. [9] showed that with an HRT of 36 h, a UASB treating frozen fishery wastewater was capable of producing 57–65% methane. Matsuura et al. [10] observed 40–50% methane in a UASB at an HRT of 12.8 h. On the other hand, Jijai et al. [11] reported that decrease in HRT would result in decreased COD removal and biogas generation. In a separate study by Rico et al. [12], an anaerobic digestion of cheese whey in UASB at HRT 2.2 d resulted in 75–50% of methane composition, whereas Saha et al. [13] demonstrated that at HRT of 1 d, a UASB treating domestic wastewater added with methanol able to generate 40–55% of methane. Recently, Nkemka et al. [14] observed 28.4% of methane during bioaugmentation with an anaerobic fungus in a two-stage process for biohydrogen and biogas production using corn silage and cattail. The literature review above showed that methane composition varies from each experimental study and depends on various factors such reactor configuration, seed sludge, type of wastewater, and operating conditions.

Many studies have been performed which combine experimental and theoretical investigations of anaerobic digestion. Fuentes et al. [15] carried out experimental and theoretical studies on anaerobic fluidized bed biofilm reactors, which were modeled as dynamic three-phase systems and included the anaerobic degradation of complex substrates and kinetic parameters selected from the literature. Step-type disturbances were applied to the inlet substrate (glucose and acetic acid) concentration and the feed flow rate, and the maximum efficiency was determined as the disturbances were applied [15]. Lima et al. [16] performed theoretical and experimental investigations of domestic wastewater treatment in a UASB reactor and showed satisfactory agreement between numerical and experimental results for the pressure and sludge concentration at the outlet of the reactor. Vafajoo et al. [17] described the theoretical and experimental treatment of polyethylene terephthalate (PET) effluent using an anaerobic hybrid bioreactor and a synthetic medium containing glucose as a carbon source to study the COD removal efficiency. Their results showed satisfactory agreement between the theoretical model and experiment data, confirming that the developed model could be used toward optimization of a real application. Recently, Nielfa et al. [18] investigated the theoretical methane composition generated by the co-digestion of the organic fraction of municipal solid waste and biological sludge and confirmed that theoretical prediction methodologies give an indication of maximum methane productivity.

Herein, the methane generation potential of an anaerobic digestion reactor is determined using complimentary theoretical and experimental test procedures. In both theoretical and experimental approaches, the effects of feed COD, COD removal efficiency, and HRT were investigated. The aim of this research was to evaluate and compare the theoretical and experimentally measured methane composition in a multi-stage Anaerobic Bioreactor. The stoichiometric empirical formula [19,20] was used as a basic formula to calculate the biogas potential of the reactor. Theoretical formula together with the experimental data provides an alternative method for the evaluation of bioenergy potential in anaerobic digestion.

2. Materials and methods

2.1. Multi-stage anaerobic bioreactor

In this experiment, multi-stages were incorporated into the bioreactor. The bioreactor contained four identical 2.75-L Plexiglas® cylinders, represented as R1, R2, R3, and R4 (Fig. 1), linked in series, with a total active reactor volume of 11 L. Each stage of the reactor had a 3-phase separator baffle placed 2 cm below the effluent ports to prevent floating granules from washing out with the effluent. Effluent from each stage of the reactor flowed by gravity to the next stage. A temperature controller and heater were installed to maintain the reactor temperature at 38˚C. A peristaltic pump (Masterflex L/S, Easy Load II Pump Head) was used to control the influent feed rate. The methane yield was determined using an optical bubble counter while methane was recovered in a Tedlar® gas bag and measured using routine analysis with a Gas Analyzer (GA2000, Geotech).

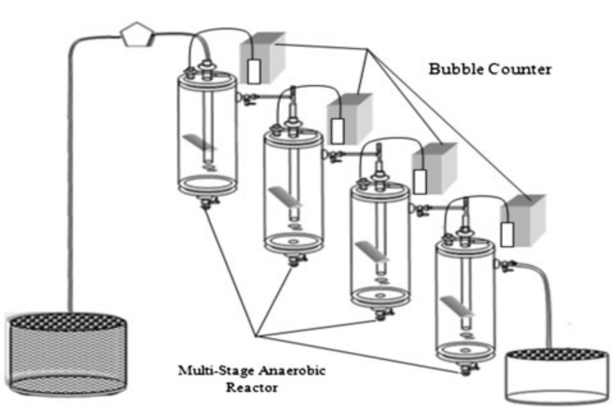

Fig. 1. Multi-stage anaerobic bioreactor.

2.2. Seeding sludge

The bioreactor was inoculated with anaerobic sewage sludge taken from Indah Water Konsortium (IWK), Bunus Sewerage Treatment Plant, Kuala Lumpur. Approximately 1.4 L of sludge sieved through 2.0-mm mesh were added to each stage of the reactor and contained 8,000 mg total suspended solids (TSS) L^{-1} and 5,000 mg volatile suspended solids (VSS) L^{-1}. The initial TSS in the reactor was reduced to 6,000 mg L^{-1} based on the settling tests performed on the sludge. The reactor was then filled with tap water to dilute the supernatant of the seed sludge, and the reactor was purged with nitrogen to remove residual air. No feed was introduced for five operational days to ensure the sludge settled. Once settled, the reactor was gradually fed with synthetic wastewater.

2.3. Feeding and analysis

The bioreactor was filled with synthetic wastewater (glucose) as feed due to its degradability and because it does not limit the rate of anaerobic biodegradation [21]. Glucose readily produces measurable intermediate metabolites and is commonly used as a carbonaceous substrate in experimental studies [22]. Nutrient deficiency in the feed was corrected using macronutrients (EnBac® N100, Bio-Systems Corporation Ltd) designed to provide the essential macronutrients and micronutrients to supplement bacterial metabolic needs for enhancing the microorganisms' growth rate in anaerobic environments [23]. Inadequate nutrients may affect the anaerobic process and biogas generation. In this study, the average ratio of COD:N:P in the wastewater was maintained at 250:5:1 [24–26]. The analysis of parameters was carried out using standard methods [27]. Sample analysis included COD, pH, alkalinity, and volatile acids (VA). The measurement of COD in this study was based on soluble COD. All feed and effluent from the reactor were filtered using GA filter paper before the COD measurement.

2.4. Methane production potential

The potential for biogas generation in wastewater can be estimated by the amount of feed utilized in the treatment process. During anaerobic digestion processes, the biodegradable fraction of the feed is converted to end products, specifically biogases; this process is described as biomethanation. It reflects complex microbial degradation of an organic compound into methane and carbon dioxide by a diverse group of anaerobes [28]. Adhering to the gas law, the determination of theoretical biogas production can be evaluated by the empirical formula of biomass used for stoichiometric product estimation. In this case, an organic compound (CnHaOb) was assumed completely biodegradable and converted by the anaerobic organism into biomethane (sludge yield is assumed to be zero). Thus, the theoretical amount of the gases produced can be calculated by stoichiometric formulas (Eq. (1), Buswell and Neave; Eq. (2), Van Der Waals Equation of State):

$$C_pH_qO_r + \left(p - \frac{q}{4} - \frac{r}{2}\right)H_2O \rightarrow \left(\frac{p}{2} + \frac{q}{8} - \frac{r}{4}\right)CH_4 + \left(\frac{p}{2} - \frac{q}{8} + \frac{r}{4}\right)CO_2 \quad (1)$$

and

$$P + a^*\left(\frac{n}{V}\right)^2 \times (V - nb^*) = nRT \quad (2)$$

where P = pressure of gas; n = number of moles of gas; 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^{-1}, carbon dioxide = 0.0000439 m^3 mol^{-1}); T = temperature (Kelvin); R = gas constant 8.31441 m^2 kgs^{-2} K^{-1} mol^{-1}.

The flowrate was calculated by measuring volume of inlet to the feed per unit time, and the pressure was calculated from the pump's horsepower (0.1 hp) and flow rate. During the experiment, synthetic wastewater (glucose) was used for generating methane biogas. This process was considered to be completely degradable.

$$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$$

The degradation pathway of substrate (glucose) results in three moles of methane and three moles of carbon dioxide. The moles of glucose used (calculated using MW = 180 g mol^{-1} and experimentally determined mass used) were used to determine the moles of gas formed, n.

In previous research, the theoretical biogas yield was predicted using the ideal gas law without considering intermolecular forces and molecular size of gases inside the reactor [29]. 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 [30].

Table 1
Van Der Waals constant for methane and carbon dioxide [30]

Substance	a^* (bar L^2/mol^2)	b^* (L/mol)
Carbon dioxide	3.658	0.0429
Methane	9.476	0.065

*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.

The degradation of carbon content was calculated based on influent COD from the beginning to the end of reactor operations. The organic loading rate (OLR) was gradually increased from 0.25, 0.34, 0.50 to 1.00 kg COD m^{-3} d^{-1} by decreasing the HRT incrementally from 4 to 1 d.

From the volume of biogas, the gas potential was calculated using Eqs. (3) and (4):

$$\text{Gas yield}(\%), \left(\frac{1}{\text{g COD}} d^{-1}\right) = \frac{\text{volume gas}}{\text{COD utilized} \times \text{volume reactor}} \times \text{HRT} \quad (3)$$

and

$$\text{Gas content } \% = \frac{\text{Gas yield}}{\text{Total gas yield}} \times 100 \quad (4)$$

3. Results and discussion

The potential of the bioreactor system for methane generation is illustrated in Tables 2 and 3. The performance of methane production was controlled by COD introduction at each operational time. The organic content (COD) was found to be 990, 1,049, 1,043, and 1,043 mg L^{-1} for corresponding operational times of 4, 3, 2, and 1 d (HRT), respectively. The methane yield increased with increasing operational time. Theoretically, the highest methane yield (measured in volume of gas generated per mass of COD digested per unit time) achievable was 0.1202 L g^{-1} d^{-1} (HRT 3 d). The maximum attained methane yield in the experiment was 0.00286 at 4 d HRT and occurred in reactor 3 (R3). Methane yields consistently decreased with decreasing HRT (0.00145, 0.00104, and 0.000948 L g^{-1} d^{-1} at HRT = 3, 2, and 1 d, respectively). The results showed that the highest organic removal (97.70%) was achieved at an HRT of 3 d and the methane yield was high (Fig. 2a). The theoretical

Table 2
Theoretical methane potential

Total removal (%)	HRT (d)	Gas properties: Volume (L)	Gas properties: Gas produced (l/d)	Methane yield (l/g COD d^{-1}): R1	R2	R3	R4	Total	Methane composition (%): R1	R2	R3	R4	Average	Ratio methane: COD
93.94	4	0.0109	0.00272	0.011	0.023	0.028	0.038	0.100	5.4	11.7	14.0	19.1	12.55	0.63
97.70	3	0.0116	0.00386	0.007	0.010	0.017	0.086	0.120	7.5	10.8	12.7	19.1	12.53	0.53
92.08	2	0.0117	0.00584	0.008	0.009	0.010	0.011	0.038	10.1	12.1	13.2	14.8	12.55	0.57
83.22	1	0.0120	0.01201	0.003	0.006	0.006	0.007	0.022	7.3	14.5	13.5	14.9	12.55	0.62

Table 3
Methane in experimental study

Total removal (%)	HRT (d)	Methane composition (%): R1	R2	R3	R4	Average	Gas yield (l/g COD d^{-1}): R1	R2	R3	R4	Total	Ratio methane: COD
93.94	4	4.35	11.80	13.35	5.90	8.85	4.86E-04	2.23E-04	2.86E-03	1.90E-05	3.58E-03	0.45
97.70	3	3.10	7.55	7.50	3.05	5.30	1.25E-04	1.04E-04	1.45E-03	3.76E-05	1.71E-03	0.22
92.08	2	2.80	7.00	7.10	2.90	4.95	1.37E-04	1.28E-04	1.04E-03	3.94E-06	1.31E-03	0.23
83.22	1	2.60	6.40	6.60	2.80	4.60	9.93E-05	1.43E-04	9.48E-04	3.11E-06	1.19E-03	0.23

methane concentration contradicted with experimental results. Theoretical results predicted that the highest average concentration was 12.55% at 1, 2, and 4 d HRT, and declined to 12.53% at 2 d HRT, whereas the maximum experimental methane composition (Fig. 2b) was 8.85% at an HRT of 4 d and decreased to 5.30, 4.95, and 4.60% at an HRT of 3, 2, and 1 d, respectively. Fig. 2c illustrates the effect of COD: HRT ratio on methane yield. The ratio shows the relationship between COD converted into methane at each HRT. The highest theoretical ratio was 0.63, whereas the highest ratio obtained experimentally was 0.45 at an HRT of 4 d. Methane generation is optimum at high HRTs and low OLRs [31]. High HRTs serve as a sufficient platform for the adaptation of microorganism to a new environment for bacterial growth.

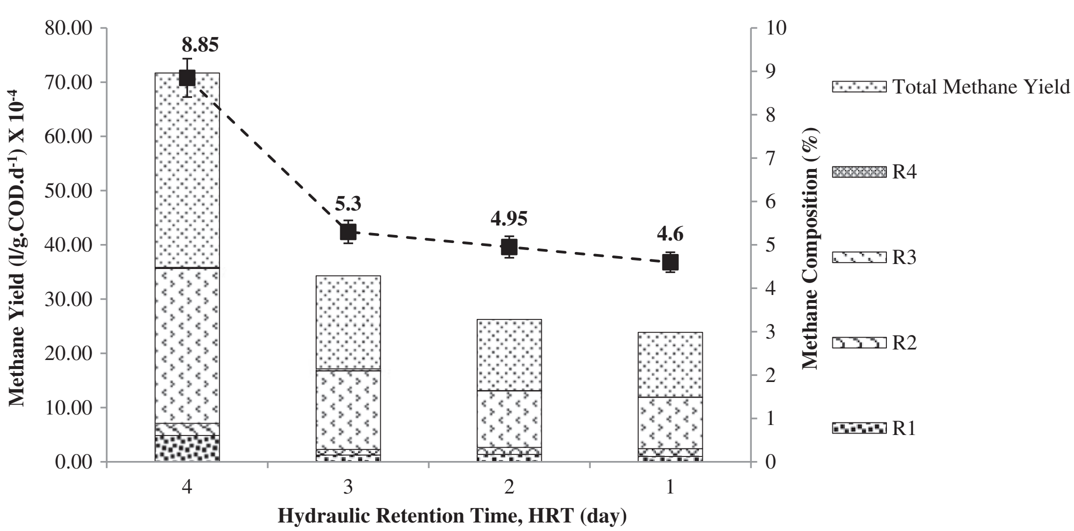

Fig. 2a. Theoretic potential in methane generation.

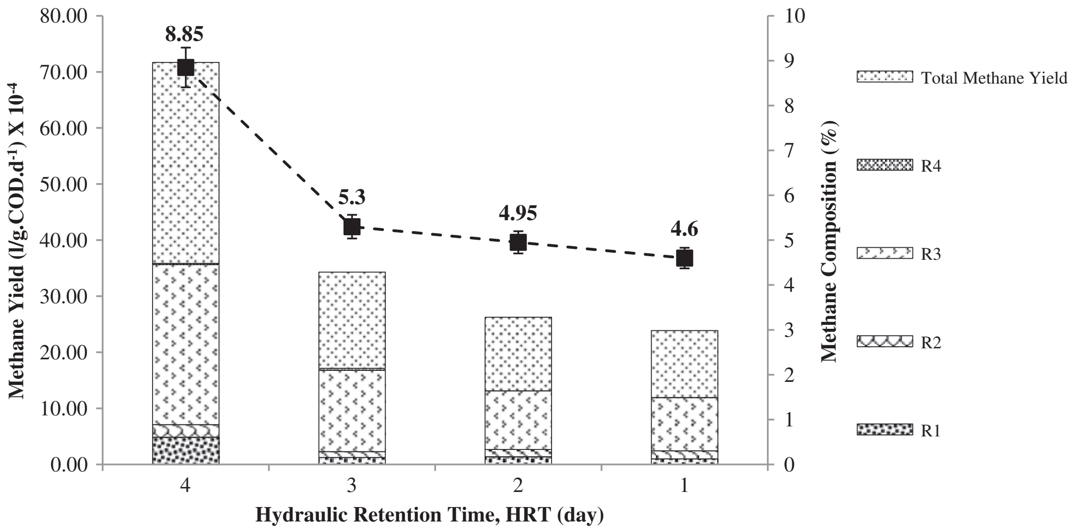

Fig. 2b. Actual methane generation (experimental).

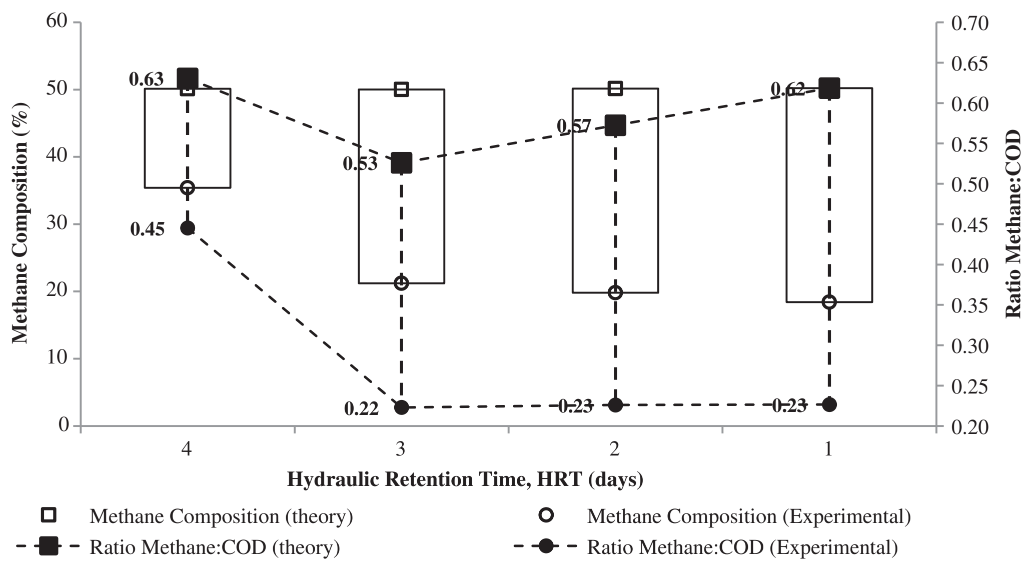

Fig. 2c. Relationship of methane potential (theoretical and experimental).

Unfortunately, high HRTs also pose problems such as the requirement of large reactor volume and high operational costs [32]. In this study, the feed (glucose) initially had a high COD value and lack of alkalinity and nutrient deficiency. As a result, the influent COD tended to acidify rapidly, and the methane yield was decreased as methanogens require optimum pH to generate methane [33].

Apparent from the experimental data, higher HRTs provide sufficient time for the anaerobic microorganisms to degrade the organic substances; this increases the treatment efficiency through high COD removal, and higher methane generation is achieved. Comparatively, the theoretical results for methane potential were not similar to the real experiment. Nallathambi Gunaseelan [33] demonstrated that the theoretical methane potential is 40% different from the actual experimental study. The theoretical methane in this study is slightly lower (around 12.55%) due to the actual temperature, pressure, and also intermolecular activity inside the reactor (not at standard temperature and pressure, STP). With short HRTs, the microorganism population was likely dominated by acidogenic bacteria (supported by the pH profile) and methanogenic bacteria were suppressed. With longer HRTs, the methane yield increased due domination of methanogenic bacteria [34,35]. With all HRTs, there was a visible trend in methane generation with reaction stage; the methane composition increased from R1 to R3 but then decreased at R4. As noted by Ghaniyari-Benis et al. [36], this phenomenon is possible due to the increase in OLR that contributed to increased acidogenic activity at lower pH. At low pH, the methanogens may not thrive well and lead to decreased methane generation. This trend was observed in this study in the total methane composition from HRT 4 to 1 d. It is also possible that the decreasing trend that was seen in each HRT after R3 could be due to the domination of denitrifying bacteria which is responsible for denitrification, as described by Hobson and Wheatley [37].

Depending on controlled conditions within the reactor, the microbial activity can affect the methane potential; for example, high removal of organic (high COD removal efficiency) is expected to contribute to high methane generation. The COD removal efficiency was 93.94, 97.70, 92.08, and 83.22% when the HRT was 4, 3, 2, and 1 d, respectively (Fig. 3). The results signify that there was no major change in the COD removal efficiency when the reactor was operated at HRT 4, 3, and 2 d. A similar trend was also observed by Ghaniyari-Benis et al. [36,38] during the treatment of synthetic wastewater (molasses) in a multi-stage anaerobic biofilm reactor. They reported that a decrease in HRT from 24 to 16 h had no effect on COD removal efficiency. However, a significant drop in the removal efficiency was observed in this study when the HRT was reduced to 1 d (83.22%). The change in HRT may have affected the process performance of the reactor where different microbial metabolic processes occur during each shift in the HRT. With a short HRT (e.g. 1 d), the reactor may have been dominated by acidogenic bacteria and in longer HRT (e.g. 4 d) the methane yield increased due to the domination of the methanogenic bacteria. In this experiment, the highest COD removal efficiency was achieved at an HRT of 3 d (97.70%), which corresponds well with the methane generation. The ratio for both theoretical and experimental data at HRT 4 d tends to show a similar result.

According to Monnet et al. [39], the metabolic pathway of glucose can be summarized into four processes; hydrolysis, acidogenesis, acetogenesis, and methanogenesis. From this pathway, one could understand that glucose, which is already a simple sugar, would skip the primary process of hydrolysis. Therefore, glucose will first undergo acidogenesis to be converted into volatile organic acids such as propionic acid or butyric acid. Next, the volatile organic acids undergo acetogenesis to form acetic acid, CO_2, and hydrogen, the branching ratios of which depend on HRT variation. Finally, the acetic acids get converted into methane during methanogenesis. Along this anaerobic digestion metabolic pathway, there is no ammonia produced. Nitrates have been added to the feed artificially in an optimal ratio (COD:N:P in the wastewater was 250:5:1), so that they will undergo denitrification and reduce to nitrogen [40] rather than generating ammonia. No ammonia was detected during the experimental testing.

In anaerobic digestion processes, pH has significant influence on the effluent composition. During digestion, acidogenesis and methanogenesis need different pH levels for optimal process control. Throughout acidogenesis, the bacteria form VAs and accumulation indeed reduces the pH levels. Subsequently, the acidogenic and methanogenic bacteria will convert those acids into biogas (CH_4 and CO_2) with an optimum pH environment within the range of 6.7–7.4 [41]; a pH below 6.7 is toxic to methanogenic bacteria [42]. As observed in Fig. 4, the pH profile across the bioreactor follows the order of $R1 < R2 < R3 < R4$. The average pH within the bioreactor was 6.0; this low pH is likely due to the use of glucose as feed. Glucose easily acidifies in a short period, consistent with the large amounts of sodium hydroxide (NaOH) required in this study to maintain the pH at a neutral level. The slightly acidic average pH is consistent with the lower than expected methane yields in the reactor due to the potential impact on methanogenic bacteria populations.

A high number of acidogenic bacteria can affect the COD removal rate in the reactor by inhibiting the growth of methanogenic bacteria [43]. The pH and VAs profiles (Fig. 4) suggest such inhibition may have contributed to the low methane content in this study. Furthermore, at lower HRT (1 d, OLR = 0.95 kg COD m^{-3} d^{-1}), the VA was high, and the pH was in an acidic range, which could be due to an active decrease of sludge in the reactors. High VA and low pH favor the establishment of the acidogenic phase and prevent the growth of methanogens [44]. The decline in VA at lower OLR (0.25 kg COD m^{-3} d^{-1}) suggests that the anaerobic bacteria were utilizing the feed well. The COD removal profile supported this, as the highest peak of total COD removal was achieved during 101–162 d; however, the decrease in methane content during this time strongly suggests that the prevalence of methanogenic bacteria was weaker. Consequently, this proved that the anaerobic process was successful, but the substrate conversion was more toward hydrogen as the end product rather than methane.

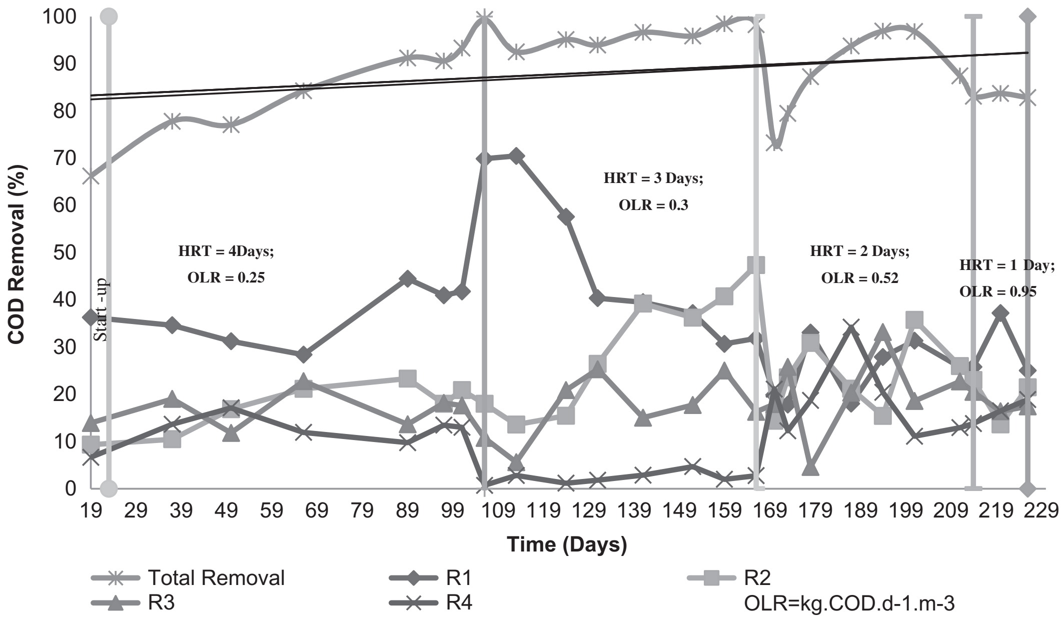

Fig. 3. COD removal profile of the bioreactor.

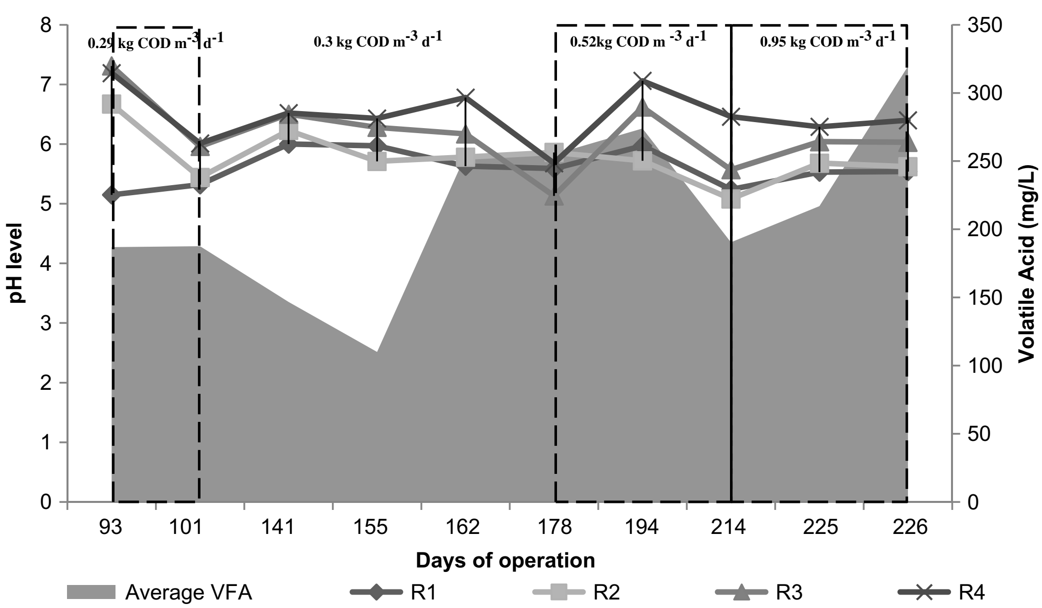

Fig. 4. VA and pH variation in R1–R4.

4. Conclusion

Even though some experimental and theoretical research has been reported on anaerobic digestion, prior to this study, there are no reported data on the multi-stage Anaerobic Bioreactor. Methane composition depends on various factors such as reactor configuration, seed sludge, type of wastewater, and operating conditions. The potential methane evaluation in the bioreactor appears to depend on the HRT sand the COD concentration in the influent. A comparison of the theoretical model with experimental data from the multi-stage Anaerobic Bioreactor was satisfactory; however, the quantity of methane present in the experimental application was significantly lower than the theoretical, likely due to the feed composition inhibiting the growth rate of methanogens as the reactor conditions rapidly became acidic.

Acknowledgment

The authors desired to show the highest gratitude to Mr Azmi who helped with the apparatus and materials for the experiments and also special acknowledgement to Universiti Teknologi Malaysia for funding this study. The authors also thank the Ministry of Education Malaysia for the Research University Grant (Vote number: 4F451).

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