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Effect of feed composition for microbial structure of thermophilically-grown granules

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**1. Introduction** Thermophilic UASB process can allow extremely high COD loading, in the range of 50-100 kg COD·m<sup>-3</sup>·d<sup>-1</sup>. On the other hand, the acceptable wastewater is very limited for thermophilic UASB process due to the difficulty or low reliability of granule formation compared with mesophilic process. For instance, failure of granulation was often observed when feeding with simple substrate (i.e., acetate, VFAs) in the start-up of the thermophilic UASB process. So the main purpose of this study was to investigate the effect of feed composition for change in microbial structure of thermophilic granules, evaluated by measurement of methanogenic activity and physico-chemical properties.

**2. Methodology** A thermophilic UASB reactor was operated at 55°C by feeding with three different substrates, i.e., phase one (1-70 days): alcohol distillery wastewater, phase two (71-281 days): synthetic acetate, and phase three (282 days~): sucrose. The total volume of UASB reactor is 2.15l. The reactor was inoculating with thermophilically-grown (55°C) granular sludge treating an alcohol distillery wastewater (20 kgCOD·m<sup>-3</sup>·d<sup>-1</sup>). The volumetric COD loading was set at 9 kgCOD·m<sup>-3</sup>·d<sup>-1</sup> with influent COD of 3000mgCOD·l<sup>-1</sup> throughout the experiment (HRT=8hr.).

Microbial structures of thermophilic granules were examined by measurement of physico-chemical properties (SVI, mean diameter) and microbial structure (SEM observation, methanogenic activity, FISH examination). The methanogenic activities were determined at 55°C in duplicate on the day 0, 60, 86, 100, 143, 272, 298, 313, 355 and 440. The test substrates used were acetate, H<sub>2</sub>/CO<sub>2</sub> (80:20, v/v%), propionate and butyrate.

**3. Results and discussion** The COD removal reached about 94% in phase one and slightly increased to 99% at phase two. Afterward, the COD removal remarkably decreased around 60% at the first period of phase three, however it is recovery to 98% within 10 days (fig. 1).

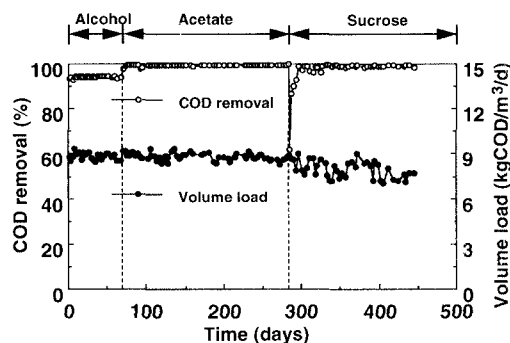


Fig. 1 Changes in COD removal and loading.

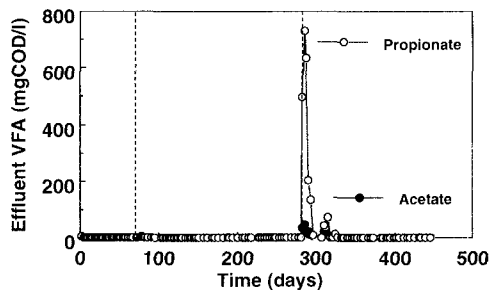


Fig. 2 Changes in effluent VFA concentration

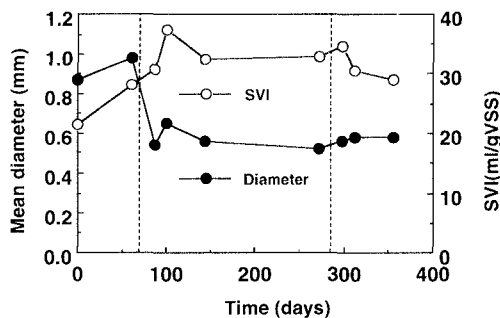


Fig. 3 Changes in SVI and mean diameter of retained sludge.

The effluent VFA concentration was kept extremely low level during phase one and two. In the first period of phase three, the effluent propionate concentration rapidly increased about 730 mgCOD.l<sup>-1</sup> due to decline of propionate-degrading acetogen in phase two (fig. 2).

The methanogenic activities (fig. 3) drastically changed by shift in feed composition in the first one month of each phase. The acetate-fed methanogenic activity increased about 2 times (day 100, 2.8 gCOD.gVSS<sup>-1</sup>.d<sup>-1</sup>) in phase two. On the other hand, hydrogen, propionate and butyrate-fed activities remarkably decreased (about 1/3-1/6 of day60). During phase three, the acetate-fed methanogenic activity reduced by half (day 355, 1.5 gCOD.gVSS<sup>-1</sup>.d<sup>-1</sup>). In the opposite hydrogen, propionate and butyrate-fed activities particularly increased to 3.9 gCOD.gVSS<sup>-1</sup>.d<sup>-1</sup> (9 times of day100), 0.54 gCOD.gVSS<sup>-1</sup>.d<sup>-1</sup> (13 times) and 0.61 gCOD.gVSS<sup>-1</sup>.d<sup>-1</sup> (6 times).

The correlation between methanogenic activity and cell density of genus *Methanosaeta* (A-MPB), *Methanobacteriales* (H-MPB) and *Eubacteria* (acidogen, acetogen and many kinds of bacteria) quantified by FISH counting in each phase (day 60, 100 and 355) presents in fig. 5. Cell density of *Methanosaeta* and *Methanobacteriales* have a good correlation with methanogenic activity fed with acetate and H<sub>2</sub>/CO<sub>2</sub> respectively.

The SVI (based on VSS) was increased by deterioration of sludge settleability and settled around 32-37 ml.gVSS<sup>-1</sup> (on the day 100) during phase two and slightly decreased to 29 ml.gVSS<sup>-1</sup> in phase three. In contrast, mean diameter of retained granules decreased from 0.9 mm to 0.5 mm in phase two (fig. 5).

In phase two, the broken granules were detected (fig.6). After feeding sucrose (phase three), granular sludges rapidly reconstructed again (fig.7). At this phase thin-long filamentous acidogen proliferating at the surface of granules, that might be promoting the improvement of physico-chemical properties of retained granules.

From these results, the presences of hydrogen utilizing methanogen and acetogenic bacteria (acidogen) have effectiveness for enhancement of thermophilic granulation.

#### 4. Reference

1) Harada H., Syutsubo K., Ohashi A. *et al.*, Realization of Super High-Rate Anaerobic Wastewaters Treatment by A Novel Multi-staged Thermophilic UASB Reactor, Environmental Eng. Research, 1997, vol.34, p327-336.

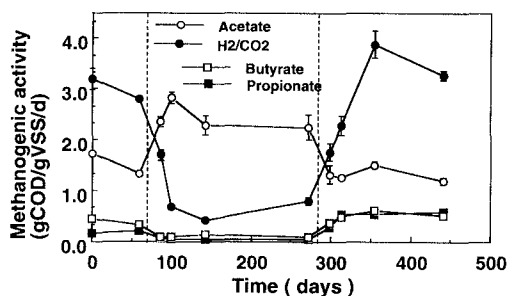


Fig. 4 Changes in methanogenic activity of retained sludge

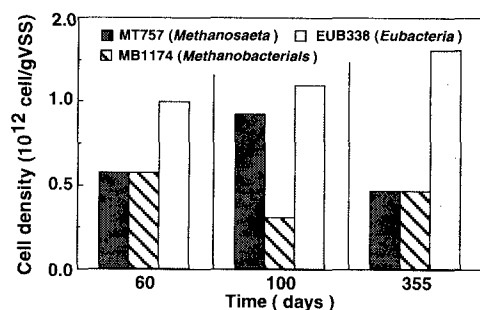


Fig. 5 Changes in cell density of *Methanosaeta*, *Methanobacteriales* and *Eubacteria* quantified by FISH.



Fig.6 SEM of retained sludge. (Phase 2 : Acetate)

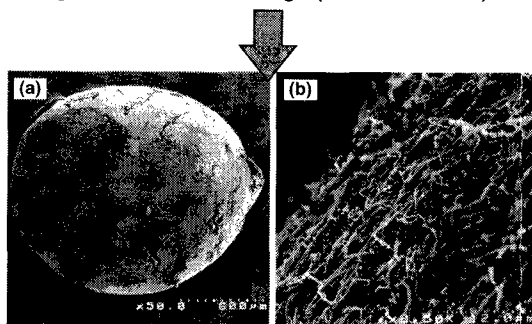


Fig.7 SEM of retained sludge. (Phase 3 : Sucrose)  
(a) Whole view, (b) Surface