Start Up And Operation Of A UASB Rector With Molasses As A Preferred Substrate – A Case Study

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Abstract: Anaerobic digestion followed by aerobic treatment has gained wide acceptability for many of the wastewater treatment. Several novel bio-reactors have been developed. All these reactors pose a problem of shock loading, when the high COD organic industrial waste is fed in to the reactor. Further, the normal substrates like glucose, sewage, citric acid may result in addition of a large volume of the substrate solution for a given organic loading. Both these problems are solved by adopting molasses, a typical by-product from the sugar industry. Among the bio-reactors to treat high strength waste waters, the Up-flow Anaerobic Sludge Blanket Reactor (UASBR), has received wide popularity for the treatment of domestic waste waters as well as for a variety of industrial wastewaters. This paper is to present a cogent and a clear picture of the startup and steady state operation of an UASB Reactor with *molasses* as the initial startup substrate for the development of well settleable sludge granules, the major feature of an UASB Reactor. The UASB reactor was fabricated in transparent Plexiglas. The reactor had a working volume of 9.75 liters with provision of ports for input, output, collection of samples, and collection of gas. Inside the settler, a gas-liquid-solid separation system using a glass funnel and a gas pressurizing plexiglass tube was provided. The aim was to granulate the sludge and acclimatizing it completely to take high organic loading. The feed was supplemented with a fixed COD: N: P ratio by adding urea for nitrogen and KH_2PO_4 for phosphorous. The HRT was fixed to be 8 hours. The reactor temperature was maintained at 35 ± 20°C. In about 55 days, the buttery seed sludge got granulated at an OLR of 8 g COD/l.day. The SEM of the granules exhibited a mixed morphology on the surface of the granules while filamentous Methanothrix proliferation could be clearly seen in the interior of the granule. Methanoceata and Methanothrix are the two dominant species found in the granules.

1. Introduction

Anaerobic digestion followed by aerobic treatment has gained wide acceptability for many of the wastewater treatment. Several novel bio-reactors have been developed. Some of these are Down flow fixed film reactor (DFFR), Upflow Anaerobic filter (AF), Anaerobic fluidized bed reactor (AFBR), and Upflow Anaerobic Sludge Blanket Reactor (UASBR). All the above types of reactors work at very low hydraulic retention time (HRT) in comparison to solids retention time (SRT). The UASB reactor conceived and developed by Lettinga and co-workers (1980) has received wide popularity for the treatment of domestic waste waters as well as for a variety of industrial wastewaters. The present study is an attempt to present a cogent and a clear picture of the startup and steady state operation of UASB Reactor for the development of well settleable sludge granules, the major feature of an UASB Reactor. Out of the several steps for the startup of a bioreactor, the direct feed of the wastewater from the industry may face the problem of shock loading. Hence the easily degradable substrates like sewage, glucose, citric acid and similar ones are to be fed in the beginning stages. However, for higher organic loadings, these substrates may pose the problem of diluting the original concentration of the liquid to be treated. For overcoming this problem, molasses, a byproduct from the sugar industry has proved to be a very good substrate for the start-up feed for both aerobic and anaerobic bioreactors. Molasses is with approximately 50% sucrose, which can be hydrolyzed to glucose and fructose by citric acid and butyric acid-producing cultures such as aerobic/anaerobic bacteria. Since this substrate is having a high COD value, it is easy to handle as it requires only a small quantity of feed liquid with calculated dilution, to get the required organic loading to be applied to bioreactors. In this paper, the typical procedure for the startup of the UASBR using the cane molasses as the starting substrate along with the obtained results of granulation of the sludge and working efficiency of the reactor has been elaborated which may help the beginners in this field.

2. Literature Review

Suneeth Kumar et al., 2023 have put forth several theoretical manipulations for the granulation to occur and concluded that the granulation theory could be considered to be analogous with crystallization kinetics and they have generalized the specific conditions for granulation including the VFA concentration, pH, OLR, macro and micro nutrients and such other parameters.

Bhatti Z.A. et al.,(2014) have conducted the study to shorten the start-up time of up-flow anaerobic sludge blanket (UASB) reactor. Two different nutrients were used during the UASB start-up period, which was designed to decrease the hydraulic retention time (HRT) from 48 to 24 and 12 to 6 hrs at average temperatures of 25-34 ºC. The removal efficiencies of the chemical oxygen demand (COD) were 80% and 98% on the 6th and 32nd day of the first and second stage, respectively. The maximum substrate removal rate of 0.08 mg COD mg-1 VSS d-1 was observed for glucose and synthetic nutrient influent (SNI) on the 8th and 40th days, respectively. When the reactor reached the maximum COD removal efficiency it was then shifted to municipal wastewater (MWW) mixed with industrial wastewater. The HRT was reduced gradually with a one week gap while treating MWW. For further cleaning, the UASB effluent was treated with 40% waste hydrogen peroxide. The whole integrated treatment process was successful to reduce the COD by 99%, total suspended solids (TSS) by 73%, total nitrogen (TN) by 84% and turbidity by 67%.

Subramanyam R and Mishra I M (2007) have studied the biodegradation of catechol through co-metabolism with glucose in aqueous solution as primary substrate in an upflow anaerobic sludge blanket (UASB) reactor. Their batch studies have indicated that the 1000 mg/(-1) glucose concentration was sufficient to co-metabolize and degrade catechol in an aqueous solution up to a concentration of 1000mg/(-1). The results have shown that the catechol was successfully mineralized in an UASB reactor in which microbial granulation was achieved with only glucose as the substrate, with a good COD removal efficiency of about 95%. They concluded that, once the reactor got acclimatized with catechol, higher concentrations of catechol can be mineralized with a minimum amount of glucose as the co-substrate, without affecting the performance of the UASB reactor.

Zhou W et al.(2006) The effects of extracellular polymer (ECP) production and electrostatic properties of the substrates on the process of granulation have been studied in UASB reactors using three kinds of substrates, glucose, skim milk and mixed volatile fatty acids (VFAs). The study confirmed the important contribution of ECP-bonding to granulation and proved the effectiveness of slight overloading in stimulating ECP production, which shortened the period of granulation. Investigations on the surface charge of the substrates and sludge revealed that the different substrates had different surface properties, which affected the granulation process directly. In the process of bacterial adhesion, the substrates functioned as external electrolytes and the ECP materials worked as the high molecular flocculants. Both surface properties and ECP content were closely related to the substrates and the running conditions. As a result, the required granulation time and the behaviour of granules were dependent on the running conditions and the substrates themselves.

Weili Zhou et al., (2005) have studied the effects of extracellular polymer (ECP) production and electrostatic properties of the substrates on the process of granulation in up-flow anaerobic sludge blanket (UASB) reactors using three kinds of substrates, glucose, skim milk and mixed volatile fatty acids (VFAs). The study confirmed the important contribution of ECP-bonding to granulation and proved the effectiveness of slight overloading in stimulating ECP production, which shortened the period of granulation. Investigations on the surface charge of the substrates and sludge revealed that the different substrates had different surface properties, which affected the granulation process directly. Based on the DLVO theory that the repulsion forces between particles can be whittled down by adding oppositely charged ions, ECP content and the surface charges of substrates were believed to be the important triggering forces for the anaerobic granulation in the UASB reactors. In the process of bacterial adhesion, the substrates functioned as external electrolytes and the ECP materials worked as the high molecular flocculants. Both surface properties and ECP content were closely related to the substrates and the running conditions. As a result, the required granulation time and the behavior of granules were dependent on the running conditions and the substrates themselves.

Yu et al., (2001) have operated six upflow anaerobic sludge blanket (UASB) reactors concurrently for 146 d to examine the effects of calcium on the sludge granulation process during start-up. Introduction of Ca^{2+} at concentrations from 150 to 300 mg/l enhanced the biomass accumulation and granulation process. The calcium concentration in the granules was nearly proportional to the calcium concentration in the feed, and calcium carbonate was the main calcium precipitate in the granules. The specific activity of granules decreased with increasing influent calcium concentration. The optimum calcium concentration for the granulation was from 150 to 300 mg/l. The addition of low-concentration calcium to the UASB reactors appeared to enhance the three steps of sludge granulation: adsorption, adhesion and multiplication, but it did not lead to a different proliferation of predominant microorganisms in the granules.

Herbert Fang and Ivan Lau (1996) have conducted detailed studies on the performances during the startup of three 2.8-litre UASB reactors operated under thermophilic condition. All the reactors were seeded with mesophilic sludges, one with the flocculent digester sludge (Reactor-F), another with UASB granules (Reactor-G), and the third with disintegrated granules (Reactor-D). The reactors were operated in parallel at 55°C and 24 hours of retention time, using sucrose and milk as substrate at COD loadings up to 10 g-COD/l·day. Immediately after temperature was step-increased from 37°C to 55°C, all reactors encountered sludge washout and deterioration of COD removal efficiency; however, the impact of temperature increase was more severe on Reactor-F. Sludge granulation took place in all reactors; first granules became noticeable after 45 days in Reactor-D, and after 90 days in Reactor-F. Reactor-G and Reactor-D were capable of removing 95% of soluble COD after 75 days, while Reactor-F after 110 days. Throughout this study, there was little difference in performance between Reactors O and D. The thermophilic granule were estimated to have a yield of 0.099 g-VSS/g-COD, and a methanogenic activity of 0.71–1.55 g-methane-COD/g-VSS·day, comparable to that of mesophilic granules.

3. Methodlogy

3.1 Analysis of the input sludge and Seed

The molasses procured to feed the reactor as substrates as well as the characteristics of the seed sludge procured from a conventional anaerobic sewage sludge digester plant were analyzed in the environmental laboratory using Standard Methods for Water and Wastewater Analysis (APHA, AWWA and WPCF).

3.2 Fabrication of the UASB Reactor

The study was conducted in a UASB reactor, having a large settling chamber at the top with gas-disengagement and gas recovery provisions. The UASB reactor was fabricated in transparent Plexiglas. As shown in Fig. 3.1 it consisted of 1200 mm high reactor portion of 100 mm diameter and a 600 mm high settler of 150 mm diameter, attached on the top of the reactor. The reactor had a working volume of 9.75 litres. Along the height of the reactor, 6 ports were provided for sampling and other measurements. The inlet system consisted of 4 ports at the bottom which in turn was attached to a common manifold for uniform distribution of the feed. Inside the settler, a gas-liquid-solid separation system using a Plexiglas funnel and a Plexiglas, gas pressurizer tube was provided. The gas collection system consisted of two aluminum drums, the top drum being inverted on the other to float-up in water with gas collection. The reactor was fed with the help of a peristaltic pump.

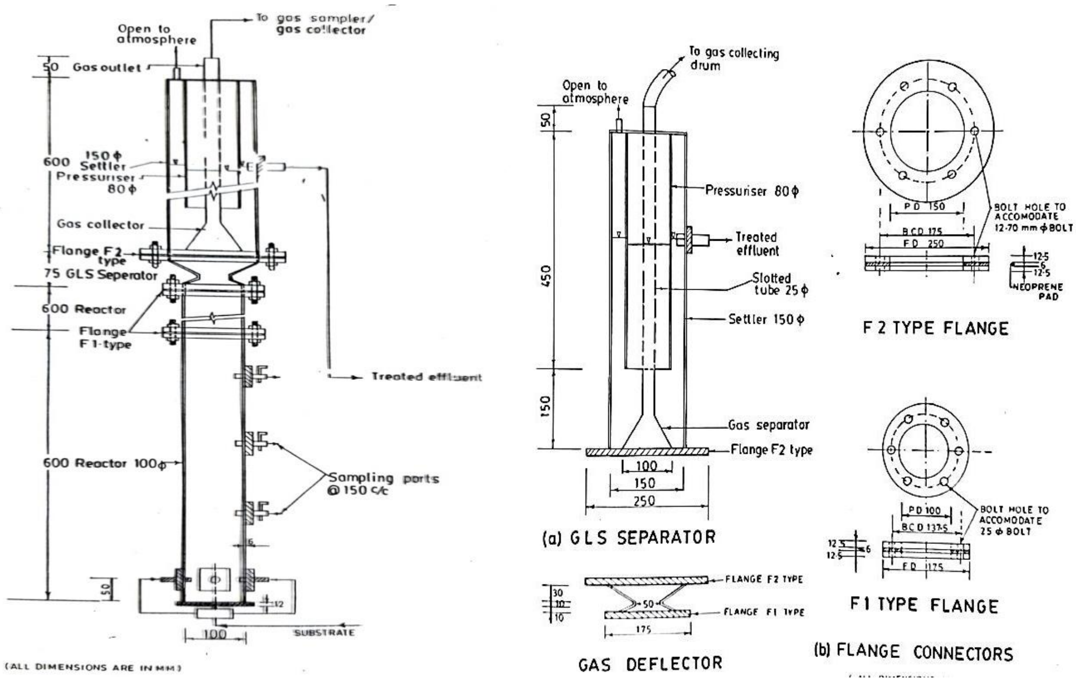


Fig. 3.1: The schematic diagram of the UASB Reactor with the fabrication details

3.3 Reactor Loading

The reactor was first seeded with the digested sewage sludge from a conventional anaerobic sewage sludge digester plant after sieving it to remove debris. To this, the micronutrients namely barium chloride, nickel chloride, zinc chloride, strontium chloride, cobalt chloride and boron, all of analytical grade were added to improve the settling characteristics of the sludge which has been suggested by Lettinga et al., 1984. The HRT was fixed to be 8 hours and the feed was supplemented with a COD: N: P, the nutrient ratio of 300:10:1 by adding urea for nitrogen and KH_2PO_4 for phosphorous. The temperature was maintained at 35 ± 2^0C. This was made possible by housing the reactor in a laminated card board environmental chamber and using a thermostat attached to a heat convector. This study consisted of three phases. During the first phase the reactor was fed with the combined substrate namely, glucose and molasses solution (50% each) so as to get the starting organic loading rate (OLR) of 1.5 g COD/l.day as suggested by Lettinga et al., 1978. Thereafter, the OLR was increased in steps. After 10 days of start-up the reactor was fed with molasses solution only. The aim was to granulate the sludge and acclimatize it to take high organic loading. The reactor was operated for 100 days and to a maximum OLR of 18 g COD/l.day.

3.4 Operation of the UASB Reactor

The substrate feeding rate was dependent on the organic loading rate (OLR) as well as HRT. The reactor was operated at a fixed OLR for a period while keeping HRT constant at 8 hours and the reactor temperature was being maintained at 35 ± 2^oC. The OLR was increased in incremental steps. Under each stepped-up loading condition, the reactor was operated till pseudo steady-state (constant COD removal efficiency and gas production rate) was attained. The reactor effluent was analyzed daily for pH, VFA and COD. The volume of the collected gas was also measured every day. At the end of each increased step-up of loading, the constituents of the VFA present in the effluent and the composition of the gas were analyzed by gas-chromatography.

4. Results And Discussion

4.1 Characterization of Molasses

The molasses procured to feed the reactor as substrate was analyzed for its characteristics using Standard Methods (APHA, AWWA and WPCF). The results of the analysis of molasses are shown in Table

4.1.1. The results show that the molasses has a high organic content of 81% and a solids content of 60% with a dark brown colour and sweet odour with a very high value of COD (9.6 x 10^5 mg/l).

Table 4.1.1: Analysis of the Molasses used

Sl. No.	Parameter	Values %
1	Total Solids	68.00
2	Organic Content of Solids	81.2
3	Inorganic Content of Solids	23.2
4	Calcium as CaO	0.44
5	Magnesium as MgO	0.57
6	Potassium as K_2O	1.86
7	Total Nitrogen as N	0.28
8	Total ash content	4.32

4.2 Start-Up and Operation of the Reactor

To begin with the reactor was fed with the solution of molasses and glucose (50% each) in tap water as substrate to cultivate and grow biomass in the reactor. The reactor was operated for 10 days initially with this substrate. The COD: N: P ratio was maintained as 300:10:1 as detailed in methodology section. The micronutrients mentioned earlier were added which improved the settling characteristics.

After the startup, the OLR was increased in several steps g COD/l.day till the reaching of the above said pseudo-steady–state was achieved (Steady COD removal and gas production rate). At the end of each increased step-up loading, the constituents of the VFA present in the effluent and the composition of the gas were analyzed by gas-chromatography. Several investigators, notably Lettinga et al. (1984), Wu et al.(1987), De Zeeuw (1980) have investigated the amount of the digested sewage sludge required for the inoculation of the reactor. Lettinga et al. (1984) have suggested an amount of seed sludge at the rate of 10 g VSS/l reactor volume. In this investigation, the seed sludge used was having a concentration of 43.5 g VSS / l. Three liters of this digested sewage sludge was fed to the reactor amounting to 13.4 g VSS / l reactor volume. The characteristics of this seed sludge are shown in Table 4.2.1. After 10 days, the reactor was fed with only molasses as the substrate. The reactor was operated and monitored continuously for a period of 100 days. The results of the performance efficiency of the UASB reactor along with the operational details during different periods of operation in the start-up period are shown in Table 4.2.2.

Table 4.2.2: Characteristics and Quantity of the Seed Sludge

Sl. No	Parameter	Value
1.	Total Solids. g/l	130.7
2.	Volatile Suspended Solids, g VSS/l.	43.5
3.	Colour of the Sludge	Dark grey
4.	Sludge Activity by Performance of the Reactor on the first day: Methane Production, l/g COD removed. Day COD removal, %	 0.25 52
5.	Height of the Sludge Bed, m	0.3
6.	Volume of the Sludge, l	3.0
7.	Sludge Occupancy, ml/g VSS (as determined in the Reactor)	23

The start-up period could be divided in to three distinct phases as explained below:

4.2.1 Phase I (OLR:≤ 2g COD / 1.day)

This phase consisted of the first 18 days from the seeding of the reactor. The reactor was operated on glucose and molasses (50% each) solution for 10 days and thereafter on molasses solution only. The COD of the reactor feed (substrate) was kept at 500 mg/l and the HRT was kept at 8h. The initial OLR was maintained at 1.5 g COD/1.day with an SLR if 0.108 g COD/g VSS.day. This phase found to have proliferating biomass with no noticeable sludge washout. At the initial OLR of 1.5 g COD/1.day, the biomass concentration was found to be 100 mg/l after 3 days of start-up. The corresponding sludge washout during this phase was 3.0 g/day which got reduced to 0.9 g/day after 15 days. This is substantially lower than the sludge washout of 48.7 g/day on the first day of operation reduced to 3.15 g/day on the 10th day as reported by Manjunath (1987). The reactor was found to have attained steady-state after 18 days of operation which is less than 22 days reported in literature (Manjunath et al., 1990). The sludge was having black colour and the original strong sewage odour persisted even after 18 days.

4.2.2 Phase II (OLR: up to 5 g COD / l.day)

During this phase of operation, the OLR of 1.5 g COD /l.day was first increased to 3.0 g COD / l.day and then to 5 g COD /l.day sequentially. The reactor was being operated at these OLRs until the pseudo-steady state was reached at each load. The average sludge washout during this phase was found to be in the range of 0.8 to 1.0 g/day. The near-absence of the sludge washout in both the above phases may be attributed to the adequate detention time (6.64 h) for the effluent in the settler at the top of the reactor and a very low up-flow velocity of the liquid ($\sim 0.068\, m/h$), giving a very high settler efficiency. The gas production rate showed continuous increase with the increase in the organic loading. The gas evolution and the bubbling action brought about rigorous mixing of the reactor contents during phases I and II. As the reactor operation progressed, the settling quality and the density of the sludge improved showing thick flocculent sludge. This is confirmed by the VSS content of the sludge which increased from 34.7 to 49.75 g VSS / I at steady-state with an OLR of 3.0 g COD / l.day. This got further increased to 51.84 g VSS / l at 5 g COD / l.day. The colour of the sludge was found to have changed from black to light grey and the initial intensity of the sludge odour was also considerably reduced.

4.2.3 Phase III (OLR: > 5 g COD / l.day)

OLR was further increased in steps of 1.0g COD / l.day. During this phase which can be considered to be the extension of the earlier phase II and could be christened as the working phase, granulation was expected to take place (Lettinga et al., 1984). After 55 days of operation, small sludge granules of about 0.5 to 1 mm size were observed in the reactor. During this period, the OLR was maintained at 8 g COD / l.day and SLR was found to be 0.51 g COD / g VSS.day. Pol et.al., (1983) and Manjunath (1987) have reported the granulation at a minimum SLR of 0.6 g COD / g VSS.day, and about 0.4 g COD / g VSS.day, respectively. Our studies further reinforce the conclusion that the granulation process may begin at a SLR in the range of 0.4 to 0.6 g COD / g VSS.day.

Manjunath et al. (1990) have obtained granulation after 48 days with molasses as feed and at an OLR of 1.5 to 5 g COD / l.day, while Jayantha and Ramanujan (1994) have reported granulation after 84 days with an OLR of 1.0 to 3.5 g COD / l.day. Low organic loadings are normally not recommended for quick setting-in of the granulation process (Lettinga et al., 1984). However, in the present study, the granulation was observed after 55 days with a much higher loading rate of 8 g COD / l.day. This may be due to improper feeding of the substrate initially and reactor – upsets due to high accumulation of VFA which forced the reactor feed to be shut down for certain periods in between. Kosaric et al. (1990) have studied the characteristics of granules from UASB reactors. They have observed that at low up-flow velocities and low OLRs, majority of the granules would be grey and white and washout of large size granules should occur. Under such conditions, the granules may undergo irreversible change in their composition and structure. Thus, the hydrodynamic conditions in the reactor influence the colour of the granules. The black granule, although more resistant to hydrodynamic and mechanical stresses, may become hollow in the core and may float in the reactor and get washed out. Similar phenomena were found to prevail in the reactor in the present investigation during this phase. However, the possible mechanisms and theory of granulation have been explained by Suneeth Kumar et al., (2023).

The loading was then increased to 9.0 g COD / l.day after which it was increased in steps of 2.0 g COD / l.day until it reached 15.0 g COD / l.day and then it was increased to 18.0 g COD / l.day. The performance of the reactor was observed to have improved with respect to the COD removal efficiency, the gas production rate and the increase in sludge concentration. The SLR got increased from 0.38 to 0.69 g COD / g VSS. Day. With the increasing accumulation of the granulated sludge, the capability of the reactor to operate with higher loadings also increased. However, the OLR increase also led to VFA build –up. The average VFA build-up during the start-up period of 100 days was about 470 mg/l with a maximum level of 660 mg/l. At each incremental increase in OLR, a sudden marginal rise in VFA was observed which got reduced with the passage of time. As and when the VFA concentration was found to be more than 500 mg/l, the inflow of the substrate to the reactor was stopped. The inflow and was restarted when the VFA level came down to around 400 mg/l. The smooth progress of the start-up of this UASB reactor and the development of good granular sludge after 55 days indicated that the loading schedule followed was appropriate. The reactor was operated and monitored continuously for a start-up period of 100 days. The above details of the startup and operation are shown in Table 4.2.2 for a period of 100days.

TABLE 4.2.2 OPERATIONAL PARAMETERS AND PERFORMANCE RESULTS DURING START-UP PERIOD

Duration days	HRT h	Feed Concen. mg/l	OLR g COD/ l.day	SLR g COD/ g VSS/l.day	VSS/ TS Ratio	Effluent pH	Effluent VFA as CH3COOH mg/l	COD removal %	Biogas production l/ l reactor volume/ day	Methane Content %	Max. Methane Prodn. Rate l/ g COD removal
0-18	8	500	1.5	0.11-0.14	0.336-0.34	7.42-6.91	140-.550	52-64	0.25-0.42	58	0.25
19-31	8	1000	3.0	0.20	0.537	7.41-6.85	240-660	63-72	0.54-0.89	64	0.26
32-46	8	1330-1660	4.0-5.0	0.31	0.544	7.61-7.23	150-450	71-80	0.11-1.54	70	0.23
47-66	8	2000-3000	6.0-9.0	0.53	0.562	7.41-6.91	270-650	71-86	1.91-2.77	75	0.27
67-75	8	3660	11.0	0.61	0.589	7.18-7.35	360-480	81-85	2.9-3.81	73	0.30
76-82	8	4330	13.0	0.69	0.605	7.42-7.21	330-440	78-84	3.91-4.61	75	0.31
83-90	8	5000	15.0	0.78	0.610	7.51-7.10	270-580	79-87	4.87-5.86	75	0.35
91-100	8	6000	18.0	0.92	0.616	7.27-7.94	400-640	81-88	3.84-6.81	75	0.32

4.3 Feeding the reactor

During phase II loading, at an OLR of 3 g COD /l.day (about 36 days from start-up), the substrate was supplied through the single port at the bottom, which was changed to two-and then to four-port system as explained in section 3.3.1. This change in the feeding pattern enhanced the COD removal efficiency of the reactor along with the enhancement in gas production i.e. COD removal got increased from 71% (single bottom port) to about 80% (4 port system) and the gas production increased from 1.11 to 1.54 l/l reactor volume.day. The earlier bottom single port feeding system could be one of the reasons for the marginal delay in granulation, indicating the importance of uniform feed distribution. This improvement in the feeding pattern might have helped later in accelerating the granulation process of the sludge.

4.4 COD Removal Efficiency

The daily variation of influent COD values and effluent COD for the different OLRs during the start-up period was noted and the COD removal efficiency of the reactor during its operation at different OLRs during the start-up period were calculated which are shown in Fig. 4.4.1.

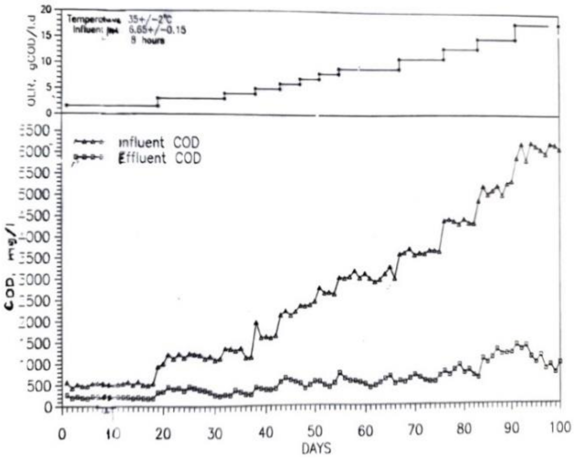


Fig. 4.4.1: Variation of daily COD

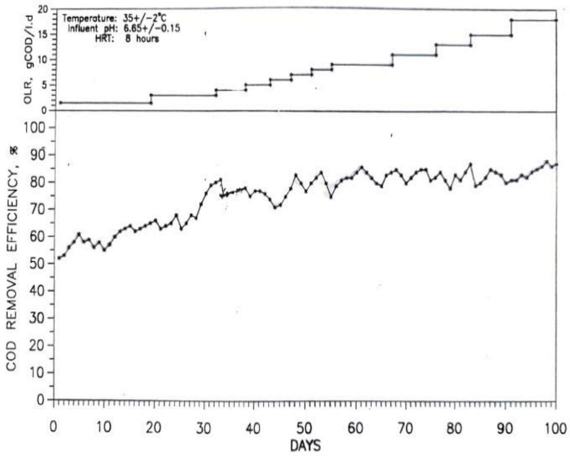


Fig. 4.4.2: Variation of % COD Removal

Fig 4.4.2 shows The COD removal efficiency increased from 52% at an OLR of 1.5 g COD / l.day to 88% at an OLR of 18 g COD /l.day. The relative decreases in efficiency at several points are due to abnormal build-up of VFA within the reactor. On an average, the COD removal efficiency increased with the step-up increase in OLR. The removal efficiency is found to vary linearly with a gradual slope after an acclimation period of about 28 days which is depicted in Fig. 4.4.3 (OLR: 4 g COD / l.day).

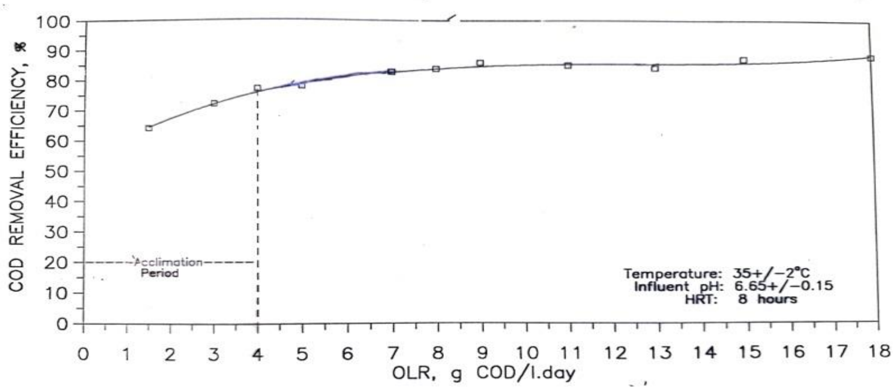


Fig. 4.4.3: Variation of % COD Removal with OLR

However, the gas production corresponding to COD conversion was markedly influenced by the reactor environment provided by the organic and sludge loading rate (SLR) that were imposed. It was observed that for every step increase in OLR, there was an increase in the gas production as well as the COD removal efficiency. However, the gas production and the COD removal efficiency reached a steady-state level in about 3-4 days for each step of OLR increase, showing the adjusting capacity of the reactor to the increasing loads, hovering around steady-state. The maximum COD removal of 88% was observed at maximum OLR of 18 g COD / l.day.

4.5 Sludge Development and Concentration

The reactor sludge defined as total solids (TS) and volatile suspended solids (VSS) varied during the reactor operation and this variation is projected in Fig. 4.5.1 along with the ratio of VSS/TS. It is found that the sludge growth diminished during the first 18 days of the operation (OLR:3g COD/l.day). However, it started increasing thereafter. This behavioral pattern is depicted by the ratio of VSS to TS. It was 34% at an OLR of 1.5 g COD / l.day which got increased to 61% at an OLR of 18 g COD / l.day. The sludge concentration in the reactor, one of the chief factors to evaluate the performance of the reactor, could not be determined accurately as the sludge bed was found to expand and contract due to the entrapped or rising gas bubbles, and the internal circulation of the reactor contents due to up-flow velocity and the gas induced buoyancy. A similar experience has been reported by Riera et al. (1985) while treating distillery wastewater. The profiles of VSS determined along the height of the reactor at various OLRs that are shown in Fig.4.5.2.

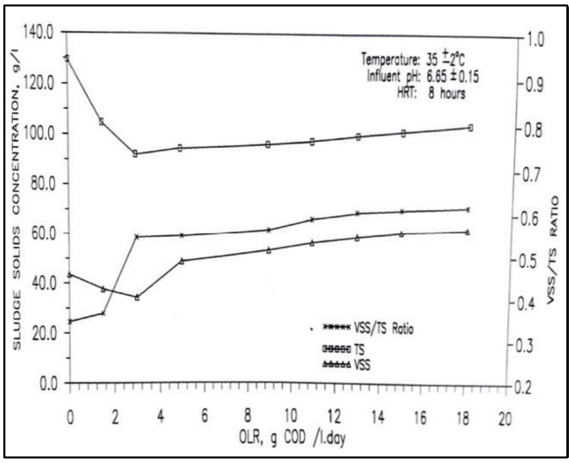


Fig. 4.5.1: Variation of % COD Removal with OLR

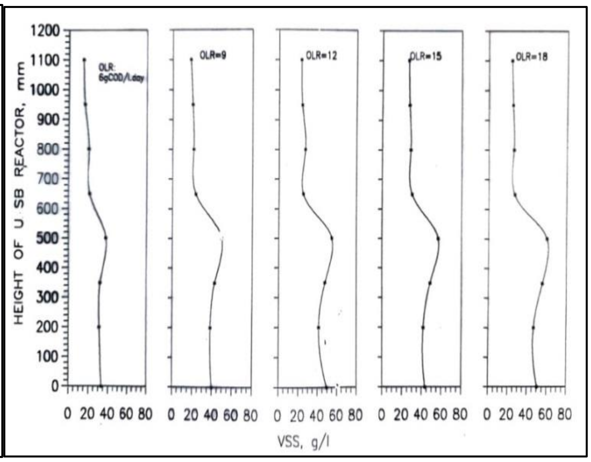


Fig. 4.5.2: VSS Profile along the height of the reactor

4.6 Granulated Sludge

The granulation of the seeded sludge is shown to be substrate dependent and it may not occur at all for some specific substrates. Pol (1983) has reported that granulation did not occur with mixed VFA as substrate with high ammonium concentration (1000 mg NH4- N/l). Further, for some wastewaters, such as cheese whey, granular sludge did not develop with non-granular sludge as seed but when inoculated with granular sludge, the granules were intact and well maintained (Yan et al., 1983). Furthermore, for some wastewaters like distillery spent wash, the inoculated granules are reported to have got disintegrated and transformed to heavy particles with high ash content (Drissen et al., 1994).

The granulation process which started after about 55 days during the start-up period showed interesting patterns. Plate 4.6.1 shows the difference in structure between the raw seed sludge and the granular sludge. Initially, the granules were of small and almost uniformly sized flaky discrete particles as can be seen in the photograph. Most of the granules were with sharp edges, characterized as spiky granules with an average size of around 1 mm x 1 mm and 0.5 mm thick. The granule size increased during phase III operation, and at the end of this phase the average size of each granule got increased to about 1 x 2 mm which are shown in plate 4.6.1. The sharp edges became smooth and the thickness also got increased to about 1–1.5 mm. Lettinga et al. (1980 and 1984), Pol et al. (1982, 1983) and Pette and Versprille (1982) have reported that the granules were varying between 0.5 and 5 mm size with an average predominating size of 1to 3 mm. These findings were for different types of wastewaters. In the present study, some of the granules were found to be almost spherical in shape with an average diameter of about 3 mm. However, the number of such granules was very small. Most of these spherical granules floated on the top of the reactor. A similar observation has been reported in literature (Lettinga et al., 1984). The sludge profile along the height of the reactor (Fig. 4.6.1), indicates that the maximum concentration of the granules was between the top of the sludge bed (Port at 500 mm from the bottom of the reactor) and the middle port (at 350 mm level) while the bottom port above the inlet (at 200 mm level) had a low concentration. This may be because of the effect of fluidization at the inlet of the reactor.

Plate 4.6.1: Difference between Seed Sludge and Granulated Sludge

4.7 VSS Profile along the Reactor

Bio-mass content of the sludge is measured as volatile suspended solids (VSS) in wastewater treatment practices. The VSS content can be considered to be a fair representative of the bio-mass as the organics present would be only of microbial origin. As shown in Fig. 4.5.1, at the end of the start-up period, the reactor could accumulate a maximum VSS concentration of 63.8 g / l in the sludge bed under an OLR of 18 g COD / l.day. The VSS profile also shows that a distinct blanket consisting of small sludge particles formed as a result of either smaller granular sludge formation / disintegration or wearing out of granules of sludge bed under the influence of rising gas bubbles. The maximum VSS concentration of the bed decreased from the initial 43.5 to 34.7 g VSS / l during acclimation and then got increased to 63.8 g VSS / l. The concentration in the blanket at the interface between the bed and the blanket was in the range of 11.42 to 26.85 g VSS / l. The VSS concentrations in the sludge bed as well as in the blanket are matching with the range of values reported in literature, although the reactors were operating on different substrates. Heertzes and Meer (1978), and Pette and Versprille (1982) have reported VSS concentration of 50 – 100 g VSS / l in the sludge bed and 5 – 20 g VSS / l in the sludge blanket. Manjunath (1987) has reported the concentration of the sludge bed of 55 g VSS / l and that of the blanket between 5 –25 g VSS / l. The sludge blanket in this study has shown a VSS profile of higher concentration. This may be due to very good efficiency of the settler provided at the top of the reactor.

4.8 Settling Velocity

The settling velocity affects the amount of sludge hold – up inside the reactor since the sludge develops under the influence of rising liquid – gas stream. The settling velocity depends on the size, shape, mass density and concentration of the granules and also on the viscosity of the liquid. The settling velocities of several granules were determined by observing the fall of granules gently released at the top of a 2 liters measuring cylinder filled with the treated effluent. The settling velocities of the granules were found to be in the range of 0.60 to 1.10 m/min and the granules varied from 0.7 mm to 2 mm in size. Pol et al. (1983), Schmidt and Ahring (1996), Pette and Versprille (1982), Dolfing et al. (1985), and Andras et al. (1989) have reported the settling velocities in the range of 0.3 – 0.83 m / min. Manjunath (1987) has reported settling velocity in the range of 0.75 – 1.0 m / min for granules of 2 – 3 mm size with molasses as the substrate. In the present investigation, the maximum size of the granules was within 2 mm and the maximum settling velocity was found to be around 1.1 m / min. This shows the presence of high density small sized granules in the reactor. The reduction in size of the granules can be attributed to the high over burden pressure of the column of water in the reactor as well as of the long settler on the top of water. The density of the granules was found to be in the range of 1060 to 1400 kg/m^3. This indicates the dense aggregation of anaerobic microorganisms together with the inorganic matter to show good settling characteristics of granules. The reactor had, at any time granules of different sizes and density. At the given linear flow velocity of 0.15 m/h (2.5 m/min), even small sized granules with high densities and large sized granules with low densities were prevalent.

4.9 Sludge Occupancy

The sludge occupancy, a measure of compactness of sludge, was observed to be about 20 – 25 ml/g in the present study. Sludge occupancy values of 20 -40 ml/g for flocculent sludge have been reported by Lettinga et al. (1980) from a reactor treating beet sugar mill wastewater. Manjunath et al.(1990) have observed the occupancy values ranging from 14 – 20 ml/g for granular sludge and 40 – 50 ml/g for flocculent sludge while treating sugar mill wastewater.

4.10 Sludge hold–up in the reactor

The sludge retention / hold–up in the reactor depends on the following interrelated factors:

- Sludge Settleability
- Sludge Washout
- Gas Production / Loading rates
- Gas – Liquid – Solid Separation and
- Design of the Reactor

A negligible amount of sludge wash out has been observed during the present study. This may be attributed to the large settler provided at the top of the reactor. The VSS concentration in the effluent during the start – up period, being of the order of 100 mg / l at the beginning, got reduced to a mere 30 mg / l after the formation of the granular bed. This indicates that the conditions inside the reactor were favourable for a good sludge hold – up. At times, a few large, fluffy, round and white pellets of about 6 mm size, were found to float on the top of the liquid surface and washed out along with the effluent. This may be due to the loose flocculent structure with low bulk density due to the entrapment of gas in the core (Kosaric et al., 1990a). Sludge washout has been reported to be less in a granular bed than in flocculent sludge bed (Lettinga et al., 1984). The increased gas production and consequent good mixing enhances the sludge thickening. Under this situation, entrapped gas gets released leading to lesser bed expansion than during low gas production rates. But excessive gas production may disintegrate the granules and / or deplete the level of the sludge bed due to washout of suspended solids. Hence for the better performance of the reactor, an optimum gas production and OLR is essential to ensure necessary sludge hold-up.

4.11 Stability of sludge bed

Sludge bed, particularly the granular bed was stable under the loading rates employed in the study. Once the granulation was set-in, there was no undue expansion of the bed. A good sludge retention has been reported by Pol et al.(1983) while treating soluble carbohydrates. They have reported that stable granules easily develop on wastewaters with high VFA.

4.12 Sludge Loading Rate

Fig. 4.12.1 shows the sludge loading rate (SLR) corresponding to different OLR and operating days. It shows a minimum value of 0.11 g COD / g VSS. day to start with, at an OLR of 1.5 g COD / l.day which increased to a maximum value of 0.69 g COD / g VSS. day at an OLR of 18 g COD / l.day. Unlike the aerobic process, the cell yield in anaerobic process is low. Hence, the SLR, normally increases with the increase in OLR. Lettinga et al. (1980, 1984) have reported SLR values in the range of 0.5 to 1.1 for beet sugar, 1.0 to 1.4 for potato processing waste, 1.1 to 1.8 for alcoholic wastes and 2.3 g COD / g VSS.day for VFA bound substrates. Jayantha and Ramanujan (1995) have reported a value of 1.6 g COD / g VSS.day at the OLR in the range of 3.5 to 4.8 g COD / l.day for distillery spent wash. Manjunath (1987) has reported SLR in the range of 0.55 to 0.58 g COD / g VSS.day for OLR in the range of 10 to 12 g COD / l.day operating on molasses solution. Thus, it is found that the values obtained in present study are slightly higher than the values reported by Manjunath (1987) and are in the range of values reported for beet sugar substrate.

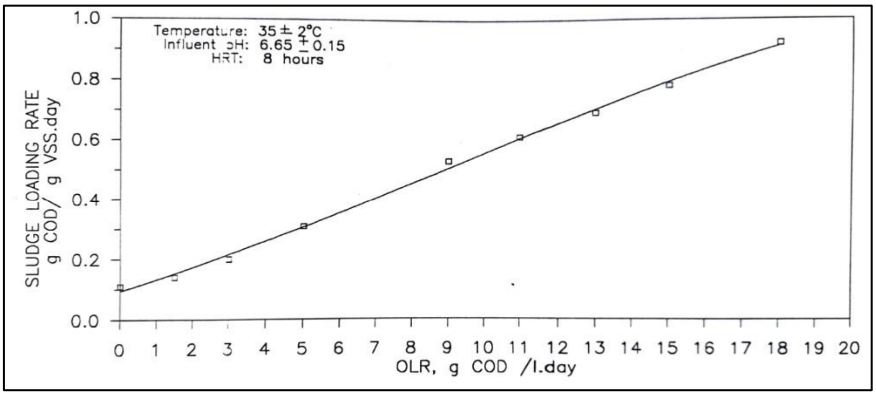


Fig. 4.12.1: Variation of Sludge Loading Rate with OLR

4.13 Gas Production

Gas production is one of the key parameters indicating the health of a reactor. At low loadings gas production is less, and the sludge bed expands due to entrapped and accumulated gas. The increase in gas production due to higher loadings brings about additional turbulence causing good mixing and consequently enhancing the sludge thickening. The gas entrapped in the bed is thereby released and the expanded bed contracts. This phenomenon continues and the sludge bed height increases. But excessive gas production may result in disintegration of granules, causing undue washout of sludge and in turn depletion of the sludge bed. Hence an optimized sludge – hold up with correct SLR would be essential with a view to have an efficient operation of the reactor.

Total gas productions on various operating days are shown in Fig. 4.13.1 for different OLRs. Due to the presence of viable microbial cells in the seed sludge for anaerobic treatment, bio-mechanization started immediately after the feeding of molasses solution into the reactor. The biogas production rate and the methane production rate were found to vary almost linearly with OLR after some acclimatization as shown in Fig. 4.13.2. The slope of this line represents the gas production in l/g COD applied. At an OLR of 1.5 g COD / l.day with 50% molasses and 50% glucose solution as feed, biogas production was found to be 0.42 l / l reactor volume.day on the 18th day of operation during the start-up period. The average methane content was 62%. Biogas production reached a maximum value of 6.81 l/ l reactor volume.day on the 100th day with an OLR of 18 g COD/ l.day.

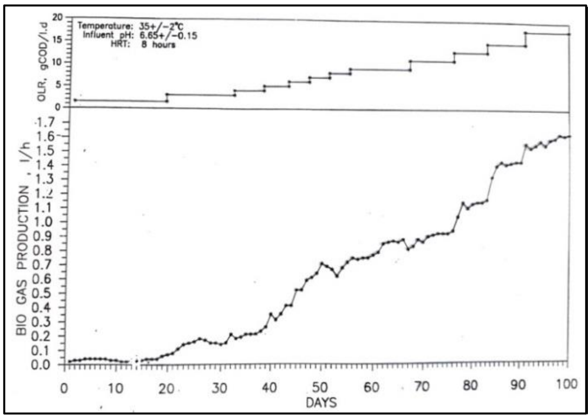


Fig. 4.13.1: Total Gas Production during operating days

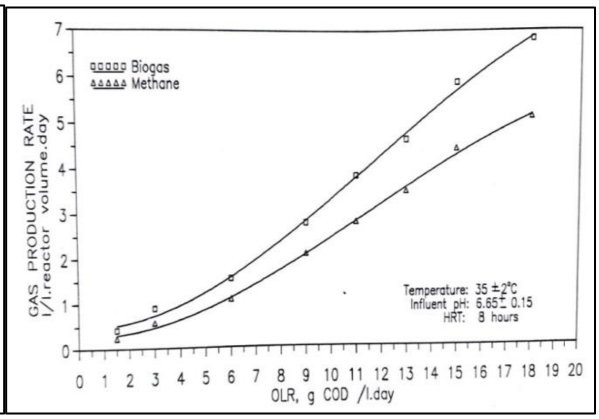


Fig. 4.13.2: Variation of Gas and Methane with OLR

The methane content in the biogas was found to be low in the initial stages. It, however, went up later on with the increasing organic loadings, reaching a maximum of 75% at an OLR of 18 g COD/ l.day. Fig. 4.13.2 shows also the methane gas yield at various OLRs. The methane gas yield was found to be varying between 0.32 – 0.35 l/ g COD removed.day, which is quite matching with the theoretical value of 0.37 l/g COD removed.day (Manjunath, 1987).

During experimentation, sometimes methane production was observed to exceed the theoretical value of 0.35 l methane / g COD removed.day at STP. Such events were generally preceded by either high loadings or disturbed digestion environment. Presumably some amount of COD would be retained in the sludge bed. Further, some excess COD may be exerted by the dead cells (Cell Lysis), and its subsequent metabolization. This cell lysis may be due to toxicity, shock loading etc. Both the above factors may lead to excess methane production. Similar experience has been reported by Li and Digiano (1983). In the range of loadings studied after the initial acclimation period, no significant effect of organic loading on methane production was found (Fig. 4.13.2) and the values hover in the range of 0.3 to 0.35 l/g COD removed at 37^{0}C and 1 atmospheric pressure.

4.14 VFA and pH Levels

The concentration of volatile fatty acids (VFA) is one of the major controlling parameters in anaerobic digestion. This was monitored and kept around 500 mg/l, the limit reported in anaerobic fermentation (APHA, AWWA and WPCF, 1989). The variation of VFA with OLR on the operating days during the start-up period is shown in Fig. 4.14.1.

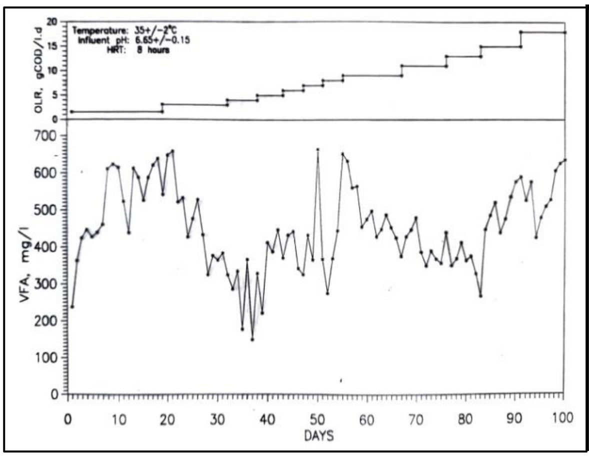


Fig 4.14.1: Variation of VFA in Reactor

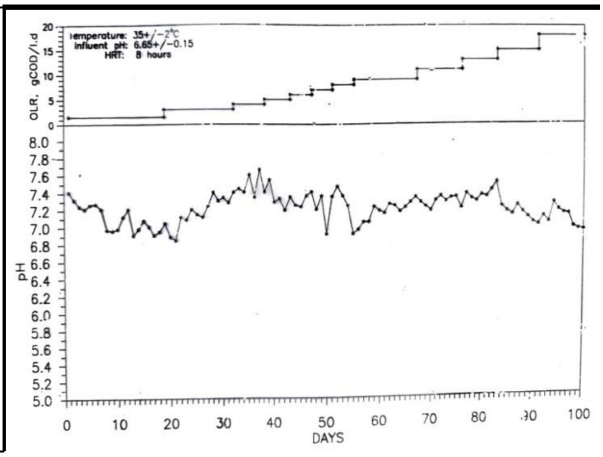


Fig 4.14.2: Variation of pH in Reactor

The level exceeded this value a few times for a short duration which was immediately brought down by stopping the feed in to the reactor so as to avoid further accumulation of VFA inside the reactor. In the conventional anaerobic digestion literature, an ultimate limit of 2000 mg VFA/l has been specified beyond which the microbial activity in the reactor stops and the reactor becomes stuck.

For the control of VFA level, the control on the influent pH is also equally important. The pH of influent molasses solution which was about 4.3 was raised and maintained between 6.5 –7.0, the optimum range for good methanogenesis, by using sodium bi-carbonate. The maintenance of a higher influent pH would result in neutralization of the VFA formed. Reduction in pH to a low level would increase the VFA to inhibitory level. The variation in the effluent pH with the different OLRs for all the days during the start-up period is shown in Fig. 4.14.2 above. As can be seen, the pH varied between a minimum of 6.85 and a maximum of 7.61 with the average at 7.2. This indicates that the reactor showed good adjustability with respect to the buffering capacity of the reactor. This may be attributed to the possible formation of sufficient alkalinity ($CaCO_3$).

4.15 Composition of VFA

The analysis of VFA in the reactor effluent was carried out using the gas NIKON Chromatograph with Chromosorb – 102 glass column and FID detector. This analysis confirmed the fact that acetic acid was utilized almost completely, at low loadings while propionic acid was the major component present in the reactor effluent. This is normally expected. The results of this analysis are shown in Fig. 4.15.1. The accumulation of propionic acid indicates that the propionic acid degradation could be the rate limiting step in the anaerobic reaction. At lower loads, almost all acids are utilized almost completely which started accumulating slowly after reaching an OLR of 9 g COD/l.day.

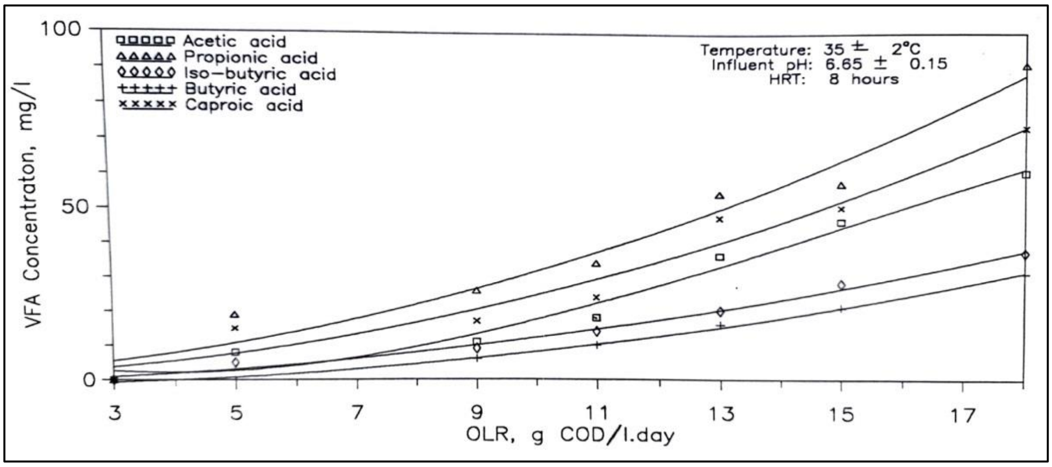
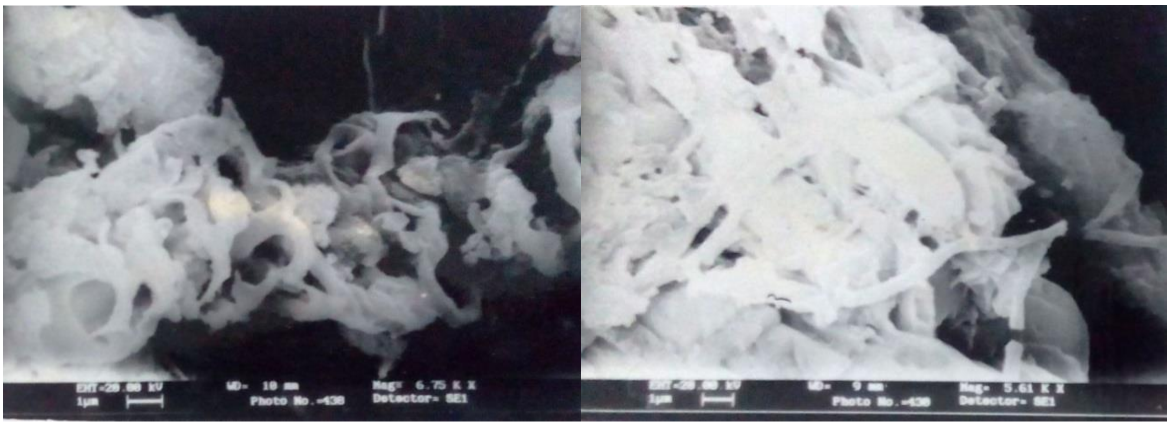


Fig. 4.15.1: Variation of VFA Component Concentration in the effluent

4.16 Microbial structure of Sludge

For this study, the microbial inoculum was procured from a working anaerobic bio-reactor treating municipal wastewater. The Scanning Electron Micrographs (SEMs) of both the raw sludge and the bisected granular sludge have been obtained (Suneeth Kumar, 1978). As shown in the plate 4.16.1, the aggregation of the flocs of methano-bacteria, intertwined with each other with profuse porous nature could be seen the seeded in the SEM of raw sludge, micrograph. Proliferation of methano-bacteria could be seen on the surface. A variety of methanogen -like bacteria can be found. The bacteria in the raw digested sludge resemble like Methano-spirillum, M. barkeri and methano bacterium.

The SEM of the surface of the bisected granule showed proliferation of a large number of Methano-seata species, densely packed. Large number of Methanobacterium (rods), some Methanococcus (Cocci) and bacilli along with some scattered colonies of Methanothrix and Methanosarcina (Methanoseata) could be seen in this SEM.

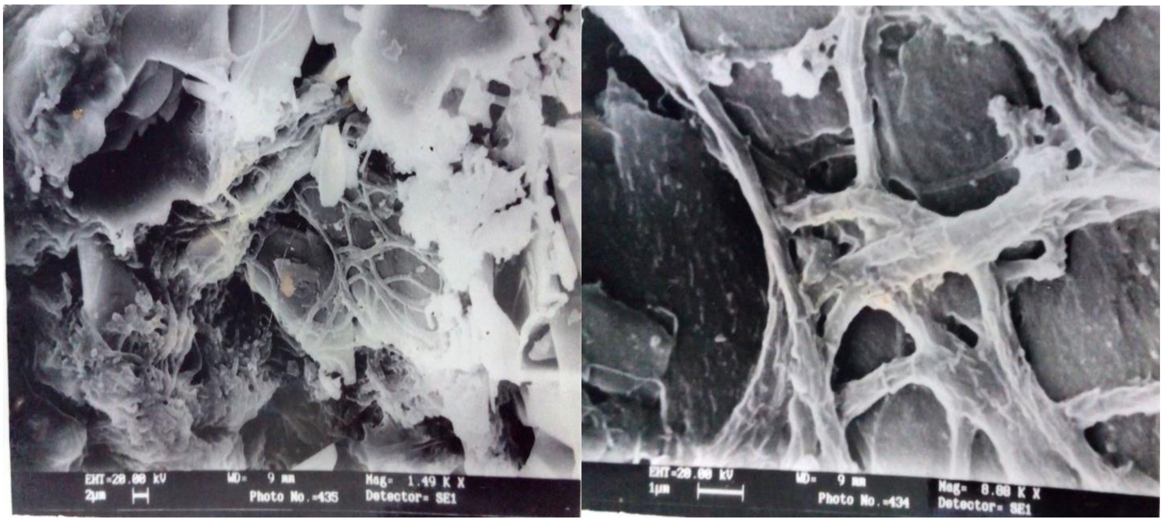


A) Proliferated methanogenic group **B) Diversified morphology with methanogens**

Plate 4.16.1: SEMs of the seed sludge

These also show networks with diffused layers of unspecified syntrophic organisms. The Plate 4.16.2A clearly exhibits the sphagetti like structure, typical of VFA grown granules (Dolphing, 1986). The presence of a number of short, blunt-ended rods depicting methanothrix is also the typical micro-structural element of a mixed VFA feed (Lettinga et al., 1984). The bands are at the edge of the plate like structure at the end of each rod as in

Plate 4.16.2B (Fang et al., 1994). These are reported to contain small carbohydrate-positive and protein negative areas (Quarmby and Forster, 1995).



A – Top-Calcium PPT; Left – Spaghetti type Center – Methanothrix **B –Methanothrix showing rods and plates**

Plate 4.16.2: SEMs of a bisected sludge granule

5. Conclusions

1. Raw sewage sludge can easily be granulated within a short period of 55 days by adopting cane molasses as the primary subtrate. The granule size varied between 0.5 to 2 mm.
2. Molasses proved to be advantagoeus for the start-up due to its high COD concentration facilitating a small volume adoption which would be easy to handle.
3. The sludge granules were angular in shape and their size initially varied from 0.5 to 1.0 mm which got incresed later to about 2mm.The granules were of density 1060 -1400 kg/m^3.The sludge occupancy of this granular sludge was between 20-25 ml/g VSS.
4. Increase in OLR could be maintained in steps of 1.0 g COD/l.day. The maximum OLR reahed in this study was 18 g COD/l.day.
5. The COD removal was quite satisfactory with a maximum of 88% at an OLR of 18 g COD/l.day.
6. The bio-gas production was 6.81 l/ l reactor volume.day with methane content of 75% which is quite comparable to many literature values.
7. The scanning electron micrographs exhibited an excellent match with the mentioned values of many authors indicating the athenticity of the work. While the outer surface exhibited a mixed morphology, the inner core showed definite presence of Methanothrix, an expected species for high VFA bound substrate.
8. The reactor exhibited excellent adoptability for the substrate change from the mixed glucose and Molasses mode to pure molasses mode, indicating the shock sustinance.

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