International Refereed Journal of Engineering and Science (IRJES)
ISSN (Online) 2319-183X, (Print) 2319-1821
Volume 2, Issue 5(May 2013), PP.46-53
www.irjes.com

Start-Up Of An Upflow Anaerobic Sludge Blanket Reactor Treating Low-Strength Wastewater Inoculated With Non-Granular Sludge

K.R.Venkatesh, M.Rajendran & A.Murugappan
Department of Civil Engineering, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar- 608 002, Tamilnadu, India.

Abstract :*The upflow anaerobic sludge blanket (UASB) process is known to be energy conservative biotechnology. Its low cost and low skill requirement render it to be a viable technology for reducing organic pollution loads. However, this system is facing a challenge in the treatment of low-strength wastewater especially inoculated with non-granular sludge. This work was performed to emphasis an in-depth understanding of an UASB reactor start-up process inoculated with digested slurry for treating synthetic wastewater of chemical oxygen demand (COD) around 700-1000 mg/L. The lab scale reactor with an effective volume of 9.97 L was operated under ambient temperature (24-35^{0}C). At the end of start-up period that lasted for 84 days, this system achieved 90.8% COD removal and a biogas evolution of 4.72 L/d (457 L / kg COD removal) at an organic loading rate (OLR) of 1.293 kg COD/ m^3 d. Concurrently, the volatile fatty acids (VFA)/ alkalinity ratio was found as 0.184. It confirms the stability of the reactor.*

Keywords: *alkalinity, anaerobic treatment, biogas, start-up, volatile fatty acids.*

I. INTRODUCTION

Wastewater may be categorized as low-strength, medium strength, high strength and very high strength based upon the concentration of chemical oxygen demand (COD), bio-chemical oxygen demand (BOD), suspended solids (SS) and nutrients such as nitrogen (N) and phosphorus (P). Generally the stronger the effluent, the greater the investment in infrastructure and energy inputs required to achieve environmentally sound treatment objective [1]. Several techniques are already available to attain the goals of environmental protection and resource conservation. In the case of wastewater treatment, combination of different methods can be used, viz. physical, chemical and biological. The last method, which constitutes the main units of wastewater treatment plants, can be divided into two classes such as aerobic and anaerobic [2].

Aerobic process, which are widely used for the treatment of wastewater have two distinct disadvantages like high energy requirement and excess sludge production, which require handling, treatment and disposal [3]. In contrast, anaerobic processes generate energy in the form of biogas, and produce sludge in significantly lower amounts than those resulting from aerobic systems. In the absence of molecular oxygen anaerobic process convert organic material into methane, a fuel that can yield a net energy gain process operation. Anaerobic processes have gained popularity over the past 20 years. It have been already applied successfully for the treatment of different type of wastewater and geared mainly towards high concentrated soluble wastewater [4]. The most common reactor type used for anaerobic digestion of wastewater is the continuously stirred tank reactors (CSTR). The main problem of this reactor is the removal of active biomass from the system that leads to long retention time. It has been overcome by immobilizing the active biomass, referred as high rate systems. A typical such reactor is upflow anaerobic sludge blanket (UASB) reactor [5].

In the UASB process, wastewater flows through bed (granular or flocculent), where different physical and biochemical mechanisms act in order to retain and biodegrade organic substances. Solubilisation and hydrolysis of SS is a slow process accomplished by extracellular enzymes excreted by acidogenic bacteria. Readily biodegradable substances are quickly acidified and then converted into methane and other biogas components [6]. The great success of the UASB process lies in its capability to retain a high concentration of active sludge due to granule formation. It allows the application of high loading rates and maintenance of high solids retention time at a low hydraulic retention time (HRT) so that efficient treatment can be carried out in a temperature climate as well [7].

Low-strength wastewater such as domestic sewage (COD concentration 500-1000 mg/L), are at present treated mainly aerobically. However, due to considerable operation and maintenance cost, studies are being conducted to develop economically more attractive high rate anaerobic systems [8]. UASB reactor system is facing a challenge in the treatment of low-strength wastewater. Nevertheless, more efficient anaerobic systems have been developed and they are being successfully applied for treatment of low-strength wastewater such as domestic wastewater, particularly under tropical conditions where artificial heating can be avoided [9]. The production and quality of granular/flocculent sludge is influenced by the composition of wastewater, reactor design and technological condition. Moreover, the formation of granular/flocculent sludge with good settling characteristics and activity is also a critical factor in dealing with low-strength wastewater [10]. In general, the operational conditions induce an ecological selection that leads to the formation of microbial aggregates, the so-called 'granules' [11]. Granules are the best inoculum for the start-up of UASB reactors due to their high specific activity and settleability. However, they are not always available. Consequently, very often the start-up process and the tentative granule formation have to be performed with an inoculum of diffuse sludge collected in conventional anaerobic digesters. There is lack of data on the operating conditions during the start-up of a UASB reactor that uses diffuse sludge as the inoculum. On the contrary, when treating wastewaters with COD values lower than 1500 mg/L, which are low- strength wastewater in the anaerobic treatment field, the start-up of UASB reactors is difficult and thus much less full-scale reactors are in operation under these conditions than in the case of medium and high strength effluents [9].

Keeping this in mind, the experimental work here described was focused on the anaerobic treatment of low-strength wastewater under tropical conditions. The main objective of this work was to look into the start-up process performance of a lab scale UASB reactor inoculated with digested slurry, which was collected from an active biogas plant. The operational parameters such as COD, upflow velocity, total alkalinity, volatile fatty acids (VFA) concentration, total suspended solids (TSS), volatile suspended solids (VSS) and pH were investigated. In addition to that, biogas generation and COD removal efficiency were also calculated.

II. METHODS

2.1 Experimental set-up

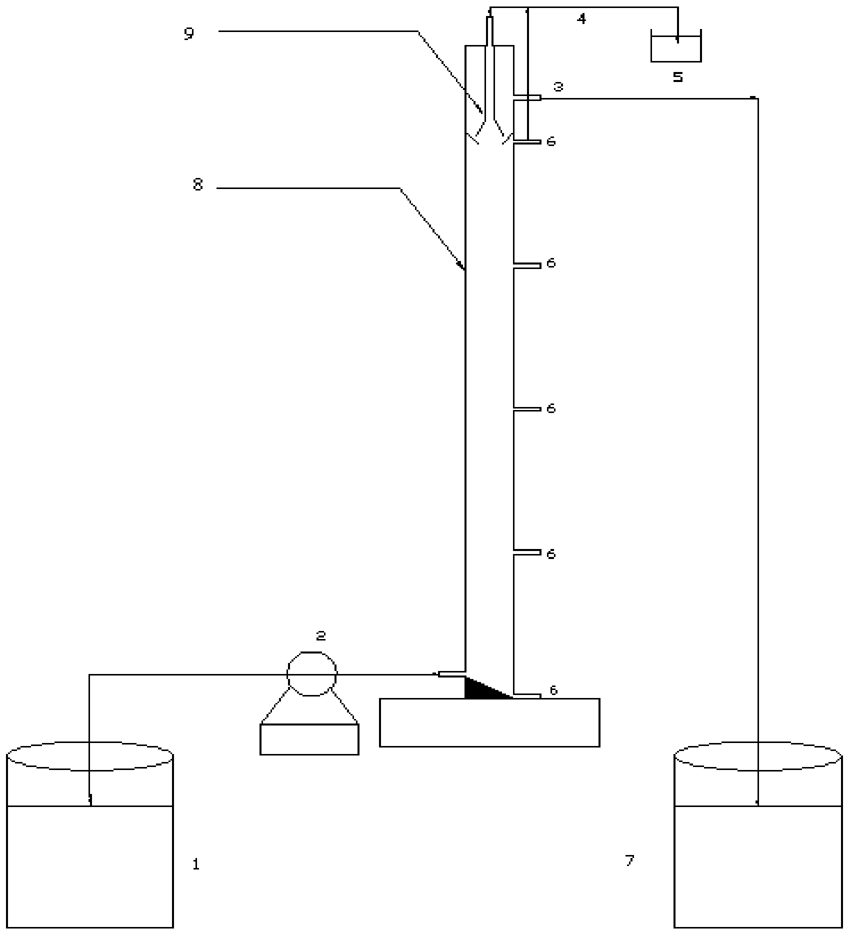
UASB reactor employed in this study was fabricated using transparent plexiglass tube of 0.1 m diameter vertical cylindrical shape with a total volume of 11.78 L. The reactor essentially had an internal effective working volume of 9.97 L and the remaining volume of 1.81 L was kept for gas liquid solid (GLS) separation arrangement. The overall height of the reactor was 1.5 m and effective height being kept as 1.27m. The reactor had one influent port at the bottom, one effluent port and five sampling ports (Figure. 1). The GLS separator attached at the top of the reactor was essentially a set of two inverted cones with intermediate overlap to facilitate effective biogas collection. Gas was collected from the outer inverted cone as well as below the dead corners of the inner cone. The treated effluent collected by a pipe attached above the GLS separator was connected to a water seal to prevent the escape of gas. A peristaltic pump (Miclins PP 15) was used for feeding wastewater as uniformly as possible over the reactor bottom, passes through the sludge bed in the reactor. The reactor was operated at mesophilic temperature (24 - 35°C).

2.2 Synthetic wastewater preparation

In this study, synthetic wastewater was prepared, in which organic COD was provided by sucrose and dried milk powder. About 1000 mg/L of COD contains 750 mg of sucrose and 250 mg of dried milk powder. The ratio of soluble to particulate COD in the feed was around 80:20. The synthetic wastewater was then neutralized to a value of pH ~ 7.2 by using sodium hydroxide (NaOH). Urea and KH_2PO_4 were added as sources of nitrogen and phosphorus respectively to maintain COD: N: P ratio. A ratio of 300:5:1 was maintained as COD: N: P during start-up period [12]. The pH in the feed tank was adjusted to a value ~ 7 after every 10 -12 hours. The fresh feed was prepared daily. Trace elements like ferric chloride, copper sulfate, magnesium chloride, calcium chloride were added appropriately.

2.3 Seed sludge

Digested slurry was collected from an active biogas plant located at Faculty of Agriculture, Annamalai University, Annamalai Nagar. After analyzing the concentration of total suspended solids (TSS) and volatile suspended solids (VSS), this sludge was used as seed. Initially about 50% reactor volume was filled up with this active sludge.



1. Feed inlet, 2. Peristaltic pump, 3. Effluent outlet,
4. Biogas collection, 5. Gas measurement unit,
6. Sampling ports, 7. Effluent collecting tank,
8. 10 cm diameter and 150 cm tall plexiglass tube,
9. Gas liquid solid separation system

Figure1. Schematic diagram of UASB reactor showing experimental setup

2.4 Analysis

During the operation of the reactor, temperature, sludge bed height and biogas production were monitored and recorded daily. Feed and treated effluent was taken for the analysis of COD, pH, total alkalinity and VFA on daily basis. TSS and VSS were determined once in a week. Liquid samples were collected from the influent, effluent and sampling ports along the reactor. Quality control was ensured using standards as well as duplicates while performing analysis. All the parameters were determined according to standard method for the examination of water and wastewater [13]. COD was determined by open reflux method. VFA was determined by distillation method and measured as acetic acid per liter. Biogas evolved was collected by water displacement method. The water in the gas collector was acidified with sulfuric acid to reduce the solubility of one of the constituents of biogas carbon dioxide (CO_2), for optimum measurement of biogas volume.

III. RESULTS AND DISCUSSION

3.1 Characteristic of seed sludge and inoculation

Initially the seed sludge (digested slurry) had a TSS content of about 70,100 mg/L and VSS content of about 28,000 mg/L. Similar type of sludge was used as seed sludge for treating sago wastewater in a Hybrid UASB reactor [14]. Lettinga and Hulshoff Pol [15] revealed that in order to avoid excessive sludge washout, the amount of seed sludge must be small enough to maintain the sludge bed within the reactor upon increasing the space-loading rate, and large enough to prevent a needless delay of the reactor start up. They also suggested, when using thick digested slurry of relatively low specific methanogenic activity, 12 to 15 kg VSS/ m^3 sludge can be used because expansion washout will be limited in that case. While using s sludge with higher specific activity, the amount of seed sludge should not exceed approximately 6 kg VSS/ m^3, because more seed sludge will be washed out due to the occurrence of sludge bed expansion. Hence, initially about 50% reactor volume was filled up with this active sludge having VSS concentration of about 28,000 mg/L. Accordingly the VSS concentration in the entire reactor at the start-up was about 14,000 mg/L (14 kg VSS/m^3). Hickey et. al., [16] recommended Sludge with 40% reactor volume might be enough to accelerate start-up.

3.2. Reactor Start-up

The start-up period is considered as the period taken for stable operation to be achieved. This is a crucial step for the stable operation of the UASB and other anaerobic reactors, at a designed organic loading rate (OLR). One of the main points stressed is the inoculation with high quality methanogenic sludge. Sludge of poor quality can also be used [17]. If self-inoculation can be effected, the start-up periods can go up to or beyond 6 months and other complications could arise [4]. In addition, operating temperature is prominent during start-up. In this work, the UASB reactor after seeding was operated at a temperature between 24^0C and 35^0C. This comes under the mesophilic range (20^0C to 45^0C). Fluctuations within this range were not enough to cause any real impact on performance and in terms of reaction kinetics [18].

To begin with, the sludge has a VSS / TSS ratio of about 0.40. Poor generation of biogas at the initial stage of start-up shows that sludge activity was initially low. At the same time, feed concentration of less than 1000 mg/L of COD helped in controlling the excessive volatile fatty acids (VFA) formation and guarded against the excessive pH drop. An inhibition of methanogenesis generally results in an increase of VFA concentration and a sudden drop in pH [19]. The upflow velocity is also an important operational parameter in anaerobic digesters. It maintains the mixing and hydraulic retention time of the substrate and biomass [20]. The upflow velocity maintained initially was low of about 0.106 m/h. No recirculation was employed to allow the lighter fraction of biomass to wash out from the reactor. The upflow velocity maintained during start-up phase is presented in Figure. 2. Accordingly, the OLR maintained during start-up phase is presented in Figure. 3.

COD removal rate during first four weeks were low in the range of 30-35% (based on total influent COD and total effluent COD) (Figure. 3). The low removal efficiency during start-up phase can possibly be attributed to the presence of unadapted seed sludge [10].Sufficient upflow velocities were maintained in the reactor, in order to facilitate sludge blanket formation offering higher area for contact between sludge and wastewaters. During period of 30 to 40 days higher upflow velocity of 0.128 m/h was tried (Figure. 2). This upflow velocity was preferred to permit the wash out of voluminous (poorly settling) sludge. This amounted to higher OLR of more than 2.1 kg COD/m^3 d (Figure. 3). However, these operating parameters made start-up operation unstable as evidenced by increase in VFA concentration in the effluent. Hence, the upflow velocity had to be controlled to a value of about 0.102 m/h after day 44 (Figure.2).

Many researchers suggest that the hydraulic retention times (HRTs) should not be allowed to less than 6 h and it should be less than 18 h during start-up period to treat any type of wastewater. At lower HRT, the possibility of washout is more prominent. This makes it difficult to maintain the effective number of useful microorganisms in the system [21]. For this reason, after day 3, the HRTs were varied between 10 h and 18 h during start-up period.

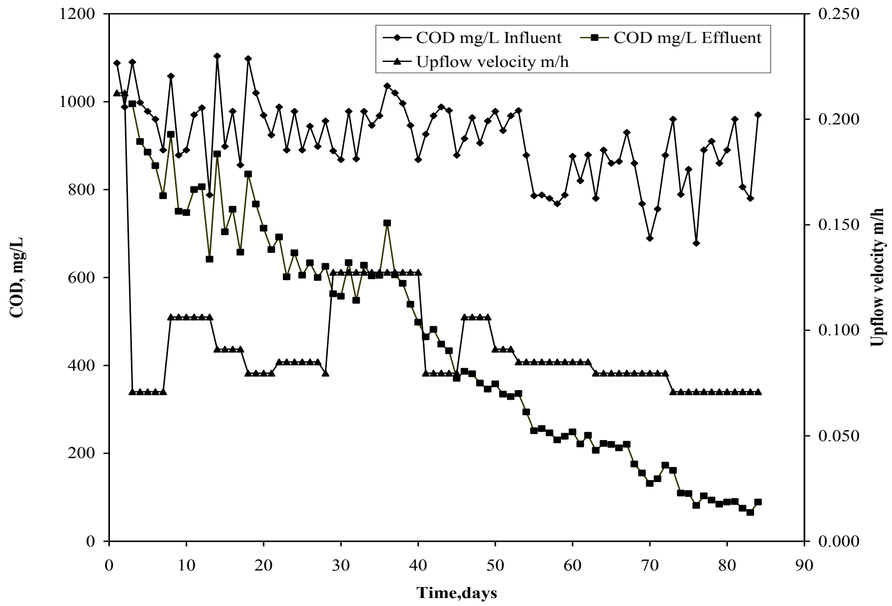


Figure 2**.** Feed, effluent COD concentrations and upflow velocity maintained during start-up period

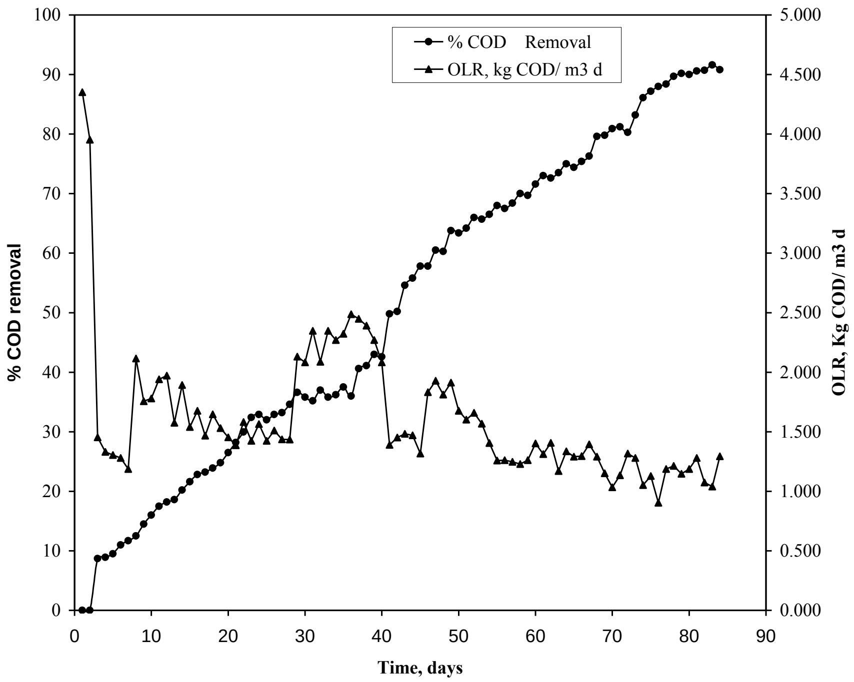


Figure 3. Organic loading rate maintained and % COD removal during start-up period

As discussed earlier during start-up period COD: N: P ratio was maintained as 300:5:1. N and P supplementation was necessary characteristics. COD: N: P should be at least 300:5:1 for efficient rapid start-up [12], [22]. Likewise, neutralization by alkali to a value of pH 7.0 - 7.2 was also necessary, particularly during start-up. In addition, optimal methanogenesis is known to take place at a neutral pH. The well-being of a reactor can often be judged by the examination of pH value of influent and the treated effluent. Variations in pH of the influent and effluent are presented in Figure. 4. The value of pH slightly fell during the initial days of operation, indicates the prevalence of acid fermentation over methanogens. However, after the initial drop, pH of the treated effluent was in the range of 7.2 - 8.3. It indicates healthy anaerobic environment, which is favorable for methanogenic organisms [23].

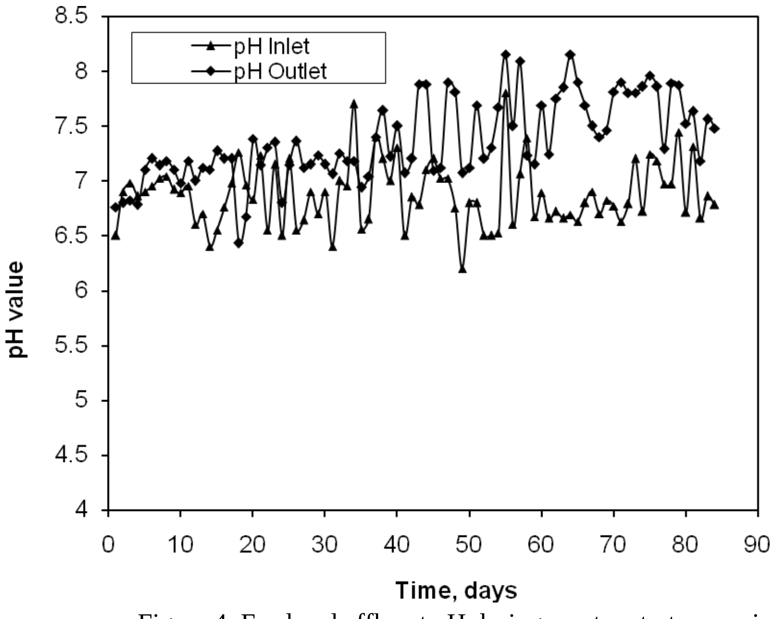


Figure 4. Feed and effluent pH during reactor start-up period

Total alkalinity, VFA concentration and pH are effective indicators of the stability of the reactor and of the correct balance between acidification and methanogenesis. The acidogenic element of the overall digestion processes will generate acids, but under normal conditions this would be buffered by the alkalinity in the reactor (expressed as calcium carbonate). Alkalinity may also be caused due to evolution of CO_2 during decomposition of organic matter [24]. In an anaerobic digester, a bicarbonate alkalinity (as $CaCO_3$) of about 1000 to 3000 mg/L was required for stable operation [25]. Disparity to this, Van Haandel and Lettinga [26] shows an average alkalinity with a range of 220-390 mg/L for the successful operation of a UASB reactor over 200 days. On this basis, sufficient alkalinity was available in the reactor. The alkalinity value of the treated effluent considerably increases as the reactor progressing towards steady state conditions (Fig. 5). Methogenisis, in particular is known to become unstable when the VFA / alkalinity ratio value is above 0.3 [27], [28]. From the beginning of the experiment, the effluent VFA values were less than 225 mg/L. The bicarbonate ion concentration or bicarbonate alkalinity is approximately equivalent to the total alkalinity for most wastes when the volatile acid concentration is very low [29]. On this aspect, total alkalinity measured during the entire period of operation represents bicarbonate alkalinity. In this work, VFA/alkalinity ratio after 40 days was 0.5. As the reactor progresses towards stabilization, the ratio gradually decreases. At the end of start-up period, the VFA/ alkalinity ratio was found as 0.184 (Fig. 5). Most researchers revealed that a value of greater than 0.40 for this ratio indicate reactor instability.

The VFA concentration (measured as acetic acid) in the effluent during first 30 days was in the range of 225-170 mg/L from that it fell down to 108 mg/L on day 50. Higher levels of VFA in the wastewaters during the initial phases of operation indicate the prevalence of acid fermentation [26]. Subsequently, the VFA in the effluent decreased and was in the range of 80 to 59 mg/L towards the end of start-up period. It indicates healthy anaerobic environment and satisfactory methanogenic activity. The overall performance of the reactor during the start-up was more than satisfactory. The selection of seed material plays a crucial role in minimizing time required for initial bioflim establishment [30], [31]. It was likely that the slurry collected from an active biogas plant and used as a seed had sufficient numbers of physiologically active microorganisms.

The biogas production up to 20 days was low, less than 1.4 L/d. Beyond day 48, however as soon as VFA concentration was controlled to less than 110 mg/L, the gas production as well as COD conversion efficiencies started showing marked improvement. By day 84, the gas production rate reached a value of about 4.92 L/d (Figure. 6) and the VFA in the effluent had reduced to less than 60 mg/L (Figure. 5). This shows that the process of hydrolysis and acidification of the organic matter took place in proper condition.

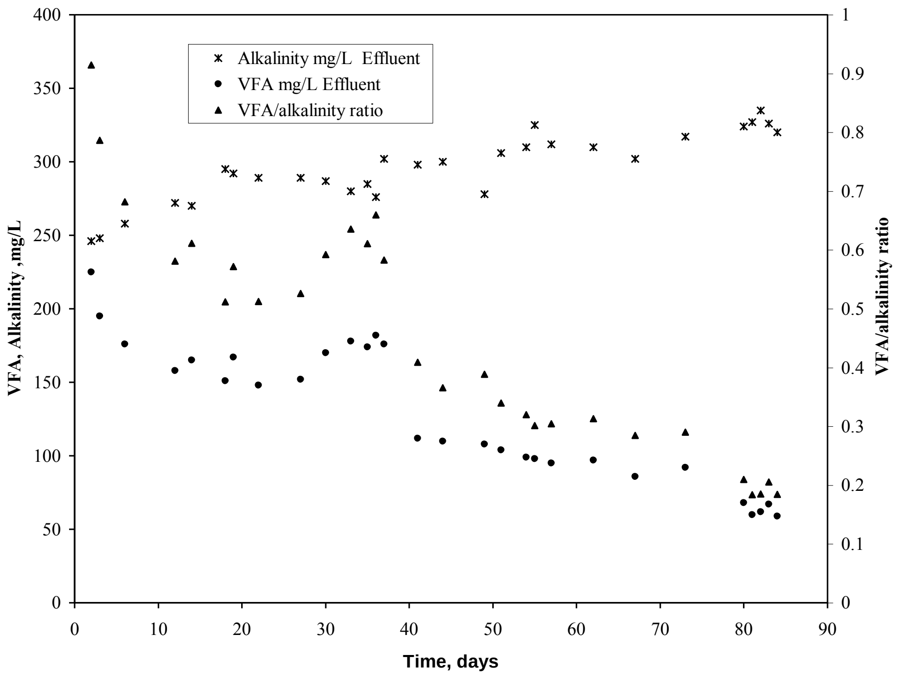


Figure 5. Effluent VFA, Alkalinity and VFA / Alkalinity ratio during start-up period.

It was assumed that the reactor had reached steady state condition merely 78 days after inoculation. This is because the results of effluent COD presented the same pattern i.e. approximately the same average COD concentrations and the same range of variations during the whole operation period after 78 days. There was no recycling of sludge during entire operation. Effluent recycle was not necessary to fluidize the sludge bed as sufficient contact between the wastewater and sludge is guaranteed even at low organic loading rate in UASB reactor [32]. The start-up period for the reactor was completed in 84 days, as the VFA level in the effluent stabilized gradually. The pH value of the treated effluent and increase in total COD removal efficiency (90.80%) indicates good start-up.

Determination of VSS/TSS ratio gives correlation to the biomass growth and its quality. The VSS/TSS ratios based on the average TSS and VSS concentrations in the reactor along with substrate utilization was observed at different periods of operation. The biomass concentration profiles were obtained by the TSS and VSS concentrations taken from sampling ports at various heights of the reactor (data not shown). At the time of reactor startup, VSS/TSS was 0.40. There was a steady increase in VSS/TSS ratio throughout the operation. The VSS/TSS ratio increased up to 0.64 by day 50 further increased to 0.80 on day 78. Initially the sludge bed height inside the reactor was 0.70 cm. On day 50 and day 78, it was 0.40m and 0.45m respectively. However, the experiment with the sludge morphological study was conducted at the end of the operational period.

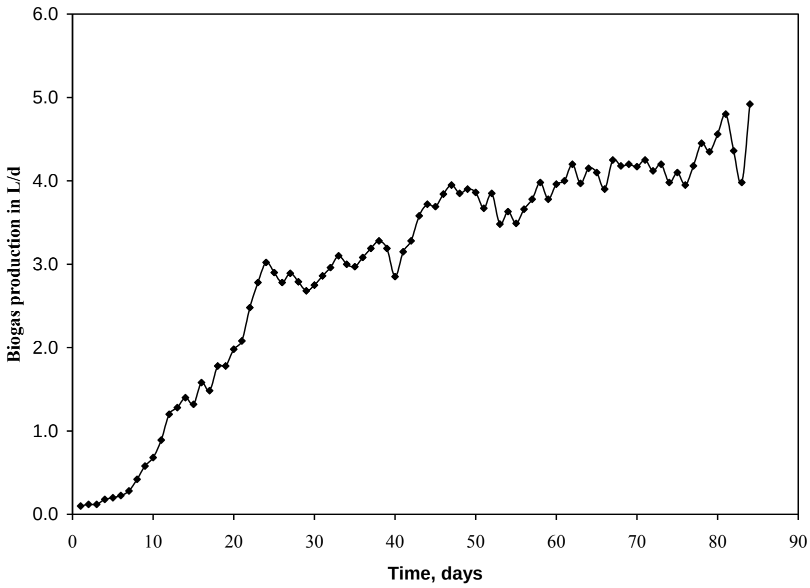


Figure 6. Biogas production during start-up period

IV. CONCLUSIONS

The attributes gathered from this study points out the feasibility of UASB reactor for the treatment of low-strength wastewater with COD values lower than 1000 mg/L, inoculated with non-granular sludge. The development of sludge bed was observed during this start-up phase. Decrease in VFA values, increase in pH values and alkalinity of the effluent, increase in biogas production rate and COD removal efficiency suggests that phase separation, process of hydrolysis and acidification of the organic matter took place in proper condition. It can be concluded that UASB reactor, inoculated by digested slurry requires 84 days for start-up and may be a robust design for treating low-strength wastewater. Further investigation should be carried out for a longer duration by varying OLR and HRTs to study the steady state and shock load performance. Effect of variation in influent parameters on sludge activity and characteristics should be further studied.

V. Acknowledgement

One of the authors (K.R.Venkatesh) is thankful to the authorities of the Annamali University for providing necessary facilities to carry out his research work.

REFERENCES

[1] Venkatesh, K.R. Biodegradation of lignin from pulp and paper industry effluents, M.E. Thesis. Department of Civil Engineering, Annamalai University, Annamalai Nagar, Tamilnadu, India.2003.

[2] Lettinga, G., Field, J., van Lier, J., Zeeman, G. and Hulshoff Pol, L.W. Advanced anaerobic wastewater treatment in the near future. Water Sci. Technol, 1997, 35(10), 5-12.

[3] Lettinga, G. Sustainable integrated biological wastewater treatment. Water Sci. Technol, 1996, 33(3), 85-98.

[4] Foresti, E. Anaerobic treatment of domestic sewage: Established technologies and perspectives. Proceedings of the ninth world congress on anaerobic digestion–Anaerobic conversion for sustainability. Antwerp, Belgium. 2–6 September, 2001, 37–42.

[5] Lettinga, G.A., van Velson, F.M., Hobma, S.W., de Zeeuw, W.J. and Klapwiji, A. Use of the Upflow sludge Blanket (USB) reactor concept for biological wastewater treatment. Biotechnol. Bioengng, 1980, 22(1), 699-734.

[6] Gujer, W. and Zenhder, AJB. Conversion processes in aerobic digestion. Water Sci.Technol, 1983, 15(1), 127-167.

[7] Agrawal, L. K., Harada, H. and Okui, H. Treatment of dilute wastewater in a UASB reactor at a moderate temperature: performance aspects. J. Ferment. Bioeng, 1997, 83(2), 179-184.

[8] Lettinga, G., Roersma, R. and Grin, P. Anaerobic treatment of raw domestic sewage at ambient temperatures using a granular bed UASB reactor. Biotechnol. Bioengng, 1983, 25(1), 1701-1723.

[9] Lettinga, G. and Hulshoff Pol, L.W. UASB process design for various types of wastewaters. Water Sci. Technol, 1991, 24(8), 87–107.

[10] Kripa Shankar Singh and Thiruvenkatachari Viraraghavan. Start-up and operation of UASB Reactors at 20^0C for municipal wastewater treatment. J. Ferment. Bioeng, 1998, 85(6), 609-614.

[11] Hulshoff Pol, L.W., de Zeeuw, W.J., Velzeboer, C.T.M. and Lettinga, G. Granulation in UASB reactors. Water Sci.Technol, 1983, 15(1), 291–304.

[12] Aiyuk, S.E., Amoako, J., Raskin, L., Van Haandel, A. and Verstraete, W. Removal of carbon and nutrients from domestic sewage using a low-cost, integrated treatment concept. Water Res, 2004, 38(13), 3031–3042.

[13] APHA. Standard Methods for the Examination of Water and Wastewater, Twentieth ed. American Public Health Association, Water Environment Federation, Washington DC, USA.1998.

[14] Rajesh Banu, J., Kaliappan. S. and Beck, D. High rate anaerobic treatment of Sago wastewater using HUASB with PUF as carrier. Int. J. Environ. Sci. Tech.2006, 3 (1), 69-77.

[15] Lettinga, G. and Hulshoff, Pol, L.W. Advanced Reactor Design, operation and economy. Water Sci. Technol, 1986, 18(12), 99-108.

[16] Hickey, R. F., Wu, W. M., Viega, M. C. and Jones, R. Start-up, operation, monitoring and control of high rate anaerobic treatment systems. Water Sci. Technol, 1991, 24(8), 207-255.

[17] Rodriguez, J.A., Pena, M.R. and Manzi, V. Application of an innovative methodology to improve the starting-up of UASB reactors treating domestic sewage. Water Sci. Technol. 2001, 44(4), 295-303.

[18] Henze, M. and Harremoes, P. Anaerobic treatment of wastewater in fixed film reactor- A literature review .Water Sci.Technol, 1983, 15(1), 1-100.

[19] Amit Kumar, Asheesh Kumar Yadav, Sreekrishnan, T.R., Santosh Satya and Kaushik, C.P. Treatment of low strength industrial cluster wastewater by anaerobic hybrid reactor. Bioresour. Technol, 2008, 99(8), 3123–3129.

[20] Muhammad Asif Latif, Rumana Ghufran, Zularisam Abdul Wahid, Anwar Ahmad. Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. Water Res, 2011, Vol. (45) 16, 4683-4699.

[21] Bal, A. S. and Dhagat, N.N. Upflow anaerobic sludge blanket reactor- A review. Indian J. Environ. Health, 2001, 43(2), 1-82.

[22] Amatya, P.L. Anaerobic treatment of tapioca starch industry wastewater by bench scale upflow anaerobic sludge blanket (UASB) reactor. M.Eng. Thesis. Asian Institute of Technology, Bangkok, Thailand. 1996.

[23] Wheatley. Anaerobic digestion- A waste treatment technology. Second edition. Elsevier Science Publishers, UK.1991.

[24] Venkatesh, K.R., Rajendran, M. and Murugappan, A. A correlation study on physico-chemical characteristics of domestic sewage. Nat. Env. Poll. Tech. 2009, 8(1), 141-145.

[25] Wilcox, S.J., Hawkes, D.L., Hawkes, F.R. and Guwy, A.J. A neural network based on bicarbonate monitoring to control anaerobic digestion. Water Res.1995, 29(6), 1465-1470.

[26] van Haandel, A.C. and Lettinga, G. Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate. John Wiley and Sons, Chichester, UK.1994.

[27] Lefebvre, O., Vasudevan, N., Torijos, M., Thanasekaran, K. and Moletta, R. Anaerobic digestion of tannery soaks liquor with an aerobic post-treatment. Water Res, 2006, 40(7), 1492-1500.

[28] Mosquera-Corral, A., Sanchez, M., Campos, J.L., Mendez, R. and Lema, J.M. Simultaneous methanogenesis and denitrification of pretreated effluents from a fish canning industry. Water Res. 2001, 35(2), 411-418.

[29] Pohland, F. G. and Bloodgood, D. E. Laboratory Studies on Mesophilic and thermophilic Anaerobic Sludge Digestion, Jour. Water Pollution Control Federation.1963, 35(1), 11-42.

[30] Bull, M. A., Sterritt, R. and Lester, J. N. Influence of COD, Hydraulic, temperature, and pH shocks on stability of an unheated fluidized bed reactor. J. Chem. Technol. Biotechnol, 1983, 33(4), 221-230.

[31] Salkinoja-Salonen, M. S., Nyns, E. J., Sutton, P. M., Van den Berg, L. and Wheatley, A. D Starting up of an anaerobic fixed film reactor. Water. Sci. Technol.1983, 15(8), 305-308.

[32] Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Lata, K. and Kishore, U.V.N. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. Renew. Sustain. Energy. Rev.2000, 4(2), 135–156.