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Full Length Research Paper

Optimization of up-flow anaerobic sludge blanket reactor for treatment of composite fermentation and distillation wastewater

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Optimization of up-flow anaerobic sludge blanket (UASB) reactor operation for treatment of a composite fermentation and distillation wastewater was achieved using a locally available thickened municipal sludge instead of imported commercial anaerobic granulated sludge. Over the first 12 days, a fed batch start-up operation was maintained and anaerobic stable sludge granules with 11.2% of extra cellular polymers (ECP) were successfully developed and further used for long-term continuous operation. Two types of granules were developed within the reactor but with very different characteristics. Granules grown in the bottom part of UASB reactor were more compact and tense than those that occurred in the upper part. The latter were fragile, irregular in shape and with much lower methanogenic activities. Bottom granules were dominated by both *Methanosarcina* spp. and *Methanosaeta* spp. whereas upper granules harbored only *Methanosarcina* spp. During continuous anaerobic treatment of composite fermentation and distillation wastewater with organic load of 24 g.l^{-1} of chemical oxygen demand (COD), a removal efficiency of up to 84% was achieved. Moreover, biogas was produced with a production rate of o.52 m^3/Kg COD removed.

Key words: Composite wastewater, up-flow anaerobic sludge blanket (UASB), anaerobic biological treatment, biogas, granulated anaerobic sludge, industrial wastewater.

INTRODUCTION

Fermentation wastewaters have a very high organic load compared to municipal wastewater (Shi et al., 2012). Their treatment to reduce chemical oxygen demand (COD) and biological oxygen demand (BOD) is essential prior to disposal on land or water bodies. The up-flow anaerobic sludge blanket (UASB) reactors have been proven for efficient anaerobic treatment systems for petroleum refinery wastewater (Rastegar et al., 2011), poultry wastewater (Glatz et al., 2011), dairy wastewater (Gotmare et al., 2011), and vitamin C biosynthesis wastewater (Shi et al., 2012). Such anaerobic treatment involves biodegradation of organic substances by exo-enzyme producing micro-organisms and simultaneous production of methane by methanogenic bacteria.

Compared with other treatment processes, USAB reactors have the advantage of their ability to retain high bio-mass with high void volume, because no support material is externally supplied (Mrunalini et al., 2013),

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Abbreviations: ECP, Extra-cellular polymers; **COD**, chemical oxygen demand; **BOD**, biochemical oxygen demand; **PVC**, polyvinyl chloride; **GLSS**, gas-liquid-solid separator; **TS**, total solids; **VFAs**, volatile fatty acids; **VS**, volatile solids.

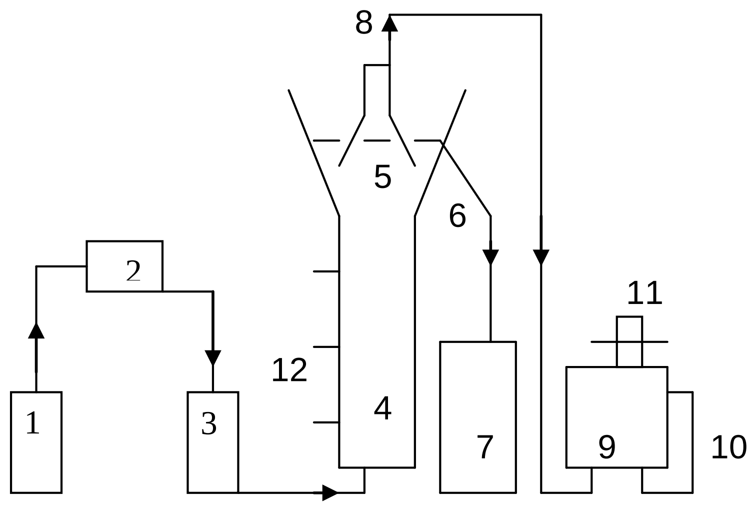

Figure 1. Schematic diagram for experimental set-up of the UASB reator. I, Waste water reservoir; 2, feeding pump; 3, heat exchange unit; 4, UASB reactor; 5, three-phase separator; 6, effluent overflow; 7, effluent collector; 8, gas outlet; 9, gas collector; 10, side-arm measuring device; 11, gas discharge valve; 12, sampling ports.

their independence from mechanical mixing of reactor contents (Ghangrekar and Kahalekar, 2003), and their ability to cope with perturbances from temperature fluctuations and high organic loading rates (Nidal 2008; Kovacik et al., 2010).

The successful operation of an UASB reactor depends on the use of highly flocculated compact sludge aggregates called granules (Najafpour et al., 2006; Narihiro et al., 2009). These anaerobic sludge granules can be supplied as commercial product or possibly formed during the start-up of UASB reactors by self immobilization of anaerobic bacterial cells, a process which is correlated with the production of extra-cellular polymers (ECP) by these cells (Schmidt and Ahring, 1994; Yoochatchaval et al., 2008).

Several researchers (Emiliano et al.2006; Vlyssides et al., 2008; Shi et al.2012) have suggested that the loose structure of filaments of methanogenic bacteria, *Methanosaeta* spp. cells and the clamps formed by *Methanosarcina* spp. cells, are the most crucial factors affecting stability of the granules. In absence of commercial preparations of anaerobic granulated sludge, development of anaerobic sludge granules starting from a locally available thickened sludge and their utilization for continuous anaerobic treatment of industrial wastewater, produced by various fermentation industries, were the objectives of the present study.

MATERIALS AND METHODS

Wastewater

The wastewater used throughout this investigation was obtained from The Company of Sugar and Integrated Industries, Hawamdia complex, Giza, Egypt. The final wastewater stream comprises of wastewaters coming from yeast, ethyl alcohol and distillation industries.

Bioreactor

The UASB reactor was made of polyvinyl chloride (PVC). The working volume of the reactor was 12 litres, consisting of 10.8 litres column portion with 15 cm in ID and 68 cm in height, and a 1.2 litre Gas-liquid-solid separator (GLSS). Three sampling ports were located at 15, 30 and 45 cm from the reactor bottom. The schematic representation of the experimental set-up is shown in Figure 1. The reactor operated in upflow mode. The biogas was collected from the top of the reactor to a flexible water-filled gasholder fitted with measurement systems for both gas and liquid flows. A top opening in the gasholder was used to discharge the biogas every time the gasholder was completely filled.

Development of stable anaerobic granular sludge

In view of the absence of locally sold commercial preparations of granular anaerobic sludge, sludge obtained from a gravity thickener at municipal wastewater treatment plant at Zenien, Giza was used to seed the reactor. Two liters of the thickened sludge with 2.8% total solids (TS) and 76% volatile solids (VS) were introduced, into UASB reactor, and stored for two weeks in order to improve its anaerobic activities. For further improvement of sludge settling capacity and methanogenic characteristics, the reactor was operated in fed-batch upflow mode at a loading rate of 0.5 g COD litre^{-1}. day^{-1} of diluted wastewater with a COD content of 12 000 ppm . Samples were collected over 12 days from both the lowest and the highest sampling ports and analyzed.

Characteristics of the anaerobic sludge granules

Compared to most probable number (MPN) technique and other cultivation methods, microscopic examination was proven as a

Table 1. Chemical composition of industrial wastewater.

Parameter	Value	Unit
pH	4.2	Ső
COD	24300	ppm
BOD	15000	ppm
Total Nitrogen	430	ppm
Total phosphorous	128	ppm
BOD/COD	0.61	-

reliable technique for anaerobic granular sludge examinations particularly those concerning with methanogenic populations (Yoochatchaval et al., 2008; Shi et al., 2012). Therefore, microscopic examination was used in this study to study different microbial groups contributed if sludge granules at the bottom and upper part of the bioreactor.

Optimization of continuous operation of the UASB reactor

Composite fermentation and distillation wastewater was fed into the reactor continuously at a rate of 4 litres day^{-1} (retention time three days) with a stepwise increase in COD content (organic loading rates of 4, 6 and 8 g COD.litre^{-1} day^{-1}). The pH of wastewater was adjusted at 7.5 ± 0.2 before being passed through an external heat exchange unit in order to control influent temperature at 35 ± 0.5. The organic loading rate was increased when the previous steady state was reached and maintained for three volume turnovers at least. Samples were taken from reactor effluent every 24 h and analyzed.

Analytical methods

The effluent from the UASB reactor was analyzed for residual COD, pH, and total volatile fatty acids (VFAs) and BOD in raw wastewater and the volatile solids (VS) of the granules according to the American Public Health Association (APHA) (1989). Acetate and propionate were determined gchromatographically (Amin et al., 1983). After thermal extraction of sludge granules at 70°C in a shaking water bath for 4 h, the water soluble ECP were separated by centrifugation at 9500 g for 10 min and analyzed for polysaccharides using the phenol/sulphuric acid method of Dubois et al. (1956), protein as Kjeldahl nitrogen (Schmidt and Ahring, 1994) and lipids by gas chromatography (Arneborg et. al., 1993), after methylation by KOH and methanol.

RESULTS AND DISCUSSION

Chemical composition of wastewater

Table 1 illustrates the chemical composition of wastewater. Clearly, wastewater contains the three major nutrients (nitrogen, carbon and phosphorous) in excess amounts than required for anaerobic metabolism. The COD: N: P ratio was 1000: 17: 5, which is considerably higher than that cited in literature (1000: 13: 3, Mrunalini et al., 2013) for optimum anaerobic metabolism. Certainly, this could be considered to be an advantage as no external nutrient addition was required throughout the whole investigation. Only pH of the wastewater had to be controlled as mentioned above.

Start-up of UASB reactor

Conversion of COD in UASB reactor

A fed-batch operation of UASB reactor was commenced after two weeks from seeding with municipal thickened as described above. Samples were taken and analyzed over 12 days. The performance of the reactor is shown in Figure 2 and Table 2. As operation proceeded, both consumption of COD and production of VFAs increased with acetic and propionic acids being the major components. Acetic acid was consumed much faster than propionic acid.

Up to 1910 ppm of propionic acid were detected on day 5 with only 230 ppm of acetic acid. Despite the high COD removal efficiency of 85%, biogass yield was very low, only 0.13 litres/g consumed COD (Table 2). This is most probably due to accumulation of high concentration of VFAs (2550 ppm). From days 5 to 9, biogas production was markedly improved as a result of breakdown of VFA indicating stimulation of methanogenic microorganisms. The best performance was observed on day 9 with a COD removal efficiency of 88% and the highest biogas yield of 0.66 liters/g consumed COD. The total VFA concentration decreased down to 1540 ppm. With further fed-batch operation, a total different performance characterized by a dramatic deterioration in COD removal efficiency was noticed. Both residual COD and concentration of VFA, particularly propionic acid increased sharply in reactor effluent and biogas production rate decreased (Table 2). Similarly several investigators have reported that propionate oxidation to acetic acid was the rate-limiting step of anaerobic methane production from various fermentative substrates (Harada et al., 1996; Kovacik et al., 2010).

The lowest COD removal efficiency of only 33% was recorded on day 12 with the highest concentration of propionic acid (2400 ppm). Acidification of reactor contents must have taken place as pH of reactor effluent decreased down below 4 (Figure 1). This resulted in a partial inhibition of methanogenic activity of developed sludge as biogas yield decreased to 0.44 litres/g consumed COD.

Development of anaerobic granular sludge within UASB reactor

It is well known that, both cell-to-cell adhesion and formation of anaerobic sludge granules are very much correlated with microbial excretion of ECP. Therefore, the amounts and chemical composition of ECP of the developed sludge granules from the bottom and the

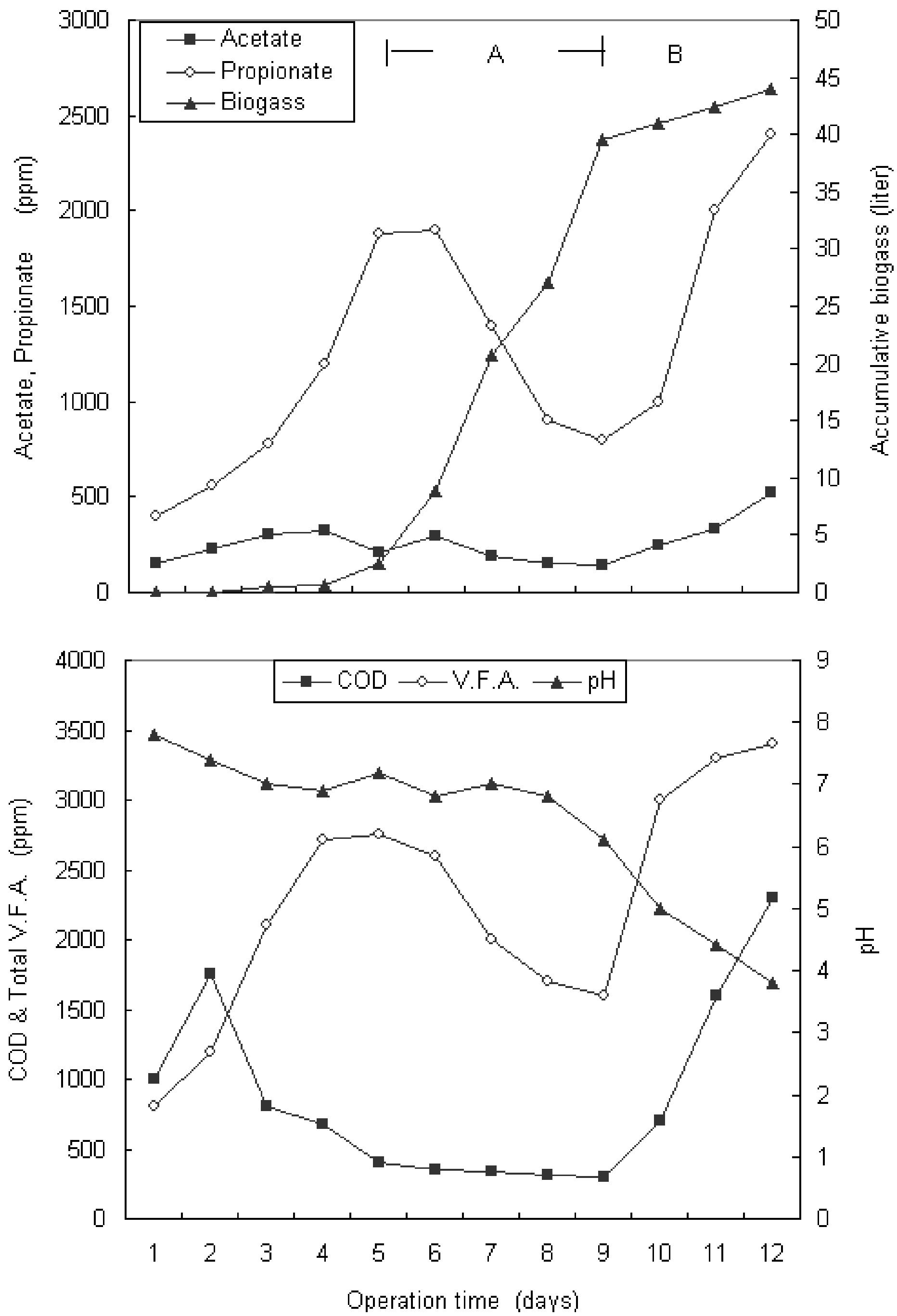

Figure 2. Performance of UASB reactor during fed-batch operation. A and B are operation stages between days 5, 9 and 12, respectively. COD value taken after excluding the amount of COD consumed in VFAs synthesis.

Table 2. Kinetic parameters on performance of UASB during fed-batch operation.

Parameter	Day 5	Day 9	Day 12
Biogas yield (liters biogas/g COD $_{cons.}$)	0.13	0.64	0.46
Concentration of VFAs (g/l)	2. 50	1.46	3.00
Removal Efficiency (%)	85.0	88.0	36.0

Table 3. Chemical composition of extra-cellular polymers (ECP) of granules formed in UASB reactor during fed-batch operation.

Type of granule	Concentration mg/g of VSS				
	Protein	Poly-saccharide	Lipid	Protein/polysaccharide	ECP
Upper granules	67.85	8.19	0.74	8.3	76.78
Bottom granules	90.09	15.3	0.204	5.9	105.59

upper part of UASB reactor was determined over the last four days of fed-batch operation and related to the dramatic change in reactor performance. Table 3 shows the results. It is very clear that granules developed in the bottom part of UASB were very much different from those formed in the upper part. The total amount of ECP was higher in former granules compared with those formed in the upper part being 105.6 and 76.8 $mg.g^{-1}$ (VS), respectively. The reported percentages of ECP in different anaerobic granular sludge are between 0.6 and 20% of VS, depending on type of wastewater, extraction and analytical methods for ECP (Emiliano et al., 2006; Gotmare et al., 2011). The obtained results show also that in both types of granules, protein contributes the major part of ECP followed by polysaccharides. The granules grown in the bottom part of the reactor had markedly higher contents of protein and polysaccharides per gram VS in their ECP compared to granules formed in the upper part.

It is suggested that the implemented fed-batch operation mode might have created an up-flow staged sludge-bed system similar to that reported by Yetilmezsoy and Sakar (2008) with various compartments along the upflow direction of the UASB reactor. The wastewater fed daily at reactor bottom might have generated a zone for fermentation of complex substances in the bottom part, which gradually becomes lower in the following upper parts, leading to more acetogenic and methanogenic substrates, such as propionic and acetic acids, in the upper part (compartment) of UASB reactor. This must be considered to explain the similarity between the obtained results and those reported by Schmidt and Ahring (1994) who found that changing the feed of an UASB reactor from a sugar-containing wastewater to a synthetic wastewater containing acetate, butyrate and proionate resulted in a decrease in both protein and polysaccharides content of the ECP of sludge granules. More recently, Kovacik et al. (2010) reported major changes in microbial diversity in biogranules in response to changes in nature and concentration of organic load fed into UASB reactor.

Lipids have also been detected in ECP of bottom and upper part granules with much lower amounts than protein and polysaccharides. However, granules grown in the bottom part had significantly lower amounts of lipids compared to upper part granules (0.204 and 0.740 $mg.g^{-1}$ VS, respectively). Similarly, Schmidt and Ahring (1994) found that granules with low content of ECP had higher amount of lipids compared to granules with high ECP content. The authors suggested that higher excretion of lipids could be a way of compensating for the lower production of protein and polysaccharides in anaerobic sludge granules.

Characteristics of the anaerobic sludge granules

Dolfing (1986) reported that microscopic examination gave up to 50% more methanogens than MPN techniques. Besides, all cultivation methods are selective and non-cultivable strains cannot be counted. In the present study, microscopic observations showed that bottom granules contained both easy detected auto fluorescent *Methanosarcina* coccoids and gas- vacuolated bamboo-shaped *Methanosaeta* filamentous rods, whereas only *Methanosarcina* cells dominated granules formed in the upper part. Narihiro et al. (2009) pointed out that *Methanosarcina* spp., unlike *Methanosaeta* spp., have the capacity to use acetate, butyrate and propionate.

Methanosaeta spp. is known to grow only on acetate. This might be considered to explain the presence of *Methanosarcina* dominated granules in the upper part of UASB reactor, when acidic conditions were developed. Microscopic examination showed also that granules grown in the bottom part of UASB reactor were more compact, and tense than those occurred in the upper part. The latter were fragile and irregular in shape.

Optimization of continuous operation of UASB reactor

In view of the above-mentioned results, it was decided to start feeding wastewater in a continuous mode in an attempt to washout acidic wastewater from the reactor, get red of fragile sludge granules in the upper part and maintain those actively growing granules in the bottom part of the USAB reactor. Optimization of the UASB reactor continuous operation for anaerobic treatment of industrial wastewater having higher organic loads was also considered. Wastewater was fed into the reactor continuously at a rate of 4 $litres.day^{-1}$ (retention time of three days) with a stepwise increase in COD content

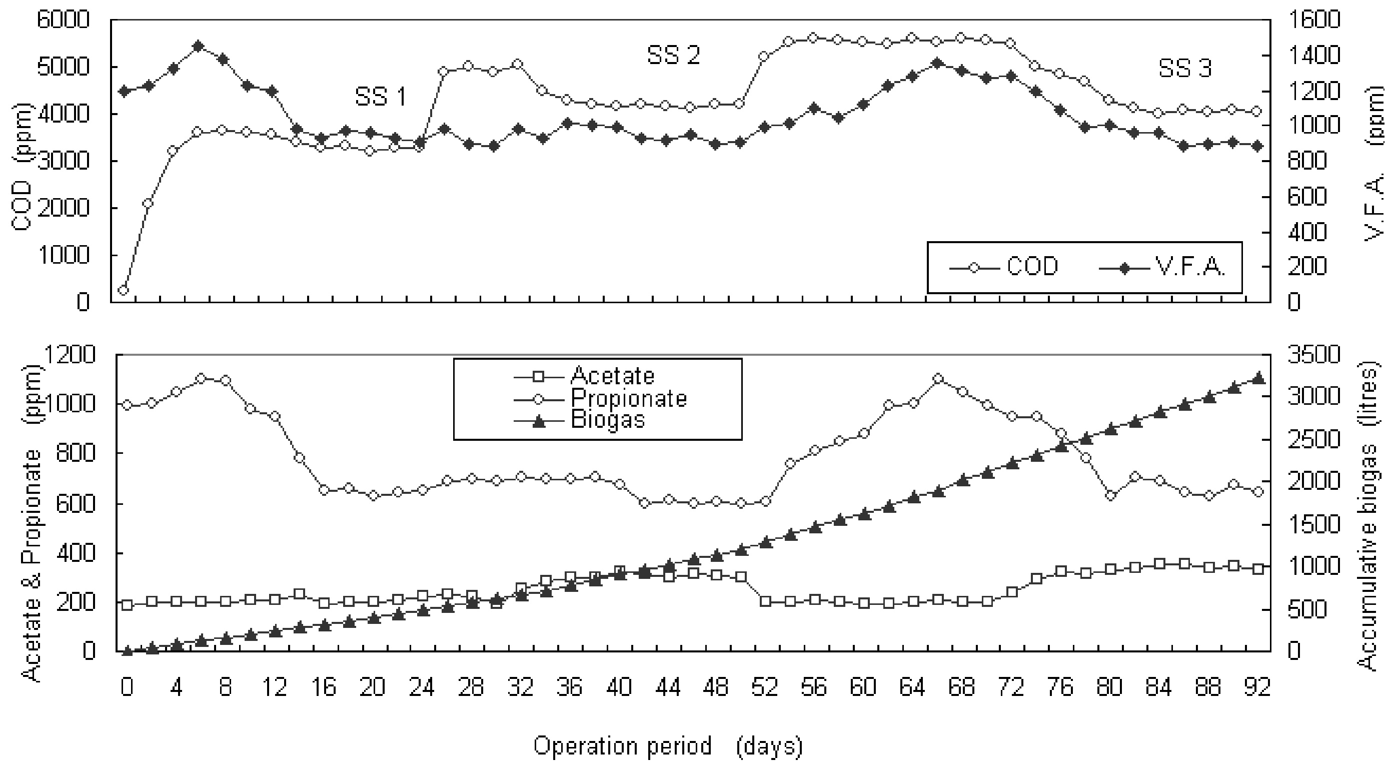

Figure 3. Performance of UASB reactor during continuous operation using industrial wastewater with different organic loading loading rates. SS1, steady state at 4 g COD/L/d; SS 2, steady state at 6 g COD/L/d ; SS 3, steady state at 8 g COD/L/d. COD values taken after excluding COD consumed in VFA synthesis.

Table 4. Removal efficiency and biogas production by UASB reactor during steady state conditions of anaerobic treatment of industrial wastewater with different organic loads.

Operation parameter	**Steady state at organic loading rate (g COD. Litre^{-1} .d^{-1})**		
	4	**6**	**8**
Final discharge COD (ppm)	3275	4170	4067
Bioreactor productivity liters biogas /g consumed COD	0.48	0.52	0.52
Liters biogas / liter of reactor volume / day	1.66	2.80	4.08
mg V.F.A. / g consumed COD	3.79	2.40	1.92
Removal efficiency (%)	73	77	84

(organic loading rates of 4, 6 and 8 g COD.litre^{-1}. day^{-1}). The organic loading rate was increased when the previous steady state was reached and maintained for three volume turnovers at least. Samples were taken from reactor effluent every 24 h and analyzed for residual COD, VFAs, acetate, propionate and biogas. Figure 3 and Table 4 show the results. For each loading rate transit increases in COD and VFA content of the reactor effluent, particularly propionate, were observed followed by a steady state. After adaptation period (the first 2 weeks), UASB reactor reached the first steady state with 73% removal efficiency of the COD. However, biogas production yield was only 0.48 liters. g^{-1} consumed COD most probably due to the high production of VFAs (3.79 mg .g^{-1} consumed COD. As loading rate increased COD, removal efficiency and biogas production yield increased in directly proportional way to give the highest biogas yield and COD removal efficiency of 0.52 liters. g $^{-1}$ consumed COD and 84%, respectively. The production of VFAs decreased to 1.9 mg. g^{-1} consumed COD. With regard to pollution control, the results show that efficiency of COD removal using UASB reactor compares very favorably with those reported by other investigators (60 to 80% by Ghangrekar and Kahalekar (2003); 59-76% by Bertin et al. (2004); 75% by Gotmare et al. (2011)). Moreover, continuous operation of UASB reactor showed that after a short transit increase, propionate concentration stabilized during each steady state at about 650 ppm level. This demonstrates the markedly improved activity of acetogenic and methanogenic bacteria, and a satisfactory balance between different microbial populations in sludge granules. This was never happened with previous fed-batch operation.

Conclusion

The successful conversion of locally obtained thickened sludge into actively growing anaerobic sludge granules with high methanogenic activity during the start-up operation provides alternative means of an effective seeding to methane reactors in absence of the expensive commercial preparations of anaerobic granulated sludge. Work is in progress to optimize a two-stage anaerobic aerobic system for more removal of organic pollutants from highly polluted industrial wastewaters, in order to reached final effluent with characteristics required by recent environmental law.

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