Impact of Solid Accumulation on Anaerobic Treatment of High Strength Molasses Ethanol Wastewater using a Two Phase Semi-Continuously Stirred Tank Reactor

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Abstract: Anaerobic treatment of molasses stillage generated from an ethanol production plant was investigated in a two phase semi-continuously stirred tank reactor (semi-CSTR) operated under mesophilic conditions (37°C). Molasses stillage used in this study was a highly polluted wastewater having 150-200 g L^{-1}chemical oxygen demand (COD), 146.48 g L^{-1} total solid (TS) and 110 g L^{-1} suspend solid (VSS). Furthermore, this wastewater contained a high amount of potassium, calcium and magnesium (9.6–9.9, 5.6–6.0 and 0.9–1.5 g L^{-1}, respectively). The two phase semi-CSTR was operated at an organic loading rate (OLR) of 1.2–3.54 kg COD m^{-3} day^{-1} and a hydraulic retention time (HRT) of 140-40 days. The results showed that the average methane yield obtained was 0.3 m^3 CH_4 kg^{-1} $COD_{removed}$ with 65% CH_4 in biogas. The COD removal efficiency decreased from 97% to 68% with an increase in OLR. When the system had been operated for 90 days or at OLR of 3.71 kg COD m^{-3} day^{-1} and HRT of 38 days, the accumulation of solid was observed in the reactors. The concentration of SS in the first and second phase reactors was 20 and 27.55 g L^{-1}, respectively. These concentrations increased by 2.49 and 3.43 times, respectively, compared to the initial solid concentration of each reactor. The ratios of VSS to SS of the second phase reactor decreased significantly while those of the first phase reactor were not significant. The ratios of VSS to SS of the first and second phase reactors decreased from 0.98 to 0.92 and 0.98 to 0.68, respectively. The solid in the first phase reactor consisted of mostly SS coming from influent feed whereas that in the second phase reactor consisted of inert material. The inert material was generated by the precipitation of calcium because of a high alkalinity inside the reactor. The average concentrations of calcium in the solid of the first and second phase reactors were 136.9 and 1,712 mg kg^{-1}, respectively. The specific methanogenic activity of the sludge in the second phase reactor decreased from 0.155 to 0.004 g COD_{CH4} g^{-1} VSS day^{-1}. The COD removal efficiency of the system dropped to 58%. The volatile acid to alkalinity ratio of the second phase reactor was higher than 0.4.

Keywords: Anaerobic digestion, Ethanol wastewater, Molasses stillage, Solid accumulation, Two phase CSTR.

1. Introduction

Bioethanol is a renewable energy source that can be used as motor fuel, mainly as a biofuel additive for gasoline. In Thailand, there are several ethanol fuel mixtures in use e.g. E10, E20 and E85 [1]. In 2009, total ethanol production of Thailand was 400.7 million liters or 1.1 mL day^{-1}. The top feedstocks for ethanol production are sugarcane molasses and tapioca-starch. Molasses account for 60% to 70% of the feedstock used for ethanol production [2]. During the distillation process of ethanol production from molasses large volumes of stillage wastewater are produced (8–15 liters per 1 liter of ethanol produced) [3]. Molasses stillage is one of the most troublesome and polluted organic industrial effluents, having extremely high chemical oxygen demand (COD) of 100–200 g COD L^{-1}. In addition, it contains high amounts of suspended solid, sulfate, potassium, calcium and magnesium [4]. Until now, anaerobic digestion is one of the most promising technologies for removing the COD of this wastewater. Traditionally, molasses stillage has been treated using anaerobic open lagoons. However, the methane emissions from these lagoons could offset a significant portion of the Green House Gas (GHG) reduction from the use of bioethanol. Recently, anaerobic closed systems have become more interesting because methane produced from stillage can be used as an energy source in ethanol production plants. This will make the entire bioethanol production more profitable and more environmentally friendly. Various anaerobic processes have been studied to improve the treatment efficiency of molasses stillage, such as up-flow anaerobic sludge blanket reactors [5-10], anaerobic filters [11-12], two-stage continuously stirred tank reactors [13-17], anaerobic hybrid reactors [18-19], etc. However, most studies investigated the treatment of low-medium strength molasses stillage. The results showed that COD removal efficiencies of >70% were achieved at OLRs in the range of 5–36 kg COD m^{-3} day^{-1} when molasses stillage was diluted to a COD concentration of <50 g L^{-1}. Therefore, to overcome inhibition of high COD, sulfate, potassium, calcium and magnesium, large volumes of water are required. In Thailand, most of the treatment plants of molasses ethanol wastewater are fed with undiluted stillage. Therefore, the anaerobic systems treating this wastewater have been operated at low organic loading rates and a long residence time due to its characteristics [20]. Until now, there are few studies that report the performance of anaerobic processes treating undiluted stillage. Therefore, the aim of this research is to study the feasibility of treating undiluted molasses stillage in two phase semi-continuously stirred tank reactors (semi-CSTR). The effect of operational parameters on the efficiencies of COD removal and methane production and also on the TVA and solid accumulation were investigated and discussed.

2. Experimental

2.1 Substrate and inoculum

The molasses stillage was obtained from the bottom of the stripper column of the ethanol production plant in Nakhon-Sawan province, Thailand. It was stored at 4°C until used. The characteristics of the feedstock are presented in Table 1. The inoculum sludge was taken from an anaerobic fixed film reactor treating cassava starch wastewater. The inoculum had 39 g L^{-1} of volatile suspended solid (VSS) and a specific methanogenic activity of 0.155 g COD L^{-1} day^{-1}.

2.2 Reactor setup and operation

The two-phase system was composed of two semi-continuously stirred tank reactors (semi-CSTR) of different volumes. The reactors were double-walled transparent acrylic cylinders that had been fitted with acrylic plates on the top and bottom. The top plate supported the mixer, mixer motor, feed tube and gas tube. The hydrolysis/acidification phase (LM1) was performed in a reactor that had a volume of 8.5 L with an internal diameter of 19 cm and a height of 30 cm. The reactor had an active volume of 7.38 L. The methanogenic phase (LM2) was carried out in a 15.37 L reactor that had an internal diameter of 24 cm and a height of 34 cm. The reactor had an active volume of 14.1 L. They were maintained at 37°C by circulating water through a water jacket from a temperature-controlled water bath. The reactors were operated in semi-continuous mode using the withdraw/feed method once a day, and they were mechanically stirred at 100 rpm using an electric motor for 15 minutes at 4- hour intervals. The two-phase system was operated with step-wise increases in the organic loading rate and at an initial OLR of 1.2 kg COD m^{-3} day^{-1}.

2.3 Analysis

The system performance was monitored by routine measurement of the parameters: pH, total volatile acid (TVA), COD, TS (Total solid), TDS (Total dissolved solid), SS (suspended solid), sulfate, TKN, ammonia-nitrogen and total phosphate following the standard methods [21]. Influent COD was measured prior to being applied to the system. Total alkalinity was measured by titration to pH 4.0, using 0.1 M H_2SO_4. Total sugar concentration, reducing sugar concentration and alcohol were determined by the phenol sulfate method, Somogyi-Nelson method and FAS-titration method, respectively, according to AOAC methods [22]. The analysis of calcium, potassium and magnesium were performed using atomic absorption spectrometry with a Shimadzu apparatus model AA-6300. The daily production of biogas from each reactor was measured using a liquid displacement system that was connected to the reactor, and the gas production was recorded automatically. The percentages of methane and carbon dioxide in the biogas were analyzed using gas chromatography (Shimadzu, Class-GC 14B, Japan), using a Porapak-N column equipped with a thermal conductivity detector (TCD). The oven, injector and detector temperatures were 70, 120 and 120°C, respectively. Helium was used as the carrier gas at a flow rate of 30 mL min^{-1}.

Specific methanogenic activity (SMA) of sludge was measured in triplicate using 120-mL serum bottles with a total liquid volume of 100 mL at 37°C under strictly anaerobic conditions. The sludge to substrate ratio used was 2:1. Acetic acid solution (1 g L^{-1}) was used as the sole substrate after being adjusted to pH 6.8–7.2 using 6 M NaOH. The basal medium used in this study contains (g L^{-1}): KH_2PO_4, 0.4; K_2HPO_4, 0.4; $MgCl_2$, 0.1; NH_4Cl, 1; yeast extract, 1; L-cysteine HCl, 0.5; $Na_2S{\cdot}9H_2O$, 0.5; $NaHCO_3$, 5; along with 10 mL mineral solution, and 10 mL vitamin solution. The mineral solution contains (g L^{-1}): nitrotriacetic acid, 4.5; $FeCl_2{\cdot}4H_2O$, 0.4; $CoCl_2{\cdot}6H_2O$, 0.12; $AlK(SO_4)_2$, 0.01; NaCl, 1.0; $CaCl_2$, 0.02; Na_2MoO_4, 0.01; $MnCl_2{\cdot}4H_2O$, 0.10; $CuSO_4{\cdot}5H_2O$, 0.01; and $NiCl_2{\cdot}6H_2O$, 0.02. The vitamin solution contains (mg L^{-1}): biotin, 2; folic acid, 2; pyridoxine HCl, 10; thiamine HCl, 5; riboflavin, 5; nicotinic acid, 5; DL-calcium pantothenate, 5; vitamin B12, 0.1; *p*-aminobenzoine, 5; and lipoic acid, 0.5. SMA was calculated from the linear range of the specific methane production rate curve using linear regression.

3. Results and Discussion

3.1 Molasses stillage characteristics

Table 1, about here, shows the analyzed composition of molasses stillage coming from the bottom of the stripper column of the ethanol production plant. The stillage had very high levels of BOD (53–77 g L^{-1}), COD (150–163 g L^{-1}) and COD/BOD ratio (2.13–2.86). Its pH value ranged between 4.25–4.3. Molasses stillage contained much higher amounts of total sugar than reducing sugar. Part of the total sugar was non-fermentable sugar, generated from the crystallization and evaporation processes of the sugarcane mill factory, which partly results in an increase in the COD/BOD ratio [4]. Stillage contained high concentrations of easily degradable organic materials, such as reducing sugar and volatile acids, indicating its suitability for anaerobic digestion. The solids content of the stillage was rather high, consisting of 146.48 g L^{-1} total solid, 110 g L^{-1} volatile solid, 36.48 g L^{-1} fixed solid, 11.85 g L^{-1} suspended solid and 10.4 g L^{-1} volatile suspended solid. Nitrogen content in the stillage was found to be sufficient for anaerobic bacteria involved in biogas production processes. However, high levels of sulfate (>6 g L^{-1}) might cause an inhibitory effect on methanogenesis. The inhibition is due to the toxicity of sulfide and the competition for common organic and inorganic substrates from SRB, which suppresses methane production [23-24]. Furthermore, molasses stillage had a high concentration of potassium (9.6–9.9 g L^{-1}), calcium (5.6–6 g L^{-1}) and magnesium (0.9–1.5 g L^{-1}). These high values might cause the inhibition of anaerobic process. For instance, excessive amounts of calcium lead to the precipitation of carbonate and phosphate, which may result in scaling of reactor and reduced specific methanogenic activity [25-26].

Table 1. The characteristics of the molasses stillage used in this study.

PARAMETERS	CONCENTRATION	UNIT
pH	4.29 ± 0.01	-
Total COD	160.00 ± 2.73	g L^{-1}
Soluble COD	148.00 ± 1.53	g L^{-1}
BOD	64.95 ± 12.00	g L^{-1}
Total sugar	48.12 ± 0.31	g L^{-1}
Reducing sugar	12.22 ± 0.05	g L^{-1}
Alcohol	0.34 ± 0.01	%V/V
Total Volatile Acid	15.00 ± 0.20	g L^{-1} as acetic acid
Alkalinity	2.29 ± 0.04	g L^{-1} as $CaCO_3$
TS	146.48 ± 0.36	g L^{-1}
TVS	110.00 ± 0.28	g L^{-1}
Fix solid	36.48 ± 0.10	g L^{-1}
SS	11.85 ± 0.52	g L^{-1}
VSS	10.40 ± 0.64	g L^{-1}
TDS	134.00 ± 0.53	g L^{-1}
DVS	100.42 ± 0.65	g L^{-1}
TKN	2,638 ± 34	mg L^{-1}
Ammonia-Nitrogen	297 ± 8	mg L^{-1}
Total phosphate	907 ± 36	mg L^{-1}
Sulfate	5,959 ± 140	mg L^{-1}
Potassium	9,751 ± 163	mg L^{-1}
Sodium	679 ± 19	mg L^{-1}
Calcium	5,833 ± 223	mg L^{-1}
Magnesium	1,260 ± 266	mg L^{-1}
Chloride	4,310 ± 32	mg L^{-1}

3.2 Performance of the two-phase semi-CSTR

Fig. 1 (A-C) shows the operational conditions and performance of the two phase semi-CSTR operated for 90 days, namely HRT, OLR, COD concentrations, COD removal efficiency, pH, TVA and alkalinity.

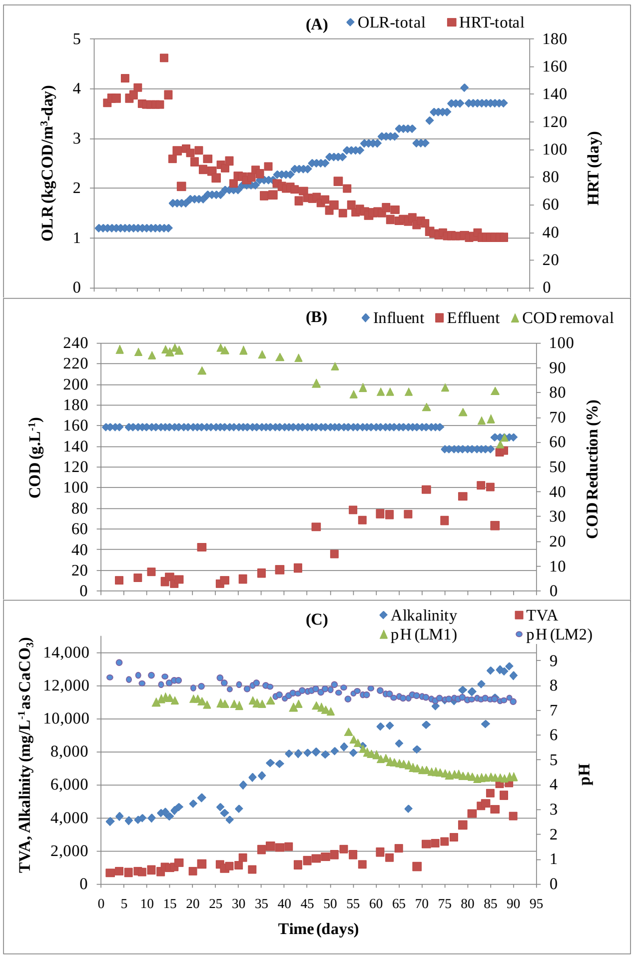

Figure 1. Variation of performance parameters with operating time of the two phase semi-CSTR: (A) COD concentration and COD removal efficiency; (B) OLR and HRT; (C) Alkalinity, TVA and pH.

The two phase semi-CSTR was started up at an OLR of 1.2 kg COD m^{-3} day^{-1} and a HRT of 140 days (Fig. 1A). The COD removal efficiency was 97% during the first 17 days of operation. Afterwards, the OLR had a stepwise increase from 1.7 to 2.91 kg COD m^{-3} day^{-1} resulting in a shorter HRT (from 92 to 54 days). The COD removal slightly decreased from 97% to 80% (Fig. 1B). When a higher OLR of 3.05 kg COD m^{-3} day^{-1} was applied by decreasing the HRT from 55 to 50 days (days 67–74), the average COD removal efficiency declined to 74% (Fig. 1B). At the same time, the TVA in the effluent rose to >2,000 mg L^{-1} (Fig. 1C). The subsequent decrease in the OLR to 2.91 kg COD m^{-3} day^{-1} resulted in a rapid recovery of the system, as can be seen from the increase in COD removal efficiency (Fig. 1B). However, a high concentration of TVA in the effluent was still observed (>2,500 mg L^{-1}) (Fig. 1C). At an OLR of 3.54 kg COD m^{-3} day^{-1} (days 79–81), the COD removal efficiency gradually decreased from 82% to 72% when the concentration of TVA increased from 3,500 to 4,200 mg L^{-1}. When the OLR was further increased to 3.71 kg COD m^{-3} day^{-1} (days 82–90), the performance of the system considerably deteriorated. The efficiency of COD removal sharply dropped to 58.7%. Simultaneously, a serious accumulation of TVA was observed in the range of 4,700–6,000 mg L^{-1} (Fig. 1C), leading to a high ratio of TVA/alkalinity (up to 0.56). The high TVA/alkalinity ratio suggested that the buffering capacity in the reactor was poor. Callaghan et al. [27] reported that at a TVA/alkalinity ratio of less than 0.4, the reactor should be considered as stable. The high ratio of TVA/alkalinity (>0.4) in the effluents was observed when the reactors had been operated for 84 days (OLR of 3.71 kg COD m^{-3} day^{-1}). Although a high ratio of TVA/alkalinity was observed, the pH of the effluent of LM2 was in the range of 7.5–8.5 throughout the experiment. The pH in the LM1 was within the range of 7.0-7.3 when the system was fed at an OLR of 1.2 to 2.51 kg COD m^{-3} day^{-1} (days 0–54). These pH values are within the optimal pH for methanogenesis. When the OLR was increased to 2.63 kg COD m^{-3} day^{-1}, the pH in the LM1 decreased from 5.8 to 4.3. The acidic pH had an inhibitory effect on the methanogenic bacteria, as a result the LM1 completely changed to be an acidification phase reactor.

Fig. 2 shows the variation of biogas and methane yields with the loaded OLR. A linear increase in the methane yield was obtained when the OLR increased from 1.2 to 2.39 kg COD m^{-3} day^{-1}. The average biogas and methane yields obtained were 0.373 and 0.245 m^3 kg^{-1} $COD_{removed}$, respectively. The methane content in the biogas was in the range of 60–65%, which was comparable to the values generally observed in molasses stillage treatment systems (65–80%) [20]. When the OLR was further increased to 2.63 kg COD m^{-3} day^{-1}, a drastic decrease in biogas and methane yields was observed, coinciding with the COD concentration of effluent sharply increasing by two times, compared to that of the previous OLR. When the pH value of the LM1 dropped to an acidic range, the methane yield was mainly produced from the LM2. The average biogas and methane yields were 0.171 and 0.118 m^3 kg^{-1} $COD_{removed}$, respectively. However, the biogas and methane yields gradually increased with an increase in the OLR (2.76–3.54 kg COD m^{-3} day^{-1}). The biogas and methane yields increased from 0.236 to 0.338 m^3 kg^{-1} $COD_{removed}$ and 0.123 to 0.224 m^3 kg^{-1} $COD_{removed}$, respectively. The obtained methane yield is similar to Cho [14] who reported that the methane yield of 0.2 m^3 kg^{-1} $COD_{removed}$ was achieved when the digester was fed with the influent COD concentration of 22.5 g L^{-1}. At higher an OLR of 3.71 kg COD m^{-3} day^{-1}, the biogas and methane yields dropped, corresponding to the deterioration of other parameters previously discussed. The biogas and methane yields were 0.298 and 0.192 m^3 kg^{-1} $COD_{removed}$, respectively.

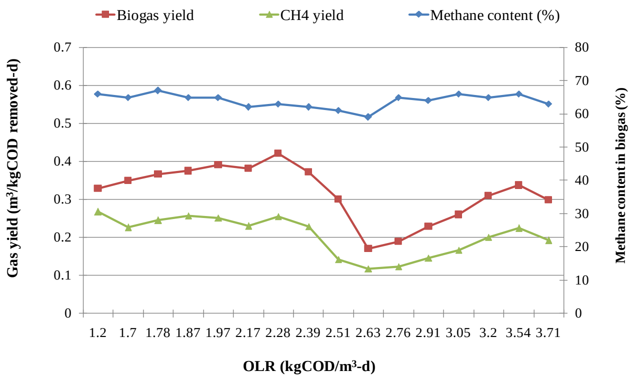

Figure 2. Specific biogas and methane yields and methane content in biogas plotted with OLRs during start up.

3.3 Solids accumulation in a two-phase semi-CSTR

Fig. 3 (A-B) show the solid concentrations in LM1 (A) and LM2 (B) plotted against the OLRs during the start up period. The solids content of the influent molasses stillage were rather high, containing 146.48 g L^{-1} total solid (TS), 110.00 g L^{-1} volatile solid (VS), 36.48 g L^{-1} fixed solid (FS), 11.85 g L^{-1} suspended solid (SS) and 10.40 g L^{-1} volatile suspended solid (VSS) (Table 1). The initial SS concentrations in LM1 and LM2 were 10.48 and 8.04 g L^{-1}, respectively. With respect to an increase in OLR, the concentration of solid in both reactors clearly increased. For example, in LM1 (Fig. 3A), where the TS concentrations were 107.44, 117.9 and 137.42 g L^{-1}, the VS were 69.4%, 81.69% and 75.29% of TS at OLR of 2.63, 3.05 and 3.71 kg COD m^{-3} day^{-1}, respectively. Regarding the SS concentrations, they increased by 4.30, 4.35 and 2.49 times initial concentration and VSS levels were 96.53%, 96.67% and 92.56% of SS at the respective OLR. At an OLR of 3.71 kg COD m^{-3} day^{-1}, a decrease of SS concentration was observed partly due to a wash out of inoculated microorganisms (data not shown). The accumulated SS mainly came from intact yeast cells in influent stillage. The hydrolysis of these cells required a much longer residence time than that used in this study [28]. In LM2 (Fig. 3B), all types of solids analyzed increased when the OLR was increased. Although some part of SS regularly washed out, the sludge bed height increased rapidly over time. At OLR of 2.63, 3.05 and 3.71 kg COD m^{-3} day^{-1}, the TS concentrations were 35.28, 44.85 and 76.86 g L^{-1} while the VS were 59.67%, 56.63% and 59.87% of TS at OLR of 2.63, 3.05 and 3.71 kg COD m^{-3} day^{-1}, respectively. SS was significantly accumulated as concentrations rose from: 14.72, 18.07 and 27.55 g L^{-1} at an OLR of 2.63, 3.05 and 3.71 kg COD m^{-3} day^{-1}, respectively. These concentrations increased by 1.83, 2.25 and 3.43 times compared to the initial concentration. The VSS were 97.83%, 92.25% and 68.06% of SS. The results revealed that the ratio of VSS to SS decreased significantly. This illustrated that inert materials were produced, especially at an OLR 3.71 kg COD m^{-3} day^{-1}. A decrease in the proportion of VSS in SS resulted from the formation of calcium precipitates in the sludge. The average concentrations of calcium in the sludge and supernatant at an OLR of 3.71 kg COD m^{-3} day^{-1} are shown in table 2. The calcium concentrations of sludge and supernatant in LM1 were 136.9±7.3 and 887.4±35.6 mg kg^{-1} while those of LM2 were 1,711.6±145.0 and 265.9±3.8 mg kg^{-1}, respectively.

Table 2. Average concentrations of calcium in sludge and supernatant at OLR of 3.71 kg COD m^{-3} day^{-1}.

Light metal ion	Reactor	Supernatant (mg kg^{-1})	Sludge (mg kg^{-1})
Calcium	LM1	887.4±35.6	136.9±7.3
	LM2	265.9±3.8	1,711.6±145.0

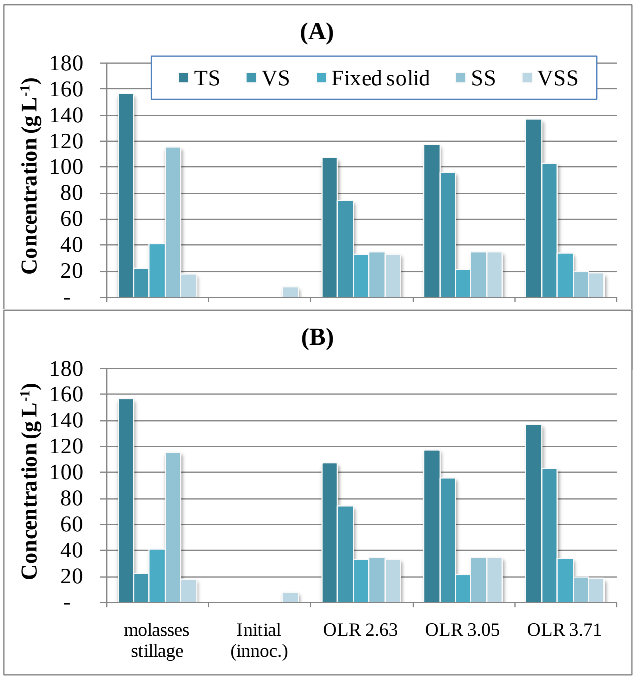

Figure 3. Solid concentrations in LM1 (A) and LM2 (B) plotted with OLRs during start up.

Van Langerak et al. [29] found that treatment of calcium-rich wastewater (780-1,560 mg L^{-1}) led to the development of anaerobic sludge containing a high level of ash content. Azbar *et al*. [30] reported that the pH gradient has an impact on the solubility of inorganic ions. For instance, calcium was found to be precipitated effectively inside an anaerobic reactor with a high alkalinity. In LM2, the pH values were within a range of 7.2–8.5 and alkalinity varied in the range of 4,000-13,000 mg L^{-1} as $CaCO_3$. Not only inert materials but also VSS, which were intact yeast cells, occupied the active volume of the reactor. The yeast cells comprising of a complex matrix of phosphomannans, glucans, chitin and protein resulted in a difficulty in their biodegradation, therefore, their presence may deleteriously affect the anaerobic digestion process [31]. The high level of solids inside LM2 resulted in the reduction of HRT and the reaction time between microorganism and substrate in wastewater. Furthermore, the inactive solids surrounding the active biomass resulted in the limitation of substrate transfer [32]. At OLR of 3.71 kg COD m^{-3} day^{-1}, the system performance deteriorated as mentioned above. In addition, the specific methanogenic activity of the sludge in LM2 decreased from 0.155 to 0.004 g COD_{CH4} g^{-1} VSS day^{-1}.

4. Conclusions

The results of this study demonstrate the feasibility of using anaerobic two phase semi-continuously stirred tank reactors for treating undiluted molasses stillage having COD of 150-200 g L^{-1}. The average COD reduction efficiency was 69% and the methane yield obtained was 0.3 m^3 CH_4 kg^{-1} $COD_{removed}$ with 65% CH_4 in biogas at OLR and HRT in the ranges of 1.2–3.54 kg COD m^{-3} day^{-1} and 140-40 days, respectively. The performance of the system deteriorated dramatically after 90 days of operation (OLR was 3.71 kg COD m^{-3} day^{-1} and HRT of 38 days). The specific methanogenic activity of sludge in the methanogenic reactor obviously decreased. This was likely due to the occupation of the reactor's active volume by the slowly biodegradable SS in molasses stillage and inert solids caused by calcium precipitates.

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