INTERNATIONAL JOURNAL OF ENVIRONMENT

Volume-9, Issue-1, 2019/20 ISSN 2091-2854

Received: 2 Oct 2019 *Revised: 28 Dec 2019* *Accepted: 1 Jan 2020*

EVALUATION OF UASB REACTOR PERFORMANCE DURING START-UP OPERATION USING GLUCOSE BEARING SYNTHETIC WASTEWATER

Revanuru Subramanyam[1,2*]

[1]Department of Civil Engineering, The Papua New Guinea University of Technology, Lae 411, Morobe Province, Papua New Guinea

[2]Department of Chemical Engineering, Indian Institute of Technology, Roorkee - 247 667, India

*Corresponding author: revanuru.subramanyam@pnguot.ac.pg

Abstract

This research article describes start-up performance of an UASB (Upflow Anaerobic Sludge Blanket) reactor in terms of chemical oxygen demand (COD) removal efficiency, biogas production, sludge loading rate (SLR), volatile fatty acids (VFA), pH, alkalinity, total solids (TS) and volatile suspended solids (VSS), fed with synthetic wastewater with increased concentrations of glucose. The reactor was loaded up to an OLR (Organic Loading Rate) of 15 kg COD m^{-3} d^{-1} and achieved a COD removal efficiency of 82 ±3%. The results showed that digested seed sludge was successfully acclimatized and transformed finally into granular sludge within a period of 120 days. An increase in the accumulation of VFA at high OLRs showed that methanogenesis could be the rate-limiting step in the reactor operation. The SLR and VSS/TS ratio were increased with an increase in OLR. During the initial stages, uniform distribution of VSS concentration and later on maximum VSS concentration were found at port number two at a height of 350 mm. The carbon balance depicts that the maximum percentage of influent COD converted to methane COD. An increase in specific methanogenic activity values with the age of sludge confirmed the transformation of the seed sludge in to a granular sludge.

Keywords: Carbon balance; Glucose bearing synthetic wastewater; Sludge loading rate; Specific methanogenic activity; Volatile fatty acids.

DOI: http://dx.doi.org/10.3126/ije.v9i1.27432

1. Introduction

Upflow anaerobic sludge blanket (UASB) reactors have been documented as a robust technology worldwide because of its ability to fruitfully treat a wide variety of industrial wastewaters and sewage (Subramanyam and Mishra, 2013a). As a matter of fact, out of 1,599 commercial scale reactors built around the globe by January 2003 for treating different kinds of industrial wastewaters and sewage, 852 (almost 53%) are UASB reactors (Kleerebezem and Macarie, 2003). The UASB process was based on a simple technique of accumulation and concentration and compaction of a huge population of bacteria in the form of granulated sludge under favorable physico-chemical and environmental conditions. The natural turbulence caused by influent flow and the biogas released by the granules keeps adequate contact between substrate and biomass. The main advantages of these systems were capable of withstanding high loadings with relatively low detention times and no support material was needed for the immobilization of microorganisms, concurrently eliminates the cost of packing material (Subramanyam, 2007). Anaerobic digestion of complex organic matters to methane occurs in four phases: hydrolysis/liquefaction, acidogenesis, acetogenesis and methanogenesis. The first phase was hydrolysis, in which complex organic matters viz., carbohydrates, proteins and lipids were broken down into monomers by the extracellular enzymes released by microorganisms (e.g., cellulose, protease, lipase and amylase). During acidogenesis phase, sugars, long-chain fatty acids and amino acids resulting from hydrolysis were used as substrates by fermentative microorganisms to produce volatile fatty acids (VFAs), such as acetic, propionic, butyric and other short-chain fatty acids, alcohols, H_2 and CO_2. In acetogenesis phase, the obligate hydrogen-producing acetogenic bacteria further degrade to acetate, propionate, carbon dioxide and hydrogen. During methanogenesis phase, the methanogens utilize mainly H_2/CO_2 and acetic acid to form methane, CO_2 and other gaseous end products (Gujer and Zehnder, 1983). Thus, two groups of anaerobic bacteria (acidogens and methanogens) exist in sludge granules and maintain a delicate stable relationship.

The start-up of UASB reactors proceeds after inoculation of the reactor fed on organic substrate with the seed material. Careful selection of the inoculum was necessary for the formation of well-formed granular sludge. The presence of carrier materials for bacterial attachment helps in the initiation of the sludge aggregation. The suggested amount of seed sludge should be charged to the UASB reactor at the rate of 10-20 g volatile suspended solids (VSS) per litre reactor volume (Hickey et al., 1991). The recommended use of digested sewage sludge of fairly poor methanogenic activity (~ 0.05 kg CH_4-COD (kg VSS.d)$^{-1}$**)** as the inoculums (Lettinga et al., 1984); initial sludge loading rate (SLR) in the range of 0.05-1 kg COD (kg VSS)$^{-1}$ d^{-1} and the sludge volume index (SVI) to be ~50 mL g^{-1} of suspended solids or lower after the washout of the finely dispersed fraction of the sludge. Earlier start-up and granulation of biomass could be achieved using mixed sludge (anaerobic digested sludge: cow dung and aerobic sludge) compared to anaerobically digested sludge (Gupta and Gupta, 2005). The addition of external additives such as natural and synthetic polymers, and vitamins may enhance the start-up and characteristics of granular sludge (Subramanyam, 2013). In addition, favorable environmental conditions viz., neutral pH, temperature and very low toxicity/inhibition should be maintained in the reactor.

A wide variety of inocula such as a partially granulated anaerobic sludge obtained from pilot scale membrane bioreactor wastewater treatment plant (Haider et al., 2018); digester sludge from municipal wastewater treatment plant (Fang and Zhou, 1997; Subramanyam and Mishra, 2007) and sludge from mesophilic anaerobic digester (Tay et al., 2000) have been used to seed the UASB reactor. The start-up involves enhancement of initial activity of the inocula by feeding it with easily degradable carbon source such as sucrose, glucose and VFA mixture. The duration of start-up varies between 30 and 300 days depending upon the nature of the seed and the operating conditions maintained in the reactor used. Other alternatives involved in start-up of UASB reactor was self-inoculation and took 100 days when treating raw sewage at 29 ºC (Kalogo et al., 2001). If seed sludge was granulated/partially granulated then acclimation/granulation takes place in less time. The granulation process of a UASB reactor fed with a mixture of glucose and VFA: VFA consisted of acetic acid and propionic acid (3:1) and its concentration in the feed varied from 15 to 80% (Brito et al., 1997). They observed fairly loose structure of the sludge bed initially which was found to have granulated with increase in VFA.

The start-up of UASB reactor for industrial and municipal wastewater treatment requires surplus amount of granular sludge from functioning UASB reactors that is desirable to reduce start-up periods and enable the system to reach high removal efficiency. However, availability of high quality granular sludge was difficult in many situations and the use of digested sludge is a traditional solution. If the seed sludge was not granulated, the start-up periods were longer (Poh and Chong, 2009) and washout of finely dispersed flocculent sludge particles was a common problem. The start-up period was a critical step in the operation of UASB reactors and generally takes a longer period of time. Many researchers reported long start-up periods of 2-3 months to 1 year (or even more) for the anaerobic reactors depending upon the source and activity of seed sludge, sequence of operation, characteristics of wastewater to be treated, initial hydraulic retention time and sludge retention time along with many other factors. A higher degree of bacterial diversity may play an important role in the performance of anaerobic reactors during the start-up period (Oz et al., 2012). An unconventional start-up approach could offer a practical solution for the inherent long start-up in UASB systems with associated saving in time and cost (Show et al., 2004).

In view of the above circumstances, as buttressed by the literature review, there was lack of information and data on the effects of various operating parameters for successful formation of granulation. The start-up of the UASB reactor is considered as a major area of the research. In the above context, the present work addresses specific and detailed aspects of the UASB reactor performance during the start-up phase by fed with glucose bearing synthetic wastewater (SWW) under different organic loading rates (OLRs). The results could be extremely helpful for designing control systems for UASB reactors.

2. Materials and Methods

2.1 UASB reactor Set-up

The bench scale UASB reactor was fabricated from the plexiglass pipes and sheets, excepting the sampling and inlet/outlet ports, which were made of brass. The details of the reactor assembly and the experimental set-up are given in the past studies (Subramanyam, 2007; Subramanyam and Mishra, 2007). The whole reactor assembly was mounted on an angled iron frame and was supported through struts to keep the reactor in vertical position. The reactor assembly comprises of two independent modules: each of 600 mm length and 100 mm diameter fitted one above the other with the help of F-1 type flanges. Neoprene pads were provided in between the flanges to make the joints leak-proof. A gas-liquid-solid separator with an internal diameter of 150 mm and a height of 600 mm was fixed on to the top of the reactor. The feed inlet system comprised of four, 10 mm size brass nozzles around the circumference of the reactor at a height of 50 mm from the bottom, which were connected to a common feed manifold. Brass sampling ports of 10 mm size had been provided along the height of the reactor, each at a distance of 150 mm from one another. The effective reactor volume, excluding volume of the settler and gas-liquid-solid separator was 9.75 L. The laboratory scale UASB reactor was housed in a constant temperature chamber of size 1.2 m x 1.2 m x 2.8 m height made of plywood sheets supported on metallic frame. The reactor temperature was maintained at 35 ± 2 ^{0}C with the help of a thermostatic heating device, operated and controlled from the outside. The biogas generated in the reactor was collected by water displacement method in a variable volume, double drum gas collection system. These drums were made of aluminium. The outer drum was of 330 mm diameter and 315 mm height. The drum with smaller diameter of 260 mm was placed inverted inside the larger drum with the base at the top. A small port was provided at the centre of the base of the smaller drum and connected to the main gas vent of the reactor through a polythene tube. Both the drums were coated with acid resistant paint and the outer drum was filled with slightly acidified water. The outer wall of the inner drum was having a level indicator ribbon to indicate the level rise of the inner drum. This in turn was used to calculate the gas production. Biogas collected in the drum was analysed for composition using a gas chromatograph.

2.2 SWW

The SWW was prepared by adding a pre-calculated amount of glucose in tap water to get the required organic loading in terms of COD value. Macro and micro-nutrient solutions were prepared and added as recommended by Tay et al. (2001). Sodium bicarbonate was also added at a concentration of 500-2,000 mg L^{-1} to the feed solution to maintain the reactor effluent pH at 6.8 ± 0.2 during the entire experimental work.

2.3 Chemicals

The glucose was of analytical reagent grade and was supplied by Himedia (Mumbai, India). The remaining chemicals used in the experimental work were all of analytical reagent grade and supplied by Ranbaxy Laboratories (New Delhi, India), Himedia (Mumbai, India), Sd fine-chem (India) and Merck limited (Mumbai, India).

2.4 Analytical Techniques

The determination of the pH, COD, SVI, SS and VSS was carried out by following the standard procedure (APHA, 1998). Methane in the biogas stream was determined using a gas chromatograph (NUCON- 5700-GC, Nucon Engineers Ltd., New Delhi, India) equipped with a FID using porapak Q column (stainless steel column of I.D. 2 mm, length 2 m, 80-100 mesh porapak Q). The temperatures of the column, the injection port and FID were maintained at 60, 200, and 220 °C, respectively. The volatile fatty acids (VFA) and total alkalinity (TA) were determined by direct titration as per procedure suggested (Dilallo and Albertson, 1961) as follows: a 50 mL of sample was taken and centrifuged for 10 min at 6000 rpm, 25 mL of the supernatant was taken in a beaker and initial pH was measured, 0.1N H_2SO_4 was added to the aliquot of 25 mL to lower the pH down to 4.3. The TA (mg L^{-1} as $CaCO_3$) was calculated by multiplying mL of 0.1N H_2SO_4 (used up to pH 4.3) and 200. The titration was continued till the pH of the sample lowered from the range of 3.5–3.3. Then, sample was gently boiled for 3 min and then cooled down to the room temperature. Subsequently 0.05N NaOH was added to it to raise the pH up to 4.0 first and then to pH 7.0. The volume of NaOH consumed in raising the pH from 4.0 to 7.0 was noted down. The VFA alkalinity (mg L^{-1} as $CaCO_3$) was calculated by multiplying mL of NaOH consumed with 100. If VFA alkalinity was less than 180, then it considered as VFA concentration in mg L^{-1} as HAc. Otherwise if VFA alkalinity was more than 180, then it was multiplied by 1.5 to get VFA (mg L^{-1} as HAc). Biogas evolved was collected by water displacement method. The specific methanogenic activity (SMA) test was conducted in accordance with the procedure suggested (Isa et al., 1993).

2.5 Carbon Balance

A carbon balance in terms of COD around the reactor can be made by considering the COD in the influent and effluent streams, gaseous CH_4-COD, dissolved CH_4-COD, COD on VFA and the COD unaccounted for on any day of operation of the UASB reactor. Average values for COD removal efficiency, biogas and methane production (L d^{-1}) and effluent VFA (as acetic acid, mg L^{-1}) can be taken for any day of operation under a specific feed condition and reactor operating conditions.

In general, the COD balance around an UASB reactor operating under steady state condition can be written as:

$$COD_{in} = CH_4\text{-}COD_{sol} + CH_4\text{-}COD_{gas} + COD_{eff} + VFA\text{-}COD + COD_{unacc} \quad (1)$$

Where,

COD_{in} = Influent COD of the UASB reactor (g d^{-1}).

CH_4-COD_{sol} = COD equivalent of methane in the solution form (dissolved) in the UASB reactor as observed in the effluent (g d^{-1}). (The dissolved methane escapes to the atmosphere when the pressure is reduced or the reactor effluent is exposed to atmosphere).

CH_4-COD_{gas} = The COD equivalent of the methane collected in the gas collection device (g d^{-1}).

COD_{eff} = COD of the effluent of the UASB reactor (g d^{-1}), as obtained from the influent COD and the COD removed in the reactor.

VFA-COD_{eff} = COD equivalent of VFA in the UASB reactor effluent (g d^{-1}).

$COD_{unacc.}$ = Unaccounted COD (i.e. COD associated with the biomass) (g d^{-1}).

COD_{in} is calculated as follows:

$$COD_{in}\ (g\ d^{-1}) = \text{Influent COD}\ (g\ L^{-1}) \times \text{Feed flow rate}\ (L\ d^{-1}). \quad (2)$$

CH_4-COD_{sol} is calculated from the partial pressure of methane in the gas phase in equilibrium with the reactor solution assuming that the Henry's law is applicable.

The methane solubility as given by Henry's Law is

$$C_{equil} = K_H p_{CH_4} \quad (3)$$

where,

C_{equil} is the concentration of CH_4 dissolved in the liquid at equilibrium (mg L^{-1}),

p_{CH_4} is the partial pressure of methane in equilibrium with the liquid,

K_H is the Henry's law constant for CH_4 at the given temperature (mg $L^{-1}atm^{-1}$), and is given as (Peavey et al., 1985)

$$K_H = -0.384T + 36.44 \quad (4)$$

Where, T is the effluent temperature in °C.

Dissolved CH_4-COD in the effluent has been computed from equation (4) at 35 °C and 1 atm.

The biogas generated in the reactor gets dissolved in the reactor liquid and attains its saturation level at the reactor temperature and the remaining biogas remains in a free gaseous state and is collected in the gas holder through a gas-liquid-solid (GLS) separator device. In the reactor effluent, the dissolved CH_4 escapes to atmosphere from the liquid (water) surface because the partial pressure of CH_4 in the atmosphere is much less than that in the gas in the UASB reactor. Owing to gas losses, the mass of collected CH_4 is lower than the actual gas amount produced. This loss is computed as follows:

CH_4-COD_{sol} (g d^{-1}) = 4 C_{equil} * Effluent Flow rate (L d^{-1})/1000…………………………… (5)

CH_4-COD_{gas} is calculated as follows:

Biogas generation at T °C (L d^{-1}) = G°

= Production rate of biogas (LL^{-1} d^{-1}) * Volume of the reactor...……………………….....…. (6)

Methane collected at T °C (L d^{-1}) = G° * (%) methane...……………………………..…...... (7)

Theoretically, 0.4 l of methane at 35 °C and 1atmosphere pressure is produced per g of COD removed.

Methane collected at T °C (g d^{-1}) = G°/0.4 = 2.5 G°………………………………………. (8)

COD_{eff} is computed as

COD_{eff} (g d^{-1}) = $(COD)_{eff}$ (g L^{-1}) * effluent flow rate (L d^{-1}) ....…..… (9)

VFA-COD is computed asVFA (g d^{-1})

= VFA concentration in the effluent (g L^{-1}) * effluent flow rate (L d^{-1}) * 1.06

(1.06 is the conversion factor for acetic acid in terms of COD as VFA-COD) ……………………... (10)

$COD_{unacc.}$ for is obtained as the balance (residual) COD from the following:

$COD_{unacc.}$ = COD_{in} – (CH_4-COD_{sol} + CH_4-COD_{gas} + COD_{eff} + VFA-COD).……………………..…. (11)

2.6 Reactor Operating Procedure

The reactor was seeded with 3 litre of screened and washed digested sludge through a peristaltic pump (Miclins, Model PP20, Chennai, India). The characteristics of the seed sludge charged in to the UASB are given in Table 1. The SWW having different concentrations of glucose concentration in terms of COD values, viz. 500, 1000, 1330, 1660, 2000, 2660, 3330, 4000 and 5000 mg L^{-1} was fed to the UASB reactor on days 1, 25, 39, 55, 69, 82, 94, 106, respectively from the day of the start of the reactor. SWW containing glucose (COD = 500 mg L^{-1}) was fed into the reactor in an upflow mode at an organic loading rate (OLR) of 1.5 kg COD $m^{-3}d^{-1}$ with a hydraulic retention time (HRT) of 8 hours. The corresponding sludge loading rate (SLR) (conventionally called F/M ratio) was 0.146 kg COD (kg VSS)$^{-1}$ d^{-1}. The start-up procedure was in accordance with the guidelines suggested by Lettinga et al. (1984). After the filling-up of the reactor with the feed, nitrogen and carbon dioxide were bubbled through the reactor for 2-3 minutes at a low flow rate once every day for 3 days to purge the reactor of its oxygen and to reduce oxygen toxicity. OLR was increased in steps (ramps) once the reactor attained a pseudo-steady state. The pseudo-steady state was assumed to have set-in, when the COD of the effluent and the biogas production rate were found to remain constant (within ± 2%) for three consecutive samples. A maximum OLR of 15 kg COD m^{-3} d^{-1} was attained within a period of 120 days.

Table 1. Characteristics and Quantity of the Sludge Charged into the UASB Reactor.

S. No.	Parameters	Value
1	Total solids (g L^{-1})	153.76
2	Total suspended solids (g L^{-1})	71.93
3	Volatile suspended solids (g L^{-1})	32.80
4	VSS / SS ratio (%)	45.6
5	Colour	Black
6	Sludge volume index (SVI) (mL g^{-1})	43
7	Volume (L)	3
8	Height of sludge bed, (mm)	350
9	Sludge occupancy, g VSS (L reactor volume)$^{-1}$	10.1
10	Sludge occupancy, L (g VSS)$^{-1}$ (as determined in the Reactor)	30.49
11	Sludge activity by performance of the reactor on the first day	
	(i) Specific methanogenic activity (SMA), kg CH_4-COD (kg VSS.d)$^{-1}$	0.0625
	(ii) COD removal, %	42

3. Results and discussion

3.1 Reactor performance during start-up of the UASB reactor

The performance of the UASB reactor operating on SWW containing glucose was shown in Fig. 1. The COD removal efficiency of the reactor was found to have increased progressively day by day, from 42% (COD_i = 495 mg L^{-1} and COD_f = 287 mg L^{-1}) on the first day at an OLR of 1.5 kg COD m^{-3} d^{-1}, to 83% (COD_i = 510 mg L^{-1} and COD_f = 87 mg L^{-1}) at the end of day 24 as shown in Fig. 1(b).The measurable biogas production was noticed on day 5 and it reached a maximum of 0.53 m^3 m^{-3} d^{-1} at the end of day 24 as shown in Fig. 1(b). The methane content of the biogas was found to be 58% on day 24 and the maximum methane production rate was found to be 0.25 m^3 (kg COD removal)$^{-1}$ d^{-1} at the end of day 24. As a result of the hydraulic load and the gradual increase in the gas production, a rise in the sludge bed from 0.35 m to 0.45 m was observed at the end of day 24.

When the COD of SWW was increased from 500 mg L^{-1} to 1000 mg L^{-1} on day 25, the COD removal efficiency declined from 83% on day 24 to 69% on day 27, but increased later on. However, the production rate of biogas increased and reached a maximum of 1.03 $m^3 m^{-3} d^{-1}$ on day 38 as shown in Fig. 1(b). With an increase in the influent glucose concentration, the COD reduction and the biogas production increased: they reached 87% and 1.86 $m^3 m^{-3} d^{-1}$, respectively, on day 54. Methane content and maximum methane production rate were found to be 70% and 0.304 m^3 (kg COD removal)$^{-1}$ d^{-1} on day 54. The gas production rate showed a continuous increase with an increase in the OLR. The accumulation of the biogas in the sludge bed resulted in bubbling action, which brought about rigorous mixing of the reactor contents from ~350 mm upwards from the bottom of the reactor during these days.

The COD removal efficiency on day 55 was found to be 85%, which was reduced to 52% on day 56 and 45.24% on day 57. This reduction was because of the failure of the temperature controller of the thermostatic chamber for about 48 h. Fig. 1(b) also shows a slump in the biogas production on day 57 to a value of 1.16 $m^3 m^{-3} d^{-1}$. The rector temperature dropped from 35 ºC to about 20-22 ºC due to failure. However, on the evening of day 57, the reactor temperature was again maintained at 35 ± 2 ºC and the COD removal efficiency was seen gradually increasing and finally reaching a value of 88% at the end of the OLR of 6 kg COD $m^{-3} d^{-1}$ on day 68.

This shows that the reactor environment markedly influenced the COD conversion efficiency. An increase in the COD removal efficiency was observed when the OLR was increased in steps up to an OLR of 8 kg COD $m^{-3} d^{-1}$. Above this OLR, the COD removal efficiency decreased marginally and the biogas production increased marginally as depicted in Fig. 1b.

The overall COD removal efficiency of the system was found between 80 and 90% during the operation time up to an OLR of 10 kg COD $m^{-3} d^{-1}$ as shown in Fig. 1b. The maximum methane content and the maximum methane production rate were noted as 75% and 0.326 m^3 (kg COD removed)$^{-1}$ d^{-1}, respectively, at an OLR of 8 kg COD $m^{-3} d^{-1}$ during the start-up phase. Afterwards, the reactor was subjected to an increased OLR of 12 and then to 15 kg COD $m^{-3} d^{-1}$. This augmentation resulted in the COD removal of 78 and 75% and the biogas production of 3.45 and 3.92 $m^3 m^{-3} d^{-1}$, respectively.

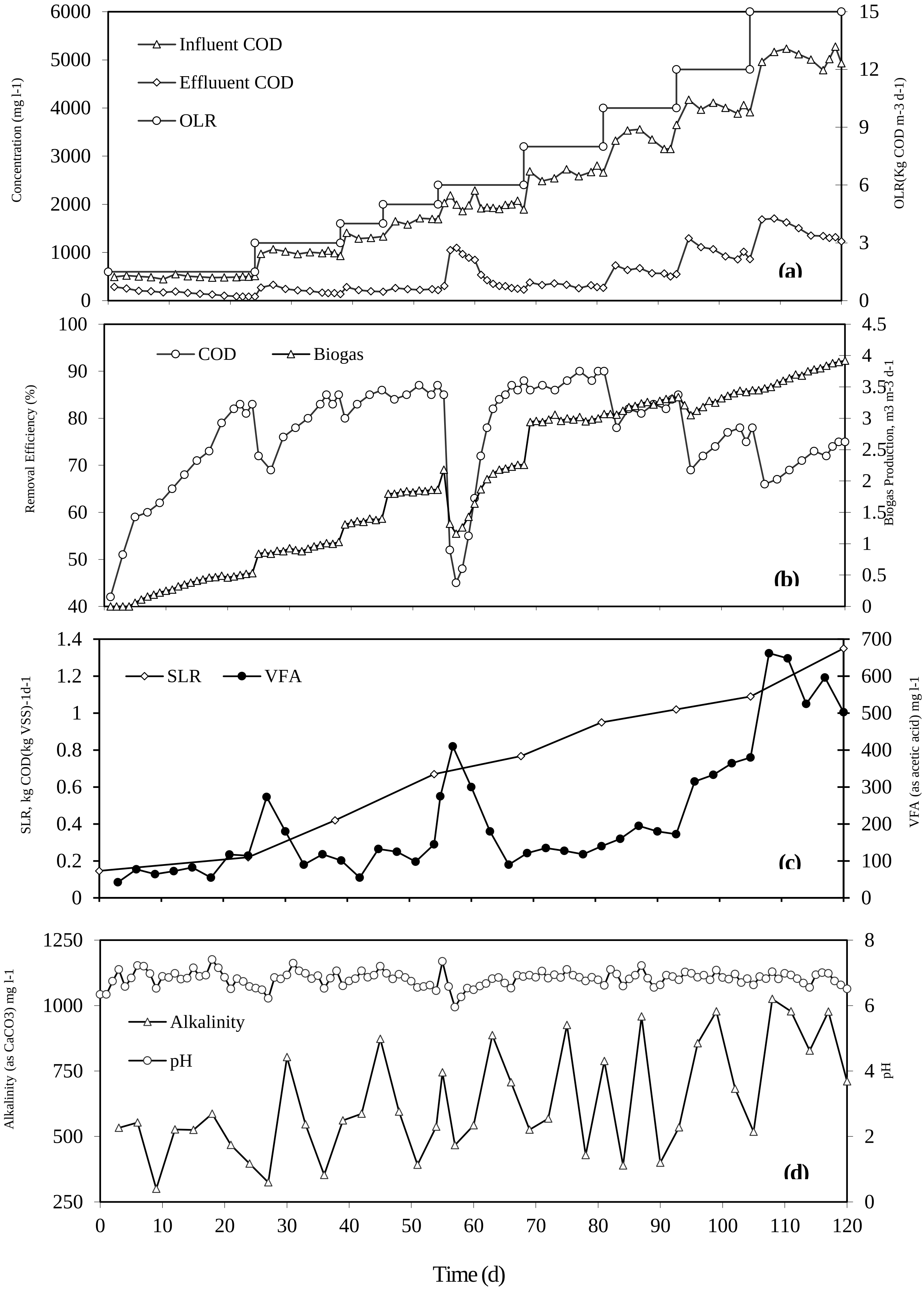

Fig. 1. Performance of the UASB reactor during start up (a) Influent and effluent COD; (b) COD removal (%) and Biogas production ; (c) SLR and VFA; and (d) Alkalinity and pH

In a sucrose-fed UASB reactor, the COD removal efficiency decreased markedly from 91% to 80%, and then to 74% when the OLR was increased from 7.9 to 10.6 and then to 13.8 kg COD m^{-3} d^{-1}. The reason for reduction of COD removal efficiency was due to high accumulation of VFAs from 202 to 632 and then to 1096 mg acetate L^{-1}, respectively according to Chou and Hwang (2005). In another research (Sivan, 1997), with a glucose concentration of 1000-5000 mg L^{-1}, corresponding to the OLR of 5-15 kg COD m^{-3} d^{-1} in the reactor feed, the COD removal efficiency could be more than 90% with less than 9 OLR. When the OLR increased to 12 kg COD m^{-3} d^{-1}, the COD removal efficiency decreased to 77% under similar conditions. During the present investigation, the VFA concentration was found to have increased about 172 mg L^{-1} at an OLR of 10 kg COD m^{-3} d^{-1} to more than 600 mg L^{-1} during the initial period of OLR of 15 kg COD m^{-3} d^{-1}. This, however, decreased to 500 mg L^{-1} at the end of the start-up operation.

The development of granular sludge was the key factor for successful operation of the UASB reactors. The analysis of characteristics of granules grown on aqueous glucose solution in this UASB reactor demonstrated that a good quality, well settling granular sludge was cultivated and retained in the reactor (Subramanyam and Mishra, 2013b). After achieving the limited objective of good acclimation and granulation of the sludge, the start-up operation was terminated with glucose feed at an OLR of 15 kg COD m^{-3} d^{-1} on day 120.

3.2 Effect on SLR

Fig. 1c shows a minimum SLR value of 0.146 kg COD (kg VSS)$^{-1}$ d^{-1} applied at an OLR of 1.5 kg COD m^{-3} d^{-1} at the start of the UASB reactor. At the beginning of the granulation process, the SLR value was in the range of 0.5-0.7 kg COD (kg VSS)$^{-1}$ d^{-1} at an OLR of 4-6 kg COD m^{-3} d^{-1} between day 45 and 62 of the reactor operation. It was obvious that because of low cell growth in the anaerobic process, continuous and linear increase in the SLR is usually observed with an increase in OLR. At the end of day 120, the SLR increased to a maximum value of 1.35 kg COD (kg VSS)$^{-1}$ d^{-1} at an OLR of 15 kg COD m^{-3} d^{-1}.

3.3 Effect on concentration of VFAs

VFAs are important intermediate compounds in the metabolic pathway of methane fermentation and cause microbial stress if present in high concentrations. This results in a decrease of pH, ultimately leading to failure of the digester (Buyukkamaci and Filibeli, 2004). The concentration of VFAs (as acetic acid) in the effluent of the UASB reactor varied between 42 and 662 mg L^{-1} during the start-up period of the reactor operation as shown in Fig. 1c. On day 27, the VFA concentration was 273 mg L^{-1} when the OLR was increased from 1.5

to 3 kg COD m^{-3} d^{-1}. On 57^{th} day, the VFA was 410 mg L^{-1} due to failure of the thermostatic assembly of the reactor chamber for 48 h. However, the VFA concentration came down subsequently once the temperature in the reactor reached about 35 ºC. High VFA concentration (> 200 mg L^{-1}) has adverse impact on the reactor performance (Morvai et al., 1992). The ratio of the volatile fatty acids/total alkalinity (VFA/TA) varied between 0.08 and 0.88, and exceeds 0.80 only twice on days 27 and 57 during the entire start–up period. The VFA (as acetic acid) levels of 202, 632 and 1096 mg L^{-1} at OLR values of 7.9, 10.6 and 13.8 kg COD m^{-3} d^{-1}, respectively, reported for aqueous sucrose solution as the feed in an UASB reactor (Chou and Huang, 2005).

In the present case, the maximum VFA concentrations of 140, 195, 380 and 662 mg L^{-1} were found at OLR of 8, 10, 12 and 15 kg COD m^{-3} d^{-1}, respectively. The VFA levels observed for sucrose solution as the feed are higher than that for glucose solution as the feed at the corresponding OLRs. The accumulation of VFAs, proving that methanogenesis was the rate-limiting step in the glucose-fed UASB reactor (Chou and Huang, 2005). In the present studies, higher VFA level was neutralized by maintaining a higher influent pH with the addition of $NaHCO_3$ to the feed solution.

3.4 Effect on pH and Alkalinity

The variation in the pH of the effluent and the alkalinity during start-up of the reactor operation was shown in Fig. 1d. The pH varied within a narrow window of 6.45-7.40 for most of the days. The alkalinity of the reactor mass varied between 300 and 1025 mg L^{-1}. The pH above 7.5 or below 6.5 may be unsafe to bacterial groups, especially to methanogens (Souza, 1986). On the first day, the pH was observed to be 6.34, on day 27, 6.22 may be due to sudden increase of glucose COD from 500 to 1000 mgL^{-1}. On 59^{th} day, the pH was 5.95 probably due to comparatively lower activity of the acidogens in the reactor at lower temperature (20-22 °C). The total acids produced during acidogenesis could not be converted by the methanogens into gas. Thus, an excess accumulation of the volatile acids was found in the reactor, resulting in a drop in pH. The successful start-up operation means the facilitation of the granulation process progressing smoothly with the stepped-up OLR along with the maintenance and control of VFA and pH level in the reactor. Sharp pH fluctuations can cause the digester to stop functioning (DiLallo and Albertson, 1961). The pH 6.8 ± 0.2 was the optimum pH value for good methanogenesis; higher influent pH would neutralize the effect of VFA formation on the pH of the reactor content.

3.5 Sludge development and concentration

Fig. 2 shows the variation of the TS and VSS concentrations and the VSS/TS ratio with OLR during the start-up period. The digested sludge used for the inoculation of the reactor had a low VSS/SS ratio of 0.456, which was typical since digesters contain more inert solids content than normal wastewater. The VSS/SS ratio was important as it indicates the amount of viable sludge in total sludge measured as SS. If the ratio is high, it indicates a larger percentage of viable sludge. The sludge concentration was found to decrease during the first 24 days of the reactor operation at an OLR of 1.5 kg COD m^{-3} d^{-1}. This was because of the extremely low SMA and the low value of the applied SLR. The expansion of sludge bed and the washout of the seed sludge results in the fall of the VSS concentration with a minimum of 21.92 g L^{-1} after 24 days of operation. This was 33% less than the initially seeded VSS concentration of 32.80 g L^{-1}. Thereafter, the sludge concentration gradually increased because of the increase in the rate of the biomass production and a decrease in the rate of washout. The increase in the sludge concentration was higher at a higher value of the applied SLR. The ratio of VSS/TS concentration, which was initially 21.33%, increased to 62.2% at an OLR of 15 kg COD m^{-3} d^{-1}. Granular sludge typically had VSS/SS ratios between 0.6 and 0.85 (Subramanyam, 2013). The VSS concentration also increased from 21.92 g L^{-1} to 35.45 g L^{-1} with the progressive step-wise increase in the OLR to 15.0 kg COD m^{-3} d^{-1}. The gas formation and the resulting bubbling action maintain the reactor in a dynamic mode with the biomass in a state of suspension.

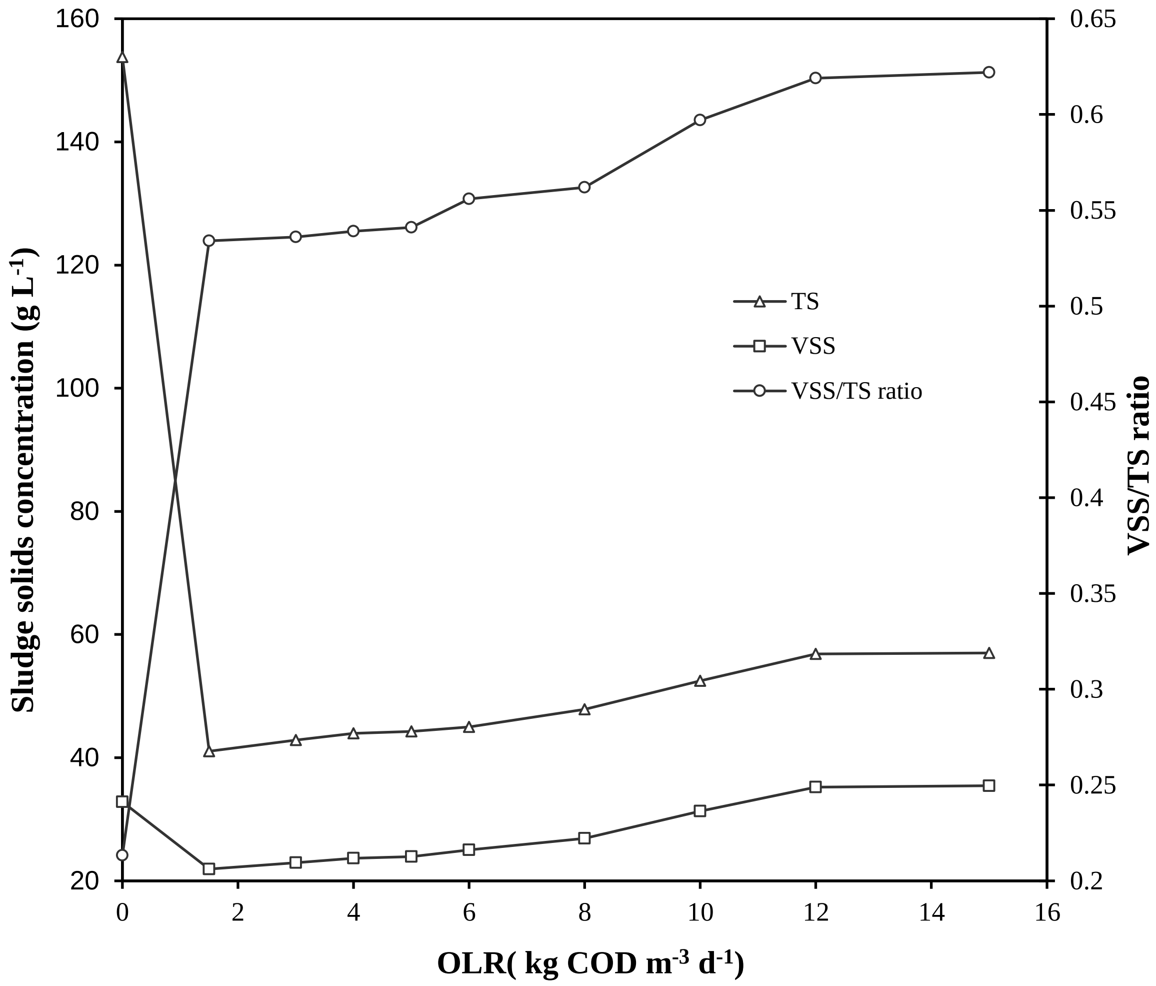

Fig. 2. Variation of sludge solids concentration of the reactor at the end of each OLR.

3.6 VSS Profile along the Height of Reactor

Existence of high concentration of active biomass in UASB as indicated by VSS concentration was an important performance indicator. To evaluate the granule concentration in the reactor, the VSS profile in the reactor was measured at different sampling days. The determination of VSS profile was not a simple task because of the constant turbulent motion and due to contraction and expansion of the sludge bed resulting from the continuous release of gas bubbles (Subramanyam and Mishra, 2008). Fig. 3 shows the VSS concentration profile along the height of the reactor during the start-up period. On the first day of the start-up operation, the VSS concentration was 32.80 g L^{-1} up to a height of 350 mm, which increased to its highest value of 35.45 g L^{-1} after 120 days of operation, i.e. at the end of the start-up period with glucose as the feed, at an OLR of 15 kg COD m^{-3} d^{-1}. The rising gas bubbles and the upflow liquid velocity generate shear forces, which act on the sludge granules causing some disintegration. The small-sized particles generated due to disintegration and the unagglomerated microbial masses are buoyed up and form a blanket of particles at the top of the reactor. The VSS profile, as shown in Fig. 3 clearly indicates the formation of a blanket. The maximum VSS concentration of the sludge bed decreased from 32.80 (day 1) to 21.92 g L^{-1} (day 24) during acclimation, and then increased from 24th day onwards along the height of the reactor with the maximum concentration being 35.45 g L^{-1}on day 120 at a height of 350 mm. The seed sludge in the reactor stayed as a loose mass initially and then expanded easily as a blanket as the granulation progressed. The reactor showed stratification of the biomass resulting in the settling of the larger bio-particles in the lower part and the formation of a suspension in the upper part of the reactor. At steady state of the granulation process, a static, dense sludge bed at the bottom and a suspended, thin sludge blanket at the upper portion with a clear interface between them could be seen in the reactor. The mixing caused by the rising gas bubbles and the up-flowing reactor mass keep the smaller aggregates of the biomass in suspension, thus, forming a sludge blanket.

3.7 Carbon Balance

The carbon balance in terms of COD was made around the reactor during the degradation of glucose-bearing SWW at each steady state for the reactor operation on day 54, 68, 81, 93, 105 and 120. The influent COD varied between 48 and 144 g d^{-1}. The methane dissolved in the effluent was found to be in the range of 1.85-1.99 g d^{-1}, i.e. 2.58-2.78 % of total methane formed. The maximum COD removal efficiency varied from 75 to 90%. Fig. 4 depicts that the influent COD converts to 50-76% CH_4-COD, 10-25% COD remains in the effluent and 5-11% COD converts to VFA-COD. The unaccounted COD varied between 3.75 and 21 g d^{-1} i.e., 8-15% of influent COD during start-up period. The maximum COD conversion to CH_4-COD (76%) was observed on day 81 and VFA-COD (11%) occurred on day 120. Harada et al. (1994) reported that 63% of the incoming COD was converted into CH_4-COD while treating low strength synthetic wastewater using a

UASB reactor.

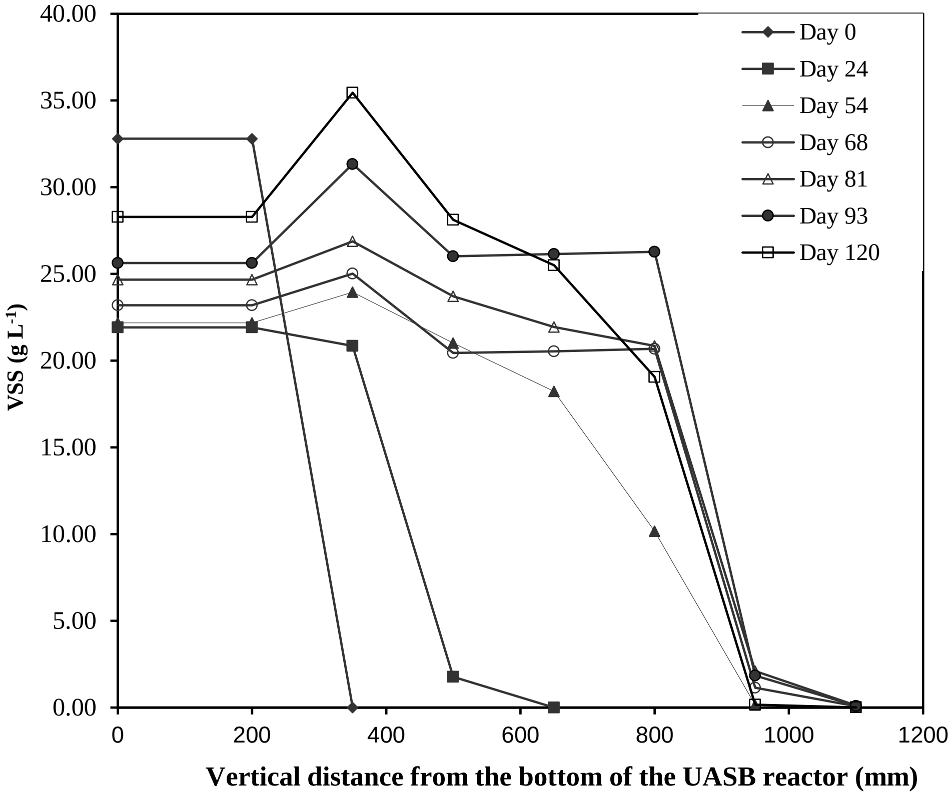

Fig. 3. VSS profile along the height of the reactor on different days.

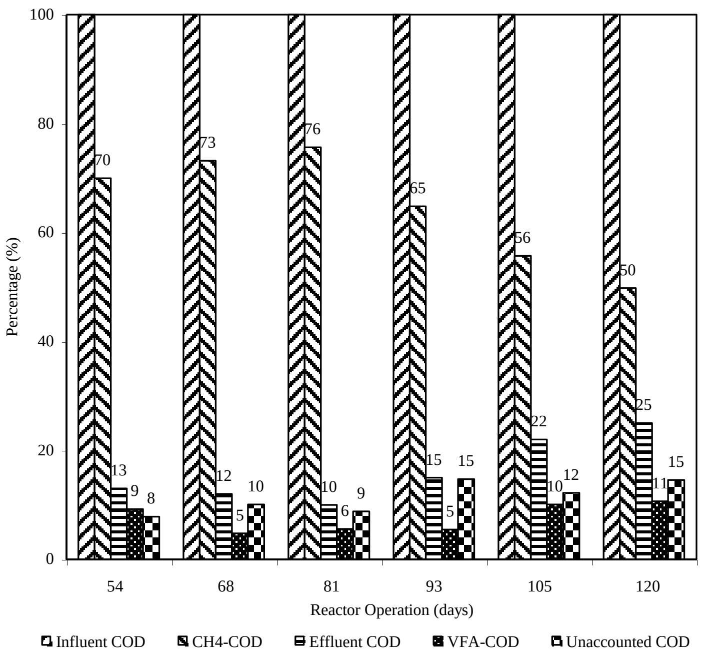

Fig. 4. Carbon (in terms of COD) balance diagram for degradation of glucose bearing SWW.

3.8 Specific Methanogenic Activity

The best method to assess the changes in the activity of the inoculum of the reactor during the biomass acclimation to feed containing glucose was the study of the specific SMA. The sludge samples were withdrawn from the UASB reactor on days 0, 54, 90, and 120 and used as seed for SMA tests in batch reactors. Fig. 5 shows the generation of methane with time in a batch reactor using the UASB reactor sludge sampled on different days. Acetate was used as a feed with a COD concentration of 2500 mg L^{-1}. The SMA value for the seed sludge was found to be 0.0625 kg CH_4-COD (kg VSS.d)$^{-1}$. This is within the range of SMA values ~0.05-0.1 kg CH_4-COD (kg VSS.d)$^{-1}$ reported by various investigators (Brito et al., 1997; Yan and Tay, 1997; Tay et al., 2001).

The batch test results showed the SMA values to be 0.253, 0.391 and 0.515 kg CH_4-COD (kg VSS.d)$^{-1}$ on day 54, 90 and 120, respectively. It is interesting to note that the SMA increased during the transformation of the seed sludge to granulated sludge. With an increase in the SMA, the agglomeration of the biomass increases, resulting in their transformation into granules. The increase in the SMA also indicates the acclimation of the biomass to the substrate feed in the UASB reactor. This changes the sludge characteristics gradually converting the seed sludge into the granular sludge. High methanogenic activity of 0.515 kg CH_4-COD (kg VSS.d)$^{-1}$ observed on day 120 indicates that the sludge mass has been converted in to granular sludge mass.

The SMA observed from the day zero, i.e. the day of inoculation to day 120, i.e. the day of completion of granulation process were 0.0625 and 0.515 kg CH_4-COD (kg VSS.d)$^{-1}$. These SMA values are within the range of 0.05-1.72 kg CH_4-COD (kg VSS.d)$^{-1}$ as reported by other investigators (Brito et al., 1997; Yan and Tay, 1997).

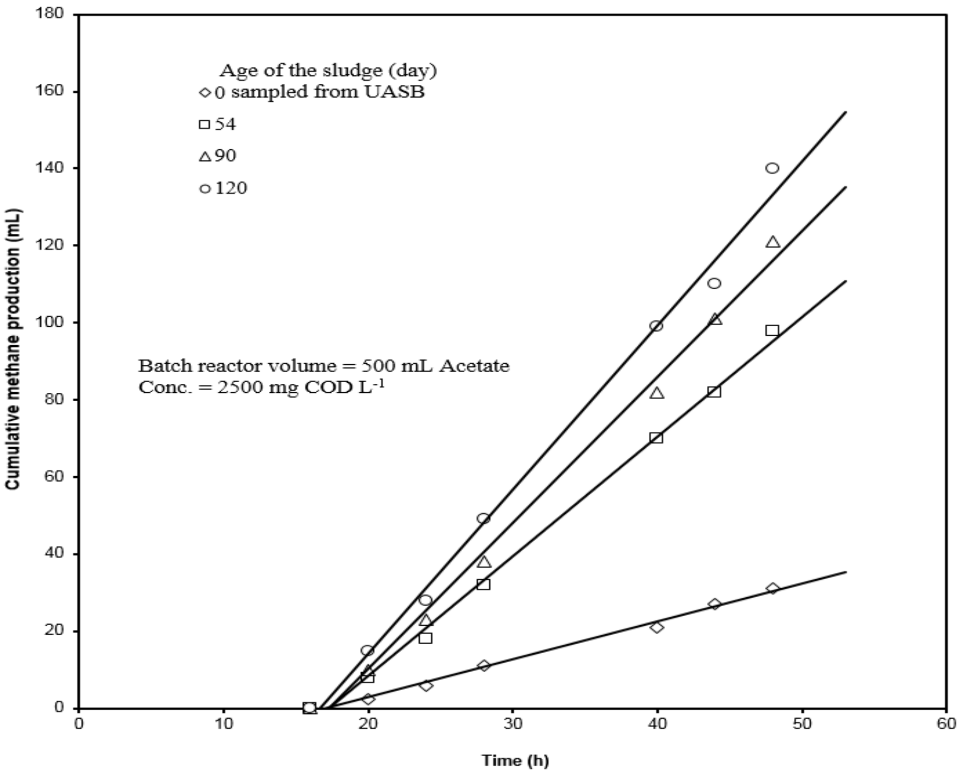

Fig. 5. Methane generation profile in the batch tests from the sludge of the different ages sampled from the UASB reactor.

4. Conclusion

The start-up of UASB reactor was feasible with digested sludge as inoculum and can be successfully cultivated into granular sludge by enhancing concentrations of glucose in SWW within a period of 120 days. Granulation was accomplished with stepped-up increase in OLR up to 15 kg COD $m^{-3}d^{-1}$ and SLR of 1.35 kg COD (kg VSS)$^{-1}d^{-1}$. The overall COD removal efficiency of the system was high (83-90%), during the reactor operation time up to OLR of 10 kg COD $m^{-3}d^{-1}$. An OLR was increased to 12 and thereafter to 15 kg COD m^{-3} d^{-1}, the COD reduction decreased to 78 and 75%, respectively due to production of high VFAs. The VFA levels as 195, 380, and 662 mg L^{-1} (as acetic acid) were found at an OLR of 10, 12, and 15 kg COD $m^{-3}d^{-1}$, respectively. The increase in the accumulation of VFAs at high OLRs showed that methanogenesis could be the rate-limiting step. The carbon balance depicts that the influent COD converts to 50-76% CH_4-COD, 10-25% COD remains in the effluent, 5-11% COD converts to VFA-COD and 8-15% unaccounted COD. The increase in SMA values with the sludge age showed an increase in the activity of biomass and the transformation of the seed sludge in to a granular sludge with an increase in the organic load of the reactor.

Conflict of interest

The author declares that there are no relevant financial or nonfinancial relationships to disclose.

Acknowledgements

The author gratefully acknowledges the financial assistance provided by the Ministry of Human Resource Development, Government of India at the Indian Institute of Technology, Roorkee (India) for pursuing his doctoral work.

References

APHA, 1998. Standard methods for the examination of water and wastewater. United Book Press, Washington, USA.ISBN: 0875532357 9780875532356

Brito, A.G., Rodringeres, A.C. and Melo, L.F., 1997. Granulation during the start-up of a UASB reactor used in the treatment of low strength wastewaters. *Biotechnology letters*, 19(4), 363-367. https://doi.org/10.1023/A:101835910

Buyukkamaci, N. and Filibeli, A., 2004. Volatile fatty acid formation in an anaerobic hybrid reactor. *Process Biochemistry*, 39(11), 1491-1494. https://doi.org/10.1016/S0032-9592(03)00295-4

Chou, H.H. and Huang, J.S., 2005. Comparative granule characteristics and biokinetics of sucrose-fed and phenol-fed UASB reactors. *Chemosphere*, 59(1), 107-116. https://doi.org/10.1016/j.chemosphere.2004.09.097

Dilallo, R. and Albertson, O.E., 1961. Volatile acids by direct titration. *Journal of Water Pollution Control Federation*, 33(4), 356-365. https://www.jstor.org/stable/25034391

Fang, H.H.P. and Zhou, G.M., 1997. Anaerobic degradation of benzoate and cresol isomers in sulfate-rich wastewater. *Water Science and Technology*, 36(6-7), 7-14. https://doi.org/10.1016/S0273-1223(97)00501-5

Fang, H.H.P., Chen, T., Li, Y.Y. and Chui, H.K., 1996. Degradation of phenol in wastewater in an upflow anaerobic sludge blanket reactor. *Water Research*, 30(6), 1353-1360. https://doi.org/10.1016/0043-1354(95)00309-6

Gujer, W. and Zehnder, A.J.B., 1983. Conversion processes in anaerobic digestion. *Water Science and Technology*, 15(8-9), 127-167. https://doi.org/10.2166/wst.1983.0164

Gupta, S.K. and Gupta, S.K., 2005. Morphological study of the granules in UASB and hybrid reactors. *Clean Technology and Environmental Policy*, 7(3), 203-212.https://doi.org/10.1007/s10098-005-0272-4

Haider, A., Khan, S.J., Nawaz, M.S. and Saleem, M.U., 2018. Effect of intermittent operation of lab-scale upflow anaerobic sludge blanket (UASB) reactor on textile wastewater treatment. Desalination and Water Treatment, *136, 120-130.* doi: 10.5004/dwt.2018.23231

Hickey, R.F., Wu, W.M., Veiga, M.C. and Jones, R., 1991. Start-up operation, monitoring and control of high-rate anaerobic treatment systems. *Water Science and Technology,* 24(8), 207-255. https://doi.org/10.2166/wst.1991.0226

Hulshoff-Pol, L.W. and Lettinga, G., 1986. New technologies for anaerobic wastewater treatment. *Water Science and Technology,* 18(12), 41-53. https://doi.org/10.2166/wst.1986.0162

Hwang, P.C. and Cheng, S.S., 1991**.** The influence of glucose supplement on the degradation of catechol. *Water Science and Technology*, 23(7-9), 1201-1209. https://doi.org/10.2166/wst.1991.0571

Isa, M.H., Farooqi, I.H. and Siddiqui, R.H., 1993. Methanogenic activity test for study of anaerobic processes. *Indian Journal of Environmental Health,* 35(1), 1-8. https://www.researchgate.net/publication/279438547

Kalogo, Y., Bouche, J.H. and Verstraete, W., 2001. Physical and biological performance of self-inoculated UASB reactor treating raw domestic sewage. *Journal of Environmental Engineering*, 127(2), 179–183. https://doi.org/10.1061/(ASCE)0733-9372(2001)127:2(179)

Kennes, C., Mendex, R. and Lema, J.M., 1997. Methanogenic degradation of p-cresol in batch and in continuous UASB reactors. *Water Research***,** 31(7), 1549-1554. https://doi.org/10.1016/S0043-1354(96)00156-X

Kleerebezem, R. and Macarie, H., 2003. Treating industrial wastewater: anaerobic digestion comes of age. *Chemical Engineering*, 110(4), 56-64. https://www.researchgate.net/publication/279555643

Lettinga, G., Pol, L.W., Koster, I.W. and Wiegant, W.M., 1984. High rate anaerobic waste water treatment using the UASB reactor under a wide range of temperature conditions. *Bio Technology and Genetic Engineering***,** 2(1), 253-284. https://doi.org/10.1080/02648725.1984.10647801

Morvai, L., Mihaltz, P. and Hollo, J., 1992. Comparison of the kinetics of acetate biomethanation by raw and granular sludges. *Applied Microbiology and Biotechnology*, 36(4), 561-567. https://doi.org/10.1007/BF00170204

Oz, N.A., Ince, G.T. and Ince, B.K., 2012. Effect of seed sludge microbial community and activity on the performance of anaerobic reactors during the start-up period. *World Journal of Microbiology and Biotechnology,* 28(2), 637-647. DOI: 10.1007/s11274-011-0857-5

Poh, P.E. and Chong, M.F., 2009. Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. *Bioresource Technology***,** 100(1), 1-9. https://doi.org/10.1016/j.biortech.2008.06.022

Show, K.Y., Wang, Y., Foong, S.F. and Tay, J.H., 2004. Accelerated start-up and enhanced granulation in upflow anaerobic sludge blanket reactors. *Water Research*, 38(9), 2293-2304. https://doi.org/10.1016/j.watres.2004.01.039

Sivan, P., 1997. The Degradation of 2-Chlorophenol in an Upflow Anaerobic Sludge Blanket (UASB) Reactor. Ph.D. Thesis, der Universit at Fridericiana ZU Karlsruhe (TH), aus Bangkok, Thailand. DOI: 10.5445/IR/1000005898

Souza, M.E., 1986. Criteria for the utilization, design and operation of UASB reactors. *Water science and technology*, 18(12), 55-69. https://doi.org/10.2166/wst.1986.0163

Subramanyam, R., 2007. Performance of an UASB Reactor for the Treatment of Catechol and Resorcinol. Ph.D. Thesis, Indian Institute of Technology, Roorkee, India.

Subramanyam, R., 2013. Physicochemical and morphological characteristics of granular sludge in upflow anaerobic sludge blanket (UASB) reactors. *Environmental Engineering Science*, 30(5), 201-212. https://doi.org/10.1089/ees.2012.0347

Subramanyam, R. and Mishra, I.M., 2007. Biodegradation of catechol (2-hydroxy phenol) bearing wastewater in an UASB reactor. *Chemosphere,* 69(5), 816-824. https://doi.org/10.1016/j.chemosphere.2007.04.064

Subramanyam, R. and Mishra I.M., 2008. Co-degradation of resorcinol and catechol in an UASB reactor. *Bioresource Technology* , 99(10), 4147-4157. https://doi.org/10.1016/j.biortech.2007.08.060

Subramanyam, R. and Mishra, I.M., 2013a. Critical Review of Anaerobic Biodegradation of Benzenediols: Catechol, Resorcinol, and Hydroquinone. *Journal of Hazardous, Toxic, and Radioactive Waste*, 17(3), 218-229. https://doi.org/10.1061/(ASCE)HZ.2153-5515.0000178

Subramanyam, R. and Mishra, I.M., 2013b. Characteristics of methanogenic granules grown on glucose in an upflow anaerobic sludge blanket reactor. *Biosystems Engineering*, 114(2), 113-123. https://doi.org/10.1016/j.biosystemseng.2012.11.010

Tay, J.H., He, Y.X. and Yan, Y.G., 2000. Anaerobic biogranulation using phenol as the sole carbon source. *Water Environment Research*, 72(2), 189-194. DOI:10.2175/106143000x137275

Tay, J.H., He, Y.X. and Yan, Y.G., 2001. Improved anaerobic degradation of phenol with supplemental glucose. *Journal of Environmental Engineering, ASCE*, 127(1), 38-45. https://doi.org/10.1061/(ASCE)0733-9372(2001)127:1(38)