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Characteristics of Granular Sludge in an EGSB Reactor for Treating low Strength Wastewater

Yoochatchaval, W.[1,4], Ohashi, A.[2], Harada, H.[3], Yamaguchi, T.[1] and Syutsubo, K.[4*]

[1]Nagaoka University of Technology, Niigata, Japan
[2]Hiroshima University, Higashihiroshima, Japan
[3]Tohoku University, Sendai, Japan
[4]National Institute for Environmental Studies, Ibaraki, Japan

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ABSTRACT: A lab-scale expanded granular sludge bed (EGSB) reactor was operated at 20°C with low strength wastewater (0.6-0.8 g COD/L) for over 200 days. Reactor was inoculated with mesophilic granular sludge. The up-flow velocity was set to 5 m/h by effluent recirculation. The COD loading was increased up to 12 kg COD/m^3/day until the day 76, resulting in hydraulic retention time of 1.5 hours. Physical properties (settleability and diameter) of retained sludge tended to deteriorate during the first 2-3 months, however sludge settleability kept sufficiently in the later part of experiment due to the reconstruction of granular sludge. The growth yield (Y_g) of retained sludge (0.13 g VSS/g COD) was about two times higher than mesophilic and thermophilic granular sludge processes while the endogenous decay constant (K_d) is very low (0.0001/day) as compared with those processes. The sludge retention time of retained sludge reduced from 100 days to 40 days by the reduction of hydraulic retention time from 4 hours to 1.5 hours. Maintenance of 40 days of sludge retention time caused the stable retainment of biomass and the significant increase of methanogenic activity of the retained sludge.

Key words: EGSB reactor, Anaerobic treatment, Granular sludge, Growth yield, Low-strength Wastewater, SRT

INTRODUCTION

Anaerobic biofilm processes, such as up-flow anaerobic sludge blanket (UASB), fluidized bed and fixed bed, are widely accepted as a proven technology for the methanogenic treatment of organic wastewater. The main advantage of this system is the high-rate treatment of wastewater, related to the good retention of anaerobic bacteria by formation of biofilm or granular sludge (Lettinga, 1995 & Speece, 1996).The UASB process, widely used for anaerobic wastewater treatment, is usually applicable for high-strength wastewater under mesophilic (30-35°C) or thermophilic (55-65°C) condition. It exhibits superior process performance due to the good maintenance of methanogenic sludge with long sludge retention time (SRT). However, most organic wastewaters are discharged with low organic concentration (less than 1 g COD/L) at ambient temperature (10-25°C) (Rebac *et al.*, 1999 & Angenent *et al.*, 2001). Thus, it is difficult to apply the UASB system to this kind of wastewater because the shortage of substrate leads to the deterioration of physical characteristics of granular sludge (Kato *et al.*, 1997, Rebac *et al.*, 1999 & Price *et al.*, 2004). Recently, the expended granular sludge bed (EGSB) system was developed. In this system, effluent recirculation was used to enhance the substrate-biofilm contact. As a result, the EGSB reactors show sufficient process performance for treatment of low strength wastewater (ethanol and volatile fatty acid-containing wastewater) with 0.7-0.9 g COD/L (Kato *et al.*, 1997; Rebac 1998).

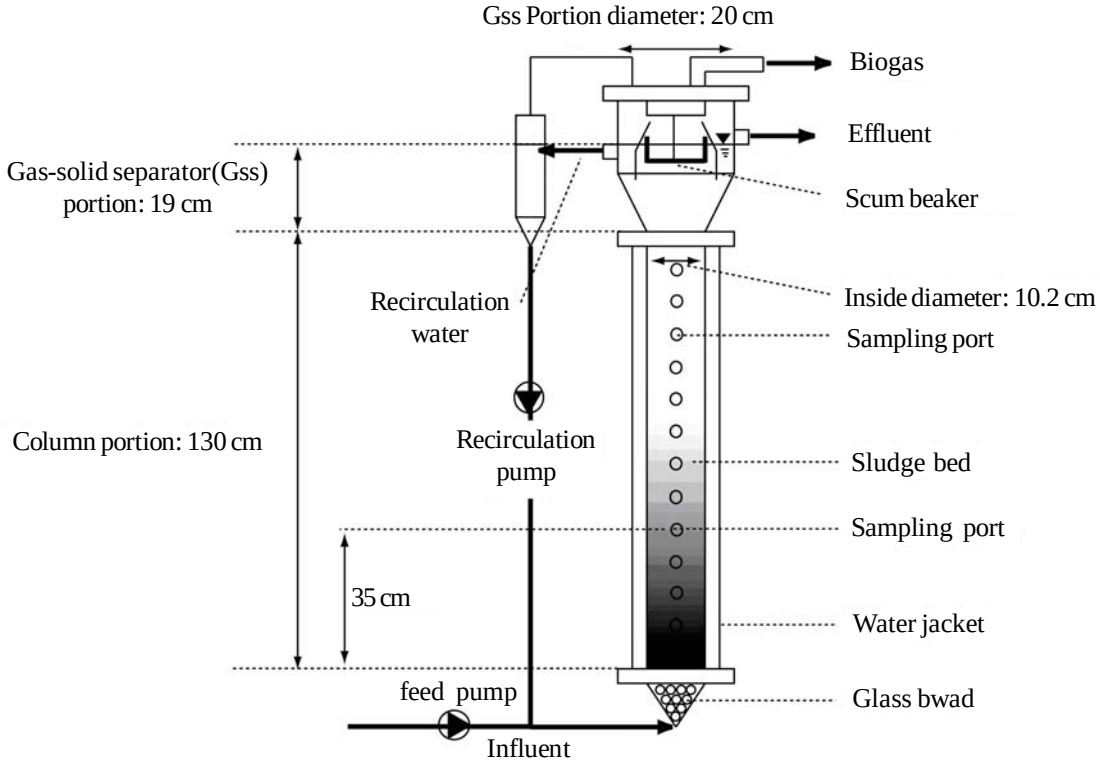
To retain a sufficient amount of methanogens in the biofilm, it is necessary to keep the SRT above

*Corresponding author E-mail: stubo@nies.go.jp

the doubling time of methanogens. This leads to the formation of granular sludge with a sufficient level of methanogenic bacteria. Usually, SRT should be maintained 2 to 3 times above the bacterial doubling time to achieve stable operation. In literature reports, sludge retention time in anaerobic bioreactors was investigated (Borja *et al.*, 1995, Syutsubo *et al.*, 1996; de la Rubia *et al.*, 2006). However, there is almost no published data with respect to SRT in the EGSB reactor system for treatment of low-strength wastewater. In order to accumulate basic knowledge on granulation in this system, we operated a lab-scale EGSB reactor fed with low-strength wastewater (0.6-0.8 g COD/L) at 20 °C, and investigated the changes in physical and microbial properties of the retained sludge. Furthermore, this is the first report of growth characteristic and retention of granular sludge in EGSB reactor for low strength wastewater treatment at 20 ºC.

MATERIALS & METHODS

A schematic diagram of an EGSB reactor is shown in (Fig. 1). The reactor consists of a column portion (11.7 L) and a gas-solid separators (GSS) portion (5.1 L). The height and inside diameter of PVC cylinder column are 130 cm and 10.2 cm. The total liquid volume of the reactor is 16.8 L including GSS. This volume was used for calculations of volumetric loading and hydraulic retention time. The EGSB reactor was operated for over 200 days under 20°C. The up-flow velocity was set to 5 m/h; effluent recirculation was set to maintain this required up-flow velocity. In previous work, it was found that inoculation of granular sludge was effective in shortening the reactor start-up period needed to maintain a sufficient SRT (Syutsubo *et al.*, 1997). Our reactor was inoculated with mesophilic granular sludge, obtained from a full-scale UASB reactor receiving sugar-containing wastewater, and started with 360 g VSS, giving a concentration of 45 g VSS/L of reactor.



Material: Polyvinyl chloride (PVC)
Shape: Culinder
Total liquid volume: 16.8 L
Temperature: 20 °c
Upflow velocity : 5 m/h

Fig. 1. Schematic diagram of an EGSB reactor

The synthetic wastewater was used as feed. Feeding of wastewater to the reactor was started immediately after inoculation with the mesophilic granular sludge. The COD concentration of this wastewater ranged from 0.6 to 0.8 g/L during reactor operation. This wastewater was composed of sucrose, acetate, propionate and yeast extract as carbon source in the COD ratio of 4.5:2.25:2.25:1. The compositions of basal minerals and trace elements were as follows (mg/L): NH_4Cl, 37; KH_2PO_4, 33; $MgCl_2 \cdot 6H_2O$, 13; $CaCl_2 \cdot 2H_2O$, 33; KCl, 10; $FeSO_4 \cdot 7H_2O$, 7; $CoCl_2 \cdot 6H_2O$, 0.17; $ZnSO_4 \cdot 7H_2O$, 0.15; H_3BO_3, 0.06; $MnCl_2 \cdot 4H_2O$, 0.42; $NiCl_2 \cdot 6H_2O$, 0.04; $CuCl_2 \cdot 2H_2O$, 0.027; $Na_2MoO_4 \cdot 2H_2O$, 0.025; $NaHCO_3$, 800 (Syutsubo *et al.*, 1997). All chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Japan). The temperature of feed was kept constant at around 20ÚC. The influent pH was ranged at 6.5 - 6.8 and the effluent pH was throughout maintained at 7.0 - 7.2. Average concentration of sulfate in the influent was 46 mg SO_4^{2-}/L, originated from tap water.

Routine analyses of effluent wastewater of the reactor and biogas were conducted 5 times per week. The influent and effluent were sampled for pH, COD, volatile fatty acid (VFA), suspended solid (SS) and sulfate analyses. Retained sludge was occasionally collected from port No.4 (35 cm height from the bottom) of the reactor for analysis of physical and microbial properties, namely sludge concentration (MLVSS), sludge volume index (SVI), granule size distribution and methanogenic activity. The COD and sulfate concentration of influent and effluent were analyzed by DR-2500 spectrophotometer (HACH Company, USA) in accordance with the manufacturer's manual.

Raw sample was used for total COD and 0.45 μm-filtered sample for dissolved COD. After sampling, effluent was homogenized for suspended solid (SS) and total COD measurement. For the measurement of effluent COD, a small amount of sulfuric acid was added to the samples followed by purging of the samples with nitrogen gas to remove sulfide. Samples for COD measurement were put in to the reagent-containing vials ($HgSO_4$, $H_2Cr_2O_7$, Ag_2SO_4 and H_2SO_4) and incubated at 150 °C for 2 hours. Then, the absorbance of reagent was measured by DR-2500 to determine the COD concentration. The COD removal efficiency was calculated by the difference between influent total COD and effluent dissolved COD. The effluent volatile fatty acids (VFA) were measured from filtrated samples and analyzed by FID (Flame Ionization Detector) gas chromatograph (GC-14A, Shimadzu, Japan), equipped with a 2.1 m × 3.2 mm (ID) glass column with Thermon 3000 (60/80 mesh). Gas production rate and gas composition were determined by wet-test gas meter and TCD (Thermal Conductivity Detector) gas chromatograph (GC-8A, Shimadzu, Japan), 2 m × 3 mm (ID) stainless-steel column with Unibeads-C (60/80 mesh).

Granule size distributions were obtained by an image analysis (Scion Image, USA) of more than 500 granules for each sample taken from port No. 4 (35 cm height from the bottom) of reactor (Syutsubo *et al.*, 1998). Sludge sample was spread to the Petri dish and then, photographed by digital camera. Granular size in both major axis and minor axis was determined by an image analysis. Then, the volume and diameter of granules were calculated. Sludge samples for scanning electron microscopic (SEM) observation was prepared according to the method described previously (Uemura and Harada, 1993). The methanogenic activities of retained sludge were determined in duplicated at day 0 (seed), 35, 104 and 254 with 122 mL serum vial bottles, according to Syutsubo in 1997 (Syutsubo *et al.*, 1997). The sludge samples for the measurement of activity were washed with 25 mM phosphate buffer to remove extra substrate and disintegrated by a homogenizer (anaerobic condition maintained by purging with nitrogen gas). The test substrates were acetate, propionate and H_2/CO_2 (80%:20%, v/v). The initial concentrations of acetate and propionate were 2 g COD/L and 1 g COD/L respectively. The vial head-space was filled with N_2 gas at 1 atm (101 kPa). For the measurement of hydro-genotrophic activity, the vial headspace was filled with H_2/CO_2 gas at 1.4 atm (142 kPa). All vials were incubated on a reciprocal-shaker (120 rpm) at four different temperatures between 10°C and 45 °C.

The microbial community structure of the retained sludge was investigated by 16S rDNA-targeted DGGE (Denaturing Gradient Gel Electrophoresis). DNA was extracted from sludge

samples by using an Isoil beads beating kit (Nippon gene, Japan). PCR (Polymerase Chain Reaction) was performed using a specific primer for amplifying either Domain Bacteria or Domain Archaea 16S rDNA (Muyzer *et al.*, 1996 and Syutsubo *et al.*, 2008). DGGE analysis was conducted using a DCode™ gel electrophoresis system (Bio-Rad, USA), on a gradient gel (35% - 55% of denaturant for Bacteria and 40% - 60% for Archaea) at 60ºC for 3.5 h. Major bands containing DNA were excised, and these nucleotide sequences were determined by a genetic analyzer (model 3100, Applied Biosystems, USA).

RESULTS & DISCUSSION

The EGSB reactor was operated over 200 days with low-strength wastewater (0.6 - 0.8 g COD/L) at 20°C. The process performance is shown in (Fig. 2). In this experiment, the COD loading increased stepwise up to 12 kg COD/m^3/ day by reducing the hydraulic retention time (HRT)

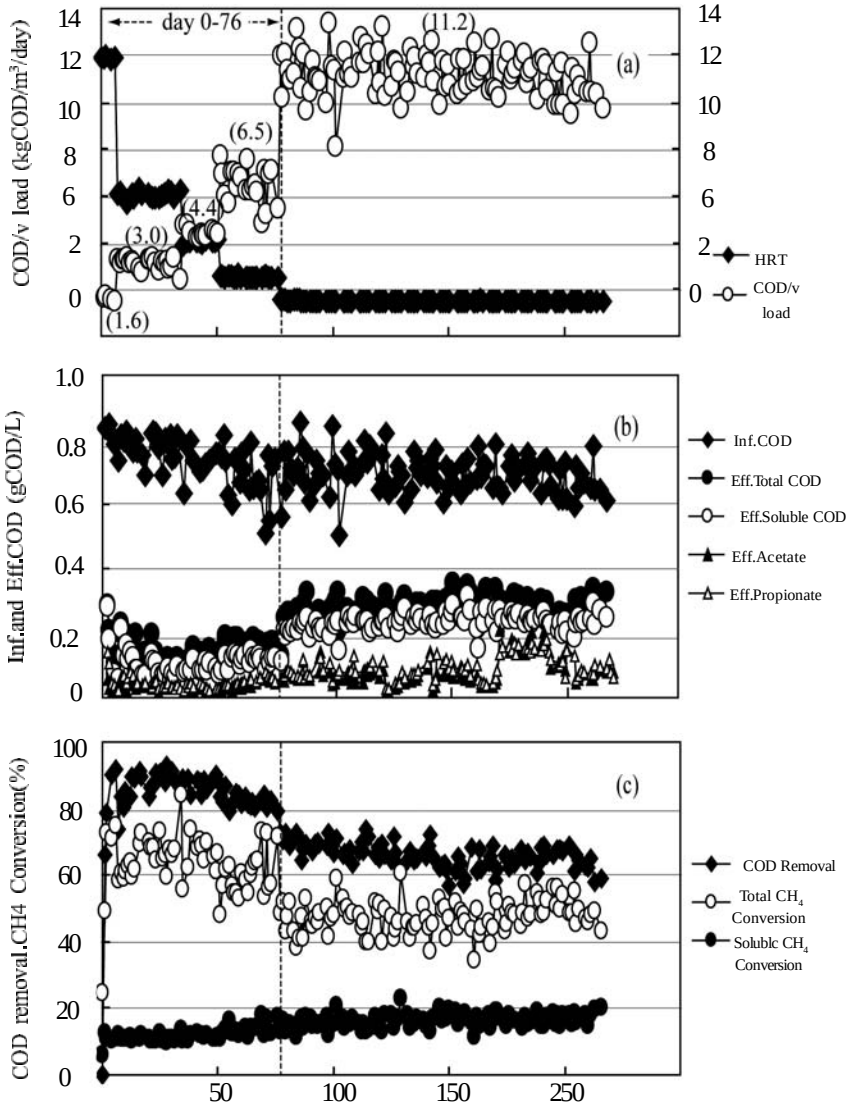


Fig. 2. Process performance of the EGSB reactor treating low-strength wastewater at 20°C. (a) HRT and COD volumetric loading; (b) Influent and effluent COD, effluent acetate and propionate; (c) COD removal, total CH_4 conversion and soluble CH_4 conversion

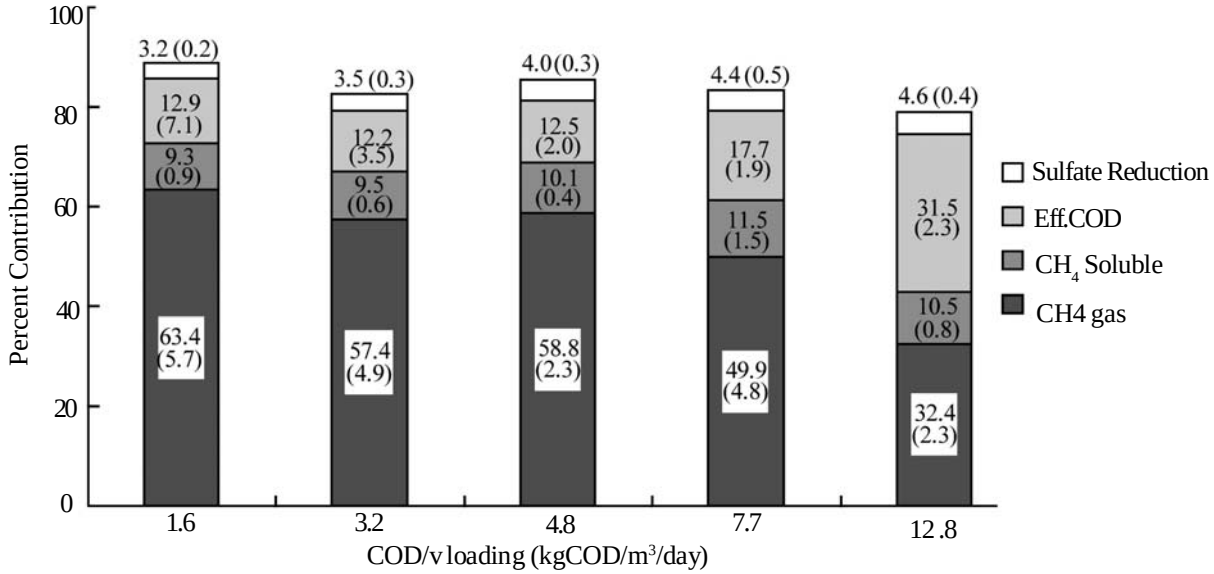


Fig. 3. COD balance, with respect to the portion of COD, for methane gas, soluble methane, effluent COD and sulfate reduction. Standard deviation is given between parentheses

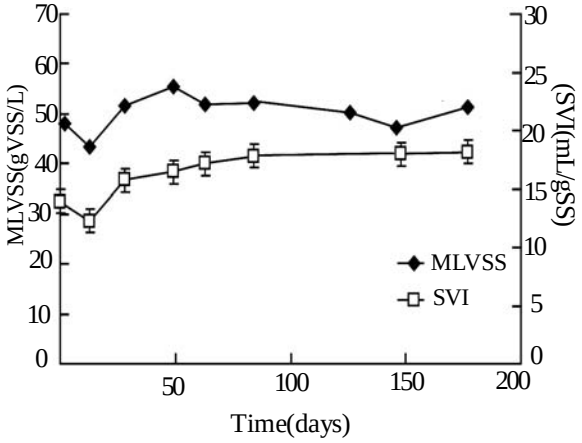


Fig. 4. Time course of MLVSS and SVI of the retained sludge

from 12 h. to 1.5 h. The COD loading reached 6.5 kg COD/m^3/day with the HRT of 2.5 h. on day 51. At this loading, the COD removal efficiency and total methane conversion based on removed COD were 82.3% and 73.2% (including 13.7% of soluble methane), respectively. On day 77, COD loading reached 12 kg COD/m^3/day by reducing the HRT to 1.5 h. The COD loading rate of the reactor was kept at this constant value over the next 140 days. As a result, average COD removal efficiency and total methane conversion were slightly reduced to 66.2±4% and 64.0±6.7% respectively.

The influent sulfate was derived from tap water. Sulfate was not detected (less than 1 mg SO_4^{2-}/L) in the effluent throughout the continuous flow experiment.

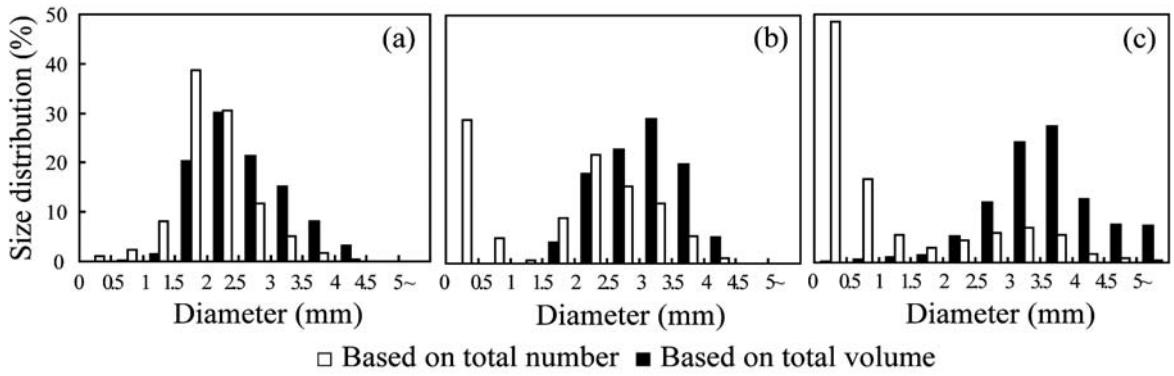


Fig. 5. Size distribution of granular sludge, expressed in percentage of the total number and the total volume of granules. (a) seed sludge (day 0); (b) day 63; (c) day 126

The contribution of sulfate reduction for COD removal was 3.2% at COD loading of 1.6 kg COD/m^3/day and was 4.6% at 12 kg COD/m^3/day, as shown in (Fig. 3).The percent contribution of methane gas production was uniform (57.4% - 63.4%) until 4.8 kg COD/m^3/day of COD loading. From then on, it decreased with increasing COD loading. At COD loading of 7.7 kg COD/m^3/day and 12.8 kg COD/m^3/day, percent contribution of methane gas declined to 49.9% and 32.4% respectively. However, the percentage of soluble methane was almost uniform at 10% for all loading-rates. This result means that the ratio between soluble methane and methane gas production increased when HRT was reduced below 4 hours.(Fig. 4). shows the behavior of sludge concentration (MLVSS) and sludge volume index (SVI) of the retained sludge. The settle-ability of retained sludge deteriorated slightly, from 12.5 mL/g SS to 17.4 mL/g SS, during the first 76 days. After that, SVI was maintained in constant at 17.3±1.2 mL/g SS in the later part of experiment. Furthermore, MLVSS concentration of retained sludge kept high, between 45 to 52 g VSS/L. The good maintenance of these key physical properties of retained sludge contributed to the stable operation of the EGSB reactor under high loading conditions.The size distribution of retained sludge is shown in (Fig.5). In this figure, size distribution is expressed in both relative number and relative volume of retained granular sludge. After the start-up period, partial disintegration of retained sludge occurred. As a result, distribution of small size granules (< 0.5 mm) based on total number was increased. However, continuous operation led to the overall growth of granular sludge. Consequently, the ratio of large size granules (larger than 2.5 mm) based on total volume was increased due to the progress of reactor operation. These results showed that the reconstruction of granular sludge occurred after 2 - 4 months of operation. We confirmed the formation of a new layer of biofilm on the surface of seed-granular sludge by scanning electron microscopic observation (Fig. 9). In order to achieve good process performance in EGSB systems, it is necessary to maintain a high concentration of methanogenic bacteria in the biofilm by maintenance of appropriate SRT. We calculated the total amount of sludge currently in the reactor to determine the SRT.

The daily change in sludge amount was defined by the balance of daily accumulation of biomass (bacterial growth) and daily washout of biomass. The amount of daily biomass washout was calculated from the flow rate (L/day) of wastewater and VSS concentration (g VSS/L) in the effluent. In order to calculate the daily growth of biomass, we determined growth characteristics (growth yield; Y_g, decay constant; K_d) of retained sludge, as described previously (Syutsubo *et al.*, 1998).

$$\left(\frac{dx}{dt}\right)_{acc,i} = -\left(\frac{ds}{dt}\right)_i Y_g - K_d \cdot X_{i-1} - \left(\frac{dx}{dt}\right)_{lost,i} \quad (1)$$

Where,

$(dx/dt)_{acc,i}$ = daily biomass accumulation rate per reactor on day (*i*); (g VSS/d)

$(ds/dt)_i$ = daily substrate consumption rate per reactor on day (*i*); (g COD/d)

$(dx/dt)_{lost,i}$ = daily biomass washout rate per reactor on day (*i*); (g VSS/d), include sampling loss

(X_{i-1}) = biomass in the reactor on day (*i-1*); (g VSS per reactor)

Y_g = growth yield coefficient of retained sludge; (g VSS/g COD)

K_d = endogenous decay constant; (per day)

The actual growth yield and endogenous decay constant of retained sludge were determined by numerical fitting between actual retained biomass (determined by measuring of sludge concentration along the reactor height) and calculated retained biomass by using assumed values of Y_g and K_d (Fig. 6). As a result of fitting (6 points), the sum total of square differences (errors) between actual value and calculated value became a minimum for Y_g and K_d of 0.13 g VSS/g COD and 0.0001/day, respectively. We found the growth yield coefficient (Y_g) was about two times those reported from mesophilic and thermophilic granular sludge processes (Pavlostathis *et al.*, 1991 & Syutsubo *et al.*, 1998). According to Rebac in 1998, the biomass yield of acidifier was very high (0.22 g VSS/g COD), while the decay rate was very low, in the two-stage EGSB reactor for treatment of sugar-containing wastewater at low temperature (8°C). These results show that the anaerobic bacteria seem to gain more energy for cell growth under less than optimum conditions.

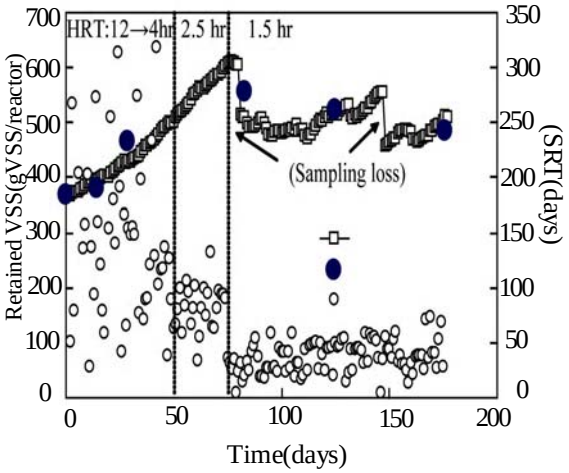


Fig. 6. Time course of total retained VSS and SRT

Figure 6 shows the changes in retained biomass (VSS) calculated from determined Y_g and K_d values. The change of SRT based on the calculated VSS is also presented in figure 6. During the first 50 days, SRT fluctuated wildly and ranged from 100 days to 400 days. Once the HRT decreased to 2.5 hours (COD loading of 6.5 kg COD/m^3/ day), SRT became more stable, ranging between 100 and 200 days. Total retained biomass increased continuously until day 76. At HRT of 1.5 hours (COD loading of 12 kg COD/m^3/day), the SRT declined further, becoming fairly constant at around 40 days.

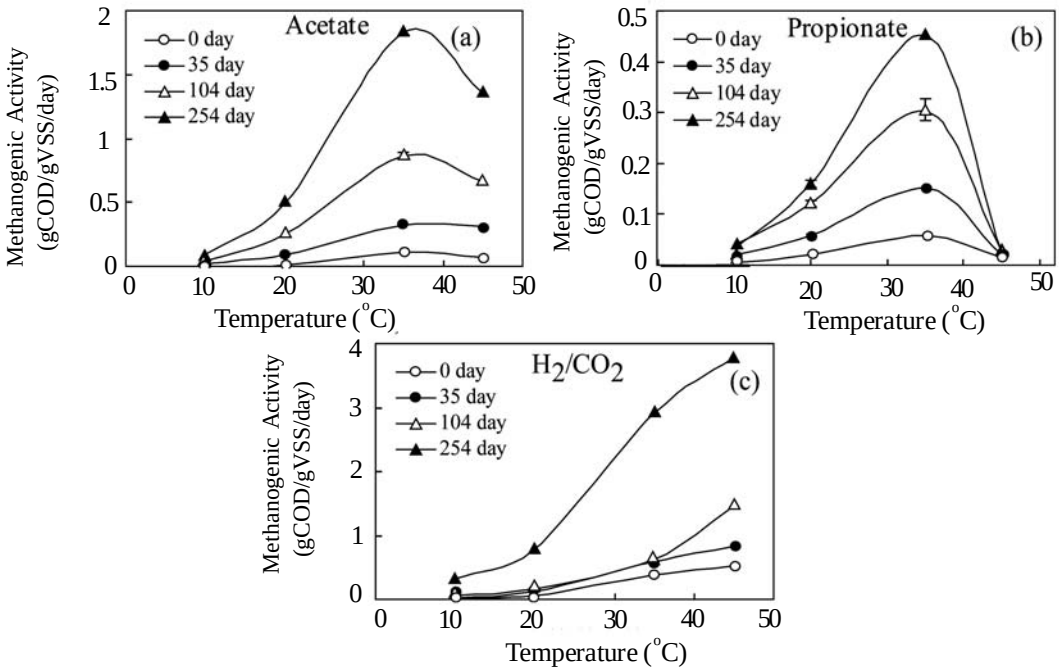


Fig. 7. Temperature dependency of methane-producing activity of the retained sludge (a) acetate; (b) propionate; (c) H_2/CO_2

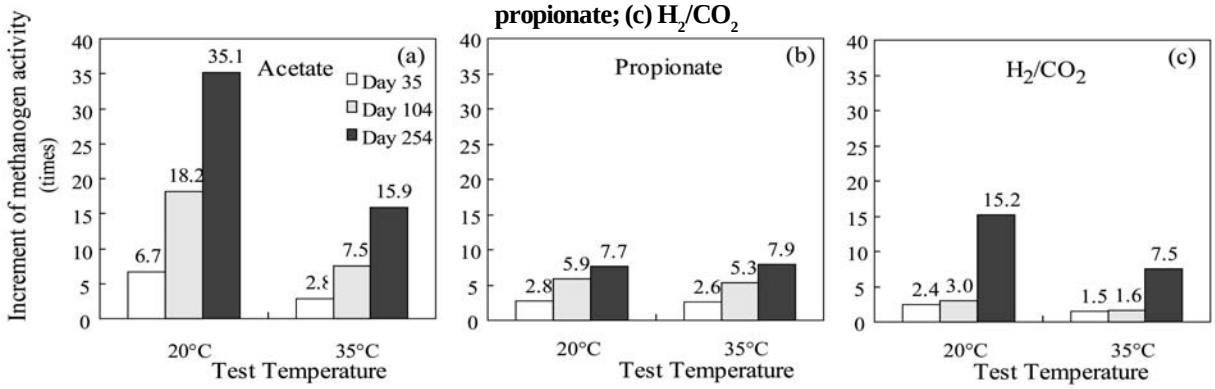


Fig. 8. The increment of methanogenic activity on day 35, 104 and 254 as compared with activity of seed sludge (day 0) at 20°C and 35°C; (a) Acetate-fed, (b) Propionate-fed, (c) H_2/CO_2-fed

When the HRT was 1.5 hours, total amount of VSS remained at a constant level (except for sampling loss). This result shows that the critical level of SRT in this EGSB system is about 40 days. After 254 days of operation, the HRT was increased to 2 hours (COD loading of 9.6 kg COD/ m^3/day) and then operated for more than 2 years with excellent process performance, with an average COD removal of 81% and SRT ranging between 60 and 80 days.

The methanogenic activities of the retained sludge were investigated at four different temperatures ranged from 10°C to 45°C. The test substrates used were acetate, propionate and H_2/ CO_2. (Fig. 7). illustrates that methanogenic activities increased as the reactor operation progressed. The reactor was operated for 254 days at 20°C; however the optimum temperature of methanogenic activities of retained sludge were unchanged. Acetate-fed activity and propionate-fed activity still showed an optimum temperature at 35C. H_2/CO_2-fed activities exhibited the highest value at 45°C. However, a sufficient level of activity increment at 20°C was observed. The 20°C activities of seed sludge (day 0) fed with acetate, hydrogen and propionate were 0.014, 0.052 and 0.021 g COD/g VSS/day, respectively.

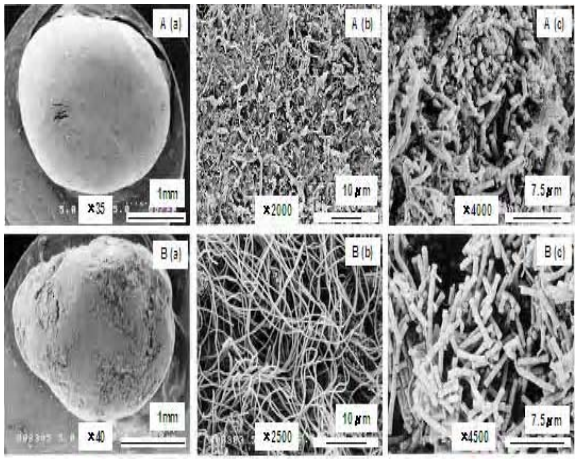


Fig. 9. Scanning electron micrographs (SEM) of retained granular sludge (day 0). A: Mesophilically grown granular sludge treating sugars used as seed. B: The granular sludge obtained from EGSB reactor on day 254. (a) Entire view of granular sludge; (b) The outer surface structure of granular sludge; (c) Interior structure of granular sludge

These activities increased to 0.50 (acetate), 0.79 (hydrogen) and 0.16 g COD/g VSS/day (propionate) by day 254. Maintenance of sufficient SRT and appropriate COD loading in the EGSB reactor might lead to a drastically increased activity at 20°C. A significant increase of methanogenic activity at 15°C-20°C was also reported in other EGSB experiments (Kettunen *et al.*, 1997, Rebac *et al.*, 1999, Connaughton *et al.*, 2006 & Scully *et al.*, 2006).Figure 8 shows the increments of methanogenic activities as compared with seed sludge-activity (day 0) at 20°C and 35°C. The increments of activity at 20°C were about 2 times higher than those at 35°C with respect to methanogenic bacteria (acetate-utilizing methanogens, H_2/CO_2-utilizing methanogens). In contrast, activity increments of propionate-degrading acetogen were almost identical between 20°C and 35°C. These results show the possibility of the proliferation of the psychrotolerant-mesophilic methanogens for 20°C incubation of granular sludge with sufficient SRT.

Microbial community structure analysis of retained sludge by 16S rDNA-targeted DGGE (Denaturing Gradient Gel Electrophoresis) shows the predominance of *Methanosaeta* as the acetate-utilizing methanogen (Syutsubo *et al.*, 2008). Mesophilic *Methanosaeta* has a doubling time of 3-4 days at 37°C (Kamagata *et al.*, 1990). Figure 7 implies that at 20°C, methanogenic activity of retained sludge dropped to 25-33% of its 35°C activity. Hence, the doubling time of methanogenic bacteria at 20°C may be prolonged to 9-16 days. This EGSB reactor exhibited stable process performance by the maintenance of SRT at around 40 days, which is about 2.5-4 times larger than the doubling time of methanogen at 20°C. Therefore, 40 days retention time of sludge is thought to be sufficient for the accumulation of the methanogenic bacteria in the granular sludge under less than optimal conditions.Morphological change of the retained granular sludge was investigated by SEM (Scanning Electron Microscopy) observation on day 254 as shown in (Fig. 9). The outer surface of granular sludge was covered with filamentous acid forming bacteria which apparently different from *Methanosaeta* (at day 254 in Figure 9 B(b)). On the other hand the proliferation of short-rods of *Methanosaeta*

was observed at the center portion of granular sludge by long time operation [Fig. 9 B(c)].

CONCLUSION

The EGSB reactor seeding with mesophilic granular sludge exhibits good process performance for the treatment of low-strength wastewater (0.6-0.8 g COD/L) at 20°C. During the operation of EGSB reactor, physical properties and SRT of retained sludge were maintained sufficiently. The 40 days SRT, that is several times longer than the doubling time of methanogenic bacteria (*Methanosaeta*), is appropriate for the maintenance of sufficient level of process performance. The good ability of sludge retainment promotes the proliferation of methanogenic bacteria in the granular sludge and the good maintenance of physical properties retained sludge. The growth yield of granular sludge developed at 20°C is apparently higher than mesophilic and thermophilic granular sludge. We conclude that seeding with granular sludge, maintenance of appropriate COD loading and SRT may be effective for the maintenance of granular sludge under less than optimal conditions.

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