Biomass Acclimatization to Sequentially Varying Substrates in an Upflow Anaerobic Sludge Blanket (UASB) Bioreactor

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In this study, an upflow anaerobic sludge blanket (UASB) bioreactor was sequentially subjected to high-strength synthetic, low-strength synthetic and domestic wastewaters. From COD removal data, supported by volumetric loading rate, hydraulic retention time, pH and qualitative biogas production data, it was observed that the biomass in the bioreactor took about twice the time required to acclimatize to a change in substrate characteristics or composition compared to a much more drastic quantitative change, i.e., more than 95% difference, in substrate concentration. As the initial experiment coincided with the bioreactor start-up, it could also be concluded that the feeding regime did not shorten the overall start-up time of a UASB bioreactor meant to treat domestic wastewater, but its eventual success was probably more assured.

Key words: UASB, start-up, acclimatization, sequential substrate change, low-strength wastewater

Introduction

Anaerobic wastewater treatment has increasingly gained attention since the 1970s. Due to stricter environmental regulations, the demand for more cost-effective treatment systems for the expanding food industry (van Lier et al. 2001) and the worldwide oil crisis at the time, fervent research had greatly improved knowledge of the microbiology and biochemistry of anaerobic microorganisms and processes. The development of modern high-rate anaerobic bioreactors resulted in the successful utilization of anaerobic processes in wastewater treatment. Their success lies in the separation of hydraulic retention time from solids retention time in the system, enabling high concentrations of biomass to be retained in the bioreactors under high hydraulic loading rates and thus establishing sufficient contact between the microorganisms and the wastewater. Of these high-rate systems, the upflow anaerobic sludge blanket (UASB) bioreactor is the most successful design for various industrial and municipal wastewaters (McCarty 2001). Investigated and developed by Lettinga and his team since 1971 (Lettinga et al. 1980), it has become the most popular and widely used high-rate anaerobic wastewater treatment system worldwide (van Lier et al. 2001; Schmidt and Ahring 1996; Hulshoff Pol and Lettinga 1986).

The most crucial step that determines the subsequent performance of the UASB system is its start-up. Described by Hulshoff Pol and Lettinga (1986) as “a fairly delicate and time-consuming process,” the start-up process will determine if a highly active biomass with good settling abilities, the two important desired characteristics, are formed in the bioreactor. During start-up, the anaerobic microorganisms required for the process are allowed to grow until a sufficiently active population is present in the biomass to enable anaerobic digestion to progress stably. The biomass also needs to be acclimatized to the substrate that it is meant to treat. According to de Zeeuw and Lettinga (1981), there are roughly three stages in the acclimatization process. In the first stage, the biomass adapts to the new substrate by forming suitable bacterial microenvironments. The second stage involves an increase in the sludge specific activity as a net effect of bacterial growth rate and the extent of washout of both active and inactive biomass. In the third stage, granulation or pelletization of the sludge takes place to produce granules that settle and form a layer of sludge blanket in the bioreactor. When a change in substrate carbon source is effected, Yang and Anderson (1993) reported that the anaerobic ecosystem changes from the entire bioreactor (when it was treating the original wastewater) to individual sludge granules with special nutritional needs, resulting in the spatial redistribution of bacterial population in the sludge after the change. In UASB bioreactors, no supporting materials are required as these granules are formed by natural self-immobilization of the bacteria (Schmidt and Ahring 1996). A careful start-up procedure entails seeding the bioreactor with available sludge if necessary, supplying proper nutrients to facilitate bacterial growth and granulation, and applying appropriate loading rates that do not exceed the maximum potential of the biomass in the bioreactor or cause biomass washout.

The long start-up period required is a major problem in the application of anaerobic digestion technology, specifically in UASB bioreactors, especially for low-
strength wastewater. This is due to the low growth rate of methanogenic organisms (Rittmann and McCarty 2001), one of the two key organisms involved in the digestion process. In low-strength wastewater treatment, the problem is compounded by bacterial washout caused by short hydraulic retention times and high organic loads when the rate of loss of active microorganisms exceeds their rate of synthesis (Rockey and Forster 1982). On the other hand, short hydraulic retention times and high organic loading rates are desirable in high-rate systems as they expedite bacterial growth and sludge granulation (Liu and Tay 2004; Francese et al. 1998). Thus, for low-strength wastewater, it is especially crucial that hydraulic retention times applied are not too short to cause excessive washout of viable biomass and at the same time not too long to be detrimental to sludge granulation due to corresponding lower volumetric loading rates. Vast literature is available on start-up procedures, guidelines and results (Behling et al. 1997; van Haandel and Lettinga 1994; Hulshoff Pol and Lettinga 1986; de Zeeuw and Lettinga 1981; Lettinga et al. 1980) and on sludge granules and granulation (Liu and Tay 2004; Francese et al. 1998; Brito et al. 1997; Schmidt and Ahring 1996; Goodwin et al. 1992).

Little has been said on biomass acclimatization behaviour alone. In this aspect, Behling et al. (1997) and Morgan et al. (1990) reported deterioration in bioreactor performance after a change of feed and attributed this to the need for the biomass to be acclimatized to the different substrates. Although these and other studies (Brito et al. 1997; Yang and Anderson 1993) were done on the effect of sequential substrate changes on a UASB bioreactor, the changes were in carbon source composition. Also, no quantitative details were provided on the acclimatization time.

In this work, the changes studied were in substrate concentrations, and then in their composition and characteristics at almost similar concentrations. From the wastewater chemical oxygen demand (COD) removal measured, the apparent biomass acclimatization behaviour in the UASB bioreactor can be deduced. As this experiment coincided with the UASB bioreactor start-up for treating domestic wastewater, it would also enlighten on this regime’s possibility of shortening the start-up time for anaerobic treatment of domestic or similar low-strength wastewater.

Materials and Methods

Experimental Setup

Figure 1 shows the experimental setup used in this research. The UASB bioreactor consisted of acrylic columns fastened together to give overall dimensions of 0.584 × 0.190 m (H × ID), with a funnel of 0.178 m OD and inclination of about 60° as the phase separator. The bioreactor working volume was approximately 13.0 L. Except for the influent tank, influent pump and gas collection bottles, the whole setup as shown in Fig. 1 was housed in a chamber controlled at 37°C. However, due to various factors, the temperature of the bioreactor body varied from 31 to 39°C throughout the earlier period of the experiment, before being stable at 37 ± 1°C for most of the experimental duration. Seed sludge and wastewater were fed into the bioreactor using a peristaltic pump (Watson Marlow, 323 E/D, U.K.) via 1.6-mm ID platinum-cured silicon tubing. Both were kept stirred gently on a magnetic stirrer plate while being pumped into the bioreactor. Pinch corks were used to divert the influent and effluent flows during sampling for analysis.

Seed Sludge

To expedite the start-up process, seed sludge was used. They were obtained from two sources:

1. From a clarifier as the sludge was being pumped into the sludge holding tank of an extended aeration sewage treatment plant; its total suspended solids (TSS) and volatile suspended solids (VSS) contents were 8.282 and 6.014 kg/m³, respectively.
2. A full-scale UASB bioreactor treating a mixture of brewery wastewater and sewage with TSS and VSS contents of 12.587 and 6.538 kg/m³, respectively.

Altogether, 2.6 L of sludge from each source had been fed for seeding. The second seed sludge was high in methanogenic activity as evident from the bubbles in the container during collection; in the first seed sludge, no such activity was observed.

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Substrate

Table 1 summarizes the substrates used throughout the duration of the reported data. The synthetic waste, with composition as shown in Tables 2 and 3, was a medium modified from that used by Praveen (1994). Prior to feeding into the bioreactor, stock solutions of 220 g COD/L medium were diluted to the desired concentration with tap water and the pH adjusted to 6.5 to 7.5 by addition of concentrated NaOH. Medium prepared in excess was stored at 4°C.

Domestic wastewater used for feeding the bioreactor was collected regularly from the Main Sewage Treatment Plant, Shah Alam, which is an activated sludge plant. The collection point was the overflow channel directing the sewage from the aerated grit chamber to the distribution box prior to the primary clarifiers. Domestic wastewater collected was filtered a few times with a makeshift sieve and autoclaved at 121°C for 20 min to sterilize it for prolonged storage. The processed domestic wastewater was stored at 4°C.

The sequence of substrates fed into the bioreactor coincided with the approach taken in developing the bioreactor for ultimate domestic wastewater treatment. The rationale of applying this regime was to first use high-strength synthetic waste to enable the biomass required to grow well and fast, then to feed the biomass with synthetic waste of low strength to gauge their ability to cope, before subjecting them to domestic wastewater, which was expected to be of low (250 mg COD/L) to medium strength (below 500 mg COD/L).

Experimental Conditions

The data reported were from Day 78 onwards, which was the start of the continuous operation period that followed an initial batch feeding at the beginning of start-up. Day 0 was designated as the day the first seeding exercise commenced. It should be noted that the two types of seed sludge were fed about 60 days apart from each other, after the first start-up exercise did not show apparent biogas production. This could be due to either process deficiency or initial setup technical error. The two seeding exercises were besieged with clogging problems and were only accomplished over a few days each.

Continuous feeding was initialized at the lowest flow rate, i.e., 3.68 mL/min. When COD removal increased, the pumping rate was increased stepwise by 20% or 5 rpm, whichever was higher. Feed rate and biogas production were monitored daily, whereas influent and effluent pH, temperature and COD concentrations were measured daily whenever possible, or otherwise after the process was deemed to have stabilized after any interruption. When the COD removal reached a near-constant maximum level above 90%, the substrate was changed to low-strength synthetic waste, and then to domestic wastewater, as summarized in Table 1. At each change of substrate, the feeding rate was reduced to the minimum, and increased stepwise again as COD removal efficiency increased.

Analytical Methods

Seed sludge biomass was characterized according to the Standard Methods for total, volatile and fixed suspended solids analyses (Clesceri et al. 1998).

<table>
<thead>
<tr>
<th>Day no.</th>
<th>Waste type</th>
<th>Theoretical</th>
<th>Duration, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-156</td>
<td>HSWa</td>
<td>11,000</td>
<td>156</td>
</tr>
<tr>
<td>157-162</td>
<td>HSW</td>
<td>12,100</td>
<td>6</td>
</tr>
<tr>
<td>163-167</td>
<td>HSW</td>
<td>13,310</td>
<td>5</td>
</tr>
<tr>
<td>168-171</td>
<td>HSW</td>
<td>11,000–19,800</td>
<td>4</td>
</tr>
<tr>
<td>172-189</td>
<td>LSWb</td>
<td>500</td>
<td>18</td>
</tr>
<tr>
<td>190-339</td>
<td>DWWc</td>
<td>250–500</td>
<td>150</td>
</tr>
</tbody>
</table>

a HSW; High-strength synthetic waste.
b LSW; Low-strength synthetic waste.
c DWW; Domestic wastewater.
d This refers to the intended or expected COD concentrations of the feed; the actual variation would be reflected in the loading rate graphs in the Results and Discussion.

e Although the feed concentration was increased, the corresponding intended volumetric loading rates for that period were maintained by decreasing the flow rates.
f The feed concentration was allowed to vary, as it was not crucial to control it during this period of transition to LSW.
Temperature and pH were measured using a pH/temperature probe (Thermo Orion, 9107BN, U.S.A.) with automatic temperature compensation. The method used in pH measurement was generally in compliance with Standard Method 4500B (Clesceri et al. 1998).

For COD analysis, Hach’s Method 8000, a combination of Reactor Digestion Method and Colorimetric Method, was used (Hach Company 1997–2000). This method is equivalent to Standard Method 5220D: Closed Reflux, Colorimetric Method (Clesceri et al. 1998). Samples were digested at 150°C for two hours with potassium dichromate to form green chromic ion (Cr$^{3+}$). The amount of green Cr$^{3+}$ was measured using a calibrated, pre-programmed colorimeter (Hach, DR/890, U.S.A.).

The actual feed rate was calculated from the wastewater volume fed and the corresponding time measured over as long a period as possible, and averaged throughout the day. It should be noted that the feed rates reported were averaged over the course of the day, regardless of the extent of fluctuations throughout. This was to give the best representation of the actual bioreactor operation, accounting also for feed interruptions due to various reasons such as influent sample withdrawal for analyses, power failures, maintenance works on the bioreactor and other unavoidable circumstances in the laboratory.

Biogas was collected by water displacement and the volume read from a calibrated gas collection bottle. Gas volume readings were recorded after four hours of the start of collection to allow the water displacement to normalize. Reported results are also the average readings of the day.

**Results and Discussion**

**COD Removal**

Figure 2 shows the time course of COD removal efficiency, volumetric loading rate and hydraulic retention time from Day 78 onwards, or 18 days after the second seeding exercise. This graph summarizes the continuous operation of the bioreactor in terms of applied loadings, both organic and hydraulic.

**High-strength synthetic wastewater experiment.** Figure 2 shows that initially, COD removal efficiency in high-strength synthetic wastewater increased steadily despite increasing volumetric loading rates and decreasing hydraulic retention times. This showed that the loading applied was below the maximum capacity of the biomass. It could also mean that the biomass was growing well as it was able to cope with the increasing volumetric loading.

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Fig. 2. Time course of COD removal efficiency, volumetric loading rate and hydraulic retention time for the reported experimental period.
From Day 130 onwards, or 70 d after the second seeding exercise, COD removal efficiency reached a maximum plateau with an average value of 96.2%. This average was obtained over a range of 39 d with a relative standard deviation (RSD) of 0.914%. The hydraulic retention time during this period varied greatly, at an average of 2.1 d (RSD 51%), with 1.36 and 8.0 d as the shortest and longest hydraulic retention times, respectively. This means that the 39 d was equivalent to at least 27 and 4 hydraulic retention times at the extreme, and at least 17 times the average hydraulic retention time during that period. In shock loading studies, researchers have adopted the assumption of reaching a steady-state condition for anaerobic processes when operating parameters and removal efficiencies remain constant over at least two to three hydraulic retention times (Nachaiyasit and Stuckey 1997; Fongastitkul et al. 1994; Polprasert et al. 1992; Grobicki and Stuckey 1991). For stable operation, Noyola et al. (1988) defined steady state as being achieved after ten times the new hydraulic retention time and a minimum of 14 d. Hence, the consistency in COD removal efficiency over the 39-d period mentioned above can be considered as an indication of steady state in the bioreactor from Day 130 onwards. The maximum COD removal efficiency plateau indicates that there was a sufficient population of anaerobic biomass in the bioreactor for anaerobic digestion to progress stably by then.

Figure 3 shows that despite the fluctuating influent COD concentration, the effluent COD concentration remained almost constant below 1000 mg/L from Day 130 onwards. This observation suffices to establish that the start-up for the UASB bioreactor was completed by then, based on the definition given by van Haandel and Lettinga (1994). They defined start-up as the accomplishment of constant effluent quality at the design load and constant presence of sludge mass, both qualitatively and quantitatively, in the bioreactor. In view of the limitations of practicable analysis in the present study, this definition was the most fitting. Moreover, the steady state in the bioreactor indicated by Fig. 2 affirms the completion of start-up.

From Fig. 4, it can be seen that COD removal rate was proportional to the volumetric loading rate, indicating that the COD loadings applied were well below the maximum capacity of the biomass. Figure 5 indicates that the COD removal rate was generally inversely proportional to the hydraulic retention time, as to be expected. This concurs with others’ findings that short hydraulic retention times expedite sludge granulation (Liu and Tay 2004).
The initial volumetric loading rate applied was only 1.2 kg COD/m³/d. Using a similar feed composition as in this work, Praveen (1994) reported a start-up time of 52 d with seed sludge from an anaerobic domestic waste treatment plant and initial volumetric loading rate of 3.8 kg COD/m³/d, and 57 d with seed sludge from an anaerobic distillery effluent treatment plant and initial volumetric loading rate of 9.4 kg COD/m³/d. In the present work, the (second) seed sludge had been obtained from an actively functioning UASB plant, and the initial volumetric loading rate was 3.2 kg COD/m³/d. The increase of volumetric loading rate before the COD removal efficiency reached 80% could not be the reason for the longer start-up time in the present work, as the COD removal efficiency continued to increase (Fig. 2) and effluent COD concentration continued to decrease (Fig. 3) despite the continual increase in volumetric loading rates. Figure 4 further proved that the biomass was obviously under no stress at the applied loading rates.

The foregoing establishment of complete start-up is important in affirming the following deductions to be made from the COD removal efficiency pattern after sequential changes of substrates.

Low-strength synthetic wastewater experiment. The next phase of experimental run using low-strength synthetic waste as substrate (Days 172–189) started with a dip in COD removal efficiency to 64%, but it increased steadily in spite of concurrent step increments in volumetric loading rates from Days 178–187 (Fig. 2). At the same time, hydraulic retention time was reduced from 4.1 d to about 1.3 d. By the end of this experiment, the COD removal efficiency reached 89.2% over a period of at least three days. This occurred from Days 185 to 187. It is evident that the highly decreased volumetric and organic loading rates compared to the preceding phase were still able to sustain the biomass in the bioreactor throughout the 18-d operation on low-strength synthetic waste. These positive indications reinforce the earlier conclusion that there was a sufficient population of active biomass in the bioreactor.

The initial drop in COD removal efficiency could be due to discharge of effluent from the previous high-strength synthetic waste experimental run. The last applied hydraulic retention time before the switch in substrate was 2.84 d, whereas the first sampling for COD analysis was done about only one day after the switch. Due to the large difference in concentration between the two substrates (between 95–97%), it is very probable that the presence of even a small volume of the old feed would raise the overall COD concentration of the bioreactor effluent during the initial stage of the new experimental run.

However, results obtained on the fourth day and beyond showed that the COD removal efficiency had not recovered to its preceding plateau level. The possibility that the greatly reduced volumetric loading rate could not sustain the biomass is unlikely, as sludge viability in UASB bioreactors can be maintained without feeding for as long as ten months (Bae et al. 1995). No observation of extraordinary biomass washout from the effluent was noted throughout the low-strength synthetic waste experimental run. As such, the possibility of viable biomass washout was unlikely. Moreover, the feed rate was at the minimum and the hydraulic retention time was considerably long throughout Days 172 to 177, i.e., before COD removal efficiency first reached 85% by Day 178. Since it was established that the biomass population was sufficient, the decreased COD removal efficiency could only be attributed to the need for the biomass to acclimatize to the vast change in substrate concentration. This took 14 d from the time the substrate was changed from high-strength to low-strength synthetic waste, where the COD removal efficiency of 89.2% achieved is considered satisfactory.

Figures 6 and 7 show the same trend as for high-strength synthetic wastewater, in relationships between the COD removal rate and volumetric loading rate and COD removal rate and hydraulic retention time. The better performance despite higher loadings indicates that the biomass had acclimatized to the new substrate. Figure 8 further reinforces this point by the decreasing effluent COD concentrations albeit increasing influent concentrations.
Domestic wastewater experiment. After the feed was changed to domestic wastewater, COD removal efficiency again decreased (Fig. 2). Influent COD data obtained within the first five days of the change was invalidated by accelerated sampling rate and so the trend up to the eighth day could not be ascertained. However, it was probable that it was greater than zero, as removal efficiency on the eighth day was 19%. It dropped to zero when analyzed from the tenth to the twelfth day after the substrate change. Thereafter, it rose slowly and unstably, and reached 63.3% within 30 d of the feed switch. This signaled a satisfactory COD removal for domestic wastewater, as it falls within the higher limit of the majority of data recorded for domestic wastewater. Thus, it shows that the biomass had acclimatized to domestic wastewater.

At the end of the low-strength synthetic wastewater run, COD removal efficiency seemed to be potentially on an upward trend, indicating that the biomass present in the bioreactor was still viable and sufficient. It can also be presumed that the sludge had granulated well enough to be able to cope with the loading imposed. Hence the earlier dip to zero removal in the domestic wastewater experiment could only be attributed to the response of the biomass to the substrate change. The drastic dip in COD degradation was most probably due to the vastly different characteristics of the two substrates. The domestic wastewater collected had high suspended solids content, whereas the only solids present in the synthetic waste was the precipitate that formed in the chemical mixture due to exposure to the atmosphere while in the influent container. The composition of pollutants and COD concentration in the domestic wastewater was also expected to be more complex and vary greater than that of the synthetic waste, which was prepared in the laboratory. Above all these, domestic wastewater is very likely to contain toxic or inhibitory substances in the form of spent detergents. Thus it was reasonable that the biomass took a much longer period to adapt to the changes, in this case about twice the time required, than when the change was from high-strength to low-strength synthetic waste.

Figure 2 also shows that it took about 36 d for the COD removal efficiency in domestic wastewater to approach 90%, whereas, for low-strength synthetic wastewater, this was achievable only 14 d after a sub-

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**Fig. 7.** COD removal rate and hydraulic retention time for low-strength synthetic wastewater.

**Fig. 8.** Time course of influent and effluent COD concentration and volumetric loading rate for low-strength synthetic wastewater experiment.
strate change, which coincided with its apparent acclimatization. This could also be an indication of the higher degree of acclimatization required in the event of a change in substrate characteristics compared to a change in concentration. However, there may be a possibility of coincidence that the domestic wastewater’s influent COD concentration was exceptionally high on that day due to fine solids that were non-biodegradable but nevertheless contributed to the COD measured in the influent. Such coincidental fluctuations were more frequent and pronounced in domestic wastewater than in the synthetic wastewaters as evident from Fig. 2. Moreover, the feed rate was also varying greatly as reflected in the highly varying hydraulic retention times, which was mostly due to clogging of the tube by the fine solids. The presence of these fine suspended solids in the influent samples resulted in extraordinarily high volumetric loading rates recorded occasionally, as can be seen on Fig. 9. These non-biodegradable solids were eventually trapped in the bioreactor, resulting in mostly clear effluent samples that were very low in COD. In such instances, the results showed very high COD removal efficiency. The relatively constant effluent COD concentration despite the highly fluctuating influent COD concentration in Fig. 9 is further testimony to this phenomenon. The highly fluctuating influent COD concentration thus blurred some concrete conclusions from the results analysis.

Figure 10 shows that the loading was well within the maximum capacity of the biomass as for the synthetic wastewaters. However, a satisfactory correlation could be obtained between the COD removal rate and hydraulic retention time only after omission of data with extraordinarily high COD removal rates (Fig. 11). The extraordinarily high removal rates were due to the presence of non-biodegradable solids in the influent mentioned earlier. The similarity in the relationship between COD removal rate and hydraulic retention time as that observed for the other wastewaters and as reported by others shows that the bioreactor was operating normally.

Other Operational Indicators

The range of influent and effluent pH and temperatures throughout the experiment are shown in Table 4. The wider range of influent pH for domestic wastewater may be due to the higher degree of non-homogeneity of the wastewater compared to synthetic waste. Since pH of the synthetic waste was adjusted prior to feeding into the bioreactor, the acidic influent might be indication of substrate hydrolysis while in the influent container due to exposure to airborne microorganisms. However, since the influent sampling port was along the same feeding tube after the influent container, the results obtained were representative of the actual feed going into the bioreactor. As such, the COD results obtained were not affected.

Despite the wider range of influent pH than expected, the effluent was always within the 6.5 to 8 pH range generally applied in anaerobic digestion (van Lier et al. 2001). This showed that the bioreactor had high buffer capacity, and that anaerobic digestion was progressing well in it.

Biogas production rate throughout the experimental period is as shown in Fig. 12 to 14. The absence of data prior to Day 129 was due to initial technical problems in the experimental setup. Generally, biogas production seemed to be very irregular, and showed no clear correlation with COD removal rate. Certain sections of the figures also show an unexpected decrease in gas produc-

![Fig. 9. Time course of influent and effluent COD concentration and volumetric loading rate for domestic wastewater experiment.](https://iwaponline.com/wqrj/article-pdf/41/4/437/230396/wqrj0410437.pdf)
TABLE 4. pH and temperature ranges throughout the experiment

<table>
<thead>
<tr>
<th>Waste type</th>
<th>Influent pH</th>
<th>Influent temp, °C</th>
<th>Effluent pH</th>
<th>Effluent temp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSW&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lowest 5.619</td>
<td>Highest 6.455</td>
<td>Lowest 21.5</td>
<td>Highest 27.7</td>
</tr>
<tr>
<td>LSW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Lowest 6.443</td>
<td>Highest 7.452</td>
<td>Lowest 24.4</td>
<td>Highest 28.2</td>
</tr>
<tr>
<td>DWW&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Lowest 5.172</td>
<td>Highest 8.688</td>
<td>Lowest 25.2</td>
<td>Highest 27.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> HSW; High-strength synthetic waste.
<sup>b</sup> LSW; Low-strength synthetic waste.
<sup>c</sup> DWW; Domestic wastewater.

**Fig. 10.** COD removal rate and volumetric loading rate for domestic wastewater.

**Fig. 11.** COD removal rate and hydraulic retention time for domestic wastewater.
tion rate with increasing COD removal rate. This may be due to several reasons:

(a) Data on the exact composition of the biogas is not available. While biogas production signals the occurrence of anaerobic digestion, the actual indicators of the metabolic status of anaerobic systems are the relative compositions of the biogas components. High volumetric production of biogas may not indicate high anaerobic degradation.

(b) The high COD removal rates were very likely due to trapping of solids in the bioreactor mentioned earlier. In this case, the biogas production rate will not be proportional to COD removal rate.

(c) There may be some measurement uncertainty because negative pressure was frequently observed in the bioreactor. This resulted in many lost data or in gas production being recorded as zero when levels read from the measurement bottle were lower than their initial values. The frequently observed ‘surge’ or suction phenomena indicating negative pressure in the bioreactor probably occurred because of negative pressure build-up resulting from utilization of any oxygen present by facultative anaerobic bacteria (Rockey and Forster 1985). However, qualitatively, the presence of biogas confirmed that anaerobic digestion took place to completion in the bioreactor.
Effectiveness of Start-up Regime

Overall, it can be established that the bioreactor’s performance in treating domestic wastewater stabilized by Day 219 when its COD removal efficiency reached 63.3%. It can then be concluded that its “actual start-up” for domestic wastewater treatment was accomplished by Day 219, i.e., slightly more than seven months after the first seeding, or slightly more than five months after the second seeding.

As such, the start-up with high-strength synthetic waste preceding domestic wastewater did not seem to shorten the overall duration of start-up. Very likely, the time for biomass acclimatization during the substrate change from synthetic to domestic wastewater accounted for this to a certain extent. However, the initial high volumetric loading rates would have assured a better growth of biomass, and this would reduce the risk of bioreactor failure when its substrate was changed to wastewaters of lower strength. Furthermore, since the hydraulic retention times applied in this study were generally long, the possibility of biomass washout was reduced. The retained biomass very likely could have aided in expediting the initial COD removal during the low-strength wastewater experiments.

Conclusion

From the COD removal data, the time required to reach a satisfactory COD removal efficiency was longer when the substrate change was from low-strength synthetic wastewater to domestic wastewater (30 d) compared to the high-strength to low-strength synthetic wastewater switch (14 d). It was apparent that the biomass took about twice the time to acclimatize to a change in substrate characteristics or composition compared to a much more drastic change, i.e., more than 95% difference, in substrate concentration. This deduction is supported by the fact that COD removal rate was proportional to volumetric loading rate for all three types of wastewaters, indicating that start-up was completed and biomass in the bioreactor was sufficiently retained and adapted to the initial substrates. The general trend of COD removal rate with hydraulic retention time for all the experiments, which agree with that reported by other researchers, showed that the bioreactor was operating normally. As such, the only probable explanation for the marked difference in the above-mentioned time lapse would be that the biomass had to be acclimatized to each of the different substrates after each feed change. Effluent pH results and observed biogas production verified that anaerobic digestion was progressing well and took place to completion in the bioreactor.

At the applied loading rates, this regime did not shorten the overall start-up time of a UASB bioreactor meant to treat domestic wastewater, but its eventual success for such low-strength wastewater treatment was probably more assured. It was very likely that the time for biomass acclimatization during substrate change contributed to the bioreactor’s overall “actual start-up time” for eventual domestic wastewater treatment.

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