# Pre and post-granulation performance of UASB System treating high-strength industrial effluent

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## ABSTRACT

Performance of a high-rate anaerobic treatment system prior to and after the formation of granular sludge was investigated. The 500-L UASB pilot was set up to receive effluent from a medium-size traditional slaughterhouse without blood recovery. The system was inoculated with mesophilic municipal anaerobic digested sludge for the startup and was fed influent with a total COD in the range of 3000-5000 mg/L. It was operated at 26-29 °C.

After six months of operation, indications of granule formation were observed. It was possible to increase OLR from 14 to 25 kg COD/m<sup>3</sup>.d (SLR of 2.3 kg COD/kg VSS.d) with the onset of granulation. At an upflow velocity of 0.8-1 m/h superior COD removal efficiencies of up to 85% was achieved with reduced potential of sludge washout. These granules were dark brownish with a diameter of 1-4 mm and a settling velocity of 20 m/hr. SS and VSS of the granules' layer were 55-68 and 45-57 g/L, respectively with a specific gravity of 1.3. SEM and TEM analyses indicated rod-shaped and filamentous Methanothrix bacteria as well as Methanosarcina cocci aggregates. Microstructures were an intertwined syntropic association between acetogenic and methanogenic microbial populations with diverse morphologies. X-ray analysis of mineral contents showed a high calcium phosphate apparently related to exracellular polymers of the shell structure.

## **INTRODUCTION**

Several studies has been conducted to evaluate anaerobic degradation of slaughterhouse effluent but none report indications of granule formation. Martinez et al (1995) reports 82% of soluble COD removal at OLR of 1.8. In another study, Ruiz, et. al(1997) report better performance of UASB at OLRs of 1-6.5 compared to anaerobic filters. Low organic loadings reported may be due to the absence of high activity granules. This study was undertaken to evaluate pre and post granulation performance of UASB system treating high-strength effluent from a traditional salughterhouse with no provision for blood recovery.

## MATERIALS AND METHODS

The 500-L effective volume square (50 cm x 50 cm) Plexiglas pilot used in this study (figure 1) was set up downstream of a medium-size traditional slaugherhouse without blood recovery. Nine sampling ports (20 and 30-cm apart at bottom and top, respectively) were provided to quantify sludge characteristic at different elevations along the reactor. Routine analyses were performed using procedures outlined in Standard Methods (APHA, 1985).

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For TEM analyses, granules washed in phosphate buffer were placed in a solution containing 5% glutaraldehyde and 0.15% (w/v) ruthenium red. The 60-mL serum bottle were sealed with oxygen-impermeable rubber stoppers and aluminum seals allowing the fixation to be completed overnight at 4 °C. The granules were embedded in 4% agar and allowed to cool. They were cut in 4 mm thick pieces and washed five times with phosphate buffer containing 0.05% ruthenium red. Post fixation was done for 2 hours in a 2% osmium tetroxide solution. After washing, dehydrating with increasing acetone concentration of water-acetone solutions, and several other intermediate steps, trimmed sections of propylene oxide resin-embedded granules were examined with Hitachi TEM.

For SEM analyses, after going through various fixation procedures, granule samples were quick-frozen in liquid nitrogen to obtain cleaved preparations. Whole and cleaved granules were dehydrated through graded series of water-ethanol and after gold-coating were examined in a Cambridge-360 SEM.

## **RESULTS AND DISCUSSION**

Performance of the reactor during the 230 days of operation can be subdivided into three distinct phases of start-up (days 1-70), normal operation (days 71-166), and maximum loading (days 167-230) after formation of granules. In the start-up phase, the system was inoculated with mesophilic digested sludge at F/M of 0.24 kg COD/kg VSS.d at a biomass concentration of 7.3 kg VSS/m3. Removal efficiency of the reactor at this point was around 30% COD. Gradual increase of loading was exercised with time until the system was adapted to receive an OLR of 6.9 kg COD/m3.d (F/M of 1) with a COD removal efficiency of 80% at the end of this period. In the second phase, the rate of loading was steeper but the system continued to perform reasonably. In the last phase, which coincided with granule formation, loading was further increased to see the maximum loading possible to the system.

**Granule formation** – Trend of effluent solids concentration is shown in figure 2. As shown in the figure, after 16 weeks of operation (period 109-130 days) sludge bed had expanded to fill the whole reactor and as a result, effluent SS reached a maximum of 1.6 g/L. This period coincided with the warmest period of the study where temperature of the reactor was 31 °C and maximum expansion of sludge bed due to the growth of filamentous bacteria had occurred. Having observed granules in the lower part of the reactor, upflow velocity was further increased to eliminate flocculent sludge trapped in between. This caused granules to become suspended in the reactor and further granule growth conditions prevail. At the initial and final phases of sludge bed expansion and granule formation, OLR of system was 14 and 25 kg COD/m<sup>3</sup>.d, respectively.

**Sludge gradient** - Existence of high concentration of active biomass in UASB as indicated by volatile suspended solids (VSS) concentration is an important performance indicator. The digested sludge used for the inoculation of the reactor had a low VSS/SS ration of 0.68 (see figure 3), which is typical since digesters contain more inert solids content than normal wastewater. This condition coupled with low upflow velocity, low gas production rate, and consequent lack of sufficient mixing in the sludge bed resulted in a steep sludge gradient at different heights of the reactor. As shown in figure 4, in sampling ports 1-3, concentration of SS and VSS were 39 and 29 g/L, respectively while in ports 4-9, they were around 1g/L (period 9-36 days). Gradual adaptation of sludge, increase of upflow velocity to 0.3-0.7 m/h during the period of 70-160 days, and higher gas production rate resulted in better mixing conditions within the reactor. Sludge gradient at this stage was less pronounced but

VSS/SS ratio had increased to 0.81. In the granule formation phase (days 167-230), a steep gradient was obvious at sampling port 3 with high concentration in the lower part (SS and VSS of 18.6 and 12.1 g/L, respectively) and minimal concentrations in the upper part of the reactor.

**Image analysis of granules** – Scanning and transmission electron microscopy analyses were conducted on the granules. SEM micrograph illustrated in figure 5 (170X, scale 200  $\mu$ m) shows a smooth surface with many holes likely used for transfer of substrate and intermediate products and a large opening believed to be a route for escape of biogas produced by methanogens. Internal layers of granules are primary composed of acetate-degrading Methanothrix recognized by the bambooshaped morphology and/or their size (1.8-2  $\mu$ m). Existence of cells similar to Methanothrix (flat-ended rods) in the central part of granules indicates that Methanothrix-like clusters may act as nuclei for granule formation.

X-ray microanalysis of dense mineral deposits shown around one of the openings showed a phosphorus and calcium content with phosphorus being dominant.

In TEM analyses microcolonies of bacteria in syntrophic association were observed. In figure 6 (4000X) streptobacillus bacteria in the top left and cocci in the lower left are shown. Other images indicate cocci in tetrad and diplococci configuration are methanosarcina.

## CONCLUSIONS

Experimental results observed in the above study indicate the feasibility of granulation in mesophilic UASB system treating slaughterhouse effluent. Prior to granule formation, the whole depth of reactor is more or less uniform with respect to VSS concentration but with time a definite gradient is developed. Upon granule formation in the reactor, it was possible to steadily increase organic loading rate up to 24 kg COD/m<sup>3</sup>.d with 75-80% COD removal efficiencies. Image analyses conducted seem to support layered structure hypotheses of granules.

Figure 1 – Schematic of UASB system receiving effluent from slaughterhouse (1 - Influent distribution, 2 - Feed tank, 3 - Recycle pump, 4 - Flow control weir, 5 - Water seal, 6 - Sampling taps)



Figure 2 – Effluent solids concentration showing gradual development of dense granular structure after washout of more flocculent sludge.



Time,day

Figure 3 – Gradual build up of active biomass concentration (VSS/SS ratio increase from 0.68 to 0.88) with time due to the adaptation of sludge and more favorable operating conditions.



Figure 4 – Transient behavior of sludge bed at different heights with time showing the formation of more dense granules in the lower part of the reactor.



Figure 5 – SEM micrograph showing smooth surface of granule with a large opening likely for biogas  $escape(170X, scale 200 \mu m)$ .



Figure 6 – TEM micrograph of syntrophic association between microcolonies of bacteria in the granule matrix (4000X). Note streptobacili at the left top and cocci at the left bottom.

