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EFFICIENCY OF TWO PHASE UASB REACTOR WITH INTERNAL PACKING COLUMN FOR THE TREATMENT OF DAIRY EFFLUENT

P.S. ArunaDevi[1], M. Saravanaraja[*2] & K. Nagarajan[3]
[1,*2&3]PG and Research Department of Zoology, Sri Vasavi College, Erode (Affiliated to Bharathiar University, Coimbatore) Tamil Nadu, India – 638 316

ABSTRACT

Generation of wastes is a riotous component of modern industrialized world. Dairy industries are one of the most considerable due to release of both solid and liquid wastes. Even though many technologies are trustworthy to adopt in dairy industry, anaerobic digestion is considered as best choice due to the better out comings. While construction of the suitable anaerobic digester is the most need to treat colossal amount of dairy waste. Objectives of the present study are physico-chemical characterization of dairy processing industrial effluent to assess it for their suitability in anaerobic treatment, designing two-phase Upflow Anaerobic Sludge Blanket(UASB) reactor with internal packing column for treating dairy waste water and to examine the maximum COD removal of dairy effluent, the reactor operated with different organic loading rate at different phases. The collected dairy water samples were analysed following the standard methods of water and waste water analysis by American Public Health Association. The UASB reactor used in this experiment was designed having the following specifications. Height: 1m, breadth: 16cm, width: 16cm, feed inlet points (sample port): 4, diameter of sample port: 4mm, aperture area: 5cm, volume of the UASB reactor: 25 l, volume of the gas separator zone: 5lt, peristaltic pump: 4-40ml/min, effective volume of the reactor: 20 l. With this reactor the dairy waste water was treated and all the physico chemical and biomass characters were observed.

Key words: *Dairy waste water – UASB reactor – biomass characters.*

I. INTRODUCTION

Total waste water generation from major water consuming industries such as agro based industries, refineries, petrochemicals, fertilizers and industrial chemicals was estimated to about 3000 million litres per day in 1997 [1]. Almost 65% - 70% of the organic pollutants released in the water bodies in the country accounted for by food and agro-based industries such as distilleries, dairies, sugar factories and pulp and paper mills.Potential environmental issues associated with dairy processing activities include the significant consumption of water for processing and cleaning, the discharge of waste water with high organic load which is higher than that of the community wastes, unpleasant odors and consumption of energy.

Several conventional aerobic treatments have been used extensively in the dairy industry; aerated lagoons, activated sludge processes [2,3], trickling filters [4] and rotating biological contactors [5]. However, the energy requirements for aeration in these installations are high and problems such as bulking and excessive growth often occur. Treatment of dairy waste waters by means of upflow anaerobic sludge blanket [6,7,8], hybrid UASB reactors [9]; expanded granular sludge bed (EGSB) reactors [10], as well as others based on anaerobic filters [11, 12,13] have been reported.

Anaerobic digestion of cheese whey offers an excellent solution in terms of both energy saving and pollution control [14]. The major advantage of this processes are low cost, higher energy efficiency and process simplicity compared to others waste treatment methods. Appropriate techniques of composting organic wastes with suitable additives can greatly improve their value as manure, and at the same time can accomplish the protection of the environment. The addition of carbonaceous materials to nitrogen-rich wastes is found to be the most effective way to reduce nitrogen losses during composting [15]. Objectives of the present study are physico-chemical characterization of dairy processing industrial effluent to assess it for their suitability in anaerobic treatment, designing two-phase UASB reactor with internal packing column for treating dairy waste water and to examine the maximum COD removal of dairy effluent, the reactor operated with different organic loading rate at different phases.

II. MATERIALS AND METHODS

The dairy effluent was collected from a nearby dairy processing industry. Waste generation in dairy processing facilities is characterized by high daily fluctuations. Therefore samples were taken at different time intervals of a day. The collected samples were analysed following the Standard Methods of "Water and waste water analysis" by APHA(1998)[16]. The seed slurry was collected from EID Parry Distillery Unit located at SIPCOT, Cuddalore, Tamil Nadu, India. The acidogenic and methanogenic seeds were collected in separate airtight plastic containers (35 litre volume). The collected seeds were stored at room temperature. The distillery slurry was presented as dispersal (flocculent) form.

The acidogenic and methanogenic reactors were inoculated with acidogenic and methanogenic slurries respectively. The seed slurries were poured into the reactors up to 35-40% and allowed to settle down. After settling, the height of the sludge beds were observed and it was 12.5cm in acidogenic reactor and 22cm in methanogenic reactor.

Construction of laboratory scale Two-Phase anaerobic digestion system
It consisted of an acidogenic reactor for the purpose of pre-acidification of the influent and an Upflow Anaerobic Sludge Blanket (UASB) with internal packing column as the methanogenic reactor. The two reactors were constructed in transparent acrylic sheet. The dimension of the acidogenic reactor was 18cm width, 18cm breadth and 50cm height. The bottom of the reactor was provided with a feed distribution system to ensure maximum contact between influent and the microbes. The diameter of the inlet and outlet nostrils was of 10mm in order to avoid clogging. The UASB reactor used in this experiment was designed having the following specifications.

Designing of UASB Reactor
Height: 1m, breadth: 16cm, width: 16cm, feed inlet points (sample port): 4, diameter of sample port: 4mm, aperture area: 5cm, volume of the UASB reactor: 25 l, volume of the gas separator zone: 5lt, peristaltic pump: 4-40ml/min, effective volume of the reactor: 20 l.

This unit was fed by the pH adjusted pre-acidified dairy waste water. The feed inlet of the UASB reactor was divided into several sub-ports. They are perforated for the even distribution of pre-acidified waste water over the methanogenic microbes.

A three phase (Gas-Liquid-Solid) separator was placed in the upper end of the reactor for separating and collecting the gas generated. The Gas-Solid-Liquid (GSL) separator was made of acrylic sheet with an aperture area of 5cm diameter. A wire mesh was fixed above this and ceramic beads of different sizes were spread over the mesh. The ceramic beads were used to enhance the effectiveness of the treatment through polishing effect and also to trap the suspended solids on the surface of the beads. The effluent above the separator overflowed into a collector, while the sludge was retained in the reactor. The gas has collected by the GSL separator. Four sampling ports were fixed along the height of the reactor, starting from a distance of 19cm from the reactor bottom. At the bottom of the reactor one more sample port with 10mm diameter was provided for sludge sampling. A plastic tube was used to connect the gas collector to an alkaline liquid (10% NaOH) displacement system, where the CO_2 got removed from methane gas. The methane gas production was measured by a wet-test gas-meter. A peristaltic pump was used to control the flow rate and to pump the influent continuously from the feed tank to the acidogenic reactor.

Biomass characterization
During the operational period, samples were periodically collected from the acidogenic and methanogenic phase sample ports. Volatile Suspended Solids (VSS) were monitored once in three days. Specific methanogenic activity of the methanogenic reactor was monitored and calculated during each phase. The presence of microbes and morphology of the bacteria were observed by light microscope through thin smearing technique.

Sampling and analysis

To assess the performance of the two phase UASB reactor, all relevant parameters were examined regularly. In the acidogenic reactor pH, VFA and alkalinity was regularly monitored at least once a day. The percentage of acidification rate in the acidogenic reactor was calculated following[17]. The formula used to calculate the percentage of acidification was:

$$\text{Percentage of acidified COD (\%)} = \frac{\text{COD of VFA (mg/l)}}{\text{Soluble COD (mg/l)}} \times 100$$

In the methanogenic reactor the parameters like pH, VFA, Soluble COD, Total COD, Alkalinity, Total Kjeldahl Nitrogen, Total Phosphorus, TSS, Biogas production and Oil and Grease were constantly monitored. In the methanogenic reactor, the pH and TSS were monitored in the reactor Sample Port 3 and after it passing through the internal packing column.

The pH, alkalinity and VFA were examined on daily basis. Other parameters like Total COD and Soluble COD levels were measured on alternate days. TSS, TKN, Total Phosphorus and Oil and Grease were measured twice a week. TSS was monitored on alternate days during the initial period of each phase.

All the parameters were analyzed according to the Standard methods for the examination of water and waste water analysis [16].ThepH was measured by a digital pH meter (Phillips). TSS was calculated by filtering the sample through No.40 Whatman filter paper and allowed to evaporate at oven temperature of 105^{o}C for 24 hours. To estimate VSS, the sample was evaporating at 550^{o}C for 1hour in a Muffle Furnase. Volatile Fatty Acids (VFA) productions were measured by a two stage titration method developed by Anderson and Yang (1992)[18].

III. RESULTS

Characteristics of dairy waste water

The results indicate that the organic strength of the dairy waste water was high, it extended from 2000-8000 mg/l of COD and 1200-500 mg/l of BOD.

Total Suspended Solids of dairy effluents were high, they might have originated from coagulated milk, curd fines or from flavoring ingredients. The nature of these suspended solid sources makes them predominantly organic. This was confirmed by the high mean VSS:SS ratio. An average, about 71% of the SS solids were volatile, even though the ratios varied over a wide range.

Dairy industry typically results in highly variable waste water pH values. Composite samples had pH values of 7.2. Whereas, periodical sample values indicate that pH ranged between 4.0 and 11. Three common forms of P (Orthophosphate, Polyphosphate and organically bound P) were present in dairy processing effluents. The total P concentrations in the sample ranged from 20-104 mg/l, the composite sample had 63 mg/l.

TKN concentrations ranged from 39 to 141 mg/l and in the composite sample 89.6 mg/l. In addition to the above, substantial amounts of K, Ca and Mg were also detected in the waste water samples. The concentrations of other elements generally were low, with average values in the µg/l range. Heavy metals, such as Cu, are inhibitory or toxic for activated sludge microbial communities at concentrations around 1mg/l. Since these elements were only present at very low level, they should not be a concern for the biological treatment of milk processing waste waters.

IV. DISCUSSION

Biomethanation

The anaerobic decomposition of Dairy organic matter into methane and carbon dioxide is a multi-step process involving a well-organized community of various microbial populations. In the first step, organic polymers (eg. Polysaccharides, fats and proteins) are hydrolyzed into smaller units (eg. Sugars, long-chain fatty acid and amino acids) by extracellular hydrolases excreted by obligate or facultative anaerobes. The products resulting from the hydrolysis are subsequently used as substrates in the next step which is often referred to as acidogenesis. This degradation pathway is often the fastest step and also gives a high-energy yield for the microorganisms. The fermentation products resulting from the acidogenic steps include acetate and reduced intermediates such as lactate, ethanol, propionate, butyrate and higher volatile fatty acids.

Conversion of these electron sinks to acetate, carbon-dioxide and hydrogen is carried out in the acetogenic steps by obligate hydrogen-producing acetogenic bacteria. Because of a low-energy yield from the acetogenic degradation, acetogenic microorganisms are very slow growing and sensitive to changes in organic load, flow rate and environmental changes. The substrates that can be used in the final methanogenic step are acetate, H_2/CO_2, methanol and formate. The most important methanogenic transformations are the acetoclastic reaction and the reduction of CO_2. Very few known methanogens can perform the acetoclastic methane production whereas nearly all known methanogenic species are able to produce methane from H_2/CO_2.

The different microbial populations involved in anaerobic digestion do not have the same requirements on operations conditions. The growth rates and pH optimal are different for acidogenic and methanogenic organisms. In a one-stage digester, the pH and the organic loading rate (OLR) are adjusted to suit the slow-growing methanogenic organisms at the expense of the relatively fast-growing acidogens and process efficiency as a whole. The operation conditions for the different microbial groups can be further optimized if the process is divided into two stages in separate reactors.

The hydrolysis and acidogenesis takes place in the first reactor. The effluent from this reactor is used as a feed stream to the second stage, a biomethanation reactor. The methanogens in the second stage can be effectively protected from overload and toxic shocks by close monitoring of the connecting stream. A monitoring parameter that indicates the intermediate products (eg. Sugars, long-chain fatty acid and amino acids) from the first stage and consequently reflect a loading of easily biodegradable organic material to the biomethanation reactor could be selected with a high priority.

Anaerobic digestion leads to the overall gasification of organic wastewaters to methane and carbon dioxide. Although anaerobic digestion processes have been carried out for decades, interest in the economical recovery of fuel methane gas from industrial and agricultural surpluses has recently increased owing to the changing socio-economical situation in the world [19]. Rapid industrialization has resulted in the generation of a large quantity of effluents with high organic contents, which if treated suitably, can result in a perpetual source of energy. In recent years, considerable attention has been paid towards the development of reactors for anaerobic treatment of wastes leading to the conversion of organic molecules into biogas.

In recent years, anaerobic process is being tried for the treatment of dairy waste waters. However, anaerobic digestion is not widespread in the dairy industry, largely due to the problems of slow reaction, which requires longer HRT and poor process stability, especially for effluents rich in components that are subject to rapid acidification. An attempt has been made by the phase separation to treat dairy waste water.

The results obtained by this two phase UASB anaerobic digestion of dairy waste water are discussed here under.

Start up: Start-up is often considered to be one of the most important as well as difficult phase in anaerobic digestion because of their instability. Its main task is to develop a highly active settleable sludge as quickly as possible. While as anaerobic bacteria are slow- growing microorganisms, major problems encountered with UASB are the typical long reactor
start-up.
In the present study, the anaerobic sludge collected in the form of active biomass from anaerobic digester of a distillery plant. Eventhough it was an active biomass, the adaptation of microbes to the new feed substrates took some time. Start-up times for UASB reactors with digested sewage sludge usually take several months [20, 21].

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Hence, long start-up period is the major deterrent to the use of UASB system. The reduction of start-up period is one of the key parameters to increase the competitiveness of high rate anaerobic reactors [22].

Series of washout was resulted with the milk powder as feed. The COD reduction rate was 30-40% during this period. The specific Methanogenic Activity assay found the performance of microbes was poor. The reactor was continuously operated with low COD of milk processing effluent. By 45th day and afterwards the COD reduction rate got increased and reached 73% removal.

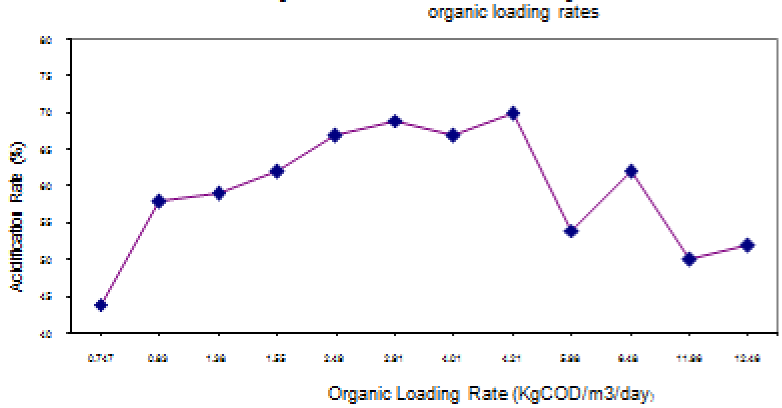
Acidogenic phase: The acidogenic phase was generally characterized by its low pH, high volatile fatty acids and low COD reduction.

In the present study, the acidogenic reactor was operated with different organic loading rates at constant HRT for a longer period of time and after attaining stable operating conditions the HRT was reduced to increase the organic loading rate. In this phase, the pH of the effluent during the operating period ranged from 3.9 to 5.2. The pH of the influent was maintained between 6 and 7.5. Acidification of milk particles in the effluent reduces the pH levels. This can be rectified by adding $NaHCO_3$.

Low pH levels within the acidogenic dominant zone of an anaerobic system play a vital role [23,24,25]. Anderson et al. (1994)also reported that pre-acidification reactor for treating Dairy waste water has the pH between 5.0 and 5.5 [18]. According to Horiuchi et al. (2002) and Liu et al. (2002)the microbial population in the acid reactor dependent on the low pH [26,27].

The low pH was mainly due to the production of volatile fatty acids. Acetic, propionic, butyric and valeric acids were commonly produced VFAs during mesophilicacidogenesis of dairy waste water [28]. The more VFA production resulted in the reduction of pH, but sometimes change of influent COD may also resulted in low pH levels (below 4.5). At higher concentrations the milk particles acidify rapidly due to the transformation of lactose into lactic acid, mainly in anaerobic conditions, also brought down the pH value. Very low pH would produce casein and suspended particles which settled at the bottom of the reactor, but soon it can be recovered by the action of acidogenic microorganisms.

Fig. 1: Acidification Rate in the Acidogenic reactor at different organic loading rates



At an HRT of 2 days, the maximum VFA production was recorded in all the phases. The maximum acidification rate was reported in Phase V, the acidification rate was 67-70% at an organic loading rate of 4.01-4.21 kg COD/m^3/day in the acidogenic phase (Fig. 1). At an HRT of 2 days, the increasing of COD concentrations results in production of more Volatile Fatty Acids and their acidification rate also were high. While reduction of HRT by 1

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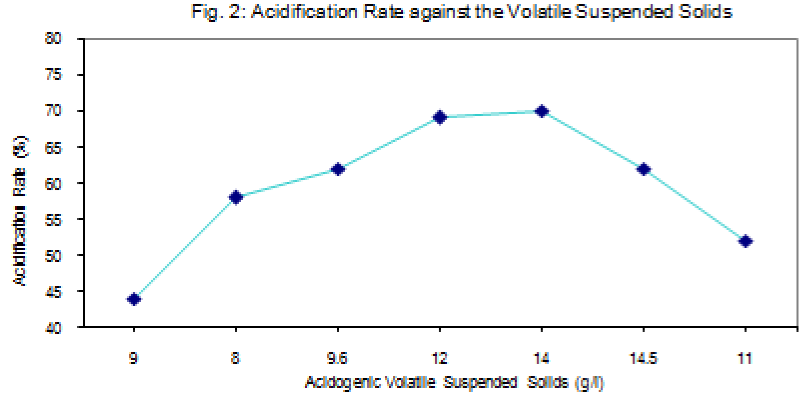
day in the Phase VI reduced acidification rate by 52% and then recovered to 62% and in Phase VII, the reduction of HRT by 12 hours, reduces the acidogenic activity, reducing the acidification rate further to 52%. This was somewhat lower than the acidification rate at an HRT of 2 days. Lowering of HRT by increasing flow rate caused turbulence of sludge bed, and affected the activity of microbes and resulted in low Volatile Fatty Acid production. According to Yilmazer and Yenguin (1999), with an HRT of 24 hours the maximum acidification was about 50% [29]. Recently Saddoud et al. (2007) used stirred tank acidogenic reactor to treat dairy waste water and they reported that at an HRT of 24 hours, the pH decreased considerably with increasing VFA concentration [30].

Cha and Noike (1997) also found that the bacterial populations in a mesophilicacidogenic reactor decreased with decrease in HRT from 48 to 6 hours [31]. The level of alkalinity in the effluent of acidogenic reactor was more than the influent alkalinity. The increasing of alkalinity was due to the production CO_2 by the acidogenic microorganisms. This CO_2 may be converted as $CaCO_3$ which increased the level of bicarbonate alkalinity.

The biogas production was not monitored at this stage. This was due to during hydrolysis, the composition of gas from the acidogenic reactor was 35% CO_2, 65% N_2 and 0% methane, 80% the COD removal during this stage was bioconversion only. In the present study, Acidogenic sludge bed was characterised by diffuse and loosely aggregated flocculent sludge. This might be due to the hydrophilic nature of acidogenic bacteria. This was confirmed by Thaveesri et al. (1995) and Daffonchio et al. (1995) [32,33].Theyfoundthat acidogens are mainly hydrophilic and thus resulted in species emigrating from the granular structure in to the liquid phase.

Fig. 2: Acidification Rate against the Volatile Suspended Solids



The height of the sludge bed had a quick increase which may be attributed to the higher growth rate of acidogenic microbes which eventually led to the increase of VSS. The VSS increased from 8 g/l during the start-up period to 14.5 g/l at the end of Phase VI. However, the VSS production declined to 11 g/l in Phase VII which was due to high flow rate and reduced Hydraulic Retention Time in this phase. High flow rate resulted in the washout of sludge and reduced HRT which did not give sufficient duration for the microbes to act on the substrate. Acitification rate against the volatile suspended solids in presented in the Fig. 2.Banerjee et al. (1999)were also of the opinion that decreases in reactor VSS concentration at 16 hours hydraulic retention time could be attributed to high flow rate and less time for settling of biomass [34].

Methanogenic phase: In order to investigate the performance of the methanogenic phase of dairy effluent waste treatment, effluent from the acidogenic reactor was fed to the UASB reactor. The loading rate in the methanogenic reactor ranged from 0.486 – 8.1 kg COD/m^3.d. The effect of influent COD concentration and organic loading rate was examined under the operating conditions of HRT 2.5 days, 1.25 days and 15 hours retention time.

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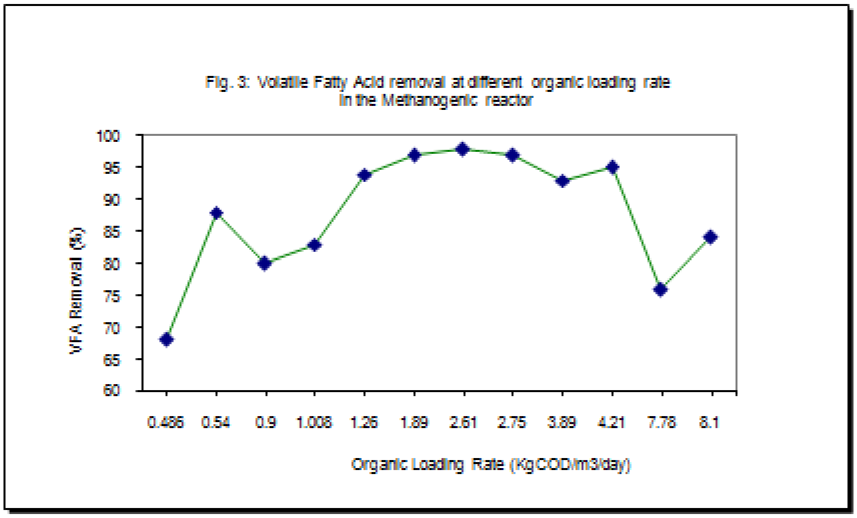
Due to the separation between the acidogenic and the methanogenic steps, VFA production was not significant in the methanogenic reactor. The VFA concentration was less than 0.3g/l. So VFA concentrations were always below the inhibitory limits, permitting the methanogenic process to be established progressively. This is the advantage of having separate reactors for acidogenic and methanogenic processes.



Percent utilization VFA in Phase II was 68% only. However, it could enhance upto 88% because of the high start-up of the reactor. High start-up was made possible by having the seed slurry from a distillery unit which was running successfully. Maximum utilization rate of about 97-98% was achieved in Phase VI but in Phase VII it came down to 76-84% due to recued HRT as already stated Fig. 3.

pH plays a crucial role for the action of methanogenic microbes. The high VFA accumulation and quick acidification of milk particles in the dairy effluent normally brigs down the pH less than 5. This inhibits the methanogens from proliferation and leads to the failure of the reactor. Single phase UASB reactors tried earlier by several investigators for dairy effluent resulted in failure because of this reason. Acidogens are active and proliferate in pH range 4.2-5.7 and methanogens require a pH range of 6.8-7.2. Separation of these two phases became the solution to mitigate this problem. This was achieved successfully in the present investigation.

The pH of the methanogenic reactor was ranged between 6.8 and 7.2. During the initial phase the pH was maintained by adding $NaHCO_{3,}$ this was continued till the Phase III, but during later phases, the stability improved by mature microbial populations in the reactor. Alkalinity was produced by the microbes during most of the operating period as the digestion of the dairy wastes resulted in the production of bicarbonate alkalinity which eventually raised the pH levels.

The importance of pH in anaerobic digestion was reported by several authors. Mudrakand Kunst (1986) stated that anaerobic reactions are highly pH dependent [35]. The optimal pH range for methane producing bacteria is 6.8-7.2 while for acid forming bacteria, a more acid pH is desirable.

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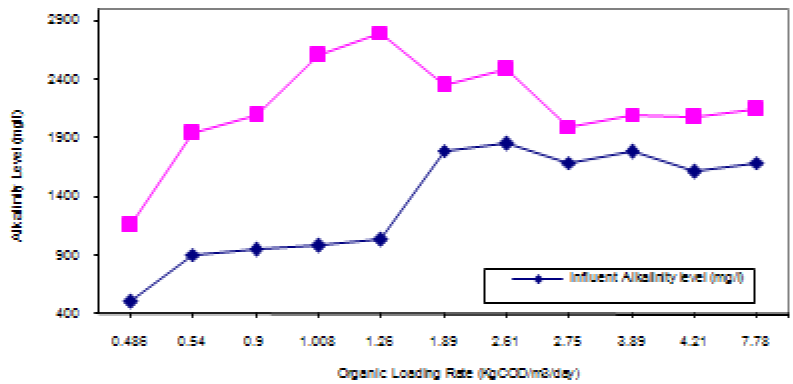
Fig. 4: Alkalinity level in the Feed and Effluent in the methanogenic reactor

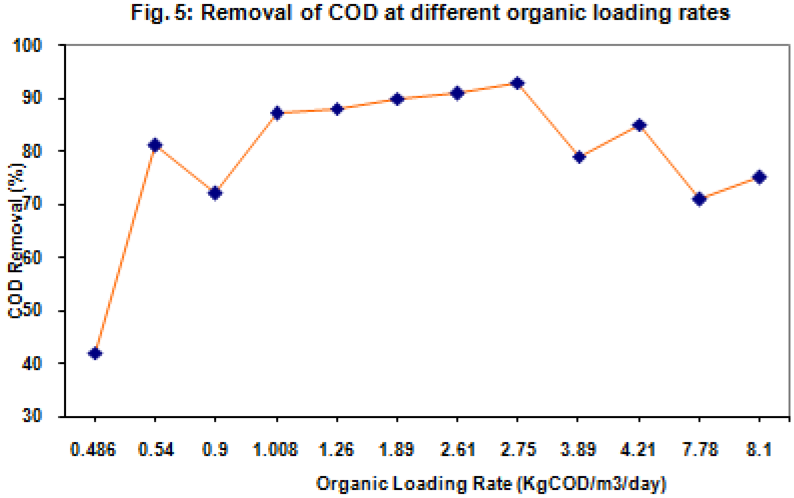


Wheatly (1991) also reported that the pH less than 6.8 and greater than 8.3 would cause souring of the reactor during anaerobic digestion [36]. Beal and Raj Raman (2000) also reported that for effective methanogenic activity, the bicarbonate alkalinity concentrations should never fall below 700 mg/l and the pH should always be maintained above 6.5 [37]. Earlier research showed that generating sufficient alkalinity from the waste and acidification aftershock loads were the most common operating problems for the anaerobic digestion of industrial wastes [38]. In the present study, alkalinity was higher from Phase III onwards and it was maintained around 2500-3000 mg/l which assured the successful operation of the reactors Fig. 4.

COD reduction:

In the present study, the increase in organic load with the increasing of COD without disturbing Hydraulic Retention Time resulted in high COD removal efficiency Fig. 5. Treatment of Dairy waste water and COD reduction was studied by many authors. Satyanarayanan et al. (1988) used a single-stage anaerobic filter for synthetic milk waste [39]. The reported COD removal efficiency ranged between 80 and 90% at an OLR of about 0.8-4 kg COD/m^3/day and HRT of 1.55-5.1 day.

Fig. 5: Removal of COD at different organic loading rates



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Laboratory experiments have also established the feasibility of UASB process for the treatment of dairy waste water. Mehrotra and Jain (1997)studied the performance of a 2.8 litre capacity UASB reactor using simulated dairy waste water [40]. The reactor was found to remove COD by 90% at an organic loading rate of 8 kg COD/m^3/day. Bench scale studies undertaken by Shastry and Kaul (1996)on a UASB reactor revealed optimum COD loading conditions to be 3.6 kg/m^3/day with a retention period of 1 day at an influent concentration of 3.6 g/l and the COD removal efficiency obtained was only 80% [41].

Roy and Chaudhuri (1996)conducted pilot scale experiments on a 20 m^3/day fixed film reactor for biomethanation of dairy waste water [42]. COD feeding into the digester was 40 kg/day at a flow loading of 20 m^3/day. However they could achieve COD reduction of 70% only after 25 days of HRT.

Young (1991) reported that HRT had an important influence on reactor performance [43]. Good flow balancing and a steady hydraulic load would be necessary to achieve a consistent effluent quality. According to his report 70-75% COD removed at a steady 24-40 h HRT and 80-90% removal at 72-96 h HRT. Kennedy and Droste (1991) reported that performance of the reactor in COD removal was between 70 and 80% at 5-15 kg COD/m^3/day at HRT of 12-24 h [44].

Saravanane et al. (2001) also reported that more HRT was preferred to increase the COD removal and to prevent the washout of inoculated biomass [45]. According to Demireland Yenigun (2006) the activity of *Methanococcus* and *Methanosarcina* decreases at the HRT of 24 hours [28]. Omil et al. (2003) conducted experiment with dairy effluent in an Anaerobic filter, the OLR maintained as 5-6 kg COD/m^3/day at an HRT of 48 hours to remove 90% COD [46]. Tawfik et al. (2007) also reported that treatment of a combined dairy and domestic waste water in an UASB reactor operated at a HRT of 24 hours and OLR range from 1.9-4.4 kg COD/m^3/day resulted in only 69% COD removal [47].

Treatment of cheese-whey with two-phase anaerobic digestion was studied by Yilmazerand Yenigun (2001) [29]. The system consisting of a continuous stirred tank reactor (CSTR) as the acidogenic reactor and an upflow anaerobic filter (UFAF) as the methanogenic reactor. The maximum acidification achieved at an HRT of 24 hours. The maximum COD removal of 90% achieved in the methanogenic reactor at a HRT of 4 days.

Nadais et al. (2005) studied the influence of HRT on COD reduction and conversion to methane of the removed COD [48]. For HRT under 12 hours continuous UASB reactors used for the treatment of dairy waste water presented conversion of methane and the COD reduction was lower than 30%. The author concluded that for maximum reduction, the reactor operated with maximum load around 3.0 g COD/l/day and the HRT must be more than 12 hours. Several other investigators also concern with the above conclusion [8, 46, 49, 50, 51, 52, 53].

However, some of the studies reported that high COD removal rate at lower HRT and in high organic loading rates. This was due to the reactors processed synthetic steady-composition wastes, with dairy wastes being simulated by powdered milk for example. These synthetic wastes contain much less Oil and Grease than real effluents. Indeed real effluents also contain additives such as disinfectant and cleansing agents that may jeopardize the biological treatment process. In the work of Ramasamy et al. (2004), for example, the authors established the feasibility of UASB reactors in treating dairy waste waters [54]. They reported COD reduction rates greater than 90% at HRT of 3 and 12 hours and their COD loading rates was 2.4 to 13.5 kg COD/m^3/day. For these experiments, powdered milk was used as effluent.

From the above discussion, it was concluded increasing of OLR by reducing HRT has much influence on the COD removal efficiency. In the present study, also the COD reduction was declined 71-78% at an OLR rate of 11.96-12.46 kg COD/m^3/day with the HRT of 12 hours for acidogenic phase and in the methanogenic phase the OLR was 7.78-8.1 kg COD/m^3/day at a HRT of 15 hours.

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In this investigation, raw effluent from one of the largest dairy processing unit was used instead of simulated ones and the COD removal of about 71-75% suggests the two phase UASB reactor could perform with higher organic load (7.78-8.1 kg COD/m^3/day) and lesser HRT (15 hours).

Other works on anaerobic treatment of dairy effluents in continuous reactors have reported a significant decrease in reactor performance or failure due to build up of organic matter inside the reactors at lower HRT [51,53,55,56,57,58,59,60] although no numerical data were presented for this accumulation.

In the present study, the better performance of the reactors than the reactors reported by other investigators can be attributed to two reasons. One definite reason is the phase separation. In the acidogenic phase rate of acidification was faster resulting in lowering of pH level (4.7-5.2) favoured VSS production which in fact served as nutrient medium for the methanation in the methanogenic phase. The other reason could be that the operation of the reactors was so planned, that the Organic Loading Rate was stepped up in a phased manner by gradual increase of the concentration of the dairy waste water. After establishing a reasonable stability more quantum of dairy waste water was sent as influent by increasing the flow rate as well as by reducing the hydraulic retention time.

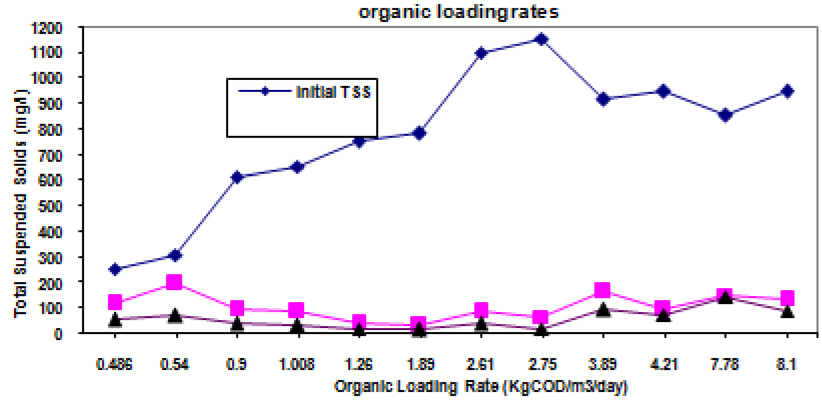
In the present study, the high COD removal rate achieved at an HRT 2 days for the acidogenic reactor at an OLR rate of 4.01-4.21 kg COD/m^3/day and in the methanogenic reactor HRT of 2.5 days at an organic loading rate 2.61-2.75 kg COD. The maximum COD of the influent was 7,250-7,650 mg/l.

This was comparatively higher than that of other reports, with single phase treatment method. The results indicate that a sharp decrease in the COD removal efficiency for loads above 2-4 g COD/l/day in the operation of sludge bed anaerobic reactors fed with dairy effluents [51, 55, 58].

V. TOTAL SUSPENDED SOLIDS

Suspended solids are in the form milk particles and washout from the sludge bed. The removal rate of TSS at the initial stage was only 54-63%, this was due to at the initial level the microbes concentrate to increase their biomass rather utilization of substrate. Moreover, a series of washout occurred in the solid particles of sludge bed due to adaptation to new feed.

Fig. 6: Removal of Total Suspended Solids at different organic loading rates



The removal rate was increased with increasing organic load, the substrate utilization rate was also increased with increasing activity of microorganisms. Thus, the reduction rate was increased in the subsequent phasesFig. 6.

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Suspended Solids in dairy effluent predominantly organic, on average of about 76% of the Suspended Solids were volatile indicating that most of the Suspended Solid particles are readily available for the utilization of microbes. Most of the suspended solids particles were retained in the acidogenic phase. The acidogenic microbes converted the solid particles into volatile fatty acid.

According to Wang (1994) COD was mainly removed through physical process of Suspended Solid retention in the sludge bed [61]. Dissolved COD was responsible for most of the methane production, but from COD balances it was apparent that some methane was also produced from the hydrolysis and fermentation of entrapped particulate organic matter.

The maximum TSS reduction was reported in Phase IV and V. In the methanogenic reactor with internal packing column and the presence of ceramic beads of various sizes at the top of the reactor contributes an important role in TSS removal by providing the surface area for the attachment of solid particles. Microbes developed with this particles and certain amount of degradation was taking place in this region also. This was confirmed by the enhanced pH at the outlet than the sample port 3.

Published works also indicate that most of the negative aspects of high rate anaerobic digestion can be overcome by restricting the supporting material at the top of the 25 to 30% of the reactor column [62]. This would further help to realize the advantages of UASB systems.

Over accumulation of sludge particles were removed at in rare occasion. The TSS removal was reduced in the VI (90-92%) and VII phases (82-90%). This was due to the increase in the flow rate of influent. The shearing force detached the Suspended Solid particles from the surface of sludge bed. This causes reduction in COD removal as well as reduction in TSS removal. Even though, the increased flow rate affected the reactor column, the internal packing column was not affected and polishing effect was done at the effluent even at higher organic loading rates.

Nutrient Removal

Dairy waste water was often characterised by high nutrient content. From the results, it has been shown that some percentage of reduction in the Total Kjeldahl Nitrogen and Total Phosphorus was observed. Utilization of organic nitrogen was markedly higher than that of phosphorus. Higher utilization of nitrogen in comparison with phosphorus during the anaerobic treatment was reported by Banu et al. (2006) [63]. The utilization rate was higher at the initial phase, thus indicating the source of Nitrogen was essential for their cell synthesis. Phosphorus was also needed but at a lesser extent.

Baloch et al. (2007) stated that high consumption (up to 16%) of influent NH_4- N was observed in the acidogenic dominant zone and it is believed that this amount of nitrogen was assimilated for the synthesis of new microbial growth [25].

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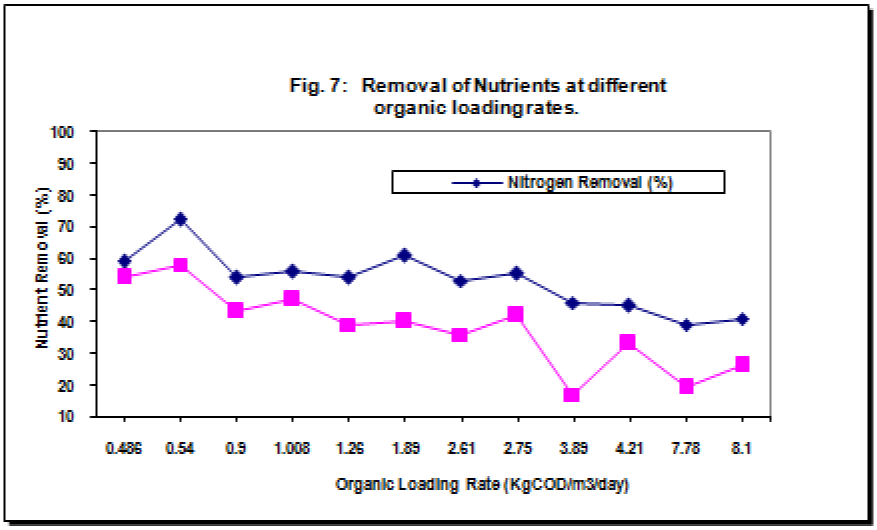

Utilization of both nutrients were high at the Phase II. The reduction was 59-72% for Total Kjeldahl Nitrogen and 54-58% for Total Phosphorus. Increased organic loading rate at the Phase III reduced the utilization of Nitrogen from 72 to 56%. Similarly the phosphorus utilization also fell from 58 to 47%. But, in the subsequent phases the reduction rate was maintained in the form 50-60% for TKN and 30-40% for Total Phosphorus. This was continued up to the organic loading rate of 4.01-4.21 kg COD/m^3/day at 2 days HRT for acidogenic phase and 2.61-2.75 kg COD/m^3/day at 2.5 days HRT for methanogenic phase Fig. 7.

The reduction rate was further reduced when the OLR raised by reducing HRT. Marked reduction was observed at the Phase VII with 12 hours HRT for the acidogenic reactor and 15 hours for methanogenic reactor. The reduction rate was only 39-41% for TKN and 19-26% for Total Phosphorus. The decrease in nutrient utilization at higher OLRs can be attributed to higher flow rate of the waste water and the consequent reduction in contact time between nutrients and microbes as well as nutrient washout.

Oil and grease

The results revealed that 100% reduction rate was observed in the Phase IV and V at the loading rate of 92-124 mg/l of Oil and Grease. The removal efficiency was 70-80% in the Phase III, but the reduction rate was decreased to 68-72% in the Phase VI and the efficiency was the least in Phase VII (42-44%)Fig. 8.

Performance of the reactor while treating the Oil and Grease containing effluents were reported by various authors. According to Omil et al. (2003), in the last two decades anaerobic reactors have been increasingly applied to treat fat based waste waters [46]. However, it is necessary to reduce the concentration of fat, oils and proteins or to eliminate these materials, altogether, in order to enable the biological treatment to proceed without any inhibition. According to Grulois et al. (1993) and Vidal et al. (2000)that in the anaerobic biological processes, Oil and Grease may solidify at lower temperatures and thus cause operational damage associated with clogging and unpleasant odours [64, 65]. Similar observations were made by Danalewich et al. (1998); Bae et al. (2003) and Carta-Escobar et al. (2005) [66,67,68].

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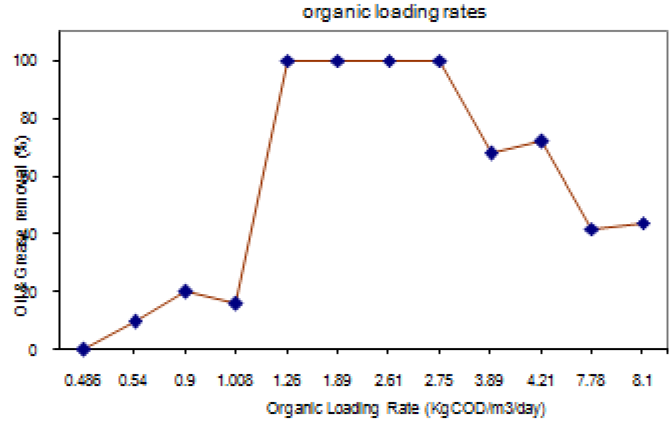
Fig. 8: Oil & Grease removal at different organic loading rates



The problem associated with the fat in anaerobic reactors is that the biodegradation of waste waters rich in fat was slower than that of waste waters with a low fat content. Thus the presence of fats in the waste water prevent the periodic production of high concentrations of VFA, which may adversely affect the subsequent processes.

In the present study, the above said problem also was mitigated by the phase separation, so that the excess of VFA production could be adjusted in the acidogenic phase itself, thereby sparing the methanogenic activity in the UASB reactor.

Moreover, the fat was not completely broken down in the acidogenic reactor itself allowing some by-products moves to methanogenic reactor. In methanogenic reactor these by-products did not affect the performance of the reactor as the pH of the reactor was maintained between 6.8 and 7.2 which enhanced the breakdown of fat in the methanogenic reactor [46, 69].

The authors also suggested that in the case of excess of Oil and Grease in the effluent (>200mg Oil and Grease/l) resulted in the heavy accumulation of fat particles in the sludge bed that adversely affect the contact between the sludge and substrate.

The result obtained by Leal et al. (2002) tested the treatment of dairy effluent with differential Oil and Grease contents and reported that high concentrations of Oil and Grease resulted in low reduction rate [70]. Manilal and Haridas (2000) studied a laboratory scale UASB operated with milk effluents [71]. Their results showed that when fat concentrations exceeds 180mg/l out of total COD of 3,500-4,500 mg/l at OLR of 3-4 kg COD/m^3/day there was a reduction in activity and incipient sludge floatation. Reactor failure was observed at fat concentration of 360 mg/l. Increasing flow rate also hinder the Oil and Grease removal rate. The problem associated with the high flow rate was, sludge floatation and fat accumulation at the top of the reactor. This was observed at the Phase VI and VII in the present study. The sludge and Oil and Grease floatation was also one of the reasons for poor acidogenic reactor performance. This led to low VFA production as well as low COD reduction. Masse et al. (2001)stated that poor activity associated with excessive amount of Oil and Grease in the waste water hinders sedimentation and causes loss of biomass through the reactor's outflow [69]. According to Perle et al. (1995) and Rinzema et al. (1993), the development and flotation of sludges with different physical characteristics or poor activity are known to cause biomass loss through the reactor's outflow decreasing its quantity inside the reactor and the treatment efficiency [72,73].

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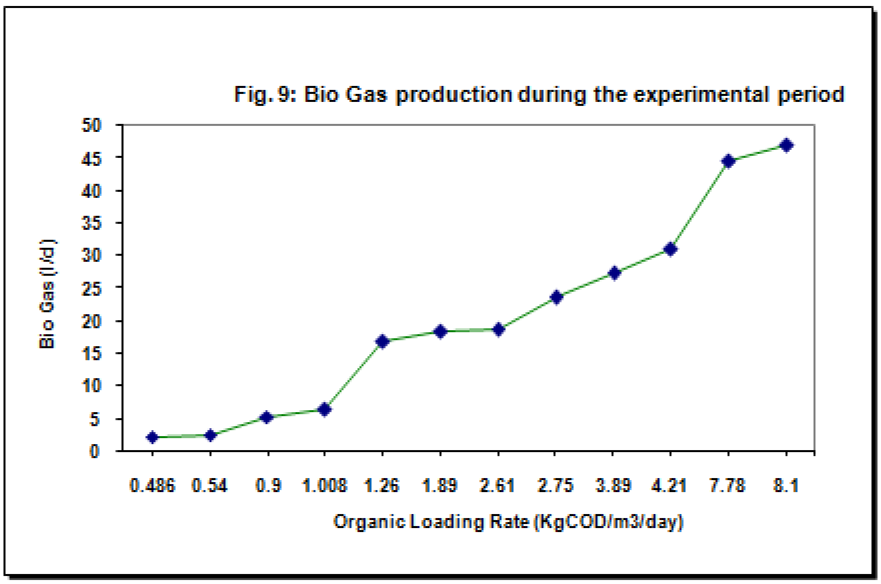
In another report by Rinzema et al. (1994)stated that the Oil and Grease adsorbed on the surface of the soluble substrates to the biomass and consequently may reduce the substrate conversion rate [74]. From the above discussion, it was concluded that the Oil and Grease level upto 124 mg/l may be treated effectively with 100% removal efficiency. The operating conditions for the maximum removal of Oil and Grease were obtained when operating waste waters with maximum organic loading rate of 4.01-4.21 kg COD/m^3/day for the acidogenic and 2.61-2.75 kg COD/m^3/day for the methanogenic phase at an influent flow range of 7.2-7.5 g COD/l. The most important factor required was HRT of 2 days for acidogenic reactor and 2.5 days for the methanogenic reactor.

VI. BIOGAS PRODUCTION

The calculated methane yield expressed as the volume of methane produced per day based on measurements from a wet-test gas meter (after the biogas passed through the alkaline solution to remove the CO_2 and H_2S, but not the water content). The results of the present investigation showed that the biogas yield was lower in the initial phase, the productivity got increased with increasing organic loading rate Fig. 9. The low rate of gas production in the initial stage indicated that part of the removed COD had not been converted. It should be noticed that biogas production was directly related to the organic matter removal rather than the organic loading. Liu et al. (2004) stated that at low OLR, bacterial will assimilate most of the organic substrates that have been fed into the reactor [27].



A slight time delay of gas production was observed initially due to the time required for activating the bacteria that converts the biomass into CH_4 and CO_2. There after the biogas production rate kept on increasing in the subsequent days. The biogas production was not affected even at lowering of HRT, indicating the two phase UASB reactor is more efficient.

Biomass characteristics

In the present study, the sludge appeared black and aggregated as flocculent sludge. This was explained as hydrophobicity has been played a key role in the aggregation of bacterial species as the methanogens are mainly hydrophobic cells [33, 75]

According to Lettinga et al. (1998), the operation of UASB reactors does not need granular sludge and it is possible to use flocculent sludge with good settleability [76]. In another study Hwu (1997) verified that in expanded granular

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sludge bed (EGSB) reactors the bacteria that degrade LCFA to VFA were found in the fine biomass particles and not in granular aggregates [77]. This might be an explanation for some results in the literature indicating a low degradation of milk fat in anaerobic reactors with granular sludge [10].

Since the performance of a UASB-reactor depends on the sludge retention and the specific methanogenic activity of the retained sludge, the sludge content in the reactors was determined regularly by calculation of the VSS profile along the reactor height. The accumulated sludge biomass in both the reactors increased along with the operation duration of the reactors. The height of the acidogenic reactor sludge bed changed from 12cm at the start up period to 13.5, 17.7-15.2, 18.9, 23.5, 24 and 21.5cm at the end of the Phase II, III, IV, V, VI and VII phases respectively. The amount of the sludge increased almost two fold due to this fast growing acidogenic microbes as well as substrate accumulation at higher organic loading rate.

In the case of the methanogenic reactor the height of the sludge bed changed from 22cm at the start up period to 19.6, 21.6, 23, 24.2, 24 and 23.2cm at the end of the Phase II, III, IV, V, VI and VII phases respectively.

Irrespective of the moderate increasing of sludge bed, the volatile suspended solid concentration increased significantly during successive phases (16.4, 19, 24, 26 and 24 g VSS/l during II, III, IV, V, VI and VII phases respectively).

Calculation of VSS was only for immediate analysing of active biomass. But this traditional VSS measurements did not distinguish between microbial biomass and any other particulate organic material which may be present in the reactor, nor did it give any indication of the potential methanogenic activity of the microbial biomass present [78].
From the stand point of the design and operation of anaerobic processes, methanogenic "activity" is of great importance. Although it has been well established that stable operation of anaerobic processes requires regular measurement of parameters such as pH, alkalinity, gas production and gas composition, removal of organic matter, these parameters only provide information concerning the current conditions inside the reactors. Therefore the use of SMA test as a control parameter could provide safer operation under field conditions i.e. under varied influent flows and variable concentrations of organic matter. The SMA test can also be used for the determination of optimum operating conditions of anaerobic reactors [79].

In the present study the amount of sludge did not significantly increase, due to sludge washout and the low sludge yield of methanogenic microbes, but the Specific Methanogenic Activity (SMA) of the sludge improved substantially.

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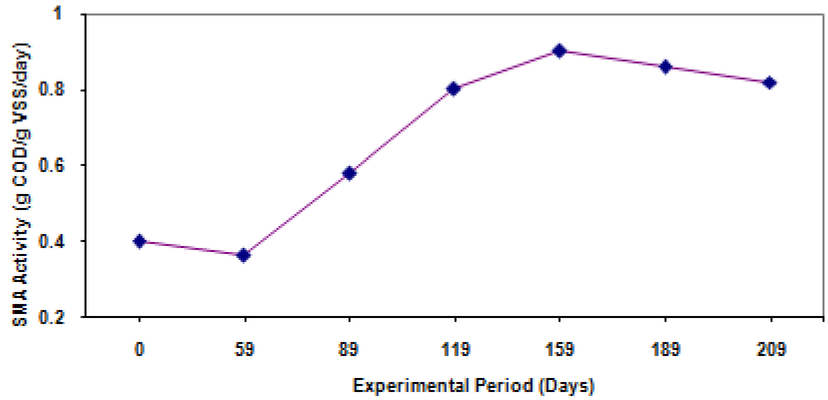
Fig. 10: Specific Methanogenic Activity during the experimental period.



The SMA test was immediately carried out after seeding the reactor with sludge taken from a distillery waste water treatment plant digester in order to determine the most acceptable initial organic loading rate. At this stage, it was 0.4 gCOD/gVSS/day, but this was reduced to 0.36 gCOD/gVSS/day at the 89^{th} day. This reduction was due to the changing of the substrate as feed. Then it was gradually increased in the subsequent phases since it permitted the increasing of organic loading rate at different time interval. The results of the current study showed that the SMA changed from 0.4 – 0.36, 0.58, 0.8, 0.9, 0.86 and 0.82 g COD/gVSS/day after 89, 119, 159, 189, 219 and 249 days of operationFig.10.

Gas production was very low (62 $mlCH_4$/gVSS/day) during the initial stage to 121, 210, 280, 360, 380 and 330 $mlCH_4$/gVSS/day at the 89, 119, 159, 189, 219 and 249 day respectively. The maximum potential methane production rate of enriched cultures cultivated on acetate has been reported to be approximately 1000 $mlCH_4$/gVSS/day [80] if all the biomass (measured as VSS) consists of acetoclastic methanogens. The potential methane production rate of 62 $mlCH_4$/gVSS/day. Obtained from this study was lower than reported value. This could be explained due to the different amounts of methanogenic population retained in the anaerobic reactors and also due to the change in feed from distillery effluent – sugar solution – milk powder solution – dairy waste water. However, the reactors performed very well, achieving over 93% COD removal efficiency at the Phase V and the gas production was also improved at this phase. At the Phase VII the increasing of flow rate slightly disturbed the methanogenic activity.

The performance of the reactors in terms of COD removal efficiency, specific gas production and SMA of reactor sludge at different organic loading rates was predict that Phase V at an OLR of about 5-7 kg COD/m^3/day showed better performance.

In the present study, the microbes were mainly of short and medium rod shaped bacteria, however, during the start-up period it was mainly of methanococcus bacteria. Changes in the microbial ecology in different feed substrate was explained by Morgan et al. (1991) [58]. They were of the opinion that the initial start-up phase, the bacterial composition of the anaerobic biomass was influenced by the waste water composition rather than reactor design.

At the later stage, the coccus bacteria were replaced by the rod shaped bacteria revealed the change in the influent substrate in the form of milk particles replacing coccus bacteria and initiating the development of rod type bacteria.

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The pH also influence the presence of particular bacteria. This was reported by Zhou *et al.* (2007) [81].The pH value in the reactor was about 6.4-7.2 which could be compatible for rod type bacteria. In low loading operation, pH value fluctuated between 7.0 and 7.5 which probably nurtured the filamentous bacteria very well.

VII. CONCLUSION

The treatment of dairy waste water by the Two Phase Anaerobic UASB reactor showed that 91-93% of COD removal efficiency at an OLR of 4.01-4.21 kg COD/m^3/day in the acidogenic reactor with an HRT of 2 days and for the methanogenic reactor the OLR was 2.61-2.75 kg COD/m^3/day with an HRT 2.5 days. A maximum acidification rate of 67-70% achieved at the influent COD of 7,250-7,650gCOD/l. Two Phase Anaerobic UASB reactor is clearly an excellent feasible system for treating dairy processing waste water. Separation of acid and methane fermentation process produces high COD reduction and methane yield irrespective of the negative aspect of the dairy effluent like pH, quick acidification, Oil and Grease content also.

A high COD reduction and methane conversion recorded even at a high loading rate of 5-7 kg COD/m^3/day with a HRT of 2.5 days, without showing any sign of instability. VFA produced by acidogenesis are effectively consumed by the methanogenic phases. The pH was maintained between 6.9 - 7.2 in the methanogenic phase after 120 days of operation without addition of any alkali, suggesting the self-sustainability of the reactors.

REFERENCES

1. *TERI.Looking Back to Think Ahead: GREEN India 2047. New Delhi, TERI. 1998. 346.*
2. *Stephenson JA. Experience in providing high level wastewater treatment at Ontario dairy. In: Proceedings of 1989 Food Process. Waste Conf., Georgria Tech. Res. Inst., Sessions.1989: 13.*
3. *Shack PA andShandhuDS. Update on filament control strategies in Sendai. Japan. 1989. 3: 192 – 195.*
4. *Walsh J, Ross Cand Valentine G.Food processing waste. Water Science Technology. 1994. 21(4): 53– 70.*
5. *Radick K. Dairy Wastes. Water Environment and Resources. 1994. 64: 417- 418.*
6. *Hansen CL and Hwang SH. Use of up-flow anaerobic sludge blanket International symposium on Agriculture and Food Processing Wastes, vol. III. Am. Soc. Ag. Eng., Chieago, 1990. 124.*
7. *Rico JL, Garcia Pand Polanco F.Anaerobic treatment of cheese production wastewater using a UASB reactor. Bio resources and Technology. 1991. 37: 271-276.*
8. *Hawkes F, Donnelly T and Anderson G. Comparative performance of anaerobic digesters operating on ice-cream waste water. Water Resources. 1995. 29: 525-533.*
9. *Ozturk I, Eroglu V, Ubay GandDemir I. Hybrid up-flow anaerobic sludge blanket reactor (HUASBR) treatment of dairy effluents.Water Science Technology. 199328 (2): 77-85.*
10. *PetruyR andLettinga G. Digestion of a milk-fat emulsion. Bioresources Technology, 1997: 61, 141.*
11. *Mendez R, Blazquez R, Lorenzo FandLema JM. Anaerobic treatment of cheese, whey: start-up and operation. Water Science and Technology. 1989.21: 1857-60.*
12. *Viraraghavan T andKikkeri S. Effect of temperature on anaerobic filter treatment of dairy wastewater. Waste Science and Technology, 1990. 22: 191-198.*
13. *Veiga MC, Pan M,Blazaquez R., Mendez R andLema JM. A double- feed anaerobic filter for the treatment of high strength wastewaters. Biotechnology. 1994. 8: 77-82.*
14. *Erguder TH, Tezel U, Guven Eand Demirer GN. Anaerobic biotransformation and methane generation potential of cheese whey in batch UASB reactors. Waste management.2001. 21: 643-650.*
15. *Witter E and Lopez RJM. The potential of sewage sludge and composting in a nitrogen recycling strategy for agriculture. Biology Agriculture and Horticulture.1987. 5: 1- 23.*
16. *APHA. Standard Methods for the Examination of water and wastewater. 20th edition. American Public Health Association, Washington, DC. 1998.*
17. *Hajipakkos C. An investigation in to volatile fatty acid production in thermophilic anaerobic digesters. Ph.D., thesis. University of Newcastle upon Tyne, U.K. 1987.*
18. *Anderson JM and Yang. Soil organic matter and organic carbon. In: Anderson J.M. and Ingram J.S.I (Eds.), Tropical soil Biology and Fertility. CAB International, Wallingford, UK. 1991. 62 – 66.*

[Saravanaraja, 5(7): July 2018] ISSN 2348 – 8034
DOI: 10.5281/zenodo.1320963 Impact Factor- 4.022

19. Naomichi N and Yutaka N. Recent Development of Anaerobic Digestion Processes for Energy Recovery from Wastes. Journal of Bioscience and Bioengineering. 2008. 103 (2): 105 – 112.
20. Versprille AT. Anaerobic treatment of potato-production wastewater. Report No. 16, IBVL, Wageningen, Netherlands, (in Dutch).1978.
21. 21. Pette KC, Vletter R, Wind E and Gils W. Development of an industrial anaerobic treatment plant for beet wastewater. Report Min of Public health and Environmental Protection, No. 33, (in Dutch).1979.
22. Weiland P and Rozzi A. The start-up, operation and monitoring of high-rate anaerobic treatment systems: discusser's report. Water Science Technology. 1991. 24(8): 257 – 277.
23. Inanec B, Matsui Sand Ide S. Propionic acid accumulation and controlling factors in anaerobic treatment of carbohydrate: effects of H_2 and pH. Water Science and Technology. 1996. 34, 317-325.
24. Azbar N, Ursillo P andSpeece RE. Effect of process configuration and substrate complexity on the performance of anaerobic processes. Water Resources. 2001.35: 817-829.
25. Baloch MI, Akunna JC and Collier V. The performance of a phase separated granular bed bioreactor treating brewery wastewater. Bioresources Technology,2007. 98: 1849-1855.
26. Horiuchi JI, Shimizu T, Tada K, Kanno Tand Kobayashi M. Selective production of organic acids in anaerobic acid reactor by pH control. Bioresources Technology.2002. 82: 209-213.
27. Liu Y, Hai-Lou X, Shu-Fang Y and Joo-Hwa T. Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor. Water Resources. 2003. 37: 661 – 673.
28. Demirel B and Yenigun O.Treatment of dairy wastewater by membrane module reactor. Bioresources Technology, 2003. 97: 1201-1208.
29. Yilmazer G andYeniguin,O.Two-phase anaerobic treatment of cheese whey. Water Science Technology.2001.40: 289-295.
30. Saddoud A., Hassairi A. andSayadi S.(2007). "Anaerobic membrane reactor with phase separation for the treatment of cheese, whey". Biores. Tech., 987, 2102-2108.
31. Cha G.C. andNoike T. (1997). "Effect of rapid temperature change and HRT on anaerobic acidogenesis". Water Sci. and Tech., 36 (6-7), 247 – 253.
32. Thaveesri J., Daffochio D., Lessens B., Vandermeren P. andVerstrate W. (1995). "Granulation and sludge bed stability in upflow anaerobic sludge bed reactors in relation to surface thermodynamics". Appl Environ Microbiol., 61, 3681-3686.
33. Daffonchio D., Thaveesri J. andVerstraete W. (1995). "Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactor". Appl. Environ. Microbiol., 61, 3676-3680.
34. Banerjee A., Elefsiniotis P. andTuhatar D. (1999). "The effect of addition of potato processing wastewater on the acidogenesis of primary sludge under varied hydraulic retention time and temperature. J. Biotech., 72, 203 – 212.
35. Mudrak K. and Kunst S. (1986). In: "Biology of Sewage Treatment and Water Pollution control". England, Ellis Horwood Ltd., 193.
36. Wheatly A. (1991). "Anaerobic digestion: A waste treatment technology". Elsevier, Sci. Publ.
37. Beal J. and Raman R. (2000). "Sequential Two-stage Anaerobic Treatment of Confectionery Wastewater". J. Agric. Eng. Res., 76, 211 – 217.
38. De Poli F., Gamboni M. andTukcge A. (1988). "Chemical and biological problems in full scale biogas plants: the Italian experience". In: "The Fifth International Symposium on Anaerobic Digestion". Bologna, (Ed.), Hall E.R. and Hobson P.N., Pergamon Oxford, 389-96.
39. Satyanarayan S., Venkataraman J. andKaul S.N. (1988). "Performance evalution of two stage anaerobic fixed film fixed bed reactor system treating dairy wastewater using special media". Indian J. Enviro. Protect., 12, 919,
40. Mehrotra Rand Jain P.K. (1997). "Treatment of dairy waste by upflow anaerobic expanded sludge bed reactor". Proceedings of the Eighth International Conference on anaerobic Digestion. 25-29 May, Sendai. Japan p.293-6.
41. Shastry S. andKaul S.N. (1996). "Kinetics of dairy wastewater treatment using upflow anaerobic sludge blanket reactor system". Ind. J. Environ Protection. 16(2) ,117 - 20.

42. Roy B. andChaudhuri N. (1996). "Appropriate treatment option for dairy waste water in India". J .Ind. Assoc. Environ. Mangt., 23(1): 1-8.
43. Young J.C., (1991). "Factors affecting the design and performance of upflow anaerobic filters". Water Sci. Tech., 24, 133-55.
44. Kennedy K.J. andDroste R.L. (1991). "Anaerobic wastewater treatment in down flow stationary fixed film reactors". Water Sci. Technol., 24, 157-77.
45. Saravanane R., Murthy D.V.S. andKrishnaiah K.(2001). "Anaerobic Fluidized Bed Degradation and the Development of a Kinetic Model for a Particulate Organic Matter Enriched Wastewater Sludge, water, Air and Soil Pollution". Volume 127, Numbers 1-4/April, 15-30.
46. Omil F., Garrido J.M., Arrojo B. and Mendez R. (2003). "Anaerobic filter reactor performance for the treatment of complex dairy wastewater at industrial scale". Water Res., 37(17), 4099 – 4108.
47. Tawfik., Sobhey M. andBadawy M. (2007) "Treatment of a combined dairy and domestic wastewater in an up-flow anaerobic sludge blanket (UASB) reactor followed by activated sludge (AS system)". Volume 227, Issues 1-3, 167-177.
48. Nadais H. (2005). "Treatment of complex fat containing wastewater by UASB reactor". Water Research. 39, 1511-1518.
49. Cayless S., da Motta, Marques D. and Lester J. (1990). "A Study of the effects of methanol in start-up of UASB reactors". Biol Wastes. 31, 123-135.
50. Goodwin J.A.S., Wase D.A.J. and Forster C.F. (1990). "Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor". Enzyme Microb. Tech. 12, 877 – 884.
51. Shin H. and Paik S. (1990). "`Improved performance of UASB reactors by operating alternatives". Biotech. Lett., 22(6), 469 - 474.
52. Borja R. and Banks C. (1994). "Kinetics of an upflow anaerobic sludge blanket reactor treating ice-cream wastewater". Environ. Technol., 15, 219-232.
53. Strydom J., Mostert J. andBritz T. (1995). "Anaerobic treattment of a synthetic dairy effluent using a hybrid digester". Water S.A., 21(2),125-130.
54. Ramasamy E.V., Gajalakshmi S., Sanjeevi R., Jithesh M.N. andAbbasi S.A., (2004). "Feasibility studies on the treatment of dairy wastewaters with upflow anaerobic sludge blanket reactors". Biores. Tech., 93(2), 209 – 212.
55. Bull M., Sterrutt R. and Lester J. (1982). "The effect of organic loading on the performance of anaerobic fluidised beds treating high strength wastewaters". Trans. Chem. E., 60, 373-376.
56. Cordoba P., Riera F. andSineriz F. (1984). "Treatment of dairy industry wastewater with an anaerobic filter". Biotech. Lett., 16(11), 753-758.
57. Motta M.D., Cayless S. and Lester J. (1990). "Process aiders for start-up of anaerobic fluidised bed systems". Environ, Technol., 11, 1093-1105.
58. Morgan J.W., Evison L.M. and Forster C.F. (1991). "Changes to the microbial ecology in anaerobic digesters treating ice cream wastewater during start-up". Water Research, 25, 639-653.
59. Gonzalez J., Valdes P., Nieves G. andGerrero B. (1990). "Application de la digestion anaerobic a los residules de industriaslacteas". Rev. Int. Decontamination ambiental. 10 (1), 37-41 (in spanish).
60. 60. Filho N., Figueiredo R. andNour E. (1996). "Anaerobic pretreatment of dairy liquid effluents". Proc. 51st Purdue Ind., Waste Conf., 323-331.
61. Wang K. (1994). "Integrated anaerobic and aerobic treatment of sewage". Ph.D. thesis, Wageningen Agricultural university, Wageningen, The Netherlands.
62. Van den berg L., Kennedy K.J. and Samson R. (1985). "Anaerobic downflow stationary fixed film reactor: performance under steady-state and non-steady conditions". Water Sci. and Tech., 17(1), 89-102.
63. Banu R.J., Kaliappan S. and Beck D. (2006). "High rate anaerobic treatment of Sago wastewater using HUASB with PUF as carrier". Int. J. Environ. Sci. Tech. Suppl. winter, 3(1), 69-77.
64. Grulois P., Alric G., Brochon J.P., Bridoux G. andManem J. (1993). "Lelimination des graisses par treatment biological aerobic (The elimination of fats by aerobic biological treatment)". Tech. Sci. Methods. 5, 247-251.
65. Vidal G., Carvalho A., Mendez R. andLema J.R.(2000). "Influence of the Content of flats and proteins on thee anaerobic biodegradability of dairy wastewaters". Biores. Tech., 74, 231-239.

66. Danalewich J.R., Papagiannis T.G., Belyea R.L., Tumbleson M.E. and Raskin L. (1998). Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal". Water Res., 32(12), 3555 - 3568.
67. Bae T.H., Han S.S. andTak T.M. (2003). "Membrane sequencing batch reactor system for the treatment of dairy industry wastewater". Pro. Biochem., 39, 221-231.
68. Carta-Escobar F. (2005). "Aerobic purication of dairy waste water in contiuousregime part-I: analysis of the bio degradation process in two reactor configaration". In: Bioche. Eng. Jl., 22, 117-124.
69. Masse L., Kennedy K.J. and Chou S. (2001). "Testing of alkaline and enzymatic pretreatment for fat particles in Slaughterhouses wastewater". Bioresour. technol., 77, 145-155.
70. Leal M.C.M.R., Cammarota M.C., Freire D.M.G., Santand Anna Jr. G.L. (2002). "Hydrolytic enzymes as coadjuvants in the anaerobic treatment of dairy wastewaters". J. Chem. Eng., 19(2), 175-80.
71. Manilal V.B. andHaridas A. (2000). "Effect of fats and solids in the performance of UASB reactor". Presented at Course on Anaerobic Wastewater Treatment, October 14, 2000, Kochi, India, organized by Cochin Univ. of Sci. and Tech., India, Delft University of Technology, The Netherlands and Wageningen Agricultural University, The Netherlands.
72. Perle M., Kimchie S. and Sheelef G. (1995). "Some biochemical aspects of the anaerobic degradation of dairy wastewater". Water Research.,29 (6), 411- 490.
73. Rhinzema A., Alphenaar A. and Lettinga G. (1993). "Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactor. Process Biochemistry 28, 527 – 537.
74. Rhinzema A., Boone M., Van knippenberg and K. Lettinga G. (1994). "Bactericidal effect of long chain fatty acids in anaerobic digestion". Water Environmental Research 66 (1), 40 – 49.
75. Grotenhuis J.T.C. (1992)."Structure and stability of methanogenic granular sludge". Ph.D thesis, Agricultural University of Wageningen, The Netherlands.
76. Lettinga G., Salih R., Sofia P., Alla N. and Alfons J.M.S. (1998). " High-Rate Anaerobic Treatment of Wastewater at Low". Appl Environ Microbiol. 65 (4),1696 – 1702.
77. Hwu C.S. (1997). "Enhancing anaerobic treatment of wastewaters containing oleic acid". Ph.D.Thesis. Agric. Univ. of Wageningen, The Netherlands.
78. Reynolds J.W. (1986). "The earthworms (Lumbricidae and sparganophilidae) of Ontario". 141. Royal Ontario Museum, Toronto.
79. Ince O., Anderson G.K. andKasapgil B. (1995). "Control of organic loading rate using the specific methanogenic activity test during start-up of an anaerobic digestion system". Water Res., 29 (1), 349-355.
80. Valcke D. and Verstraete W. (1983). "A practical method to estimate the acetolasticmethanogenic biomass in anaerobic reactors. J. WPCF EE, 1191 – 1195.
81. Zhou W., Tsuyoshi I., Masao U., Fusheng L. and Akira Y. (2007). "Effect of loading rate on the granulation process and granular activity in a bench scale UASB reactor". Biores. Tech., 98, 1386 – 1392.