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Full Length Research Paper

Performance of an innovative multi-stage anaerobic reactor during start-up period

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Start-up of an anaerobic reactor is a relatively delicate process and depends on various factors such as wastewater composition, available inoculum, operating conditions and reactor configuration. Accordingly, systematized operational procedures are important, mainly during the start-up of an anaerobic reactor. In this paper, the start-up performance of an innovative multi-stage anaerobic reactor using synthetic wastewater at various organic loading rates (OLRs) was investigated. In Phase 1 of the experimental study, the reactor was operated at hydraulic retention time (HRT) of 1 day with corresponding OLR of 1.07 kg COD.m^{-3}.d^{-1}. Thereafter, the reactor was operated at intermittent feeding (Phase 2), with HRT of 1.4 day and OLR of 0.82 to 2.45 kg COD.m^{-3}.d^{-1}. Results showed up to 71% COD reduction in the Phase 1 of the experimental study. However, in Phase 2, when the reactor was operated at intermittent feeding, the COD removal efficiency increased from 75 to 92%. It can be concluded that the multi-stage anaerobic reactor system performed better at intermittent feeding, indicating that the reactor required low loading rate and sufficient HRT for gradual acclimatization for reactor start-up. The reduction of the period necessary for the start-up and improved operational control are important factors to increase the efficiency the reactor system.

Key words: Anaerobic reactor start-up, biomass, glucose wastewater, intermittent feeding, multi-stage anaerobic reactor.

INTRODUCTION

Anaerobic digestion has proven to be a stable process for a variety of wastewaters when operated properly. It has several advantages over the aerobic and physic-chemical process such as low sludge production, higher loading potential, low operating cost and methane production (Foresti, 2001). Anaerobic methanogenic digestion an effective method for treatment of many organic wastes is a topic of increasing interest throughout the world. A number of designs and their performance have already been described by several researchers (Anderson and Yang, 1992). However, the fact remains that anaerobic process has not been utilized as widely as aerobic process. Until now, the technology of anaerobic digestion has not been able to meet the predicted expectation to its potential. Compared with other processes, its advantages are less energy requirement, high treatment efficiency and usable gas production.

During anaerobic reactor start-up, the biomass is acclimatized to new environmental conditions, such as substrate, operating strategies, temperature and reactor configuration. Moreover, the methanogens and certain acetogens may be greatly outnumbered by the fast-

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Abbreviations: ABR, Anaerobic baffled reactor; **COD,** chemical oxygen demand; **HRT,** hydraulic retention time; **OLR,** organic loading rate; **SS,** suspended solid; **SUR,** substrate utilization rate; **TSS,** total suspended solid; **UASB,** up-flow anaerobic sludge bed; **VFA,** volatile fatty acid; **VSS,** volatile suspended solid.

growing acidogens (Massé et al., 2001). Consequently, an accumulation of volatile fatty acids (VFAs) and dissolved H_2 will occur. Anaerobic reactors are usually started at the optimum temperature range for methanogen growth, which is between 33 and 40°C. Salkinoja-Salonen et al. (1983) suggested that it would be difficult to start reactors at 20°C, because of excessively low methanogen growth at that temperature. Start-up procedures will depend on various factors, including wastewater composition and strength, available inoculum, reactor operating conditions, and reactor configuration (Weiland and Rozzi, 1991). Shorter start-up time can be obtained by using wastewater low in particulate organics; for example, up-flow anaerobic sludge bed (UASB) reactors. The objective of start-up is to develop an active granular biomass with good settling capacity. The organic loading rates (OLR) should be increased only when the COD and VFA concentrations have been reduced by 80% (Lettinga, 1995).

The reduction of the period necessary for the start-up and improved operational control of the anaerobic processes are important factors to increase the efficiency and the competitiveness of the high-rate anaerobic systems (Chernicharo, 2007). In general, high-rate anaerobic processes can be operated with organic loads much higher than those of the conventional anaerobic reactors, but frequently, these highly efficient processes require longer start-up periods, better operational control and more qualified operators. Systematized operational procedures are very important, mainly during the start-up of high-rate systems (Chernicharo and Nascimento, 2001). The start-up of anaerobic reactors is determined by the initial transient period, marked by operational instabilities.

The unique multi-stage anaerobic reactor is similar in design and application to the anaerobic baffled reactor (ABR) (Barber and Stuckey, 1999). Each stage of the reactor system represents a separate compartment. A stage reactor can provide high treatment efficiency since recalcitrant substrates will be in an environment more conducive to degradation (Speece, 1996). The design of the reactor system results in the separation of acidogenesis and methanogenesis, which has potential benefits for reactor performance. With no moving parts or mechanical mixing, no requirement for biomass with unusual settling properties, and a high degree of stability to hydraulic and organic shock loads, the stage reactor has the potential to be applied economically as a pre-treatment system for many trade effluents (van Lier et al., 2001). There are many publications on the start-up of anaerobic reactors (Escudié et al., 2011; Alvarado-Lassman et al., 2010; Dong et al., 2010; Shanmugam and Akunna, 2010; Vadlani and Ramachandran, 2008; Zhiyi et al., 2008). However, there is no reported study on the start-up performance of staged anaerobic reactors.

In staged anaerobic wastewater treatment, a physical separation in the sludge bed is introduced in order to optimize the locally prevailing conditions for the anaerobic bacteria and to enhance specific conversion reactions (van Lier et al., 2001). Historically, physical separation of the different species involved in the anaerobic degradation process has been studied for carbohydrate wastewaters under mesophilic conditions. In this case, the advantage of "staging" is attributed to the high biomass yield of carbohydrate-fermenting bacteria (0.2 g vs. g COD^{-1}) compared to acetogenic bacteria and methanogenic archaea (0.03 to 0.05 g vs. g COD^{-1}) (van Lier et al., 2001). Consequently, pre-acidification of the carbohydrates in the first stage results in a high volumetric fraction of methanogenic archaea in the second stage. A number of benefits from staging are given by Speece (1996), for example, staging can dramatically improve the anaerobic treatment of carbohydrates and other pollutants which yield propionic acid and hydrogen intermediates. Furthermore, he stressed that the arrangements can easily double the activity of the anaerobic biomass resulting in the need for only half as much biomass to be provided.

In summary, start-up is often considered to be the most unstable and difficult phase in anaerobic digestion. Therefore, the main objective of this study was to observe and evaluate the start-up performance of a multi-stage anaerobic reactor system using synthetic wastewater (glucose) at various OLRs. The uniqueness of this study was mainly attributed to the mode of operation of the pilot scale anaerobic reactor during start-up (continuous and intermittent feeding). The performance of the reactor system was evaluated based on the COD removal efficiency, solid washout, pH stability and gas composition. It should be pointed out that there is no reported study on the start-up performance of a multi-stage anaerobic reactor system at continuous and intermittent feeding strategy. Most of the literature data on anaerobic treatment are in continuous operational mode and less on the intermittent feeding. Accordingly, this study will help in understanding more thoroughly the start-up strategy, especially during the intermittent feeding.

MATERIALS AND METHODS

Multi-stage anaerobic reactor

The multi-stage anaerobic reactor consists of four units of transparent identical cylindrical plexiglas compartments (stages), and 160 mm internal diameter by 1100 mm height with head plate (Figure 1). The active volume of the reactor system was 90 L (4 stages of 22.5 L). The flow diagram of the reactor system design is presented in Figure 1. Each stage of the reactor had a three-phase separator baffle placed below the effluent ports, to prevent floating granules from washing out with the effluent (Figure 2). Effluent from each stage of the reactor flowed by gravity to the next, as each stage was placed on stepped platform. Each stage of the reactor had a temperature controller to maintain the reactor temperature at 37°C. Peristaltic pumps were used to control the influent feed rate to the first stage of the reactor system. Gas production was

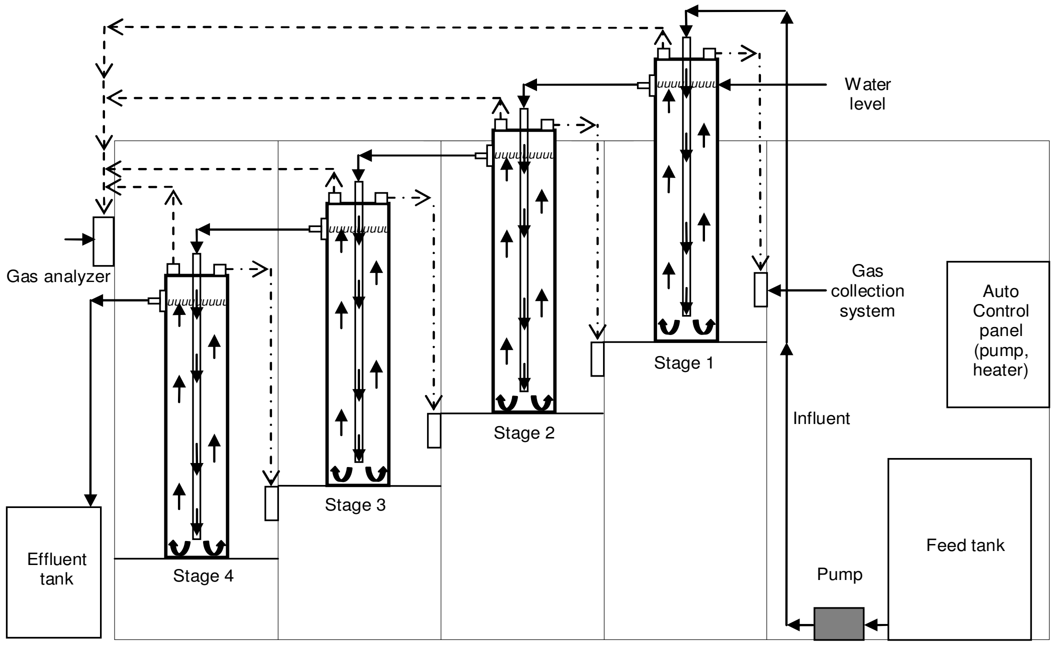

Figure 1. Innovative multi-stage anaerobic reactor system and flow regime.

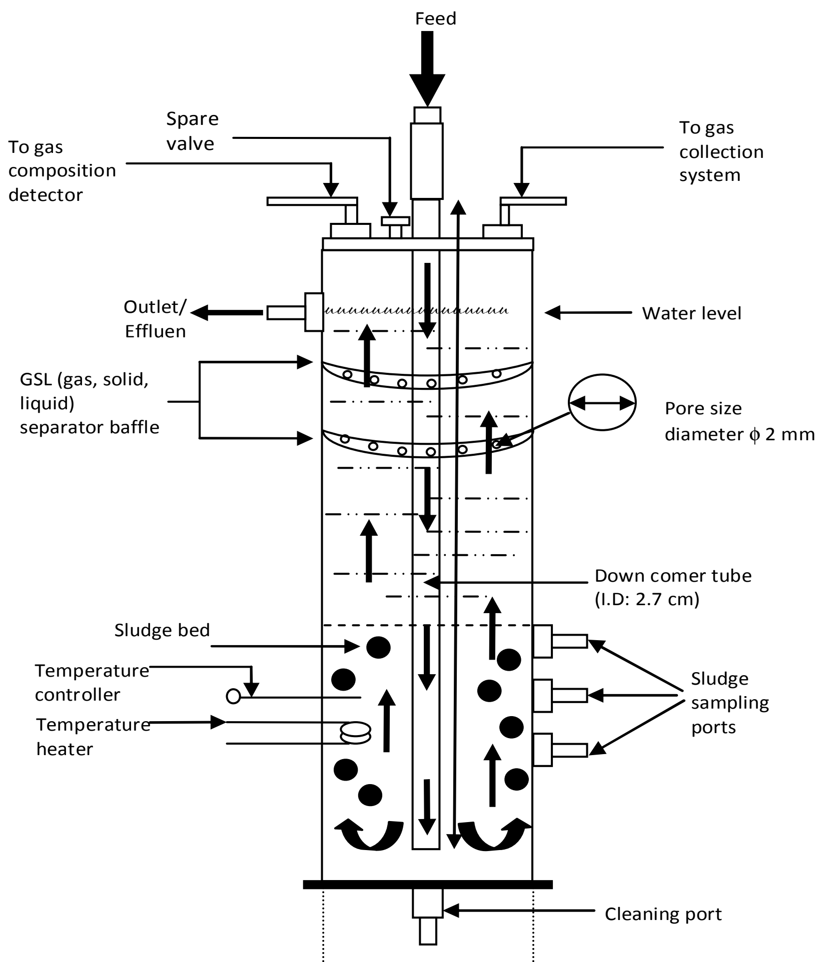

Figure 2. Detail design of an individual stage.

monitored separately for each stage using gas-water displacement method.

Feed and nutrients

To start-up the multi-stage anaerobic reactor, glucose was used due to its ease of degradation and high COD value. Glucose was used since it is a readily degradable, soluble carbohydrate that does not, itself, limit the rate of anaerobic biodegradation (Noike et al., 1985). It produces readily measurable intermediary metabolites in anaerobic digestion and is commonly used as a carbonaceous substrate in many experimental studies (Stronach et al., 1986). Nutrient deficiency was corrected by using macronutrients N100 (from Bio-Systems Corporation Asia Pacific, Malaysia). The composition of the macronutrients N100 is given in Table 1. The alkalinity was maintained in all reactor stages at 1000 to 2000 mg.L^{-1} as $CaCO_3$.

Seed sludge

The multi-stage anaerobic reactor was seeded using anaerobic digested palm oil mill effluent (POME) sludge (Felda Palm Industries Pt. Ltd. Malaysia). The sludge was sieved through 2 mm mesh giving solid contents of 53,750 mg TSS.L^{-1} (41,500 mg VSS.L^{-1}). About 7.5 L of sieved sludge was added to each reactor stage, the remaining volume been filled with tap water. Throughout the experiment, the reactor was supplied with synthetic wastewater (glucose) as a substrate. After seeding, the head plates were attached and the headspace above each reactor was flushed with nitrogen gas to displace residual air in the system before introducing the feed. The reactor was allowed to stabilize at 37°C for 24 h in seven days without further modification.

Sampling and analysis

Supernatant liquor, gas and sludge samples were taken separately for each stage. In addition, gas production rate was determined separately for each stage. Sample analysis included chemical oxygen demand (COD), pH, alkalinity, suspended solids (SS), volatile suspended solids (VSS), all according to standard methods (APHA, 1998). Gas composition (CO_2 and CH_4) was determined using a gas analyzer (Model GA, 2000). The measurement of SS and VSS was adapted from the procedures described in section 2540-D and 2450-E of standard methods (APHA, 1998). In order to determine SS, GF/A filter papers were placed in an oven at 104°C for 15 min and then heated at 550°C in muffle furnace for 10 min before taking the weight. A suitable sample was filtered and placed in an oven at 104°C for 1 h and the resulting weight was recorded for the SS. As for the VSS measurement, the filter paper and contents from the above SS analysis was placed in furnace at 550°C and the final weight was then recorded on removal from the furnace.

Reactor operation

The multi-stage anaerobic reactor was operated in continuous mode of operation with influent COD concentration of 1000 mg.L^{-1} for a period of 34 days (Table 2). A synthetic (glucose) wastewater substrate was prepared daily during reactor start-up and sampling of effluents was taken every two days throughout the operational period. The intermittent operation was performed with a feed period of 12 h with HRT of 1.4 day, followed by 12 h without feed for sludge stabilization. The influent COD of the reactor was varied from 1000 to 3000 mg.L^{-1} in order to obtain a series of OLR in the multi-stage anaerobic reactor system (Table 2). This intermittent feeding strategy was also recommended by Lettinga and Hulshoff Pol (1991) for complex wastewater.

The intermittent operation consists of an interruption of the reactor feeding during a certain amount of time (feed less or stabilization period), allowing a more complete biological degradation of the substrates accumulated in the sludge bed during the feed period (Nadais et al., 2005).

Table 1. Composition of macronutrient N100.

Parameter	Concentration
Crude protein (min)	5%
Crude fat (min)	2%
Crude fibre (max)	8%
N.free extract	45%
Calcium	2%
Phosphorus	1%
Magnesium	0.50%
Sulfur	2%
Potassium	2%
Salt	2%
Iron	0.08%
Iodine	0.03%
Boron	0.018%
Cobalt	0.0008%
Copper	0.0005%
Fluorine	0.015%
Riboflavin	8.00 mg
Manganese	0.09%
Molybdenum	0.0012%
Selenium	0.00002%
Zinc	0.005%
Vitamin A	50,000 IU
Vitamin D	3,000 IU
Vitamin E	150 IU
Vitamin K	1.00 mg
Vitamin B12	0.04 mg
Ascorbic acid	1500.00 mg
Biotin	0.30 mg
Choline	50.00 mg
Folic acid	0.30 mg
Niacin	25.00 mg
Panthothenic acid	0.20 mg
Thiamin	3.00 mg

RESULTS AND DISCUSSION

The influent substrate concentration in the multi-stage anaerobic reactor was in the range of 1000 to 3000 mg COD.L^{-1} (Figure 3). The effluent COD concentration in all stages of the multi-stage anaerobic reactor fluctuated corresponding to the OLR applied. Figure 4 shows the

Table 2. Reactor operating conditions during the start-up of multi-stage anaerobic reactor.

Operation mode	COD* ($mg.L^{-1}$)	HRT (d)	OLR ($kg\ COD.m^{-3}.d^{-1}$)	Operating duration (d)
Phase 1 (continuous)	1000	1.0	1.07	34
Phase 2 (intermittent)	1000	1.4	0.82	14
	1500	1.4	1.22	14
	2000	1.4	1.63	14
	3000	1.4	2.45	14

*Provided by glucose.

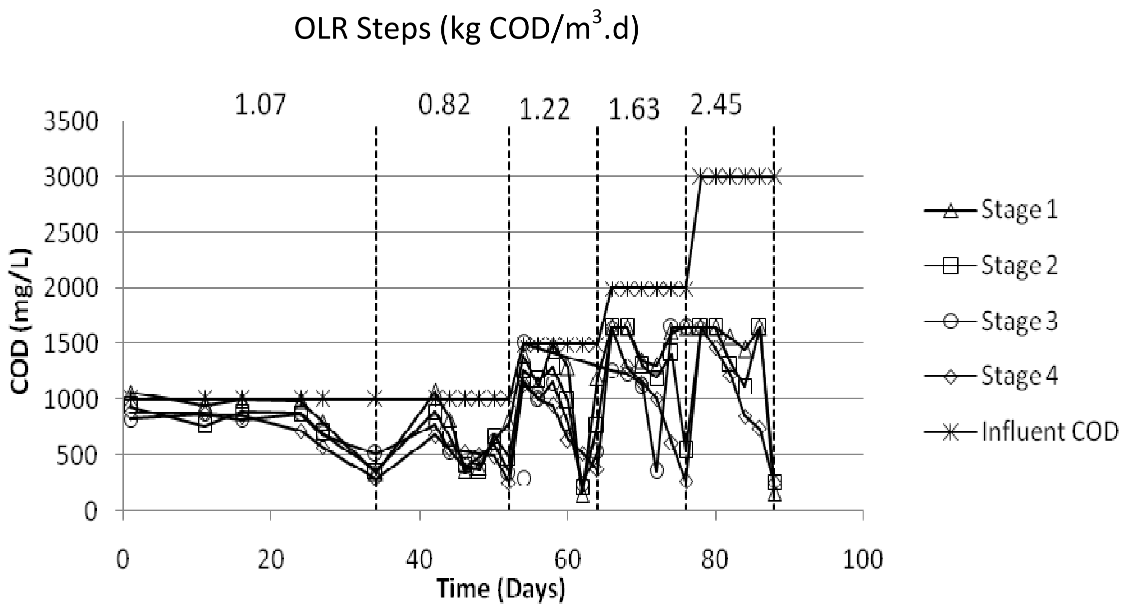

Figure 3. COD profile in each stage of reactor system at different OLR.

total COD removal efficiency and effluent pH levels during the reactor start-up. When the reactor was operated with continuous feeding (Phase 1), up to 71% COD removal efficiency was observed in the reactor system. It was found that the continuous feeding method during reactor start-up could not achieve the desired COD removal. Accordingly, it was thought that the reactor could perform better at intermittent feeding and therefore subsequent operations on the effect of OLR was carried out using this operational mode. During this intermittent feeding (Phase 2), the COD removal efficiency increased from 75 to 92% (at highest point), indicating better reactor performance when the OLR was increased gradually from 0.82 to 2.45 kg $COD.m^{-3}.d^{-1}$. This result clearly indicates that the intermittent operation of the multi-stage reactor led to a more complete biological degradation of the organic matter, and a better adaptation of the biomass for the degradation of the substrates. Similar observation was also reported by Nadais et al. (2011) during the intermittent treatment of synthetic wastewater using an UASB reactor, where the methane production rate was higher with the intermittent operation than with the continuous mode.

The COD removal profile across the reactor followed the order Stage 1>Stage 2>Stage 3>Stage 4. Most of the COD removal in the reactor system occurred in Stage 1, with smaller amounts occurring in the subsequent stages, which is a common pattern in staged anaerobic treatment, for example, in an ABR treating industrial wastewater (Uyanik et al., 2002; Bell, 2002).The highest COD removal efficiency (up to 92%) was achieved when the reactor was operated at OLR of 2.45 kg $COD.m^{-3}.d^{-1}$. A steady state of COD removal of more than 80% is considered acceptable for anaerobic reactor start-up and acclimatization (Enright et al., 2005; Buitrón et al., 2003).

One important observation is that the pH levels (Figure 4) in all stages of the reactor system showed significant fluctuation (pH 4 to 9), indicating difficulties in maintaining the desired pH levels (6.6 to 7.7) for an anaerobic reactor (Rittmann and McCarty, 2001). In order to maintain the pH levels, sodium hydroxide (NaOH) was added to the reactor system; however, this did not help recover the

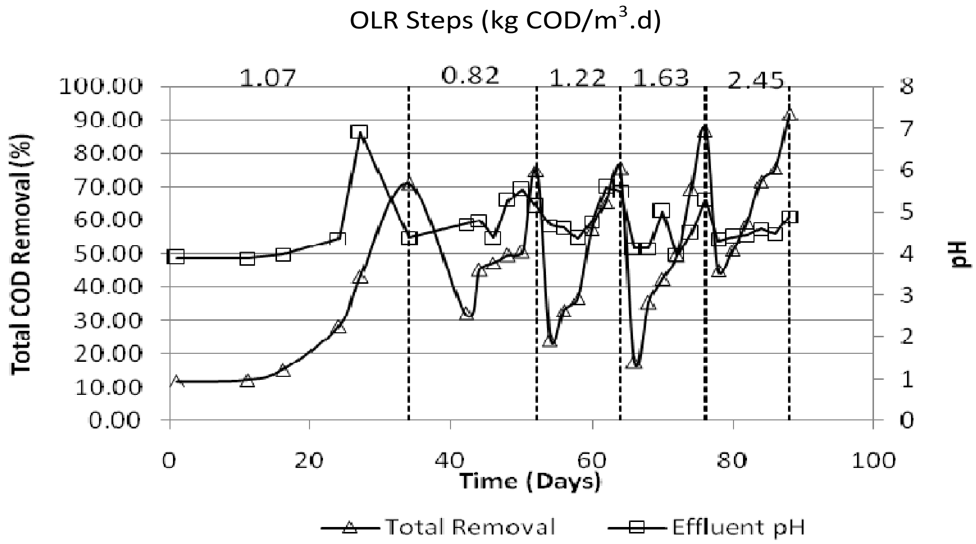

Figure 4. Total COD reduction (%) and effluent pH of multi-stage anaerobic reactor at different OLR.

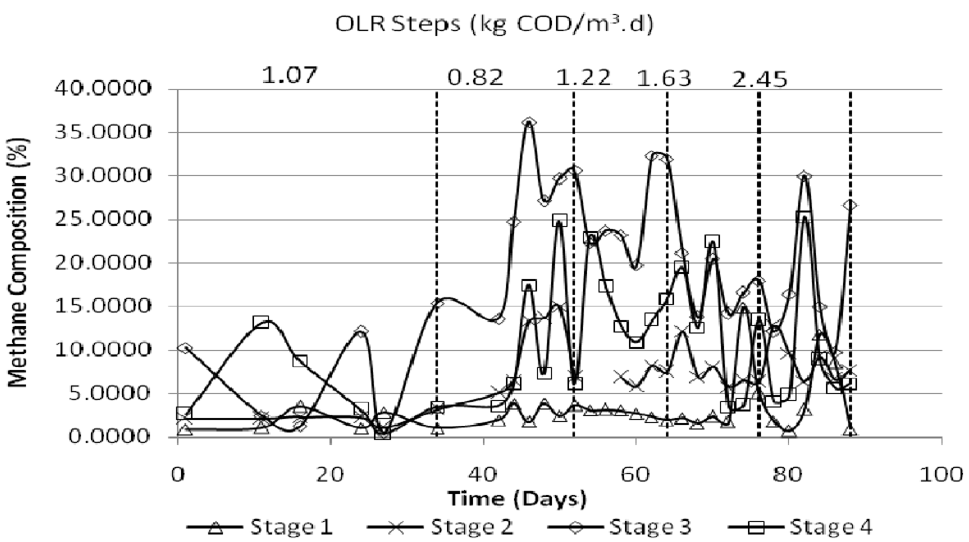

Figure 5. Proportion of CH_4 (%) in the biogas in each stage of multi-stage anaerobic reactor at different OLR.

required pH values. Even though low pH levels were noted during the operational period, high COD removal efficiencies confirm the ability of the multi-stage anaerobic reactor configuration to overcome the adverse effect of pH. One possible explanation to this could be due to the low HRT (1 to 1.4 d) applied to the reactor system. A short contact time between the substrate and biomass has been shown to favour acidogens which have faster growth kinetics and adapt better to reduced pH than the methanogens (Nachaiyasit and Stuckey, 1997a, b). In addition, excess VFA concentrations in the effluent may have contributed to improper balance between acidogenesis and methanogenesis owing to the dominance of the acidogenic process and suppression of methanogenic activity (Deng et al., 2008).

In theory, the reactor system should contribute phase separation; acidogenesis occurring in the up-stream stages and methanogenesis in the down-stream stages. However, this was not observed in the reactor, and all the stages were dominated by acidogens. Even though it was expected that the multi-stage anaerobic reactor would be stable at high OLRs, it was not able to withstand the short HRT (1 to 1.4 d).

Figure 5 shows the methane composition in each stage of the multi-stage anaerobic reactor. The methane composition of the reactor system fluctuated in all stages,

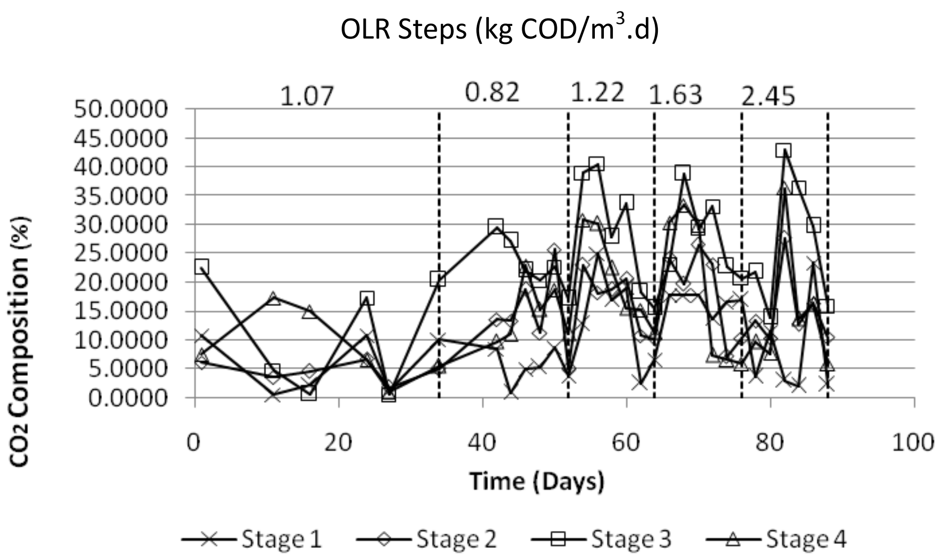

Figure 6. Proportion of CO_2 (%) in the biogas in each stage of multi-stage anaerobic reactor at different OLR.

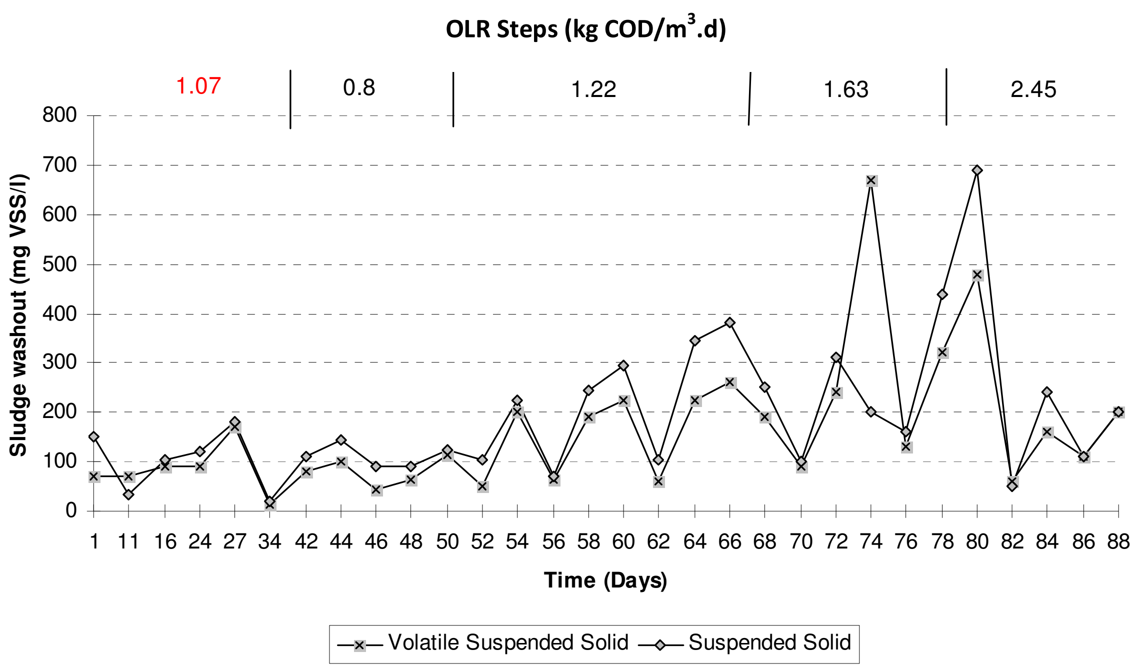

Figure 7. Solid washout from multi-stage anaerobic reactor at different OLR.

with stage 1, having the lowest composition. The highest methane composition was produced in stage 3 of multi-stage anaerobic reactor (36.1% at OLR 0.82 kg COD.m^{-3}.d^{-1}). Carbon dioxide (CO_2) composition showed similar pattern to those in methane composition profile (Figure 6). The highest CO_2 composition (42.9%) was found in stage 3 at an OLR of 2.45 kg COD.m^{-3}.d^{-1}. The presence of CO_2 in the reactor will increase the acid concentration in sludge and may cause drop of pH value (Gerardi, 2003). High level of the CO_2 composition can affect the pH profile. Moreover, higher CO_2 content may results from lack of proper balance among food supply, temperature and digestion time (Stronach et al., 1986). The lower levels of methane composition may be due to the effect of pH in the reactor system, which was not stable.

The sludge washout from the reactor system was measured frequently during the experimental period and Figure 7 shows the profile of VSS and SS in the effluent during reactor start-up. The average solid washout (VS. during the entire operational period (1.07 to 2.45 kg

Table 3. Substrate utilization rate (SUR) at different organic loading rate (OLR) of multi-stage anaerobic reactor during intermittent feeding

SUR (kg COD.kg VSS.d^{-1})	OLR (kg COD.m^{-3}.d^{-1})
5.1	0.82
3.8	1.22
3.2	1.63
3.8	2.45

COD.m^3.d^{-1}) was 150 mg.L^{-1}, confirming that the three phase separator baffle prevented solids washout from the reactor system. However, there was a major increase in the solid washout during the period of higher OLRs (675 and 695 mg.L^{-1} at OLR of 1.63 and 2.45 kg COD.m^3.d^{-1}, respectively) due to irregular flow rate (technical problem with the feed pump) during this period.

Table 3 shows the substrate utilization rate (SUR, kg COD.kg VSS.d^{-1}) during the intermittent feeding process at various OLR. When the reactor was operated at OLR of 0.82 kg COD.m^{-3}.d^{-1}, the SUR was 5.1 kg COD.kg VSS.d^{-1}. However, when the OLR was increased to 1.22 and 1.63 kg COD.m^{-3}.d^{-1}, the SUR showed some reduction (3.8 and 3.2 kg COD.kg VSS.d^{-1}, respectively). Nevertheless, this was not permenant; as the OLR was increased further (2.45 kg COD.m^{-3}.d^{-1}), the SUR increased back to 3.8 kg COD.kg VSS.d^{-1}. This confirm that although the solid wash out during this period of high OLR was substantial (Figure 7), the high SUR indicated the effectiveness of the sludge that was used in the treatment system to degrade the substrate.

Conclusions

The intermittent feeding during reactor start-up shows better performance compared to continuous feeding. At an OLR of 2.45 kg COD.m^{-3}.d^{-1}, up to 92% COD removal efficiency was observed in the multi-stage anaerobic reactor, indicating optimum operational condition for reactor start-up. It has been suggested that intermittent operation causes a forced adaptation of the biomass towards the degradation of the substrates. However, low pH values affected the performance of the reactor during each step increases in the OLR. To improve the performance, it is always a good practice not to let the pH in the anaerobic reactor reduced to a value less than 6.5. Maintaining a suitable and stable pH within the reactor should be a major priority for ensuring efficient methanogenic digestion. Although COD degradation efficiency might be affected by the lower pH, long HRT in the reactor system can lessen these effects. In addition, the load values applied during the start-up depend on the type of seed sludge employed and on its acclimatization to the wastewater to be treated. The initial load should be gradually increased according to the efficiency of the system. Further work will be carried out to increase the performance of the multi-stage anaerobic reactor.

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