Effects of low strength ultrasonication on the physico-chemical characteristics of methanogenic granules


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Abstract
The physico-chemical characteristics of methanogenic granules are one of the most important properties for the successful operation of upflow anaerobic sludge blanket, since they could directly affect the substrate and nutrient transportation into granules. In this study, the effect of low strength ultrasonication on the settling velocity, permeability, porosity, and fluid collection efficiency of the methanogenic granule were investigated, and then morphological changes were investigated using scanning electron microscopic technique. The experimental results indicate that low strength ultrasonication increased both permeability (37%) and specific surface area (230%) of the granules through the generation of grater craters and cracks on the granular surface compared to the control granule. As results, the penetration of nutrient and substrate into granules could be enhanced, and then more favorable conditions seemed to be given to the ultrasonicated granules toward to the higher anaerobic performance.

Keywords
Low strength ultrasonication; UASBr; permeability; specific surface area; SEM

INTRODUCTION
Positive effects of ultrasonication on the anaerobic digestion (AD) can be split into high and low strength based on where ultrasonication is irradiated to. High strength ultrasonication (HS-ultrasonication) can be called when ultrasonication is irradiated to feedstock as a pretreatment, while low strength ultrasonication (LS-ultrasonication) is irradiated to the reactor itself for the targeting of microorganisms involved in the AD process (Cho et al., 2012). The majority of research has predominantly focused on the HS-ultrasonication. However, relatively a little attention has been dedicated to investigate the application of LS-ultrasonication in the AD system. Elbeshbishy et al. (2011) applied ultrasonication (0.25 W/mL, 1 sec per 1 min) to hydrogen production using continuous stirred tank reactor. Enhanced hydrogen production rate (2.8 L/L/d → 5.6 L/L/d) and hydrogen yield (1.0 mol H₂/mol glucose → 1.9 mol H₂/mol glucose) were reported at 32.1 g COD/L/d of organic loading rate. In our previous research, the first report applying ultrasonication to upflow anaerobic sludge blanket reactor (UASBr) for the methane production, 43% higher methane production was achieved under continuous ultrasonication (0.05 W/mL, 1 sec per 1 min) (Cho et al., 2012).

Most explanations of enhanced biogas production after LS-ultrasonication have been only focused on the biological point of views. However, there has been no attempt to explain the enhanced biogas production after LS-ultrasonication by the changes of physico-chemical characteristics of microorganism, even though they could directly influence on substrate and nutrient transportation in anaerobic microorganisms.

In this study, firstly, the effect of LS-ultrasonication was investigated during the continuous
operation of UASBr. Then, the effect of LS-ultrasonication on the settling velocity, permeability, porosity, and fluid collection efficiency of the methanogenic granule were investigated to elucidate the correlation between enhanced biogas production and changed physico-chemical characteristics of methanogenic granule after LS-ultrasonication. In addition, scanning electron microscopy (SEM) and specific surface area of methanogenic granule were analyzed to see the effect of LS-ultrasonication.

**MATERIAL AND METHOD**

*System setup and operating conditions at UASBr.* The methanogenic granules used in this study was obtained from a full scale anaerobic plant treating brewery wastewater located in Cheongwon, Korea. 5 L of UASBr (lower part: 690 mm height x 85 mm inside diameter (i.d.); upper part: 165 mm height x 130 mm i.d.) installed with four vibrators (50 W, 20 kHz) was prepared for the tests (picture not shown). In the continuous operation of UASBr, 5 g COD/L of acidified mixture (food waste and livestock waste; V:V = 6:4) was fed at an OLR of 2.5 g COD/L/day.

*Determination of granule permeability by settling experiment.* The properties of methanogenic granules such as diameter, density, fractal dimension, permeability, and porosity were calculated through the settling experiments. In addition, specific surface area was analyzed by the Brunauer Emmett Teller (BET) surface area analyzer (Sorptomatic 1990 Surface Area Analyzer, Thermo Fisher Scientific Inc., Waltham, MA, USA). The surface morphology of granule was characterized using an environmental scanning electron microscope (LEO 1455 VP-SEM, Leo electron microscopy Ltd, UK) without any pretreatment.

**RESULTS AND DISCUSSION**

*Positive effects of LS-ultrasonication on the AD performance*

Compared to the control (un-ultrasonicated UASBr), COD removal rate was enhanced from 72% to 85% after irradiation. And daily CH$_4$ production and specific CH$_4$ production rate were also enhanced by 43% from 2,254 to 3,219 mL/day and from 180 to 258 CH$_4$ mL/g COD$_{added}$, respectively (Cho et al., 2012). In the previous research, explanations of enhanced AD performance after LS-ultrasonication were only focused on the changes of biological characteristics of methanogenic granule by measuring the dehydrogenase activity and ATP content. However, the changes of physico-chemical characteristics of methanogenic granule after LS-ultrasonication should be also considered since they are highly related to the transportation of substrate and nutrient.

*Physico-chemical properties of methanogenic granule*

*Settling experiments.* As shown in Fig. 1, the size of granules was varied from 1.5 to 6 mm and a dry mass of granules was ranged up to 4 mg for both granules. No significant difference was observed after LS-ultrasonication. However, slightly lower density was observed from the ultrasonicated granule (1.055 g/cm$^3$) than the control granule (1.075 g/cm$^3$). Interesting physical features of methanogenic granules (similar size and dry mass but the lower density) was observed compared to the control granules, suggesting that the structure of ultrasonicated granules was somehow expanded or loosened than that of the control granule since density is defined as mass per volume.

The fractal dimension is an index for describing the degree of aggregate compactness and how the particles are packed. Lower fractal dimension of the ultrasonicated granules (3.89) than the control granules (4.31) clearly support the aforementioned suggestion since a lower fractal dimension value implies a looser and more porous aggregate.

The settling velocities of the control and ultrasonicated granules were varied from 1.46 to 3.26 cm/s with an average of 2.44±0.43 cm/s and from 1.76 to 3.60 cm/s with an average of 2.62±0.51 cm/s,
respectively. Slightly higher settling velocity was observed from the ultrasonicated granules than that of the control granules, it will be discussed with other settling parameters. In addition, settling velocity was increased with increase in granule diameter for both granules as shown in Fig. 2(a) and 2(b). Values from this study were much bigger than that of activated sludge floc (0.17-0.42 cm/s) and hydrogen producing granule (up to 2.32 cm/s) due to their larger size and higher density.

Figure 1. Settling experimental results: (a) dry mass of granule, (b) fractal dimension of control granule and (c) ultrasonicated granule.

Figure 2. Observed and predicted settling velocities of (a) the control granule and (b) ultrasonicated granule; (c) ratio of the observed and predicted settling velocities of the granule; (d) fluid collection efficiencies of the granule; (e) permeabilities of the granule.

The fluid collection efficiency of the control and ultrasonicated granule were in a range of 0.02-0.48 with an average value of 0.15±0.14 and 0.02-0.47 with an average value of 0.16±0.11, respectively as shown in Fig. 2(d). They were decreased with the increase in the diameter possibly due to the pore clogging by higher EPS excretion from the bigger size of methanogenic granules.

Settling behavior of bio-aggregates is highly relies on the drag force and permeability through their interior. The characteristic behaviors of the ultrasonicated granules (slightly lower densities and fractal dimensions but slightly higher settling velocities and fluid collection efficiencies) compared to the control granules implied the increase of methanogenic granule’s permeability after LS-ultrasonication. The permeabilities of both granules were ranged up to 0.0025 cm², average values of the control and ultrasonicated granules were 0.0008±0.0006 cm² and 0.0011±0.0006 cm², respectively as shown in Fig. 2(e). 37% higher permeability of methanogenic granule was observed.
after LS-ultrasonication. In short summary, LS-ultrasonication increased the permeability of methanogenic granule. As results, the penetration of nutrient and substrate into granules was enhanced, then more favorable conditions seemed to be given to the methanogenic granules toward to the higher CH$_4$ production.

**Scanning electron microscope and specific surface area.** Multiple cracks, the paths of the produced biogas from the core to the surface of the methanogenic granule, were observed as shown in Fig. 3, however much bigger and deeper cracks were observed from the ultrasonicated granules. In addition, many additional craters were only observed from the ultrasonicated granules. It seemed that propagated ultrasonic wave generated the many craters on the surface of methanogenic granules, then the greater amount of produced biogas resulted in the much bigger and deeper cracks than the control granules. As results of more rough and uneven surface, 2.3 times higher specific surface area was observed from the ultrasonicated granules ($0.5337 \pm 0.0036$ m$^2$/g TS) than the control granules ($0.624 \pm 0.0025$ m$^2$/g TS) which were in a range of previously reported value ($0.02-0.074$ m$^2$/g TS). Higher specific surface area, implying the increase of the contact frequency between substrate and microorganism, seems to be more favorable conditions for the microbial reactions.

![Figure 3. SEM images of the anaerobic granule: (a) control granule, (b) magnified control granule (x 150), (c) magnified control granule (x 300), (d) ultrasonicated granule, (e) magnified ultrasonicated granule (x 150), (f) magnified ultrasonicated granule (x 300).](image)

**CONCLUSION**

The physico-chemical changes of ultrasonicated granules such as increased permeability (37%) and increased specific surface area (230%) seem to be very supportive of the fact that LS-ultrasonication positively affects the methanogenic granules toward higher AD performance.

**REFERENCES**
