ULSAN NATIONAL INSTITUTE OF
SCIENCE AND TECHNOLOGY

Master's Thesis

Quick freezing of sewage sludge using CO_2 gas hydrates-dissociation energy and its application to cell lysis and dewatering

Woojeong Kim

Department of Urban and Environmental Engineering
(Environmental Science and Engineering)

Graduate School of UNIST

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Kwon
Advisor
Young-Nam Kwon

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Woojeong Kim

This certifies that the thesis/dissertation of Woojeong Kim is approved.

1/08/2018 of submission

signature

Kwon

Advisor: Young-Nam Kwon

signature

Changsoo Lee: Thesis Committee Member #1

signature

Yongwon Seo

Yongwon Seo: Thesis Committee Member #2

Abstract

Sludge from wastewater treatment induces the increase of handling capacity and biological pollution due to its high water and microorganism content. This study investigated the effect of quick freezing method induced by gas hydrates-dissociation energy (QFGD) on microbial rupture and related dewatering effect of sludge. QFGD is the quick-freezing method induced dissociation energy of gas hydrate. Temperature, pressure, and guest molecule composition were analyzed for finding out the mechanism. Organic materials concentration, particle size distribution, Capillary suction time, filtering test, and coagulation-settling test were tested on dissociation of sludge and its related dewatering effect. The disinfection effect was examined by Confocal laser scanning microscopy with the Live/dead *Baclight*™ bacterial staining kit. As results, QFGD makes cell rupture in sludge and its related dewatering effect. At 0.2 ℃, CO_2 gas hydrate was formed in sludge sample with over 20 bar pressure. When CO_2 gas hydrates was formed, Temperature in the system increased significantly and when gas hydrate was dissociated, the temperature decreased significantly with sludge frozen. Concentration of soluble organic materials from sludge sample treated QFGD increased significantly than from control. Average particle size from sludge sample treated QFGD decreased than from control. Conductivity and osmotic pressure of supernatant from sludge sample treated QFGD increased significantly than from control. Although these results show sludge dissociation, CST and TTF increased after QFGD than control. This was because fine particles from sludge block the filter pore and more compact sludge cake was formed by filtering. Particle size distribution shifted toward left after QFGD than control. Cake resistance was the major reason to form total resistance. However, as sludge particles were disintegrated into fine particles, the settled volume was reduced and the organic concentration in supernatant increased through QFGD. As treatment loading could be reduced and the supernatant could be used as organic source for growth, QFGD treatment made sludge easy to be treated. Last, live cell ratio decreased after QFGD than control. Therefore, with sludge dewatering effect and disinfection effect, QFGD could be considered as a new dewatering method with disinfection.

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1 Introduction

1.1 Research background

1.1.1 The importance of wastewater treatment

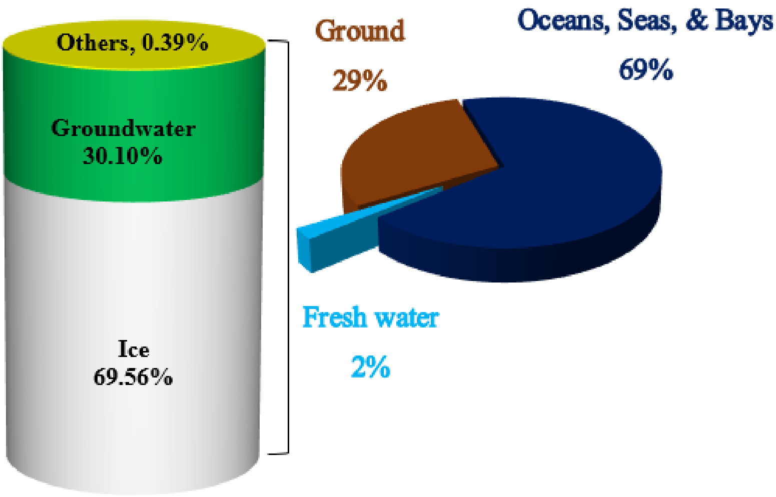


Figure 1. World water distribution (Source: Igor Shiklomanov's chapter "World fresh water resources" in Peter H. Gleick (editor), 1993, Water in Crisis: A Guide to the World's Fresh Water Resources (Oxford University Press, New York).)

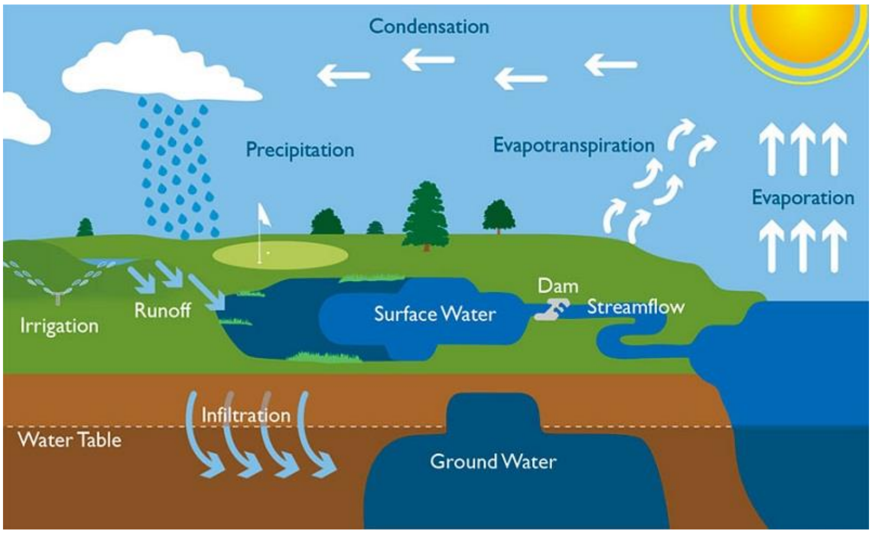


Figure 2. Circulation of water in nature[1]

Water could have been a limited but naturally purified resource. About 71 % of earth surface is covered with water and about 96.5 % of total water is seawater. As seawater is highly concentrated aquatic solution with several kinds of salts and microorganism, Seawater is not proper to be used by life with relatively low concentration. Fresh water, 3.46 % of total water can be used by life with relatively low concentration. As 65.56 % of fresh water is a form of ice, only 30.10 % of fresh water, 0.6 % of total water can be consumed by most life.[7] the reason why life could live for long time with only 0.6 % of total water is the circulation of water with natural purification like Figure. 2. When wastewater is released into nature, wastewater passes soil with screening effect and is distillated into vapor without pollutant. Then purified water is collected or precipitated into water pond and life can consume again. Therefore, with the water circulation, life can obtain pure water.

However, with industrialization and explosive population growth, as wastewater becomes more polluted and the amount of wastewater released becomes more and more large, natural purification is not sufficient to fulfil the qualitative and quantitative demand of water. In 2015, Shanghai, one of cities in China, found that 85 % of water in its rivers is not sufficient to drink and 56.4 % of water is not sufficient to be used to any purpose.[8] So, to solve this problem, wastewater treatment is developed. Wastewater treatment is the treatment which, for prevent wastewater from polluting environment, wastewater is treated by several processes for removing pollutants and then purified water changed from wastewater can be released into environment. Wastewater treatment is composed of pretreatment, primary treatment, secondary treatment, and pollutant treatment facility. And it also can be composed of physical, chemical, and biological treatment by treatment method. When wastewater is released into wastewater treatment plant, during passing to primary clarifier, big substances like sand, plastics are removed from wastewater by screening. In primary clarifier, settleable solid particles are settled then the supernatant sends to aeration tank. In the aeration tank, aerobic microbes consume oxygen and organic materials in the supernatant. When aerobic microbes grow enough, the supernatant sends to final settlement basin. In the final settlement basin, after the microbes which grow in aeration tank becomes settled, concentrated part and supernatant part are divided. The supernatant is pass through several filtrations and then released. Purified water produced from wastewater treatment can be released into environment or treated more for proper water quality to several usages. Through these overall processes, life can obtain water with proper quality. The concentrated pollutant from overall processes is sent to thickener. In the thickener, the pollutant is concentrated. Concentrated solid from the thickener is sent to digester. In digester, anaerobic microbes digest the concentrated solid anaerobically. After the product from the digester becomes dehydrated in dewatering part, the dried solid is wasted.

1.1.2 Sludge

Figure 3. Steps for London protocol application in South Korea.[2]

Sludge is produced from wastewater treatment and occurs several problems. Sludge is the highly concentrated organic, inorganic, and microbial mixture separated from wastewater in wastewater treatment. Released amount of sludge becomes more and more increased. In South Korea, wastewater treatment plants are released 32 % more than amount of sludge in 2013(10946 tons/day) than in 2008(7446 tons/day).[3] Sludge is treated by ocean dumping, landfill, and incineration. South Korea had treated large part of sludge as ocean dumping. This was because landfill needs large land and incineration needs high cost. In 2010, 15.2 % of sludge is treated by landfill, 15.8 % is treated by incineration, 27.4 % by recycling like fertilization, and 41.6 % by ocean dumping. However, as in 2009, South Korea joined the 1996 protocol, the revised version of London Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (1975), ocean dumping is banned gradationally. By the protocol, treated amount of sludge by incineration and landfill becomes more and more large. However, the more amount of sludge is treated, the wider land is needed for landfill. As toxic leachate is concentrated through wastewater treatment and it can be released into soil, landfill can cause environmental pollution. And, as sludge has high water content, about 99.5 %, incineration has lower energy efficiency. As 100 ℃, 1 mole of water needs 40.67 kJ/mole of energy to evaporate, this high water heat capacity makes sludge treatment cost increase. Therefore, new sludge treatment method is needed to solve the concentrated pollutant and high water content. Many research has been studied for improving sludge treatment. Figure. 4 shows the sludge treatments developed.

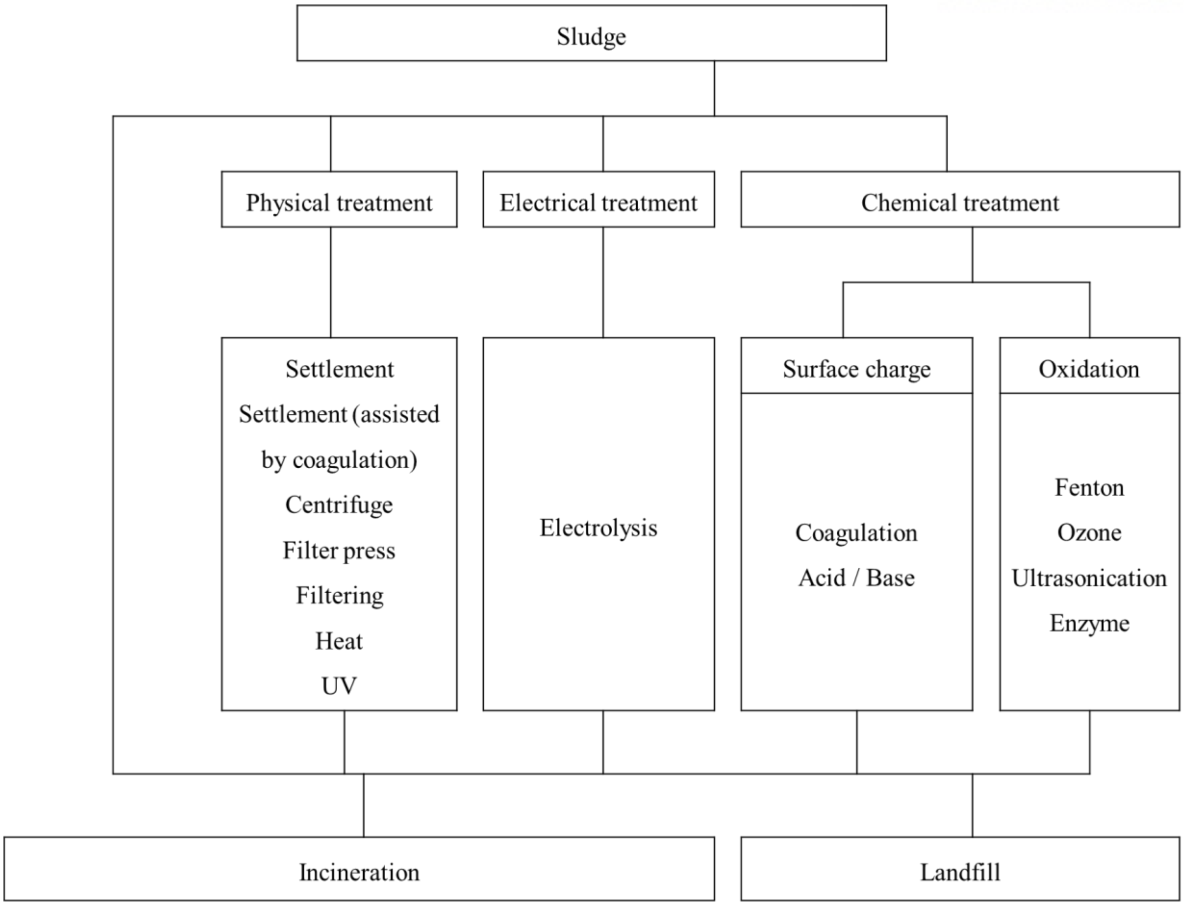


Figure 4. Several sludge treatments developed.

Landfill and incineration are the basic sludge treatment. Landfill is the method which concentrated sludge after wastewater treatment is just buried. This method is the simplest and easiest sludge treatment. Incineration is the method which the concentrated sludge is heated and the produced energy from high organic concentration can be used. However, these two methods have severe problems. As sludge becomes concentrated, toxic materials becomes more and more concentrated. This concentrated toxic material can be released to soil and cause environmental pollution. And, as sludge has high water content and water has high heat capacity, low energy efficiency is the main obstacle for incineration to treat sludge. To solve these problems, many research about sludge pretreatment has been studied for improving the treatment efficiency. Sludge pretreatment can be divided into 3 big categories, physical, electrical, and chemical pretreatment. Physical sludge pretreatment is the method to dewater sludge by physical force and includes Settling, coagulation-assisted settlement, centrifuge, filter press, filtering, heat and ultraviolet (UV) treatment. Settling, coagulation-assisted settlement, centrifuge, filter press and filtering can reduce water content of sludge but as they can only remove free water, they can reduce to the limited water content. UV and heat treatment needs large amount of energy. Electrical pretreatment is the sludge dewatering method to disintegrate sludge particles by electrical oxidation.

Electrolysis, one of the electrical sludge pretreatments, is the method, which when electricity is charged to two electrolytes in sludge, oxidation is occurred, and then this oxidation makes sludge disintegrated and dewatered. However, as Electrolysis needs high energy for producing electricity, it needs low energy efficiency. Chemical sludge pretreatment can be divided into 2 small categories by disintegration method, based on surface charge and based on chemical oxidation. Chemical sludge pretreatment based on sludge surface charge is the method to utilize the negatively charged sludge surface and dewater sludge. Coagulation, acid or base treatment are its examples. Coagulation is the method which cationic ion have connected with negatively charged sludge surface and sludge can coagulate, following with dewatering. Acid treatment makes proton neutralize the negatively charged sludge surface. Neutralized sludge surface can make sludge coagulation and dewatering. However, the remaining chemicals and changed pH in sludge can cause environmental pollution when they are released without treatment. So, the treatment cost become increased. Chemical treatment based on chemical oxidation is the method for chemically made radical to attack and dewater sludge. Fenton oxidation, Ultrasonication and ozone treatment can be chemical treatment for sludge dewatering by oxidation. In Fenton oxidation, Fe^{2+} and H_2O_2 are reacted and form ·OH radical. This ·OH radical oxidizes organic materials in sludge and then sludge dewaterability become increased. Ultrasonication treatment is the method which radical made by cavitation attacks organic materials in sludge. Ozone treatment is the method for ozone, one of the highly oxidizing agents to oxidize organic materials in sludge. Enzymatic treatment uses an enzyme to oxidize organic materials in sludge and help sludge dewatered. However, as oxidization occurs in acidic condition, acidic sludge needs post treatment for neutralizing sludge pH.

Freezing method can physically dewater sludge. At low temperature, sludge becomes frozen and inner and outer water are changed into ice. The formed ice helps sludge dewatered by the following mechanisms.

1. Express : As temperature decreases, water out of sludge changes into ice. As ice structure is composed of only water molecules, ice excludes and pressurize sludge to dewater. Excluded liquid is changed into ice. Therefore, sludge can be dewatered.
2. Elute : When ice is formed out of sludge, highly concentrated layer is formed on the surface of sludge. Due to osmotic pressure, water in sludge can be released. Therefore, sludge can be dewatered.
3. Cell rupture : When sludge is cooled and temperature in cell decreases to freezing temperature, ice is formed inside of microbial in sludge. This makes inner volume of sludge increase. When inner volume of sludge increases to fatal volume, cell membrane cannot endure, and cell ruptured. As the result, as liquid in microbial is released, sludge can be dewatered.

Freezing can be applied in cold region just by storing in cold temperature. In other region, cooling is the main obstacle to make treatment cost more expensive. Therefore, it is important to develop the method which improve dewatering and energy efficiency.

1.1.3 Gas hydrates

In high pressure and low temperature condition, gas hydrate is a crystalline structure formed by water molecules which connected by hydrogen bond around guest molecules. Gas hydrate can be divided three structures, sI, sII, and sH structure. As CH_4 gas hydrates in nature has twice more than total fossil fuel resource, gas hydrates can be considered as an effective energy resource. However, in gas transportation pipe, gas hydrates can be formed by moisture and gas and cause the pipe cracked. This phenomenon can cause economical loss. To solve this problem, gas hydrates has been investigated on inhibition of gas hydrates formation and as gas hydrates has similar crystalline structure with ice and gas hydrates can be formed more energy effectively, gas hydrates can be the alternative of freezing treatment. Gas hydrates treatment can be applied into concentration and desalination process and promotion of gas hydrate formation can help increasing treatment efficiency. Therefore, promotion and inhibition of gas hydrates has been investigated.

1.1.4 Quick freezing induced by Gas hydrates-dissociation energy

Quick freezing induced by Gas hydrates-dissociation energy (QFGD) is a recently developed quick freezing method. When gas hydrate is formed, exothermic process is occurred by releasing energy from molecules. And, when gas hydrate is dissociated, endothermic process is occurred by absorbing energy from system. QFGD method freezes sample by a driving force of endothermic process. One of the conventional gas hydrate treatment as the alternative freezing treatment is that the concentrate and ice must be separated in high pressure condition. As gas hydrates structure maintains in gas hydrates stabilization condition, the separated solid and liquid part can be mixed when gas hydrate becomes separated and spontaneously dissociated. This separation process needs high energy consumption. Han, S. W. et al. (2016)[9] solved this problem by developing QFGD. In 0.2 ℃ temperature and over 25 bar pressure Carbon dioxide (CO_2), CO_2 gas hydrates can be formed in seawater. And, seawater can be frozen by gas hydrate dissociation. The removal efficiency of cation was about 66.9 %. This removal could be obtained by spontaneous quick freezing after gas hydrate dissociation. Therefore, quick freezing induced by gas hydrate dissociation can substitutes conventional quick-freezing method.

In this study, it is investigated the effect of QFGD on cell rupture and dewatering effect in sludge. In low temperature, sludge is pressurized by high pressure of guest molecules. And then, gas hydrates can be formed and after gas hydrates becomes stabilized, gas hydrate is dissociated by shifting the condition. When gas hydrate is dissociated, sludge becomes frozen spontaneously. By this process, sludge can be frozen like the conventional quick-freezing process. But, although sludge has to be cooled indirectly, it is solidified by CO_2 directly. Therefore, energy efficiency can be improved more than the conventional freezing method. As the greenhouse effect of CO_2 is less than CH_4, CO_2 gas hydrate is less harmful to environment than CH_4 gas hydrate. Also, chemicals are not applied different with other chemical treatments and QFGD does not need post-treatment. Therefore, QFGD can be the new quick-freezing method to dewater economically and eco-friendly.

Gas hydrates treatment	**Quick freezing caused by gas hydrates-dissociation energy**
Gas hydrates has to be formed fully (100 % of sample).	Gas hydrates does not need to form fully (100 % of sample) if the endothermic energy is sufficient to freeze sample.
Gas hydrates form only the place where guest molecule can be contact with water.	Even though gas hydrates are not formed in some place where guest molecule cannot be contact with water, endothermic effect can have some influence on much wider area and temperature in the area decreases. Therefore, much more sample can be treated by quick freezing than gas hydrates treatment
To keep the structure, high pressure has to be maintained for full time of reaction	To keep the structure, high pressure has to be maintained for only part of reaction time.

Table 1. Comparison of gas hydrates treatment and quick freezing caused by gas hydrates-dissociation energy treatment

Table 1 shows the differences between gas hydrates treatment and quick freezing caused by gas hydrates-dissociation energy. As gas hydrates treatment makes salt exclusion induced by gas hydrates formation, gas hydrates has to be formed fully. As quick freezing caused by gas hydrates-dissociation energy uses endothermic process induced by gas hydrates dissociation and freezes sample, 100 % formation of gas hydrates is not essential and partial gas hydrate formation is enough to freeze sample if the endothermic energy is enough to freeze all the sample. As gas hydrates can be formed only in the place where guest molecules can be contact with water, gas hydrates treatment is efficient for limited condition. However, as QFGD treatment is induced by the endothermic energy and have an influence on wider area, it is efficient for relatively unlimited condition. At last, although gas hydrates treatment has to keep the sample in high pressure for maintaining its structure, QFGD treatment can maintain the structure for some time after pressure decrease. Therefore, QFGD treatment is more energy efficient method than gas hydrates treatment.

1.2 The objective of the study

In this study, it is investigated the effect of QFGD on microbial rupture and dewatering effect of sludge. QFGD is reacted with sludge in low temperature and CO_2 in high pressure. It is studied to analyze the organic concentration, particle size distribution, and rheology for changing sludge properties by QFGD and to measure CST, filtering test, and settlement test for comparing sludge dewatering effect. Particle size distribution and resistance test were studied for finding out the effect of sludge on filtering test. And, the fluorescence test was experimented for the disinfection effect of QFGD.

2 The related research

2.1 Sludge treatment

2.1.1 Introduction

With industrialization and increasing population, the amount of sludge produced from wastewater treatment increases more and more. The cost for sludge treatment also increases more and more. In sludge, high water content causes the increase of sludge volume, high heat capacity, and highly concentrated toxic materials. Therefore, many researchers have studied many sludge treatments to improve dewatering effect.

2.1.2 Water distribution in sludge

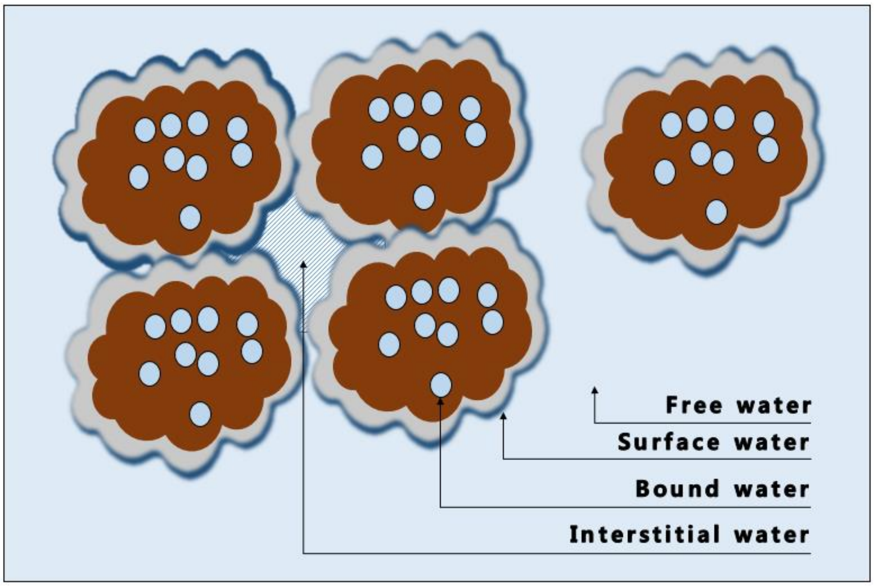


Figure 5. Water distribution in sludge

To understand the sludge dewatering, it is essential to find out water distribution in sludge. Sludge is composed of organic pockets containing liquid like Figure 5. Liquid in sludge can be divided into 4 parts. Free water is the water which has a little interaction with solid part. Interstitial water is the water which is trapped between sludge flocs. Surface water is the water which bound on the surface of sludge flocs. And, Bound water is the water which positioned inside of sludge flocs.[10-13] Typically, water in sludge distributes 70 % as a free water, 20 % as an interstitial water, 7 % as an adsorbed water, and 3 % as a bound water.[14] as free water has less interaction with solid part, it can be separated easily.

To improve the dewatering effect, liquid in organic pockets has to be released. However, as interstitial water is trapped between sludge flocs, it is essential to disintegrate sludge flocs for dewatering. Surface water is difficult to be separated from sludge flocs due to its strong interaction. Bound water is most difficult to be separated because it is packed by organic pockets. Therefore, considering the water distribution in sludge, sludge treatment has to be developed.

2.1.3 Sludge treatment

2.1.3.1 Physical treatment

A. Settlement

Settlement separates solid and liquid parts by different settling rate of particles in sludge. As this method separates solid and liquid parts gravitationally, it does not need the additional energy. In plants, this method has been applied to concentrate sludge sample and reduce the amount of sludge. However, as water is trapped, packed, and bound to solid, this method has limitation for sludge dewatering. And, as sludge has to be stored for certain time, the treatment needs large space and its efficiency is not high.

B. Filter press, Centrifuge, and filtering

In plants, sludge needs to be more dewatered for increasing dewatering efficiency. Mechanical dewatering was developed. This method uses mechanical force to pressurize solid part and dewater. Filter press, centrifuge, and filtering are the examples of mechanical dewatering. Filter press is the method that sludge is pressurized by rolling two plates. Centrifuge is the methods to separate solid and liquid part by centrifugal force. Filtering separates solid and liquid parts by particle size difference. Mechanical dewatering method can improve the dewatering effect with chemical pretreatment. G. Mininini et al. (1983)[15] compared the effect of centrifuge and filter press on dewatering effect in sludge from which phosphorus is removed by ferrous sulphate or aluminium sulphate. Before dewatering process, sludge was treated by mixing ferrous sulphate or aluminium sulphate. In centrifuge method, after sludge was mixed with polyelectrolytes (polyacrylamide 100% cationic), a screw type centrifuge machine was used to dewater sludge. In a filter press method, after sludge was mixed with aluminium chlorohydrate, a pilot filter press was applied to dewatering. As the result, when phosphorus is removed by ferrous sulphate, sludge was dewatered to 17.7 % of solid concentration by centrifuge and 6.5 % by filter press. And, when phosphorus is removed by aluminium sulphate, sludge was dewatered to 28.5 % of solid concentration by centrifuge and 23.7 % by filter press. However, as mechanical dewatering needs machine, the large capital base and maintenance expenditure are essential. And, as mechanical dewatering method can only remove free water, it has the limited water removal efficiency.[16]

C. Heat treatment

L. Wang et al. (2017)[17] investigated the effect of heat treatment on sludge dewaterability. Heat treatment makes sludge structure changed and sludge dewaterability improved. When sludge is heated, the negatively charged surface of sludge particles become positive and hydrophilicity was reduced. This modification makes the interaction between water and sludge flocs weakened and the interaction between sludge flocs stronger. Therefore, surface water can be released to interstitial water or free water. In this paper, when sludge was heated to over 180 ℃, pore structure and morphology was changed from rough to smooth over 120~150 ℃ and the interaction between water and sludge flocs became weakened and bind energy decreased over 180 ℃. And, free water content became increased due to release of surface water. Therefore, it is reasonable that the heat treatment over 180 ℃ is effective to dewatering.

Freezing and thawing treatment is the method, which sludge freezes and maintain at the freezing temperature for certain time. Wang et al. (2001)[18] studied the effect of freezing process of freezing and thawing treatment on sludge dewaterability. As freezing rate decreases, sludge could be filtered faster and its settling rate became faster. Though freezing and thawing method including slow freezing, the number of viable bacteria decreased to about 91 %. Therefore, by decreasing freezing rate, freezing and thawing treatment can improve sludge dewaterability and disinfection effect. Hu, K.et al. (2011)[19] investigated the effect of thawing process in freezing and thawing treatment on sludge treatment. Sludge dewaterability was determined by freezing process. In thawing process, as ice grows in the frozen sludge, organic materials were additionally released. As high soluble organic materials could be a source for anaerobic digester, it was found that the freezing and thawing treatment makes sludge easier to digest anaerobically. H. Saveyn et al. (2009)[20] found out the effect of freezing and thawing treatment on sludge dewaterability. After sludge was pretreated to about 70 % of water content by centrifuge, the dewatered sludge pellet was frozen slowly and maintain at the freezing temperature for long time. As the result, the water content decreased to about 50 % by the freezing and thawing treatment. However, as this treatment needs to cooling process, this process has low energy efficiency due to high water content.

2.1.3.2 Electrical treatment

Electrolysis is the method, which after radical is formed electrically, the radical attacks organic materials in sludge and increases sludge dewaterability. Yuan, H.-p., et al. (2011)[21] found out that after electrolysis treatment in the condition of 15~20 V electricity and 15~20 minutes, sludge particles were disintegrated and sludge dewaterability such as CST and SRF increased. Also, through scanning electronic microscopy (SEM) analysis, the electrolysis treatment could cause cell lysis. But, as treatment becomes stronger, sludge dewaterability seems to be decreased because organic materials from sludge makes sludge viscosity increase. However, as electrical sludge treatment uses electricity, it is dangerous and low energy efficiency.

2.1.3.3 Chemical sludge treatment

A. Surface charge

a. Coagulation

Sludge is the complex materials mixed with organic, inorganic, and microbial. On the surface of sludge, anionic functional groups like carboxylic group and phosphate are positioned and sludge becomes negatively charged.[22] Coagulation is the method to interact cation with negatively charged sludge flocs, and accumulate aggregation. Kakii et al. (1989)[23] studied that the coagulative effect of cetyltrimethylammonium bromide(CTAB) on the settling property of sludge. After sludge was mixed with CTAB, CTA^+ ion interacts with organic materials in sludge and sludge flocs were coagulated into aggregates. As the result, zone settling velocity increased, settled sludge volume after 30 minutes decreased. Matsumoto, K et al. (1980)[24] researched the effect of cationic polymer (polyethyleneimine) on the settling property of sludge. Mixing of polyethyleneimine and sludge makes sludge flocculated strongly and the settling rate increase. Therefore, chemical treatment based on the surface charge makes organic materials removed and sludge dewaterability increase. However, as the remaining chemicals in sludge can cause environmental pollution, it is essential to remove the remaining chemicals in post-treatment.

b. Acid and base treatment

Also, by changing sludge surface charge, sludge flocculation becomes promoted. For example, acid or base treatment are included in sludge dewatering treatment by changing surface charge. Ruiz-Hernando, M., et al. (2013)[25] investigated the effect of 0.784 ~ 235 g NaOH/kg TS of sodium hydroxide on sludge dewaterability. In low concentration of NaOH, as sludge surface charge become more negative, sludge flocs become more disintegrated and in high concentration of NaOH, sludge flocs become more disintegrated due to hydrolysis. Chen, Y.(2001)[26] studied the effect of acid treatment on sludge dewaterability. When pH of sludge changes from 10 to 0 by mixing sludge and sulfuric acid, sludge dewaterability showed different trends. In centrifuge test, as pH decreases, solid volume decreases. In the filtering test, as pH decreases, the amount of filtrate increases. From this test, sludge becomes more compact after filtering. When pH decreases to 2.5, water content of sludge cake decreased. However, as pH decreases to lower than 2.5, water content of sludge cake increased. This is because fine particles from sludge could block the filter pore and prevent filtrate from passing the pore. As sludge was mixed with betaine, the pore blocking become solved by solubilizing cell materials. Finally, water content decreased to 74 %. Yang, Xue, et al. (2014)[27] found out sludge dewaterability improves through mixing sludge and methanol. As methanol disintegrates organic materials and cell wall in sludge, water was released from cell. As the result, sludge water content decreased to about 13.4 %. However, as chemical treatment makes sludge acidic or basic, the post treatment has to be essential.

B. Oxidative treatment

a. Chemicals

Oxidation makes sludge dewaterability increase. Lee, K.-M., M.S. Kim, and C. Lee(2016)[28] improves sludge dewaterability by using the strong oxidative, persulfate. Through some kinds of persulfates, peroxymonosulfate (PMS) and peroxydisulfate (PDS) was reacted by heating and reacting in basic condition, Sulfate radical anion was produced as the sulfate radical anion ($SO_4^{\cdot-}$) with high redox potential and it oxidized organic materials in sludge. As the result, CST increased and dewaterability based on filtering also decreased. However, in centrifuge test, centrifuge weight reduction increase. This means sludge dewaterability increased and dewaterability based on filtering can be measured wrong by pore blocking. M.-C. Lu et al. (2003)[29] improved sludge dewaterability by Fenton oxidation. In fenton oxidation, as hydrogen peroxide (H_2O_2) reacts with Fe^{2+} and ·OH radical is formed, ·OH radical is utilized for oxidizing organic materials. In this study, 6000 m/l of Fe^{2+} and 3000 mg/l of H_2O_2 was mixed with sludge. This treatment reduced SRF than control. The water content decreased to 75.2 %. D.-Q. He et al. (2015)[30] investigated the substitution of Fe^{2+} with Fe^{3+} ion for Fenton oxidation in sludge dewatering. This method was named as Fenton-like reaction. As Fe^{3+} ion reacted with H_2O_2, HO_2 radical was formed and this radical oxidizes organic materials in sludge. As the result, water content decreased from 80.0 % to 66.1 % and dried solid content decreased from 12.9 to 10.6 g/L. The toxic materials are reduced with cell liquid by cell rupture. As Fe^{3+} cation acts to encourage plant growth, the remaining Fe^{3+} content could help sludge utilized as a fertilizer. However, as Fenton oxidation occurs in acidic condition and changes sludge acidic, post treatment for neutralizing sludge is essential.

b. Ozone and Ultrasonication treatment

Ozone and Ultrasonication are also employed for sludge dewatering. As oxygen reacts in the electric field, oxygen is dissociated into radical due to the increase of kinetic energy of free electron in oxygen. The radical forms the highly oxidizing agent, ozone. If ozone is contact with sludge, it oxidizes organic materials and protein composing of cytoplasmic membrane in sludge. As oxidation makes damages on cell membrane, ozone can enter into cell and damage nucleic acid in cytoplasm and chromosome. The process releases water originally positioned inside cell from sludge and makes sludge dewaterability improved. This method has a benefit for environment by not forming toxic materials.[31] Jian Zhang, et al.(2016)[32] researched the effect of ozone treatment on sludge dewatering. Through the increase of soluble organic concentration in supernatant and decrease of organic concentration in sludge pellet, ozone treatment can disintegrate sludge particles. In SEM analysis, ozone treatment makes sludge flocs disintegrated and then dispersed into microbial. Because of pore blocking by disintegrated fine particle, CST increases. Ultrasonication treatment disintegrates sludge through changing physical, chemical, and biological properties of sludge.[33] Ultrasonication ejected into sludge shows two phenomena, compression and rarefaction alternately. When Ultrasonication is ejected into sludge, this phenomenon is compression. When Ultrasonication is removed, this phenomenon means rarefaction. In the rarefaction, microbubble becomes formed from sludge and it becomes larger by repeating compression and rarefaction process. When microbubble becomes larger until unstable size, it is bursting and emits shock wave, such as temperature of around 5000 °C and pressure of 500 atmospheres at a lifetime of few microseconds. Due to the shock wave, sludge becomes disintegrated. Guo, S., et al. (2015) [10] found out that Ultrasonication reduces the average particle size to 39.76 % and improve filterability with very small increase. The reason is due to structure change of sludge cake. Ultrasonication makes sludge cake more compact during filtering test and this structure makes prevent filtrate from flowing through cake. Through coagulant was employed for flocculation and agricultural waste was employed for water channel formation, sludge dewaterability was improved. Although Ultrasonication and ozone treatment can oxidize sludge without chemicals, it consumes large amount of energy.

C. Enzymatic lysis

Z. Chen et al. (2015)[11] improved sludge dewaterability through mixing sludge and enzyme. When α–amylase was mixed with sludge and then protease was mixed, sludge solubilization become maximized. This was because α–amylase could lyse polysaccharides before protease lyse α–amylase. However, enzymatic lysis makes the release of large number of biopolymers from sludge and prevents the filtrate from passing through the pore during sludge filtering test. In this paper, the problem could be solved by mixing inorganic coagulation, $FeCl_3$ and water content decreased from 96 % to 82 %. Wu, Boran et al.(2016)[34] explained the mechanism for enzyme to improve sludge dewaterability through organic materials analysis. When the enzyme mixture of protease, liqidase, and anaerobic bacteria, named as Enviro-zyme 216 was mixed with sludge, 1mg/g DS of Enviro-zyme could reduce CST to 48.8 %. And, it also found out that as CST was affected by the amount of TB-EPS and Enviro-zyme makes hydrophilic part of TB-EPS soluble, micro-structure of sludge was dissociated and water was released out. Although enzymatic lysis improves sludge dewaterability, the remaining enzyme also causes environment pollution.

2.1.4 Summary

To solve the problems due to low sludge dewaterability, many research has been investigated. In sludge, water distributes into 4 parts with different bind energy, free water with little bind energy, interstitial water trapped between sludge flocs, surface water bound on the sludge flocs, and bound water positioned in microbial cell with strongest bind energy. To achieve higher sludge dewaterability, more strongly bound water has to be removed. Sludge treatment includes mechanical dewatering, such as settlement, filter press, centrifuge, filtering, UV, and heat treatment, electrical treatment, such as electrolysis, and chemical treatment, such as coagulation, surface charge modification, oxidation by radical or enzyme. The synergistic effect of these treatments has been investigated. However, it is still essential to improve dewatering efficiency in the aspect of energy saving and protection of environment.

2.2 Gas hydrates and its application

2.2.1 Principle of gas hydrates

Natural gas hydrates are crystalline solids which the gas molecules (guests) are trapped in water cavities (host).[35] in the crystalline structure of gas hydrates, there is no direct connection between guest molecules and host molecule. The structure is composed of hydrogen bond between water molecules and guest molecules are spinning in the structure.[5]

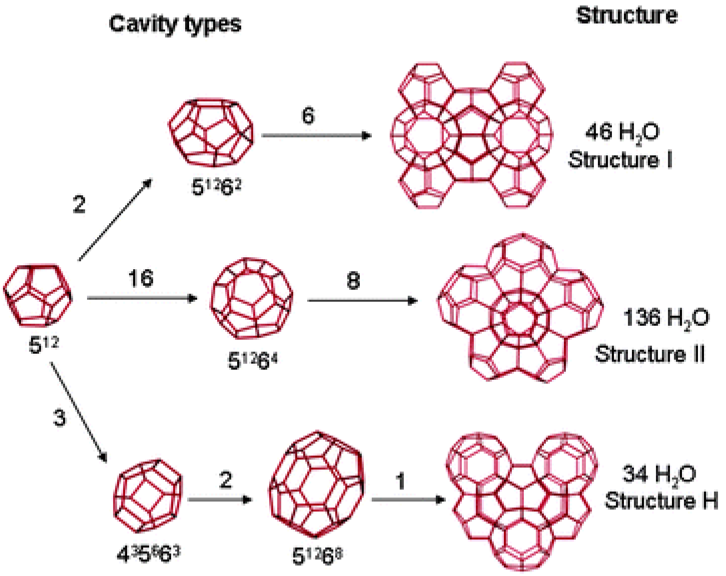


Figure 6. 3 structures of gas hydrates and their cages of which each structures are composed.[4]

Gas hydrates can take sI, sII, and sH, 3 different structure with different size and composition of guest molecules.[36] Each structures contain different kinds and ratio of cages. sI structure is a body-centered cubic lattice composed of 46 water molecules which form 6 large cages and 2 small cages, and it can be formed with CH_4, C_2H_6, H_2S, and CO_2 as a guest molecule. sII structure is a face-centered cubic lattice composed of 136 water molecules which contain eight large cages and sixteen small cages and can be formed with C_3H_8, i-C_4H_{10}, and N_2 as a guest molecules.[5] sH is a hexagonal lattice containing 34 water molecules and three cage types: three small 5^{12} cages, two medium $4^35^66^3$ cages, and one large $5^{12}6^8$ cage and can be formed with methylcyclohexane as a guest molecules.[5]

Property	Ice	CH_4 hydrate
Density (g/mL)	0.916	0.912
Heat of fusion (kJ/mol H_2O)	6	9
Thermal conductivity at 263 K (W/(m K))	2.25	0.49
Dielectric constant at 273 K	94	~58
Young's modulus at 273 K (10^9 Pa)	9.5	~8.4
Longitudinal sound velocity (km/s)	3.8	80
Compressibility at 273 K (10^{-11} Pa)	12	14
Index of refraction	1.3082	1.3485

Table 2. Comparison of ice and CH_4 gas hydrates[5]

When water in liquid phase reacts with guest molecules in high pressure, some water molecules are arranged around guest molecules. As temperature becomes lower and pressure becomes higher, the arrangement grows stable and gas hydrates is formed finally.[36] In general, water is frozen and ice crystal is formed below the freezing temperature. Although ice and gas hydrates are a crystalline structure composed of only water molecules in common, there are some differences. Figure 7 represents the different properties of ice and CH_4 gas hydrates. As there is no direct connection between water molecules and guest molecules unlike ice, gas hydrates have lower heat and electric conductivity than ice.

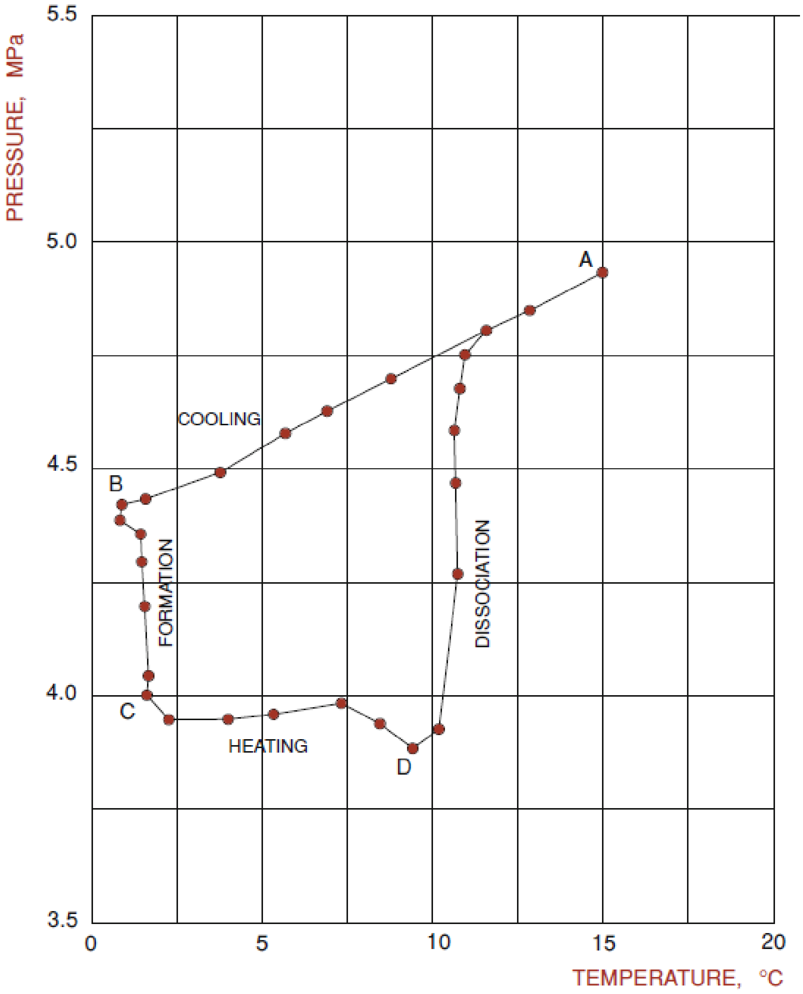


Figure 7. Determining the actual hydrate stability conditions through isochoric cooling and slow heating[5]

Gas hydrates stabilization condition can be found out experimentally like Figure 7. For example, when water at the temperature and pressure condition of point A becomes cooled to the condition of B, gas is consumed continuously and finally reaches to the gas hydrates stabilization condition. When the water is in the gas hydrates stabilization condition, pressure becomes suddenly dropped due to gas hydrates formation and temperature increased suddenly due to exothermic gas hydrate formation(B→C). Through this process, the gas hydrate formation condition can be determined. After gas hydrates are formed and stabilized, temperature is controlled and increase gradually(C→D). In this step, gas hydrates become more and more instable and finally dissociated with pressure increase and temperature decrease, and the gas hydrates stabilization condition can be determined. Therefore, gas hydrates are stable at higher than gas hydrates stabilization pressure and lower temperature than gas hydrates stabilization temperature.

2.2.2 Research field of gas hydrates

In 1934, Hammerschmidt found out that gas hydrate is one of the reason causing pipeline blocking.[37] when pipeline is positioned under the sea floor, water can enter through the crack of pipeline made by deterioration. As cooled water and pressurized gas meet, gas hydrates can be made in the pipeline. The gas hydrates in pipelines prevent gas from flowing, damage the pipeline and finally makes economical loss. Therefore, at first, many researchers were trying to inhibit the gas hydrate formation in the pipeline. Many researches are investigated on the structure, stabilization condition, promotion and inhibition of gas hydrates.

To control the formation of gas hydrates was researched to proper gas hydrates formation. The formation of gas hydrates is divided into 2 categories, thermodynamic hydrate promotion and kinetic hydrates promotion. Thermodynamic hydrates inhibitor such as tetrahydrofuran(THF)[38], promotes gas hydrates formation by shifting thermodynamic curve. Kinetic hydrates promoted such as sodium dodecyl sulphate (SDS)[39] forms micelle using guest molecules, makes its solubilization, and finally encourages gas hydrate formation. Gas hydrates formation can be inhibited thermodynamically and with low dosage of chemicals. Thermodynamic hydrates inhibition prevents gas hydrate formation by mixing chemical such as alcohol, glycol and changing phase equilibria to higher pressure and lower temperature. However, thermodynamic hydrates inhibition consumes large quantity of inhibitors and this process has to be followed the inhibitor recovery step. For reducing consumption of chemicals, kinetic hydrates inhibition using PVCap[40] and anti-agglomeration using n-Alkyl-tri(n-butyl)ammonium Bromides[41] are developed. Kinetic hydrates inhibitors can slow down gas hydrate formation by covering the interface of water and gas. Anti-agglomerants can prevent gas hydrates from changing hard by maintaining slurry forms of gas hydrates. As gas hydrates with the form of slurry can flow through the pipeline, they are not harmful. However, as it is difficult to predict the kinetics of gas hydrates formation with inhibitors, it is essential to study more on the application of kinetic hydrates inhibition.[42] Dual function inhibitors acts as both inhibitor and promotor, such as ionic liquids and amino acids. For example, Kim, Ki-Sub, et al. (2011)[43] found out the effect of pyrrolidinium cation-based ionic liquids on gas hydrates formation. N-(2-hydroxyethyl)-N-methylpyrrolidinium tetrafluoroborate ([HEMP][BF_4]) and N-butyl-N-methylpyrrolidinium tetrafluoroborate ([BMP][BF_4]) are applied on gas hydrates formation. When [HEMP][BF_4] was applied on gas hydrates formation, hydrogen bonding between water molecules are inhibited by hydroxyl group of [HEMP][BF_4]. And, when both [HEMP][BF_4] and [BMP][BF_4] are applied on gas hydrates formation, gas hydrates formation was promoted thermodynamically by shifting phase equilibria to lower pressure and higher temperature. Therefore, pyrolidinium cation-based ionic liquids has dual effect on gas hydrates formation.

The application of gas hydrates on other fields has been studied. As gas hydrates has similar structure with ice, gas hydrates have been studied on the alternative of freezing method. Gas hydrates was applied on food conservation. Takeya, S., et al. (2016)[44] studied on the application of gas hydrates to the conservation of fruits and vegetables. When fruits and vegetables are pressurized for 46 h in the condition of 20 ~ 30 bar CO_2 gas at 3 ℃, sI structure of CO_2 gas hydrates was formed inside of tissues. As gas hydrates formation in tissue makes food destroyed, weaker treatment could be better for food conservation. And, as carbonated substance released during gas hydrates-dissociation also helps food conservation, gas hydrates treatment can be the alternative of freezing method. Gas hydrates treatment could be applied on desalination. Kang, K.C., et al. (2014)[45] found out the desalination effect of gas hydrates. After CO_2 gas hydrates was formed in seawater in the condition of 29 bar and 280 K, gas hydrates became pelletized. As the results, gas hydrates treatment achieved 71~94 % of cation removal and 73 ~ 83 % of anion removal efficiency. They also found that ion removal efficiency has a trend like $K^+ > Na^+ \approx Mg^{2+} \approx Ca^{2+} > B^{3+}$ and this is because of ionic hydration based on different ionic charge and size. Therefore, gas hydrates treatment has desalination effect induced by different ionic hydration.

2.2.3 Quick freezing induced by gas hydrates-dissociation energy

Quick freezing induced by gas hydrates-dissociation energy is the quick-freezing method using endothermic process caused in gas hydrates-dissociation. When gas hydrates are formed, heat is released from molecules due to crystallization and it is exothermic process. When gas hydrates are dissociated, molecules absorb, and it is endothermic process. QFGD obtains the driving force from the endothermic process. The conventional freezing treatment has to be followed by separation process with high pressure as the separated concentrates and pure water can be mixed during gas hydrates-dissociation. As QFGD freezes sample right after gas hydrates-dissociation, it does not need high pressure for separation. Han, S. W. et al. (2016)[9] developed QFGD and found out the application of QFGD on the pretreatment of reverse osmosis (RO) process. When seawater was pressurized in the condition of 0.2 ℃ temperature and over 25 bar CO_2, CO_2 gas hydrates was formed in seawater. After the gas hydrates became stable, the system was depressurized, and the gas hydrates was dissociated in a short time. In the same time, sea water was frozen suddenly due to the endothermic process. As the result, the ion removal efficiency is about 66.9 %. If this process was applied to the pretreatment of RO process, the ion removal improved to 99 %. In addition to separation process, as CO_2, the freezing agent, is directly contact with sample and a coolant is also indirectly contact with sample, QFGD is the intermediate method between direct and indirect quick-freezing. Therefore, QFGD method has higher energy efficiency.

3 Experimental Method & Materials

3.1 Sludge preparation

Activated sludge (Figure 9-a) was sampled from a wastewater treatment plant in Ulsan, South Korea. To prevent microbial growth in sludge, the sludge was stored in 4 ℃ after sampling. All experiments and analysis were carried out in 3 weeks for sludge properties not to change. As the sampled sludge consumed very large energy due to high water content, the sludge was concentrated by the following steps (Figure.8). After the sampled sludge was thickened gravitationally for 12 h at 4 ℃, the thickened part was separated by pouring supernatant. The thickened part was centrifuged (3100 rpm, 15 min, 20 ℃) for removing more water. In this study, all centrifuge process was done by High Speed Refrigerated Centrifuge (VS-24SMTi, VISION SCIENTIFIC CO.LTD, Korea) at 20 ℃ for preventing sample from freezing. The pellet was separated by pouring the supernatant. Finally, the sludge mixture (Figure 9-b) was prepared by mixing the pellet and the supernatant from the gravitational settling with the ratio of 5 :1. The sludge mixture was tested with 24 h.

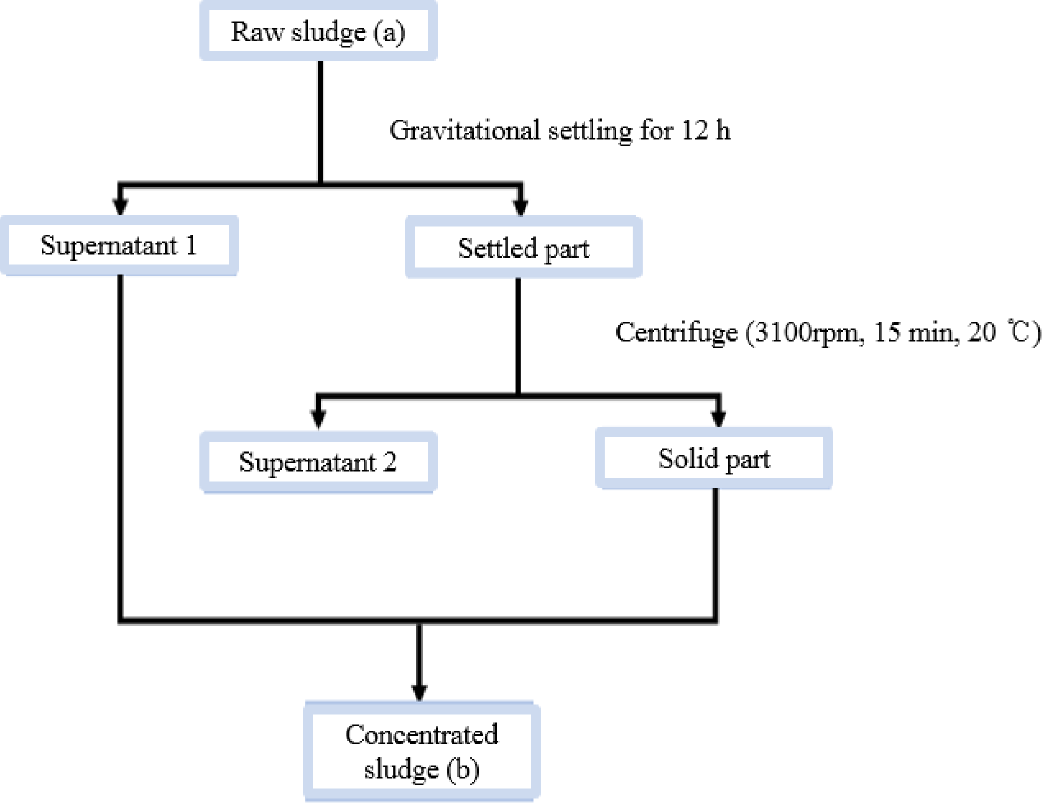


Figure 8. Preparation of the sludge mixture by concentration

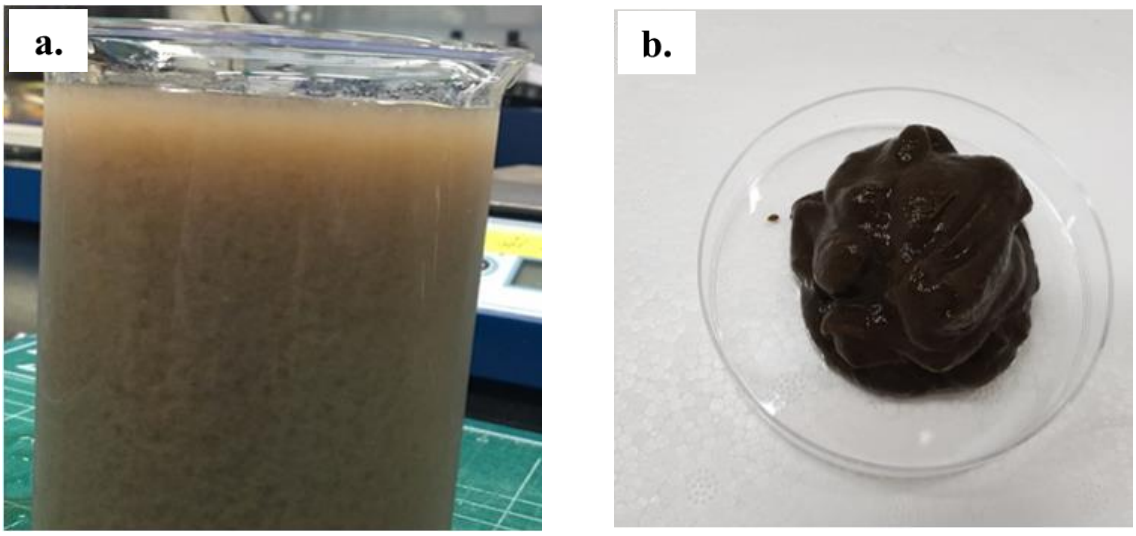


Figure 9. Images of the sampled sludge and the sludge mixture (a: the sampled sludge, b: the sludge mixture)

3.2 Quick-freezing induced by gas hydrates-dissociation energy

Guest molecules were carbon dioxide (Purity 99.999 %, Korea SEM) and nitrogen (Purity 99.999 %, Korea SEM). QFGD was done in the reaction system (Figure 10) by following the process (Figure 11). At first, 500 g of sludge was set in the reactor and cooled to 0.2 (±0.1) ℃ with mixing at 150 rpm. After the sludge mixture became stable at 0.2 (±0.1) ℃, purging was done for replacing atmospheric gas to the guest molecules in a gas layer. The guest molecules flew through the reactor in 1 (±0.3) bar for 10 min. After the temperature became stable at 0.2 (±0.1) ℃, the sludge mixture was pressurized in the condition of reaction pressure. And, the pressure was maintained for 4 h. After 4 h, the sample was depressurized to 0 bar for a short time. Frozen sample was separated from the reactor and stored in a refrigerator with 4 ℃ to melt. The exact reaction condition was explained in Table 3.

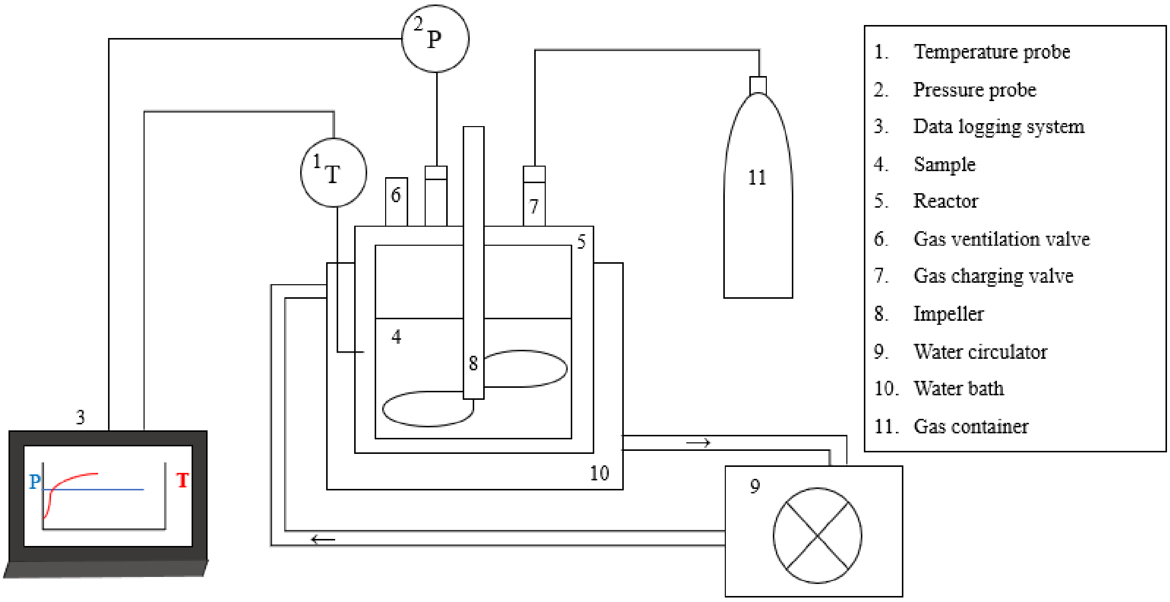
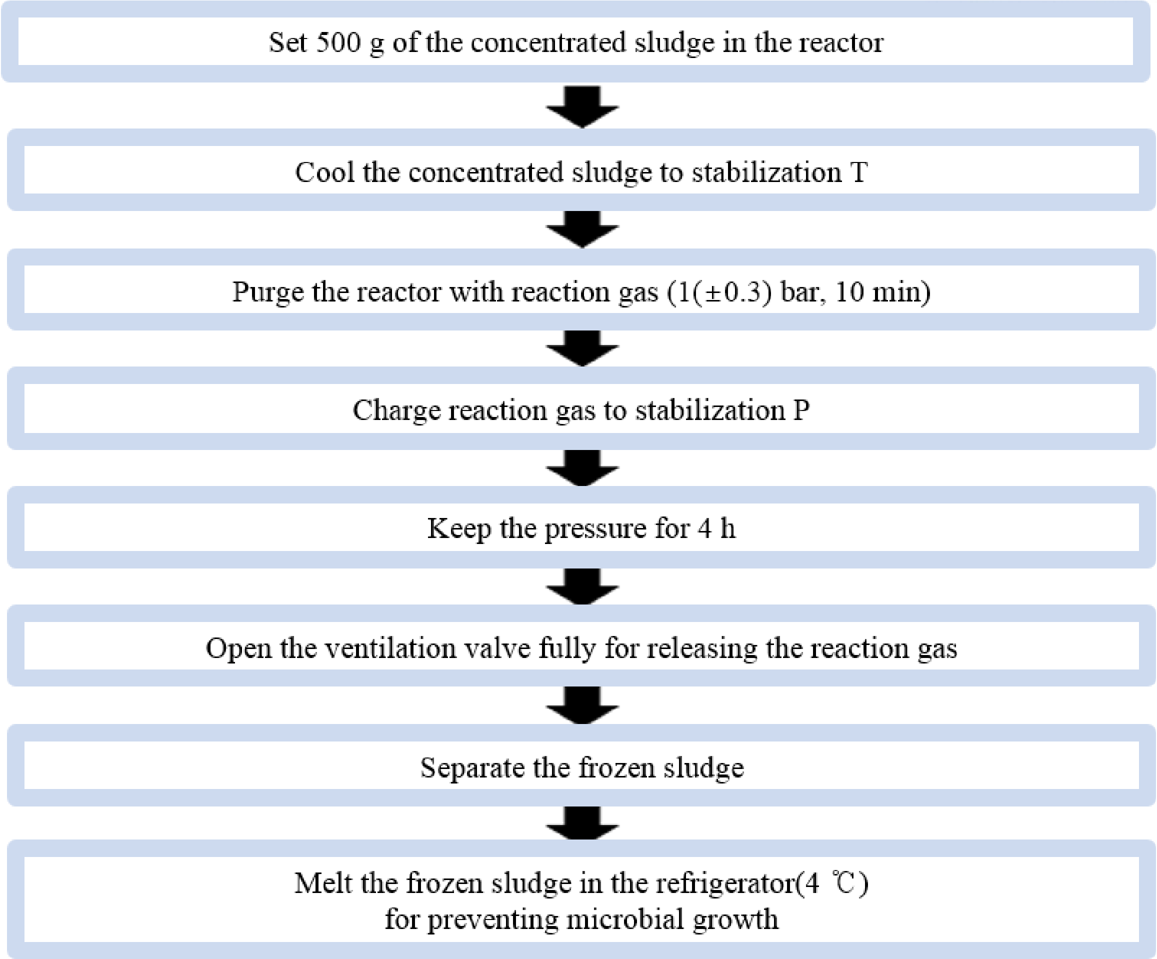


Figure 10. Schematic of the QFGD system



Set 500 g of the concentrated sludge in the reactor

Cool the concentrated sludge to stabilization T

Purge the reactor with reaction gas (1(±0.3) bar, 10 min)

Charge reaction gas to stabilization P

Keep the pressure for 4 h

Open the ventilation valve fully for releasing the reaction gas

Separate the frozen sludge

Melt the frozen sludge in the refrigerator(4 ℃)
for preventing microbial growth

Figure 11. Process for quick-freezing induced by gas hydrates-dissociation energy

Sample Name	Control	N2 35 150	CO2 10 150	CO2 20 150	CO2 35 150	CO2 35 300
Pressure, bar	-	35	10	20	35	35
Guest molecule	-	N_2	CO_2	CO_2	CO_2	CO_2
Temperature, ℃	-	0.2	0.2	0.2	0.2	0.2
Stirring rate, rpm	-	150	150	150	150	300

Table 3. The condition for CO_2 gas hydrate induced short-time freezing treatment

3.3 Analysis

3.3.1Organic material distribution measurement

To compare the change of organic concentration in the sludge sample, organic concentration of gradually separated supernatants was measured. The supernatant was separated gradually by the following steps (Figure. 12) all centrifuge process was done by High Speed Refrigerated Centrifuge (VS-24SMTi, VISION SCIENTIFIC CO.,LTD, Korea) at 20 ℃ for preventing sample from freezing. Phosphate-Buffered saline was a commercial product (10010 - PBS, pH 7.4, Gibco® by life technologies™). Ultrasonication was done by Cole-Parmer 500- and 750-Watt Ultrasonic Processors (EW-04711-65, Cole-Parmer Instrument Company, LLC, United States). The mixture of PBS and sludge pellet was mixed using Vortex Mixer (KMC-1300V, VISION SCIENTIFIC CO.,LTD, Korea).

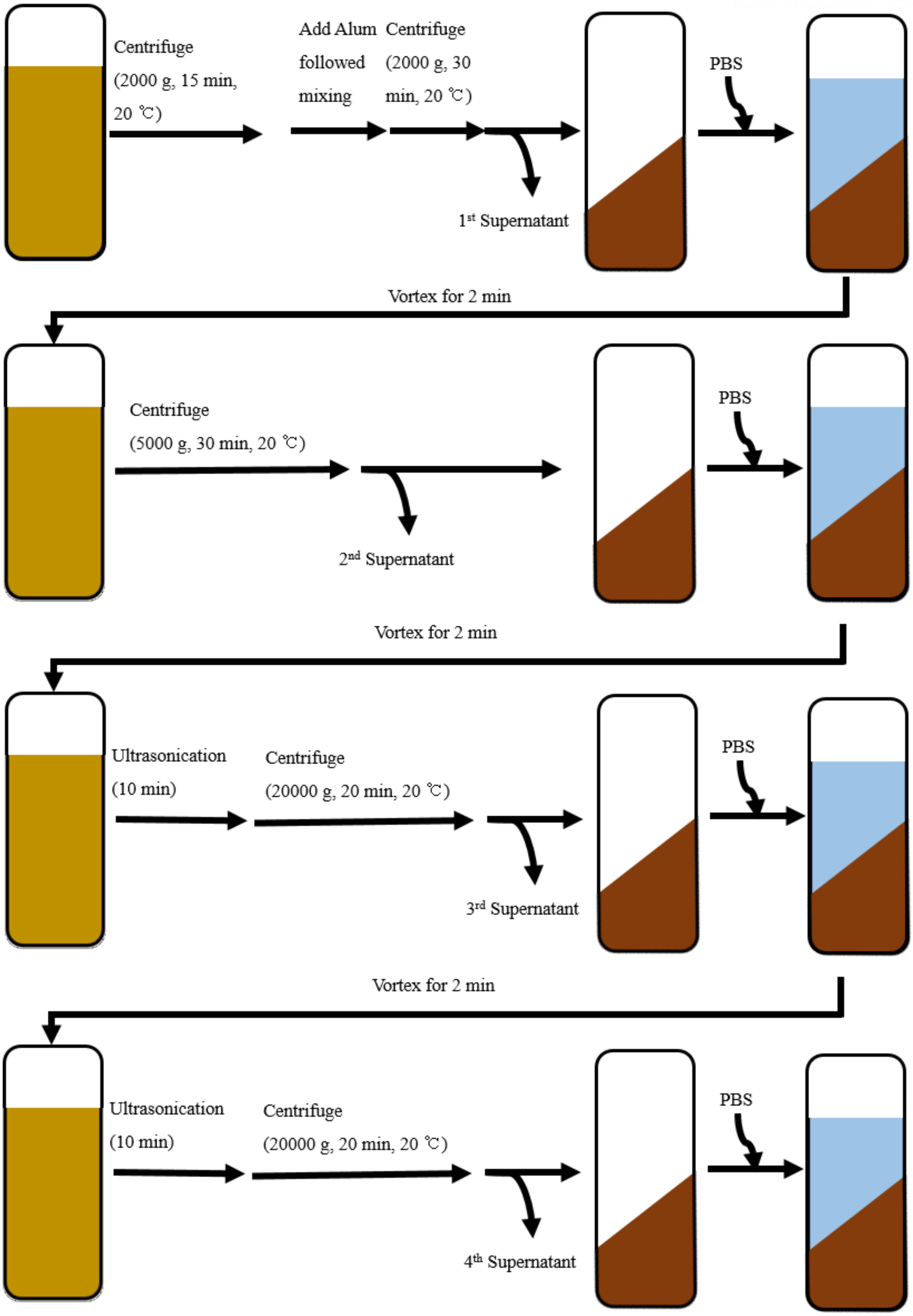


Figure 12. Gradual separation of supernatant from sludge

Step 1 After 30 g of a sample was centrifuged (2000 g, 15 min, 20 ℃), 0.2 g of aluminum sulfate ($Al_2(SO_4)_3$, Sigma-Aldrich) was added, and the mixture was centrifuged (2000 g, 30 min, 20 ℃). The supernatant was separated and filtered using 0.45 μm Polytetrafluoethylene (PTFE-H) syringe filter (SH25P045N, Hyundai micro CO.,LTD, Korea). The filtrate was named as Alum.

Step 2 Adding PBS (phosphate buffered saline, pH 7.4) into the remaining pellet (from Step 1) to 30 g, the mixture was mixed by a vortex. And, it was centrifuged (5000 g, 30 min, 20 ℃). The supernatant was separated and filtered using 0.45 μm syringe filter. The filtrate was named as Centrifuge 5000 g.

Step 3 Adding PBS (phosphate buffered saline, pH 7.4) into the remaining pellet (from Step 1) to 30 g, the mixture was mixed by the vortex mixer. And, it was treated by Ultrasonication (288000 W, 10 min) within ice for preventing sludge from heated. The treated sample was centrifuged (20000 g, 20 min, 20 ℃) and filtered using 0.45 μm syringe filter. The filtrate was named as Centrifuge 1st Ultrasonication.

Step 4 Using the remaining pellet from Step 3, Step 3 was repeated. The supernatant was named as 2nd Ultrasonication.

Filtration using 0.45 μm filter was done to prevent large particles from blocking a line in analyzers. Each filtrate was measured in polysaccharide, protein concentration, total organic carbon and total nitrogen. Protein concentration was measured by the Lowry method[46] using Total Protein Kit, Micro Lowry, Peterson's Modification (TP0300, Sigma) with BSA as a standard. Polysaccharides concentration was measured by the phenol-sulfuric acid method[47] with glucose (D(+)-Glucose, anhydrous, 98 %, Samchun Pure Chemical) as a standard. Total organic carbon (TOC) and Total nitrogen (TN) were measured by TOC analyzer (TOC-VCPH, Shimadzu Scientific Instruments, North america).

3.3.2 Chemical properties of the supernatant

Soluble organic materials can be measured by zeta potential, conductivity and osmotic pressure in supernatant. After the sludge mixture was centrifuged (5000 g, 30 min, 20 ℃) using High Speed Refrigerated Centrifuge (VS-24SMTi, VISION SCIENTIFIC CO.,LTD, Korea), the supernatant was filtered by 0.8 μm Cellulose Acetate filter (EW-81054-40, Advantec). Zeta potential was measured by a zetasizer (Marvern Instrument Ltd., United Kingdom). Conductivity was measured by a conductivity meter (ULTRAMETER II™ 6P, Myron L® Company, USA). Osmotic pressure was measured by the Vapor Pressure Osmometer (Model 5600, ELITechGroup, USA).

3.3.3 Rheology

Rheology represents the inner structure of sludge. In this study, integrity of inner structure in sludge was investigated through the thixotropic loop and viscosity analysis. the test was done by the rheometer (Haake MARS Ⅲ - ORM Package, Thermoelectron, USA) with a PP35 Ti sensor (cone diameter 34.004 mm). The experiment referred to Jibao, et al. (2016)[12]. About 1 ml of sample was charged on the plate. The shear stress was forced on sample within a region from 0.01 to 1000 s^{-1} shear rate, and equilibrate at the maximum shear rate for 30 s. After then, shear stress decreased from 1000 to 0.01 s^{-1} shear rate. During the process, shear rate and shear stress were recorded, and viscosity was calculated automatically. The area of thixotropic loop means the change of inner structure by rheology measurement. As the area increases, larger part of inner structure is modified and recovered during rheology measurement. That is, larger part is originally intact. If inner structure is more intact, viscosity increases, and modification of viscosity is also larger.

Figure 13. Rheometer (Haake MARS Ⅲ - ORM Package, Thermoelectron, USA)

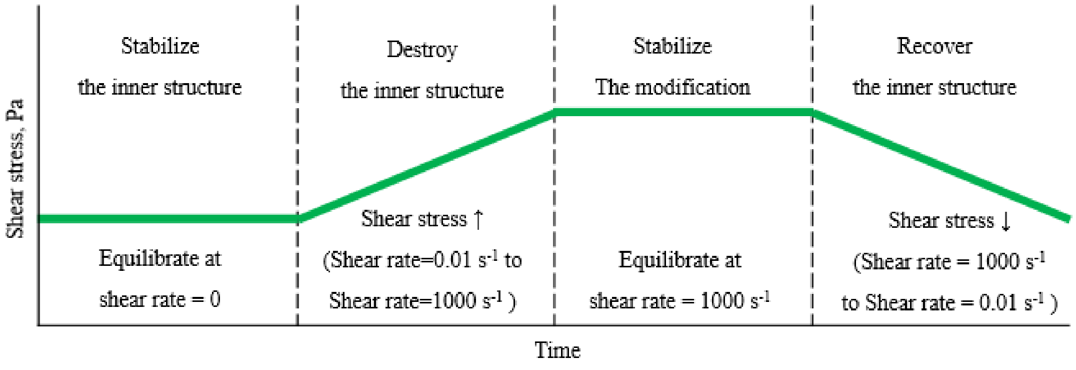


Figure 14. Process for thixotropic loop and viscosity measurement

3.3.4 Dewatering effect

Sludge dewatering effect was tested by two methods, dewaterability based on filter, and settling test. First method is capillary suction time (CST), time to filter (TTF) and filtering test. CST is the time for liquid to pass horizontally through filter in a certain distance. If sludge is dewatered poorly, CST increases. And, if sludge is dewatered well, CST decreases. In this study, CST measurement kit (Type 304B Capillary suction timer, Triton Electronics Ltd., United Kingdom) was used. About 5 g of a sample was loaded in the cylinder on the filter and the time for a liquid to pass through the filter was measured. The filtering test system was Figure 15. based on Guo, S., et al.(2015)[10] In the filtering test, about 20 g of sample was loaded on the filter in the cell and was pressurized using 1(±0.2) bar N_2 gas. During filtering test, the weight of filtrate was recorded every 5 minutes. TTF was the time when the weight of filtrate reaches to half weight of initial sample. If sludge is dewatered poorly, TTF increases. And, if sludge is dewatered well, TTF decreases. Second method is settling method. Settlement is the method to use for reducing sludge and needs less force. Therefore, it is important to improve the efficiency of settlement. And, the density of sludge can be compared by settling test. If sludge is dewatered, the density of sludge increases and settling becomes improved. Settling efficiency was measured by two methods and each settled volume index(SVI) was calculated.

First method	30 g of centrifuged sludge is mixed with 1 L of the supernatant separated gravitationally. Then, the separation image is analyzed during settlement.
Second method	50 g of sludge is mixed with 1 L of 0.041 M aluminum sulfate solution. Then, the separation image is analyzed during settlement.

Figure 15. Dead-end filtration system

3.3.5 Understand of sludge dewatering test based on filtration

Sludge dewatering effect is measured by comparing the time to filter or the amount of filtrate during filtration. Therefore, the pore size is very important to determine sludge dewaterability. Based on water distribution in sludge, to disintegrate sludge particles can improve sludge dewaterability. However, when sludge particles are disintegrated, sludge dewaterability seems to be poor in sludge filtering test. This is because fine particles disintegrated from sludge can block the filter pore and prevent filtrate from flowing through the filter pore. Therefore, for understanding about sludge dewatering effect, other methods have to be considered together. In this study, the effect of sludge on filter pore was studied. First, particle size distribution was measured. In μm particle size distribution, Particle size analyzer (Mastersizer 3000, Malvern Instrument, United Kingdom) was used to measure particle size distribution from 0.01 to 3500 μm. In nm size particle size distribution, after sludge was centrifuged (5000 g, 30 min, 20 ℃) and the supernatant was filtered using 0.8 μm filter, the particle size distribution of the filtrate was measured by Zeta-potential analyzer (Nano ZS90, Malvern Instrument, United Kingdom). The measured range was from 0.4 to 10000 nm. And, to investigate the effect of sludge on filtering test, resistance test was done.

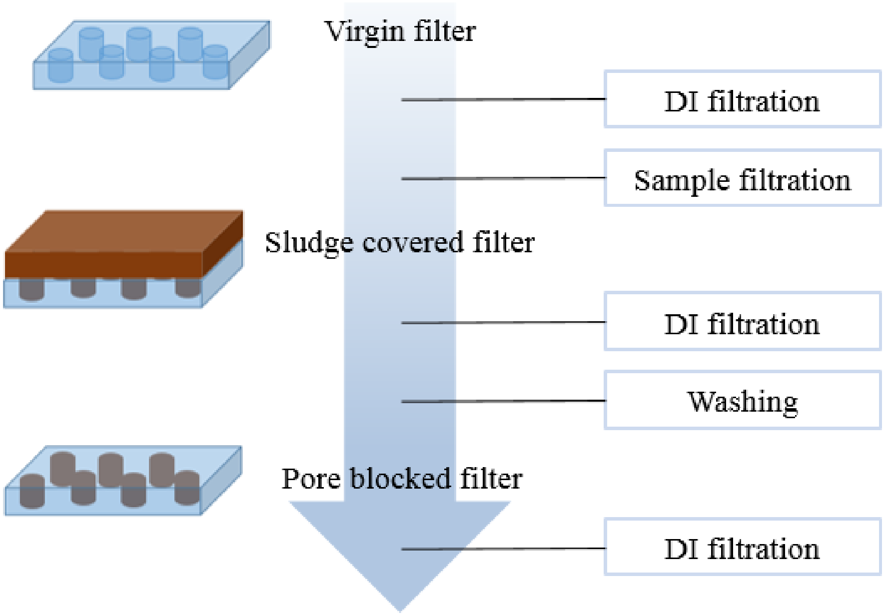


Figure 16. Process for the resistance test

It was assumed that total resistance can be divided into 3 resistances, resistance by membrane, resistance by sludge cake, and resistance by pore blocking. The equipment like Figure 16 was used. The weight of filtrate was recorded every 10 seconds. At first, for compaction, a filter was submerged in a deionized (DI) water for 24 h. After the compacted filter was installed to the equipment, filtration was done using DI water at 1(±0.2) bar of nitrogen gas. Then, sample was set on the filter and was pressurized to 1(±0.2) bar of nitrogen gas for 3.5 h. Using the filter, DI filtration was done with 1(±0.2) bar nitrogen gas. After the filtration, the filter was washed by shaking with 150 ml DI water in the condition of 100 rpm and 5 min using the shaker (NB-101MT, N-BIOTEK, Korea). using the washed filter, DI filtration was done with 1(±0.2) bar nitrogen gas. As the result, as only DI waster was filtered by the filter, the resistance from first DI filtration was named as the resistance by membrane. After the sludge filtration was done, the filter has sludge cake and pore blocking by fine particles. Therefore, after sludge filtration, the resistance by second DI filtration was the sum of resistance by sludge cake and by pore blocking. And, after washing the filter, sludge cake was removed. Resistance by third DI filtration was named as the resistance by pore blocking. Resistance by sludge cake can be calculated by substitution resistance by pore blocking to resistance from the second DI filtration.

$$\mathrm{Flux} = \frac{Applied\ \ pressure}{Solution\ \ viscosity \times Resistance(R)}$$

3.3.6 Disinfection test

The effect of QFGD on sludge disinfection was studied. The live/dead *Baclight*™ bacterial staining kit (L7012, Invitrogen™, USA) was used. This kit is composed of two dyes, SYTO®9 and propidium iodide (PI). As SYTO®9 can enter through membrane, it can stain nucleic acid in cell to green color. However, as PI cannot pass through membrane, it can stain nucleic acid out of cell to red color. Therefore, by counting green and red colored particles, disinfection effect can be compared. In this study, colored particles were measured by confocal laser scanning microscopy (FV1000, OLYMPUS, Japan) and counted by a software (Imaris, Andor Technology Ltd., United Kingdom).

4. Results & Discussion

4.1 The mechanism of QFGD in sludge application

Temperature and naked observation are the key factors which can show the gas hydrates formation and dissociation. As QFGD is the method based on gas hydrates-dissociation, it is important to recognize gas hydrates formation and dissociation through temperature profile and naked observation. As gas hydrates formation is exothermic process, the significant temperature increase can be the evidence for gas hydrates formation. And, when gas hydrates-dissociation is endothermic process, the significant temperature decrease can be the evidence for gas hydrates-dissociation. In this study, the mechanism of QFGD application on sludge was investigated. Temperature profiles during QFGD were divided into 2 parts, gas injection (Fig. 17 above) and depressurization (Figure. 17 below).

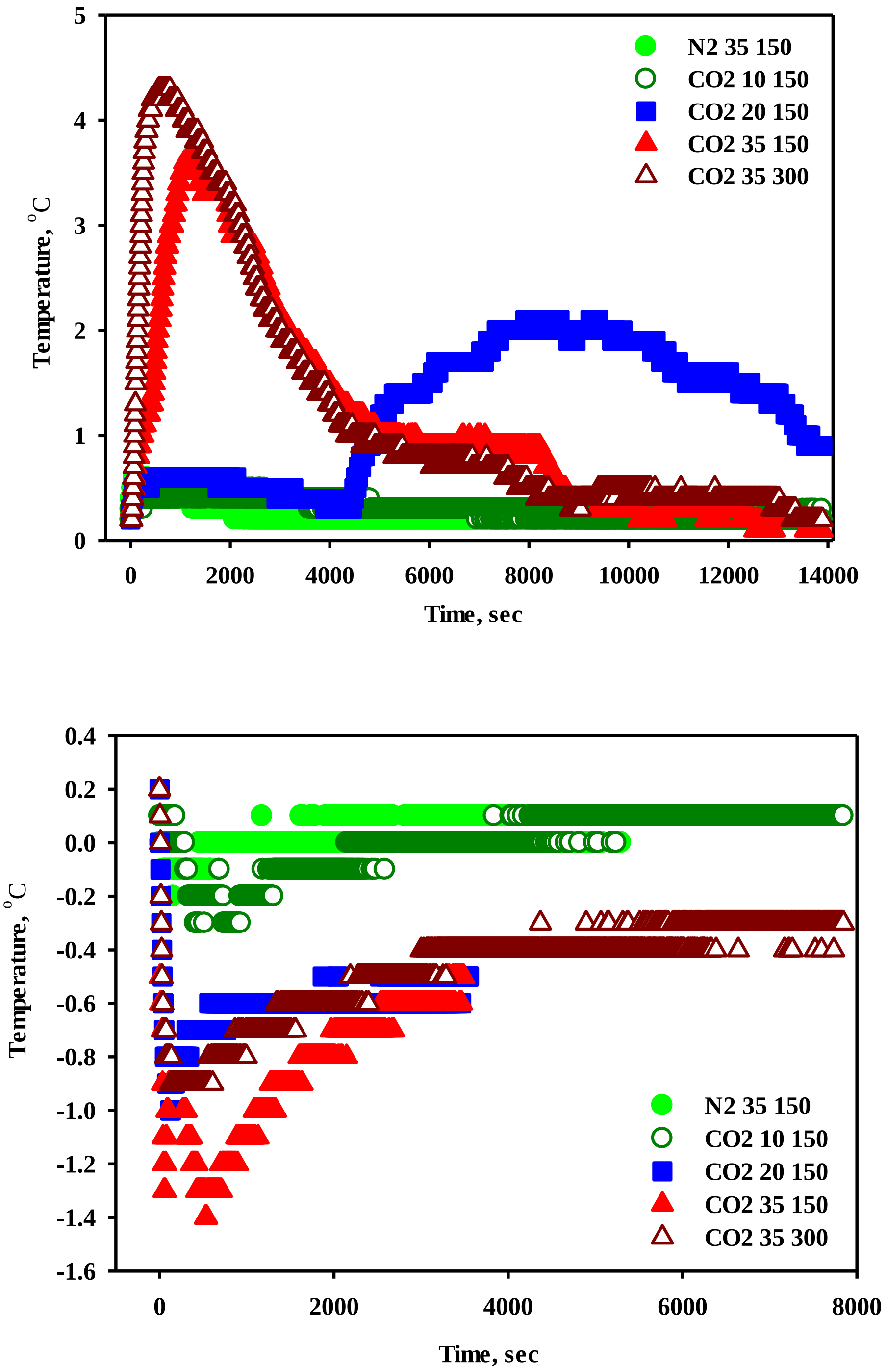


Figure 17. Temperature diagram during the application of QFGD on sludge with different guest molecules, pressure, stirring rate (Above: temperature diagram during gas injection, Below: temperature diagram during depressurization) (Control (◆), N2 35 150 (●), CO2 10 150 (○), CO2 20 150 (■), CO2 35 150 (▲), CO2 35 300 (△))

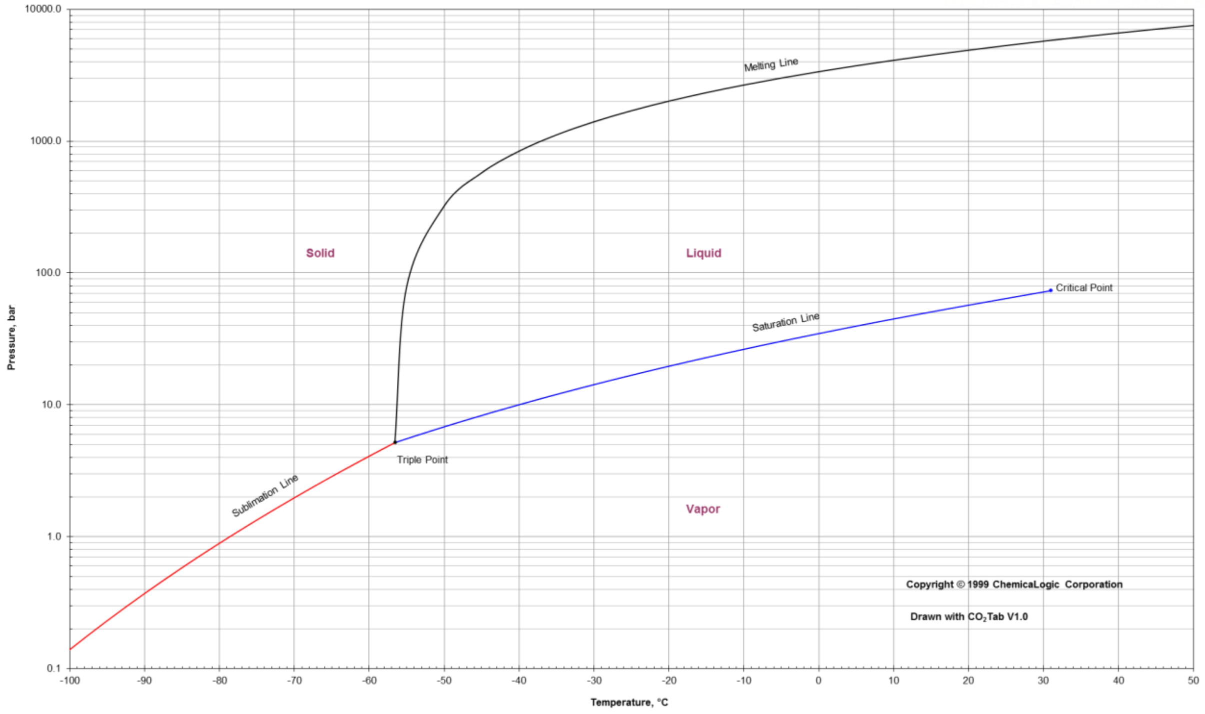


Figure 18. Phase diagram of CO_2 in H_2O[6]

In a gas injection part, as gas was injected into sludge, temperature increased in all cases. Temperature profile in the sample named as N2 35 150 showed the smallest temperature increase to 0.6 ℃. As the solubility of nitrogen gas has much smaller to water, this temperature increase is due to the Joule-Thomson effect of nitrogen gas. Temperature profile in the sample named as CO2 10 150 had a small temperature increase to 0.5 ℃. As CO_2 can dissolve into water, the temperature increase is caused by dissolution of CO_2 and the Joule-Thomson effect. Temperature profile in the sludge named as CO2 20 150 showed higher temperature increase to 2.1 ℃. In this case, as CO_2 gas hydrates were formed, the temperature increase was due to the Joule-Thomson effect, dissolution of CO_2, and gas hydrates formation. Temperature profile in the case named as CO2 35 150 showed significant temperature increase to 3.6 ℃. As CO_2 gas hydrates was also formed and sludge was pressurized at more higher pressure, the temperature increased more higher than CO2 20 150. Temperature profile in the case named as CO2 35 300 showed significant temperature increase to 4.3 ℃ due to the joule-Thomson effect, CO_2 dissolution, and CO_2 gas hydrates formation. In a depressurization part, as gas was depressurized, temperature decreased in all cases. Temperature profile in the case named as N2 35 150 showed the smallest temperature decrease to -0.2 ℃. As the solubility of nitrogen gas is much smaller than of carbon dioxide, this increase is due to the Joule-Thomson effect of nitrogen gas. Temperature profile in the case named as CO2 10 150 showed a small temperature decrease to -0.3 ℃.

As CO_2 can dissolve into water, the temperature increase is caused by regasification of CO_2 and the Joule-Thomson effect. Temperature profile in the case named as CO2 20 150 showed higher temperature decrease to -1.0 ℃. In this case, as CO_2 gas hydrates were dissociated, the temperature decrease was due to the Joule-Thomson effect, regasification of CO_2, and gas hydrates-dissociation. Temperature profile in the case named as CO2 35 150 showed significant temperature decrease to -1.4 ℃. As CO_2 gas hydrates was also dissociated and sludge was de-pressurized from higher pressure, the temperature decreased more significantly than CO2 20 150. Temperature profile in the case named as CO2 35 300 showed significant temperature decrease to -0.9 ℃ due to the Joule-Thomson effect, CO_2 dissolution, and CO_2 gas hydrates-dissociation.

The result could be understood by dividing the following two cases.

Case 1. The effect of different guest molecules on QFGD

In the same pressure and stirring rate condition, N2 35 150 and CO2 35 150 had a kind of guest molecules as only one difference. N2 35 150 had nitrogen gas as guest molecules and CO2 35 150 had carbon dioxide as guest molecules. Temperature change in CO2 35 150 was significantly larger than N2 35 150. This was because of the difference of guest molecules solubility. As CO_2 gas has higher solubility than N_2, CO_2 could be solved into water and change into gas hydrates forms easily than N_2. Therefore, due to CO_2 the dissolution and gas hydrate formation, temperature difference of CO2 35 150 was significantly larger than N2 35 150.

Case 2. The effect of different pressure on QFGD

In the same guest molecules and stirring rate condition, CO2 10 150, CO2 20 150, and CO2 35 150 had different pressure condition. Through the naked observation, CO2 10 150 was the sample without CO_2 gas hydrates formation and CO_2 gas liquefaction, CO2 20 150 was the sample with CO_2 gas hydrates formation and without CO_2 gas liquefaction, and CO2 35 150 was the sample with CO_2 gas hydrates formation and CO_2 gas liquefaction. As CO_2 gas is soluble to water, CO_2 gas can be dissolved into water contained by sludge when CO_2 was pressurized. When CO_2 gas was pressurized to 10 bars like CO2 10 150, CO_2 gas was dissolved in water without CO_2 gas hydrates formation. When CO2 gas was pressurized to 20 bars like CO2 20 150, CO_2 gas was dissolved in water and formed CO_2 gas hydrates. Therefore, it was found out that the CO_2 gas hydrates formation condition in the sludge is the condition with over 20 bar at 0.2 ℃. The samples with CO_2 gas hydrates formation, such as CO2 20 150 and CO2 35 150 had significantly larger temperature change during QFGD than without CO_2 gas hydrates formation, such as CO2 10 150. This was because of the significantly large energy transfer due to CO_2 gas hydrates formation and dissociation. However, there was no difference between the samples with and without CO_2 gas liquefaction, such as between CO2 20 150 and CO2 35 150. Therefore, gas liquefaction did cause too small energy transfer to change temperature.

Considering CO_2 gas hydrate formation and CO_2 gas liquefaction, the samples could be separated to 4 cases.

Class I.	Control
Class II.	N2 35 150, CO2 10 150
Class III.	CO2 20 150
Class IV.	CO2 35 150, CO2 35 300

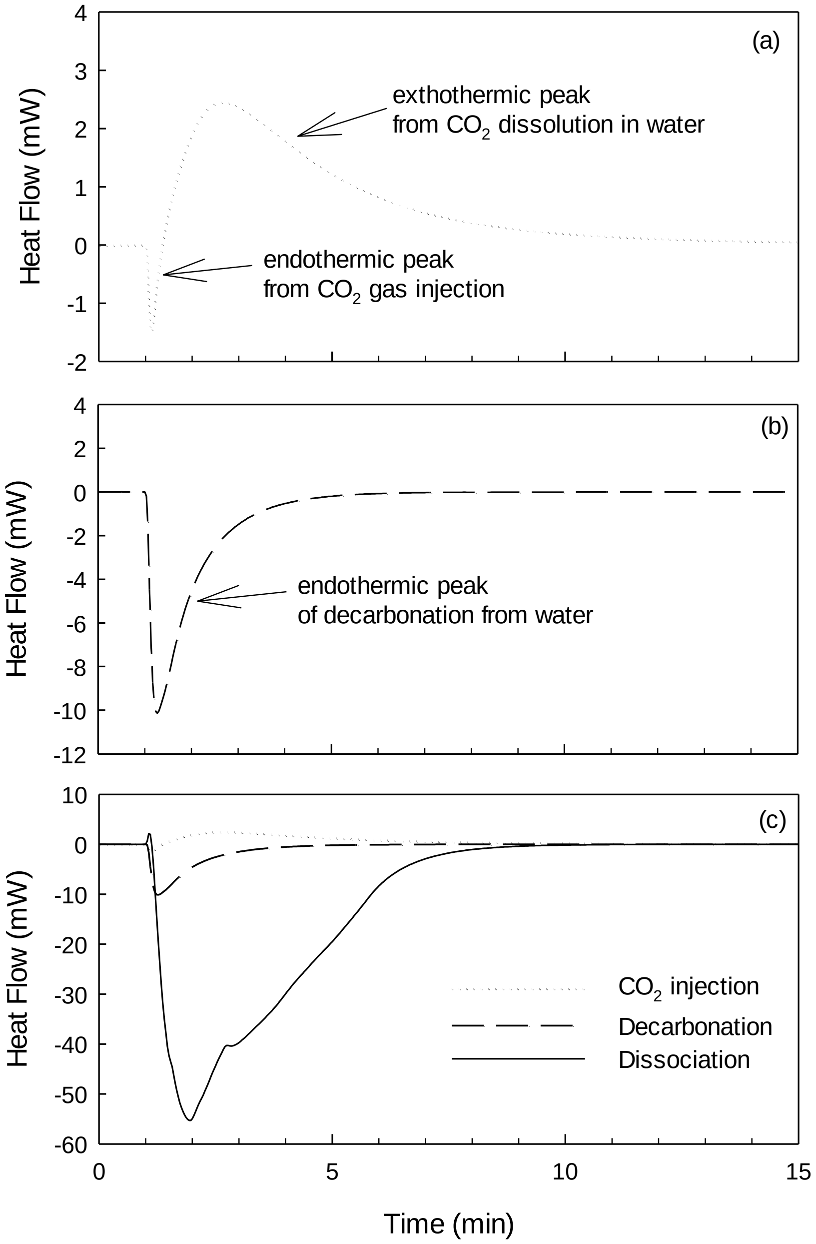


Figure 19. Changes in the heat flow during (a) CO_2 injection followed by CO_2 dissolution, (b) evacuation followed by decarbonation, and (c) comparison with evacuation followed by CO_2 hydrate dissociation at 2.0 MPa and 275.15 K[9]

S. Han et al.[9] compared the heat caused by CO_2 injection, CO_2 dissociation, and decarbonation. In figure 19, the changes in the heat flow during (a) CO_2 injection followed by CO_2 dissolution, (b) evacuation followed by decarbonation, and (c) comparison with evacuation followed by CO_2 hydrate dissociation at 2.0 MPa and 275.15 K. the heat for CO_2 gas hydrates dissociation

(500.13 J/ g water) is much higher than for decarbonation (34.48 J/g water), and the heat caused by Joule-Thomson effect is negligible. Therefore, gas hydrates dissociation energy is the main driving force for quick freezing. In my study, quick freezing was occurred in class III and IV (with CO_2 gas hydrate formation and dissociation). The quick freezing of sludge might be mainly caused by CO_2 gas hydrate dissociation energy.

4.2 The effect of QFGD on organic materials in sludge

4.2.1 Organic materials distribution

The activated sludge is formed by a connected structure of biological micro-flocs, which are composed primarily of three-dimensional, gel-like, highly hydrated extracellular polymeric substances (EPS).[48] Considering water distribution in Figure 5., water can be categorized into 4 parts, free water with a little interaction between water and sludge flocs, interstitial water trapped between sludge flocs, surface water bound on the sludge surface, and bound water positioned in microbial cell. The binding strength between water and sludge flocs increases: free water < interstitial water < surface water < bound water. For improving sludge dewaterability, sludge flocs have to be disintegrated and water with higher interaction has to be released. As sludge is the complex compound with organic materials, sludge disintegration causes organic materials released. Therefore, it is an indirect indicator to compare the distribution of organic materials. In the conventional freezing method[18], when ice is formed out of microbial cell, cell becomes dehydrated due to express and elute effect. In express effect, ice out of microbial cell grows and pressurizes microbial cell. By this pressurization, water in the cell can be released. In elute effect, when ice is formed on the surface of sludge, ice can grow by forming ice using water drawn from the cell. This process makes cell dehydrated and organic materials released[18]. If ice is formed inside of cell, cell is ruptured with inner volume increase and cell is dehydrated. Therefore, the release of organic materials is the indirect indicator of dewatering in sludge freezing process.

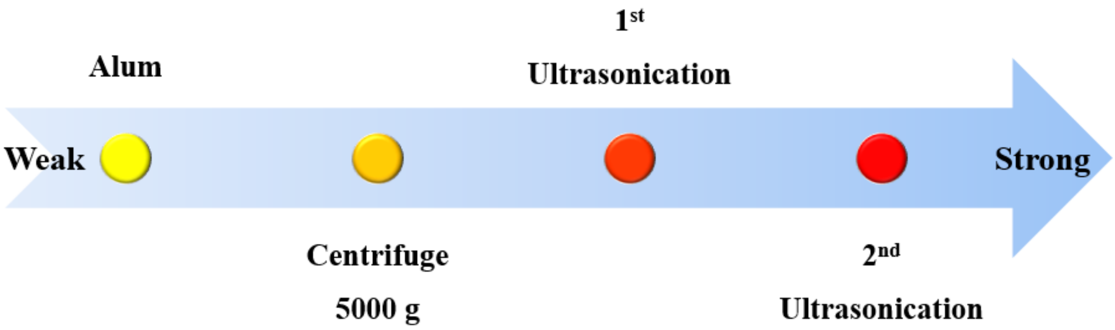
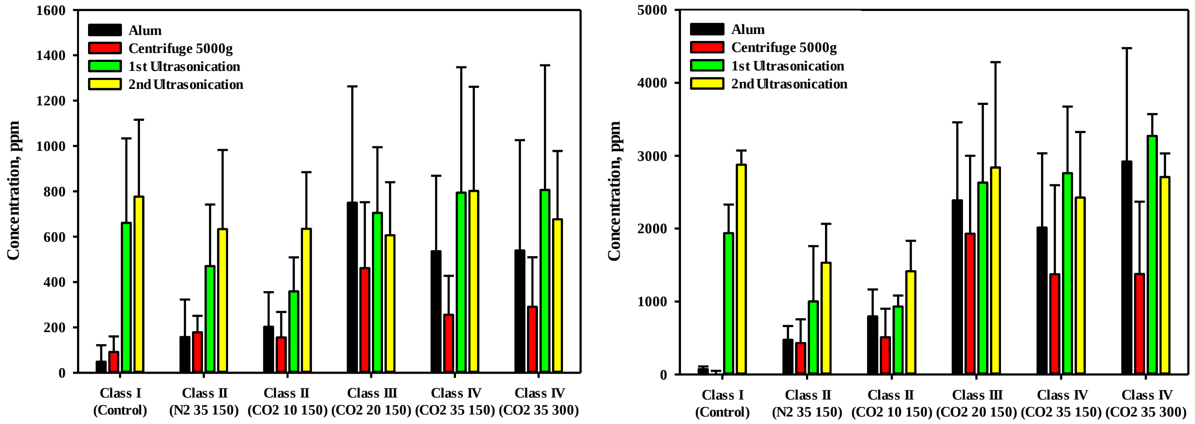
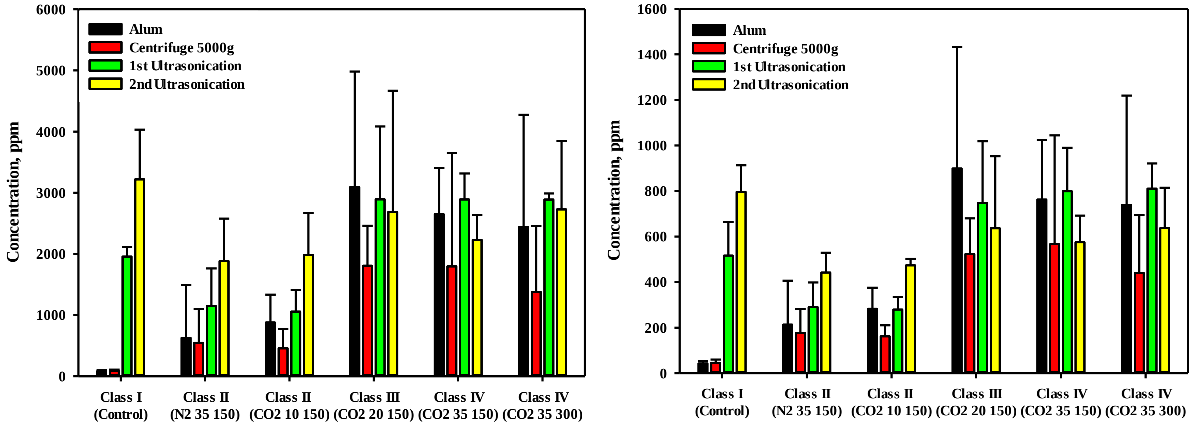


Figure 20. 4 separation steps applying different separation strength



a. Polysaccharides

b. Protein



c. Total organic carbon

d. Total nitrogen

Figure 21. Distribution of organic compounds in the concentrated sludge. (The separation strength increased: Alum, Centrifuge 5000 g, 1st Ultrasonication, 2nd Ultrasonication)

To compare the concentration of organic distribution in sludge, organic solutions were gradually separated with increasing separation strength (Figure 20): Alum step was the separation using centrifuge (2000 g, 45 min, 20 ℃) with alum coagulation, Centrifuge 5000 g step was the separation using centrifuge (5000 g, 30 min, 20 ℃), 1st Ultrasonication was the separation using Ultrasonication (288000 W) and centrifuge (20000 g, 20 min, 20 ℃), and 2nd Ultrasonication was the separation using Ultrasonication (288000 W) and centrifuge (20000 g, 20 min, 20 ℃). Mechanical dewatering can separate only free water. This means mechanical dewatering cannot attack sludge and release internal water. Therefore, mechanical dewatering, such as Alum step and Centrifuge 5000 g step cannot separate tightly-bound organic materials. As Ultrasonication method has been used for sludge disintegration, 1st Ultrasonication and 2nd Ultrasonication disintegrate sludge and make tightly-bound organic materials released. Figure 21-a. shows the concentration of polysaccharides in each separation step. Figure 21-b. shows the concentration of protein in each separation step. Figure 21-c. shows the concentration of total organic carbon in each separation step. Figure 21-d. shows the concentration of total nitrogen in each separation step. All kinds of organic materials shows same trends: as separation strength increases, the separated organic concentration also increased or was stable. In class I, same as Control, the organic concentration separated from Alum and Centrifuge 5000 g steps was low and the organic concentration separated from 1st Ultrasonication and 2nd Ultrasonication increased significantly. This means that organic materials are distributed into low concentration as soluble organic materials and high concentration as tightly-bound organic materials. In class II, including N2 35 150 and CO2 10 150, the organic concentration separated from Alum and Centrifuge 5000 g steps was higher than class I and the organic concentration separated from 1st Ultrasonication and 2nd Ultrasonication increased slightly. This means class II treatment, including stirring, pressure shift, and CO_2 dissolution, causes the release of tightly-bound organic materials into soluble organic materials. In class III, including CO2 20 150, the organic concentration separated from Alum and Centrifuge 5000 g steps was significantly higher than class I and II. And, the organic concentration separated from 1st Ultrasonication and 2nd Ultrasonication slightly increased or was stable. This means the release of tightly bound organic materials was caused by class III treatment including stirring, pressure shift, CO_2 dissolution, and CO_2 gas hydrates. In class IV, including CO2 35 150 and CO2 35 300, the organic concentration separated from Alum and Centrifuge 5000 g steps was similar with class III and the organic concentration separated from 1st Ultrasonication and 2nd Ultrasonication was also similar with class III. This means the release of tightly bound organic materials was caused by class IV treatment including stirring, pressure shift, CO_2 dissolution, CO_2 gas hydrates, and CO_2 liquefaction. As there was no significant difference between trends of organic materials in class III and class IV, CO_2 liquefaction did not affect the change in organic material distribution.

When sludge was disintegrated and released water, organic materials distribution was also changed. the distribution of organic materials is the indirect indicator to show the sludge disintegration. According to Figure 21-a~d., there were some differences with different treatment. Compared to class I, class II has slightly higher concentration of soluble organic materials and class III and IV had significantly higher concentration of soluble organic materials. As class II is the treatment including stirring, pressure shift, and CO_2 dissolution, some sludge particles can be disintegrated and release as soluble organic materials. In cases of class III, as large amount of organic materials was released, and CO_2 gas hydrates were formed, large amount of sludge flocs were disintegrated by CO_2 QFGD. In class IV, as the distribution of organic materials was similar with class III, large amount of sludge flocs was disintegrated similar with class III. As there was no difference between class III and class IV, sludge disintegration by CO_2 gas liquefaction was negligible. Therefore, it was found out that QFGD causes sludge disintegration and gas liquefaction had little effect on sludge disintegration.

4.2.2 Chemical properties of supernatant

The release of organic materials was detected by the change of sludge properties, such as surface charge, osmotic pressure and conductivity.

Sample	Raw	Control	N2 35 150	CO2 10 150	CO2 20 150	CO2 35 150	CO2 35 300
Average	-13.10	-11.12	-15.40	-15.30	-16.63	-21.00	-15.73
Standard deviation	1.127	0.743	0.400	1.916	0.833	2.258	0.666

Table 4. Zeta potential of sludge particles treated by each treatment (Sample preparation: After centrifuge (5000 g, 30 min, 20 ℃), the supernatant was filtered.

As sludge is the complex with various organic, inorganic, and microbial, sludge has surface charge and surface charge affects sludge flocculation and disintegration. In this study, surface charge of sludge particles was measured by zeta potential. Zeta potential is a diffuse double layer on the surface of particles. It can be measured based on electrophoretic mobility in electric field. If the surface charge of particles has different surface charge, particles has different velocity in the electric field and the surface charge can be calculated from its velocity. The sample was the filtrate prepared with centrifuge (5000 g, 30 min, 20 ℃) and filtration (0.8 µm syringe filter). in the filtrate, sludge fine particles smaller than 0.8 µm was used. In class I, Control, the zeta potential was -11.12 mV. The reason why surface charge of the control particle is negative was explained by Y. Liu, et al. (2003)[22]. Sludge is composed of organic materials. Due to the ionization of anionic functional group in the organic materials, sludge has negative surface charge. In class II including N2 35 150 and CO2 10 150, surface charge was more negative (N2 35 150: -15.40 mV, CO2 10 150: -15.30 mV) than class I. this is because stirring, pressure shift, and gas dissolution makes sludge disintegrated and release organic materials. The released organic materials presented around sludge and made surface charge more negative. In class III including CO2 20 150, surface charge became more negative (-16.30 mV). This surface charge was due to released organic materials by stirring, pressure shift, gas dissolution, and QFGD. And, in class IV including CO2 35 150 and CO2 35 300, the surface charge also became negative (CO2 35 150: -21.00 mV, CO2 35 300: -15.73 mV) due to stirring, pressure shift, gas dissolution, QFGD, and gas liquefaction. As there is little difference between surface charges of class III and IV, gas liquefaction seems not to be effective to sludge disintegration. Therefore, stirring, pressure shift, gas dissolution, QFGD, and gas liquefaction caused sludge surface charge more negative.

In general, acid treatment causes proton to neutralize sludge surface charge. As the neutralized surface has less attraction with water and reduces electrostatic repulsion between sludge flocs, acid treatment was effective to sludge flocculation. [12, 49] In the other hand, base treatment makes sludge surface charge more negative. As more negatively charged sludge flocs makes the electrostatic force increase, sludge flocs can disintegrate easily.[50] In this study, QFGD makes sludge surface charge more negative. As sludge surface charge becomes more negative, the particles are trying to be separated and interstitial water can be released. Therefore, QFGD makes the surface charge more negative by sludge disintegration.

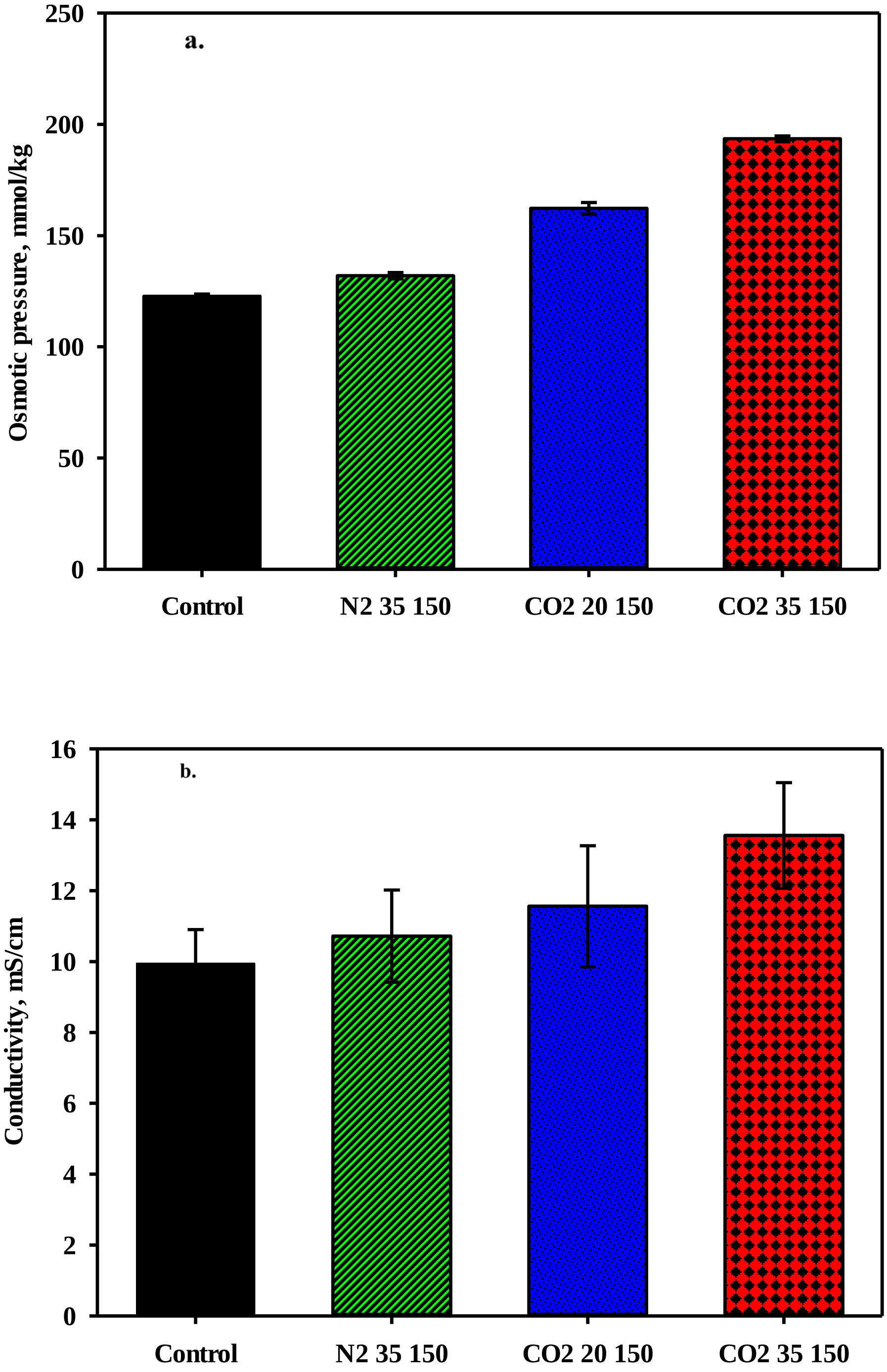


Figure. 22. Osmotic pressure and conductivity of the filtrate separated from the sludge samples (Sample preparation: After sludge sample was centrifuged (5000 g, 30 min, 20 ℃), the supernatant was filtered using 0.8 μm syringe filter)

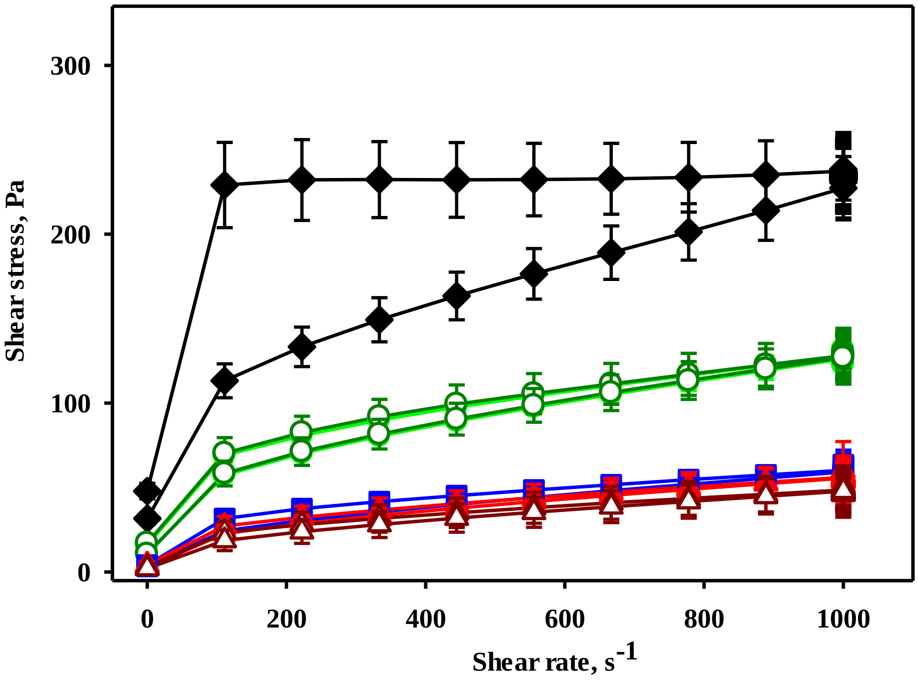
The release of organic materials was detected by the change of osmotic pressure and conductivity in the supernatant. In Figure. 22-a, osmotic pressure in class I, Control was the lowest

(122.8 mmol/kg). Osmotic pressure of class II, N2 35 150 (132.0 mmol/kg) was higher than osmotic pressure of class I. The released organic materials induced by stirring, pressure shift, and CO_2 dissolution caused the osmotic pressure of supernatant increased. Osmotic pressure of class III, CO2 20 150 (162.3 mmol/kg) was much higher than class II. This is because QFGD causes more severe sludge disintegration. Osmotic pressure of class IV, CO2 35 150 (193.5 mmol/kg) was much higher than class III. This result shows CO_2 gas liquefaction makes osmotic pressure of the supernatant increase.

In Figure. 22-b, conductivity in class I, Control was the lowest (9.836mS/cm). Conductivity of class II, N2 35 150 (10.571 mS/cm) was higher than conductivity of class I. The released organic materials induced by stirring, pressure shift, and CO_2 dissolution caused the conductivity of supernatant increased. Conductivity of class III, CO2 20 150 (11.390 mS/cm) was much higher than class II. This is because QFGD causes more severe sludge disintegration. Conductivity of class IV, CO2 35 150 (13.388 mS/cm) was much higher than class III. This result shows CO_2 gas liquefaction makes conductivity of the supernatant increase.

From the analysis of osmotic pressure and conductivity, it was found out that the released organic materials make the supernatant have higher osmotic pressure and conductivity. Through stirring, pressure shift, and CO_2 dissolution, the organic concentration in the supernatant increased. Through QFGD, the organic concentration in the supernatant increased much more. However, there was no effect of CO_2 liquefaction on osmotic pressure and conductivity in the supernatant. Therefore, it was found out that QFGD causes much more release of organic materials.

4.3 The effect of QFGD on sludge rheology



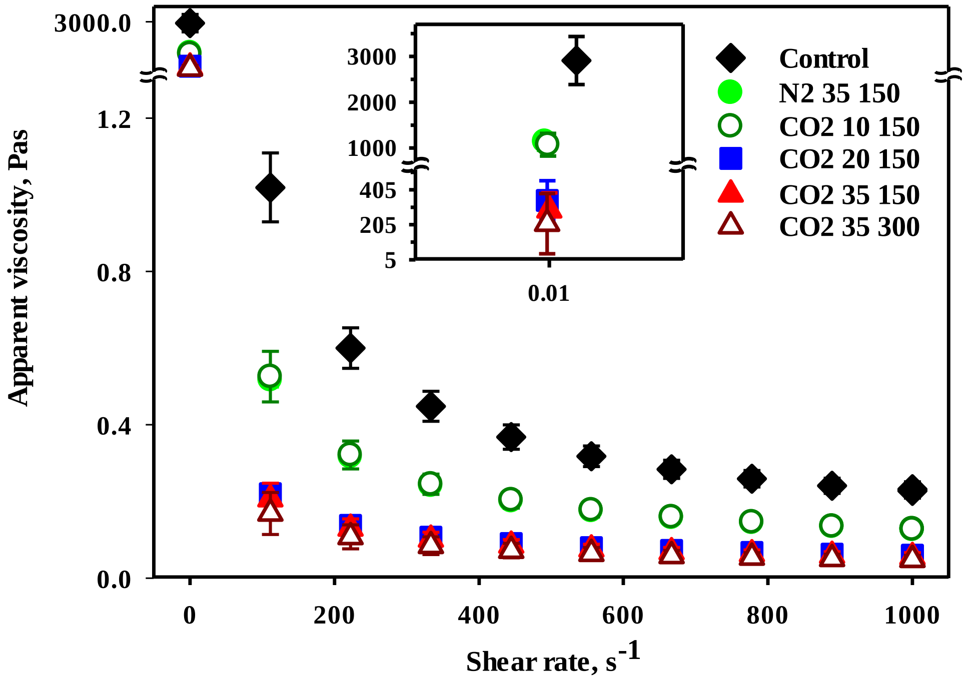


Figure 23. Hysteresis loop and viscosity of sludge for rheology analysis (Measurement process: Shear stress was modified: equilibrate when shear rate keeps at 0.1 s^{-1}, increase until shear rate becomes 1000 s^{-1}, equilibrate when shear rate keeps at 1000 s^{-1}, and decrease until shear rate becomes 0.1 s^{-1})

Sludge has complicatedly connected organic pocket containing bound water. For releasing bound water, the structure of organic compound has to be damaged. Jibao, et al.[12] was found out that microwave and acid treatment causes the inner sludge structure destroyed. In this study, the effect of QFGD on inner sludge structure though rheology. Hysteresis loop was formed by collecting shear rate and shear stress with changing shear stress. Hysteresis loop means thixotropy. When sludge is applied by external force, the inner structure of sludge becomes destroyed. And, after the maximum stress was reached and the external force was removed, the inner structure was recovered. As sludge is non-instantaneous, hysteresis loop is formed. The area of hysteresis loop means the integrity of inner structure in sludge.

In Figure. 23, as sludge is non-Newtonian behavior, all sludge samples have a nonlinear shear rate- shear stress graph. Sludge is elastoviscosity, which has both viscosity and elasticity. As shear stress increases, shear rate of sludge increases. As shear stress decreases, shear rate of sludge decreases. This is because of sludge's viscosity. In the same shear rate, shear stress in the shear stress increase period is bigger than in the shear stress decrease period. This is because of sludge's elasticity; sludge tends to maintain its state with the resistance to modification. In the same shear rate, shear stress in class III and IV was bigger than in class I and II. The area of hysteresis loop increases: Class I > Class II > Class III ≒ Class IV. Apparent viscosity also increases: Class I > Class II > Class III ≒ Class IV. As the inner structure changes was affected by micro-structure changes, it is less sensitive to modification. Through stirring, pressure shift, and CO_2 dissolution, intact inner structure was destroyed and thixotropy decreased. Through QFGD, as much more inner structure breaks down and thixotropy also decreased. Therefore, it was found out that QFGD destroys the inner structure.

4.4 The effect of QFGD on sludge dewatering

In the organic materials distribution, it was found out that sludge was disintegrated, and organic materials was released through QFGD. As the release of organic materials represents sludge disintegrated and sludge disintegration causes inner water dewatered, it was found out that QFGD disintegrates sludge flocs and improves sludge dewatering. Commonly, sludge dewatering could be measured by 3 methods: settlement test, filtering test, and centrifuge test. As free water is included in mechanical processes and mechanical process separates only free water, these analytic methods can measure sludge dewatering effect by measuring how sludge can be separated into free water and solid parts.

4.4.1 Dewatering test based on settlement

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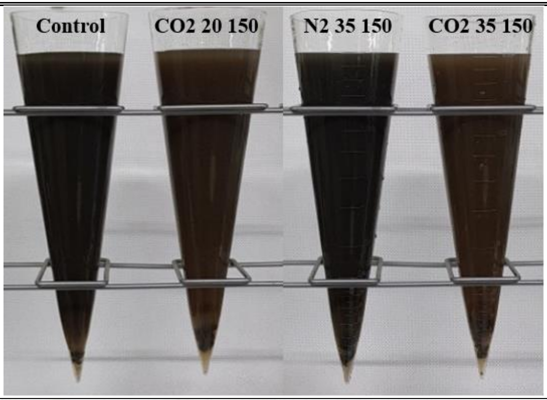
<tr><td>1 min</td><td>
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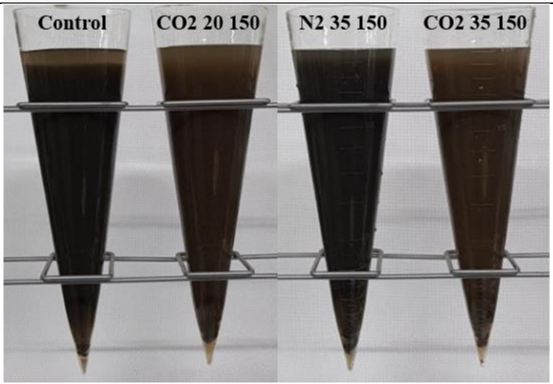
<tr><td>2 min</td><td>
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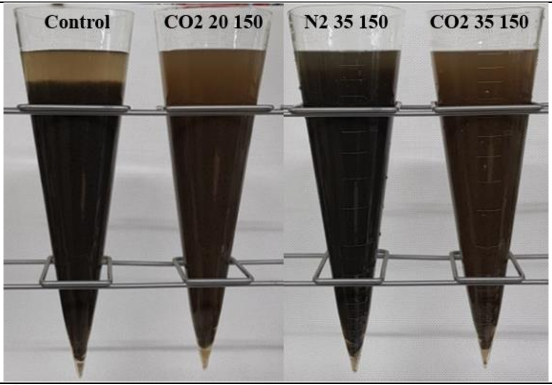
<tr><td>3 min</td><td>
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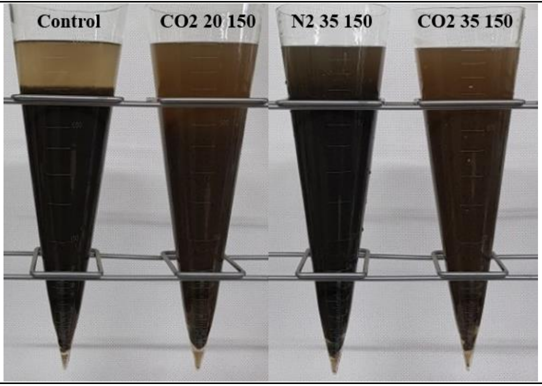
<tr><td>4 min</td><td>
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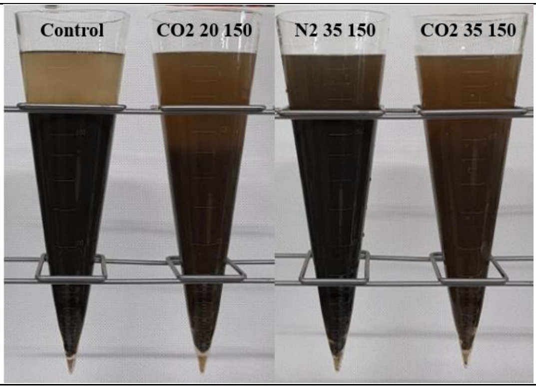
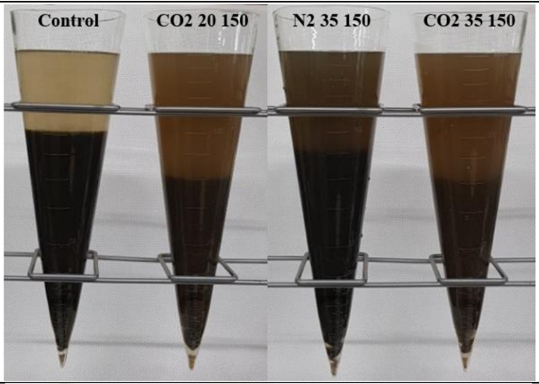
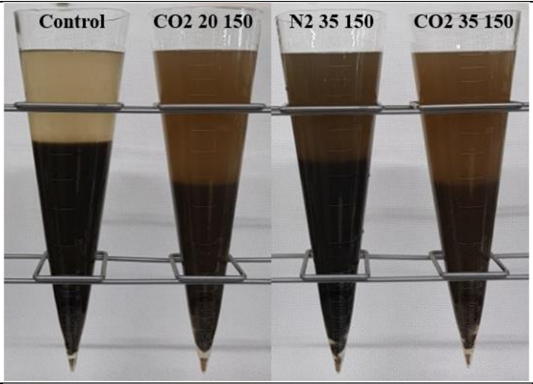
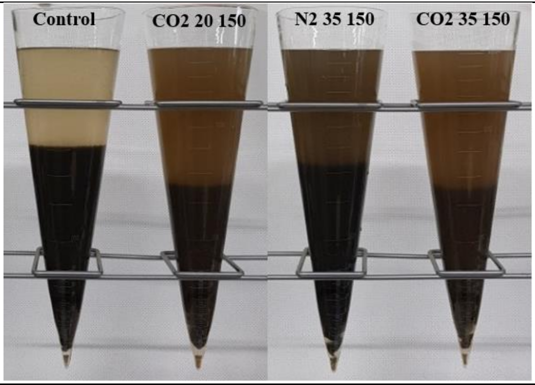
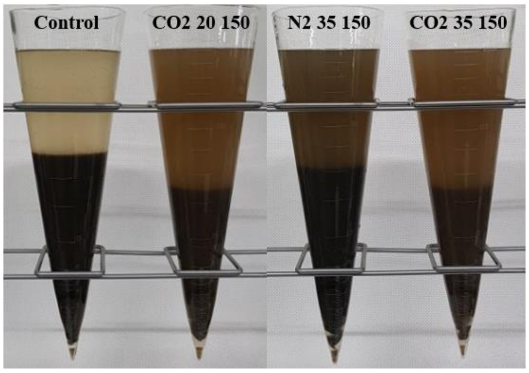
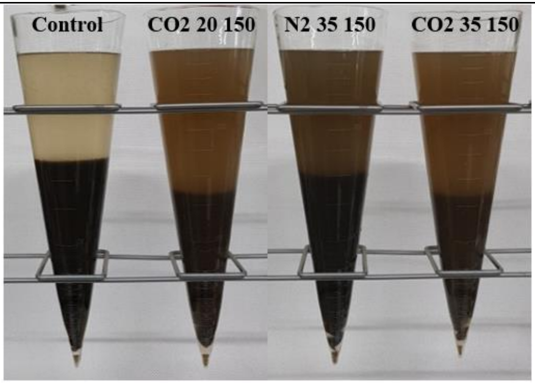
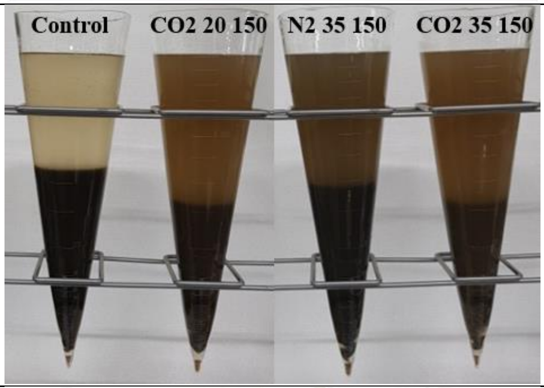
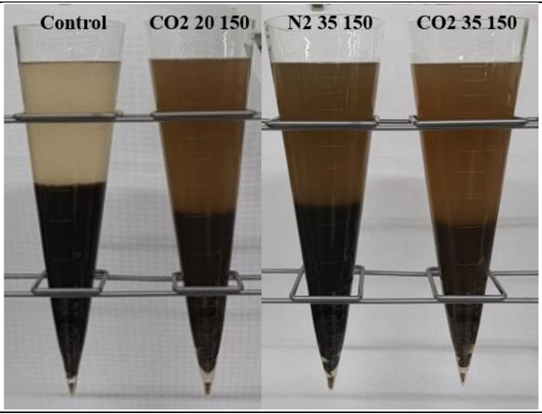
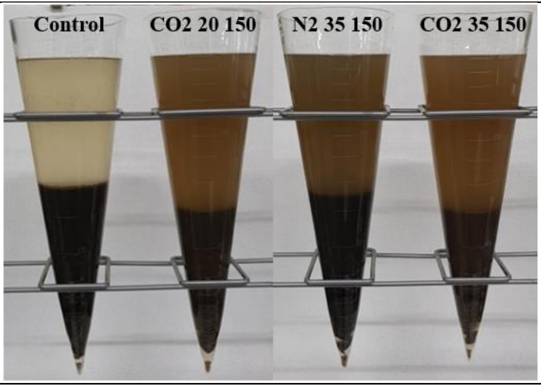
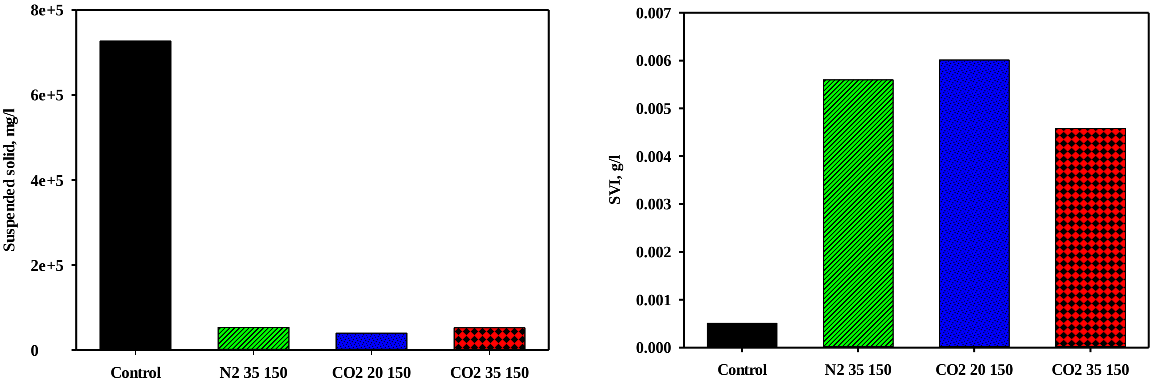
5 min	
10 min	
15 min	
20 min	

25 min	
30 min	
40 min	
60 min	

70 min	

a. Images during gravitational settling using centrifuged sludge pellet and the supernatant(sludge pellet preparation: centrifuge(5000 g, 30 min, 20 ℃); the supernatant preparation: gravitational settling: 12 h gravitational settlement)

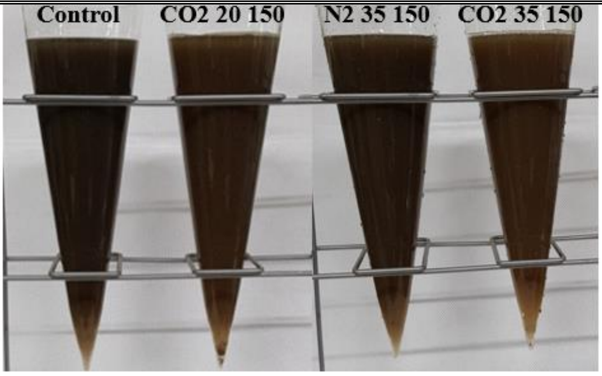
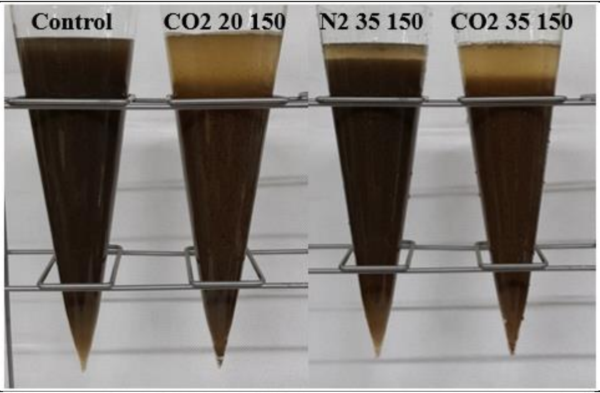
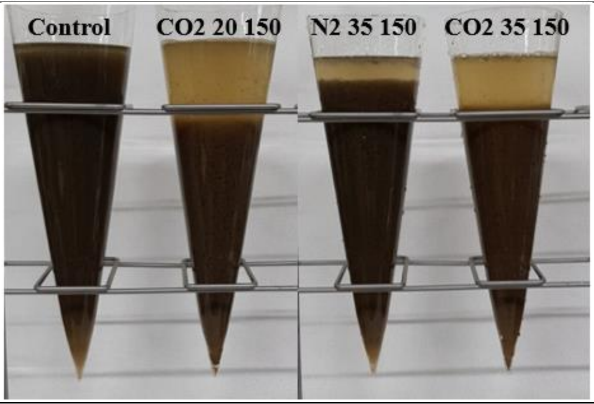


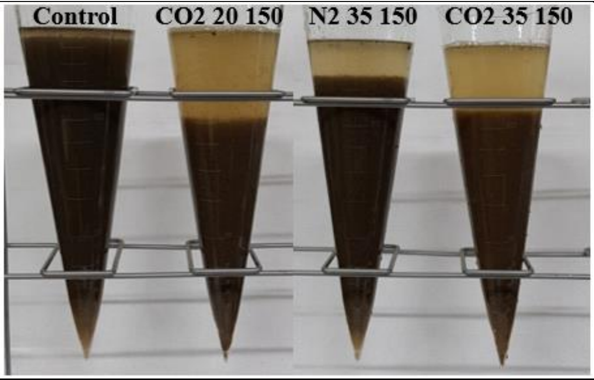
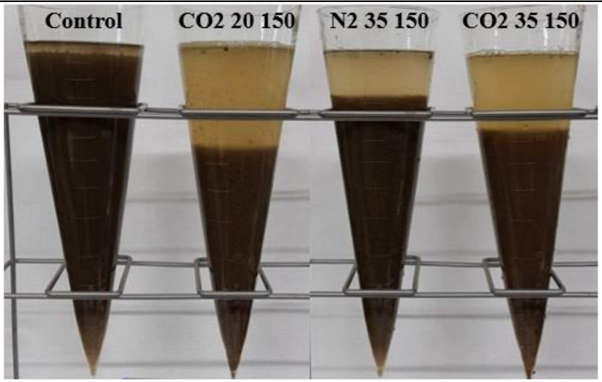
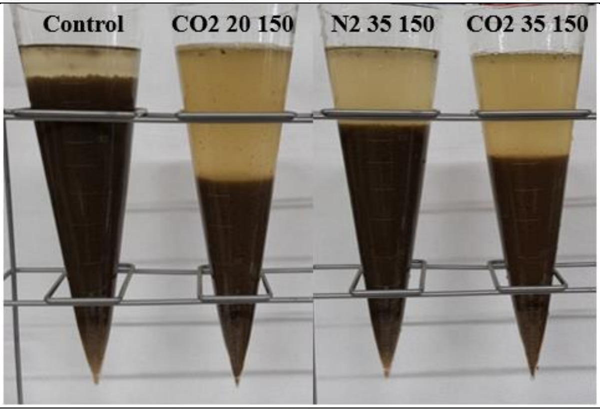
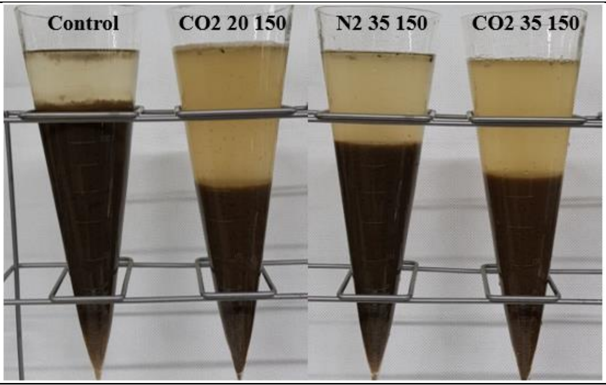
b. Suspended solid for each samples (vacuum filtration, the amount of sample: 0.5 g, filtration time: 10 min, pore size of filter: 0.8 μm)

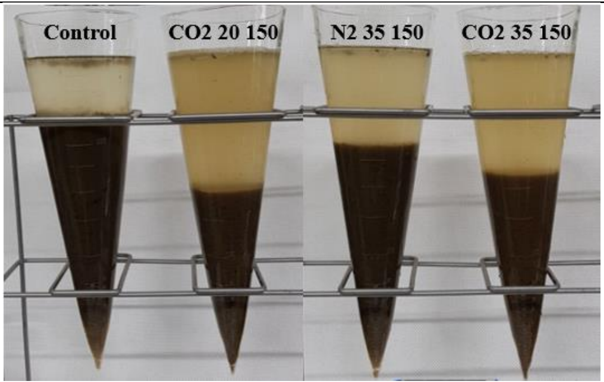
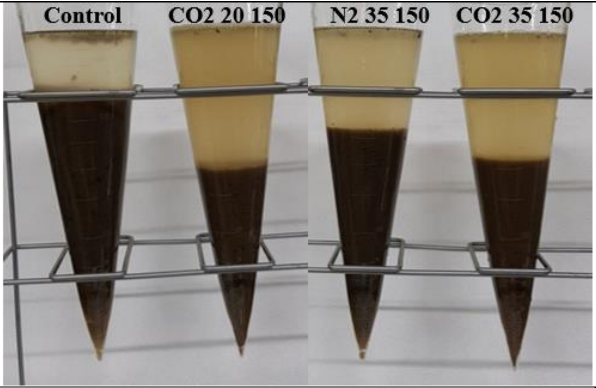
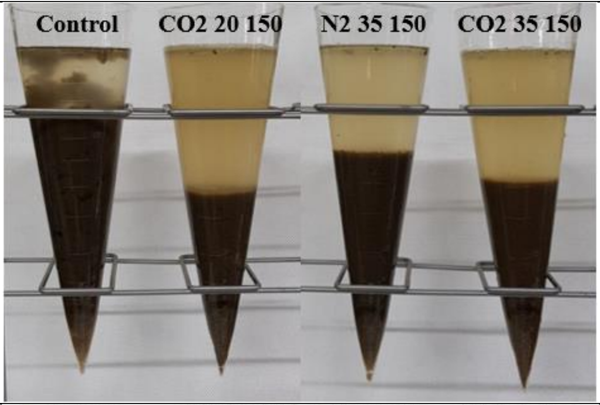
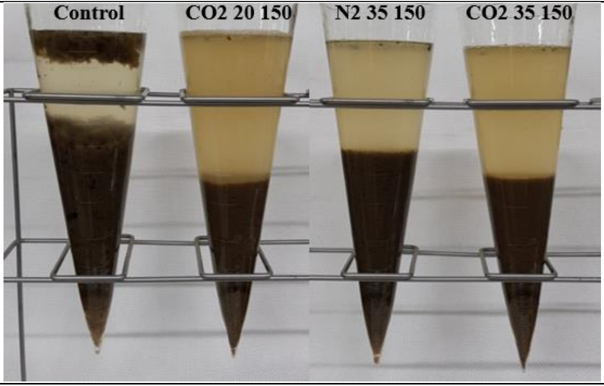
c. SVI as the result of gravitational settling using centrifuged sludge pellet and the supernatant

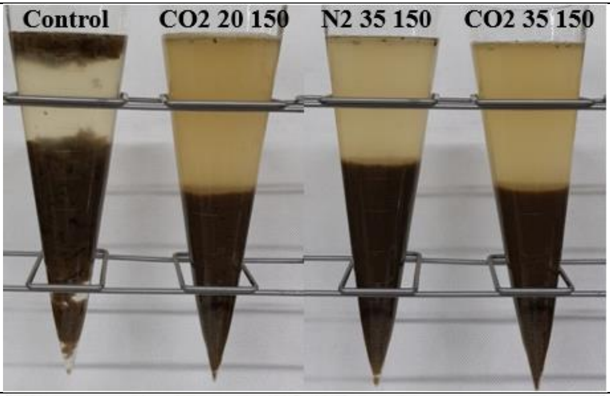
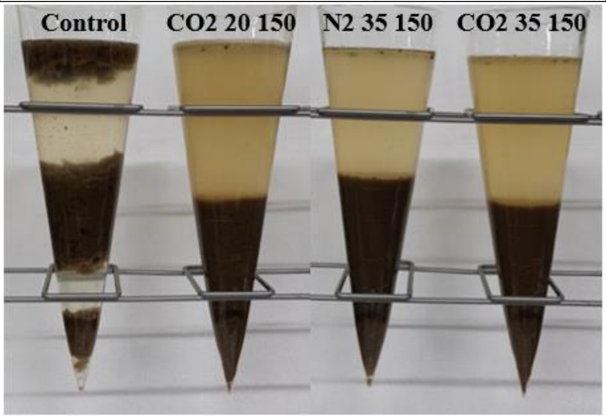
Figure 24. Settlement analysis of the mixture of centrifuged pellet and gravitationally settled supernatant.

First analysis for sludge dewatering was free settling test. Free settling test is the method which compares solid volume during settlement. Among sludge treatments, settlement is used for reducing the amount of treated sludge and it has an advantage to lower energy consumption. Therefore, sludge settlement is highly related with dewatering efficiency. In this study, the mixture of centrifuged solid and gravitational supernatant was settled. As sludge was centrifuged, the released organic materials and water was removed. In figure 24-a. the images for solid-liquid separation were compared by different settling time. In the same settling time, the settled volume for class II (N2 35 150) was higher than class I (control). On the other hand, in the images for class III and IV (with QFGD treatment) for each settling time, the settled volume lowered than other treatment. As QFGD treatment divided sludge particles into fine particles and they could be dispersed into supernatant, the settled volume for class III and IV were smaller than class I and II. Based on the image when settling time is 30 min, SVI was calculated in Figure 24-c. SVI for class I (control) was significantly lower than for other treatment. This can be understood by considering suspended solid (Figure 24-b). As suspended solid for class I (control) is significantly higher than other treatment, SVI, which is calculated by dividing settled volume into suspended solid value, was much lower for class I (control) than for other treatment.

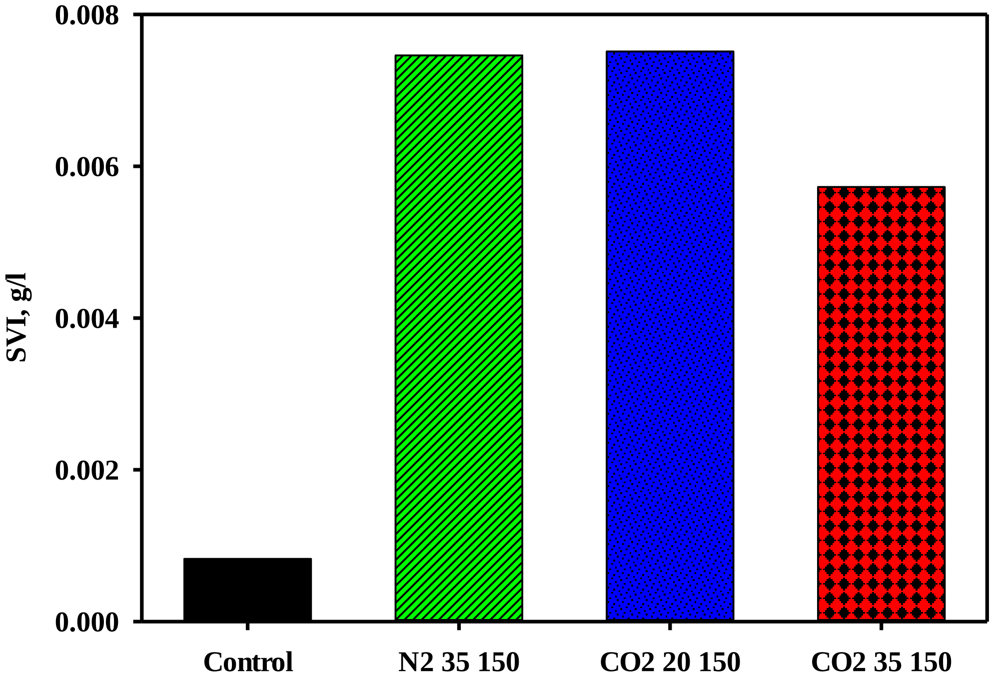
Settling time	Image
1 min	
2 min	
3 min	

4 min	
5 min	
10 min	
15 min	

20 min	
25 min	
30 min	
40 min	

60 min	
70 min	

a. Images during gravitational settling with alum coagulation



b. SVI for each sample as the results of gravitational settling-alum coagulation

Figure 25. Images of the coagulated solid and supernatant separation and SVI during the coagulation – settlement test

Second settlement test was done with alum coagulation. Coagulation has been used to improve settlement. In this test, after sludge was mixed with 1 L of about 14 g/L aluminum sulfate (Alum) aquatic solution, the mixture was gravitationally settled. The table in Figure. 25-a showed that the images for solid and liquid separation by gravitational settling with alum coagulation. Settled volume for class III and IV treatment were lower than class I and II for each settling time. This was because fine particles, separated from sludge flocs, were released and dispersed into the supernatant. In Figure. 25-b, the SVI values for each treatment was calculated. The trend between SVI values for each treatment was similar with the result for first settlement test. SVI for class I (control) was significantly lower than for other treatment.

As the result from 2 settlement tests, as QFGD treatment made sludge flocs disintegrated and fine particles, separated from sludge flocs, were dispersed in the supernatant, the final settled volume was much lower than class I and II. And, the supernatant containing high organic compound can be utilized for cultivation. Therefore, QFGD treatment makes treatment loading decrease caused by settled volume reduction and produces the liquid with high organic compound, which can be helpful for utilizing as a fertilizer.

4.4.2 Sludge dewatering based on filtration

Sludge dewaterability has been quantified by filter-based dewatering test. CST[12, 21, 28, 32, 34, 51, 52] and filtering test[10, 11, 19, 53-55] has been used to measure the sludge dewatering effect.

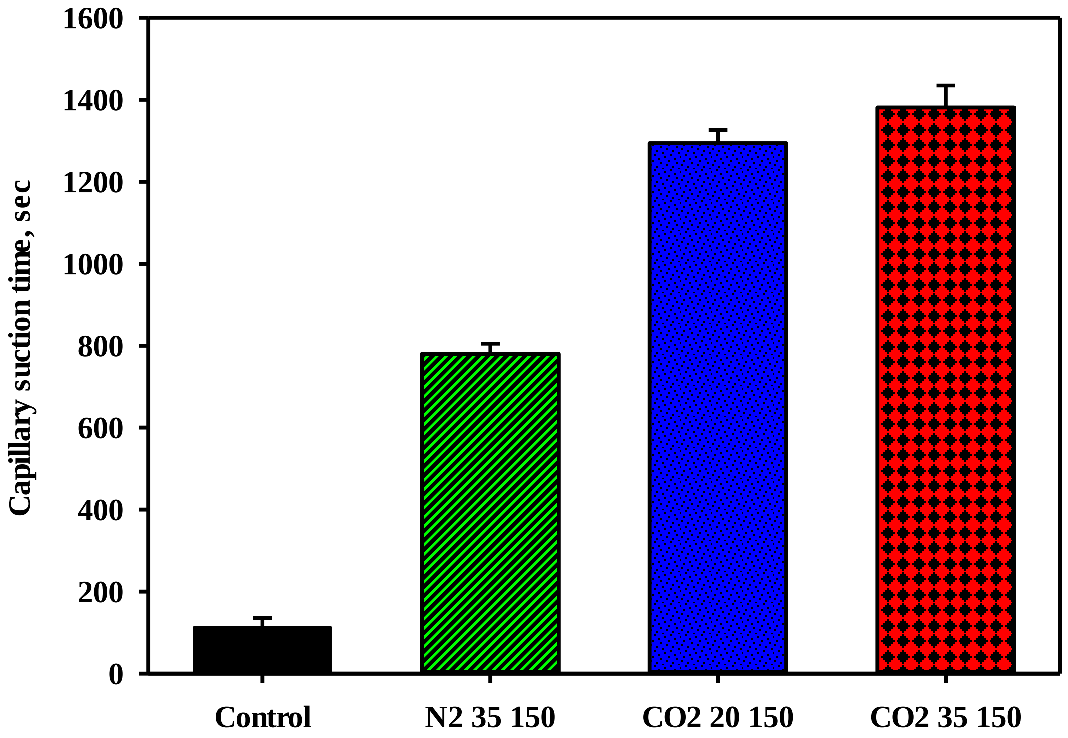
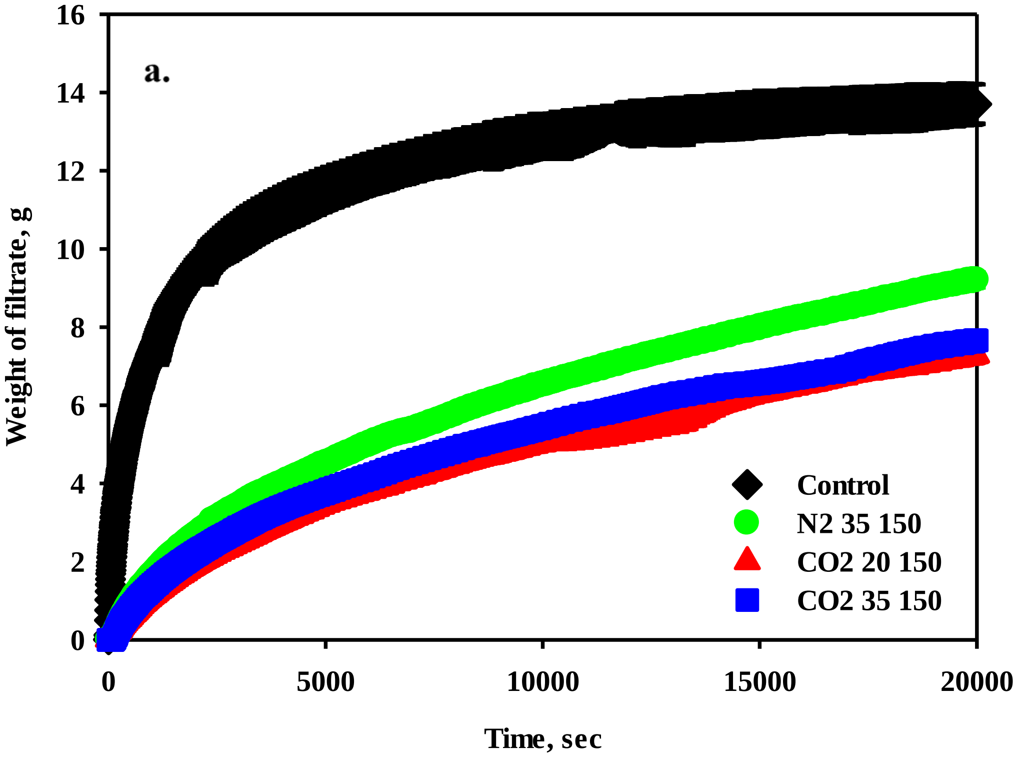


Figure 26. CST of sludge samples

CST has been used for measuring sludge dewaterability. CST method is the method, which the liquid separated from sludge passes through a filter and the time for the liquid to pass from one point to the other point. sludge the time was measured. As CST increases, it means that the liquid takes more time to pass the filter and the sludge has higher bind strength between water and sludge floc. In Figure. 24, CST of class II (N2 35 150, 780.233 sec) increases than of class I (Control, 113.17 sec). CST of class III (CO2 20 150, 1294.267 sec) increases than of class I and II. And, CST of class IV (CO2 35 150, 1381.100 sec) slightly increases than of class III. This result was because of sludge disintegration.[22] As sludge was disintegrated, organic materials were released, and fine particles were released. As organic materials were released, the viscosity of sludge increased, and it prevented liquid from flowing through the sludge and filter. The fine particles disintegrated from sludge can block the filter pore and makes the filtrate pass the filter pore. As Class II, including stirring, pressure shift, and

CO_2 liquefaction, released some organic materials, the viscosity increased, and more fine particles can be formed by sludge disintegration. As the result, CST increases. In class III, as QFGD made much more sludge disintegration, it can cause sludge viscosity increase and more fine particles formed. As the result, CST increased significantly than class I and II. In class IV, CST was similar with CST of class III. This means CO_2 liquefaction did not an effect on CST. Therefore, although stirring, pressure shift, and CO_2 liquefaction cause CST increase, QFGD could makes CST significantly increase.



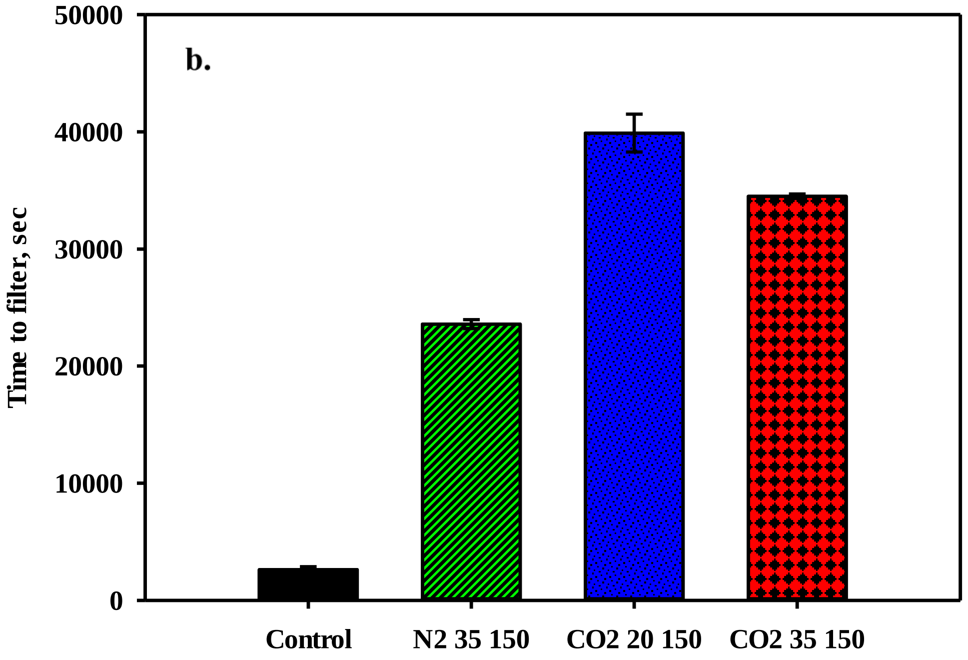


Figure 27. The weigh of filtrate with filtering time and TTF of sludge samples through the filtering test with 0.45 µm filter. a: The wieght of filtrate change with time, b: TTF from the filtering test)

Filtering test and TTF were done for measuring sludge filtering rate. The faster the filtrate is separated, and the larger sludge filtering rate is. In Figure. 27-a, the weight of filtrate increased with filtering time in all classes. The filtrate of class I (Control) was separated significantly faster and more filtrate was collected than the other classes. However, the filtrate of class II (N2 35 150) was slower than class I. And, the filtrates of class III (CO2 20 150) and class IV (CO2 35 150) were separated much slower than class I and II. This results also because of sludge disintegration. As sludge is disintegrated and organic materials are released, it makes sludge viscosity increase and fine particles released. In Figure. 27-b, TTF of class I (Control) was the smallest. TTF of class II (N2 35 150) showed significant increase than class I. And, TTF of class III (CO2 20 150) and IV (CO2 35 150) were much smaller than of class III. As sludge disintegration makes organic materials released causing sludge viscosity increase and fine particles separated from sludge, the filtrate separation rate increased in class II. The filtrate separation of class III and IV increased much faster due to more significant sludge disintegration. Therefore, QFGD makes sludge filtration slower due to increase of sludge viscosity and pore blocking.

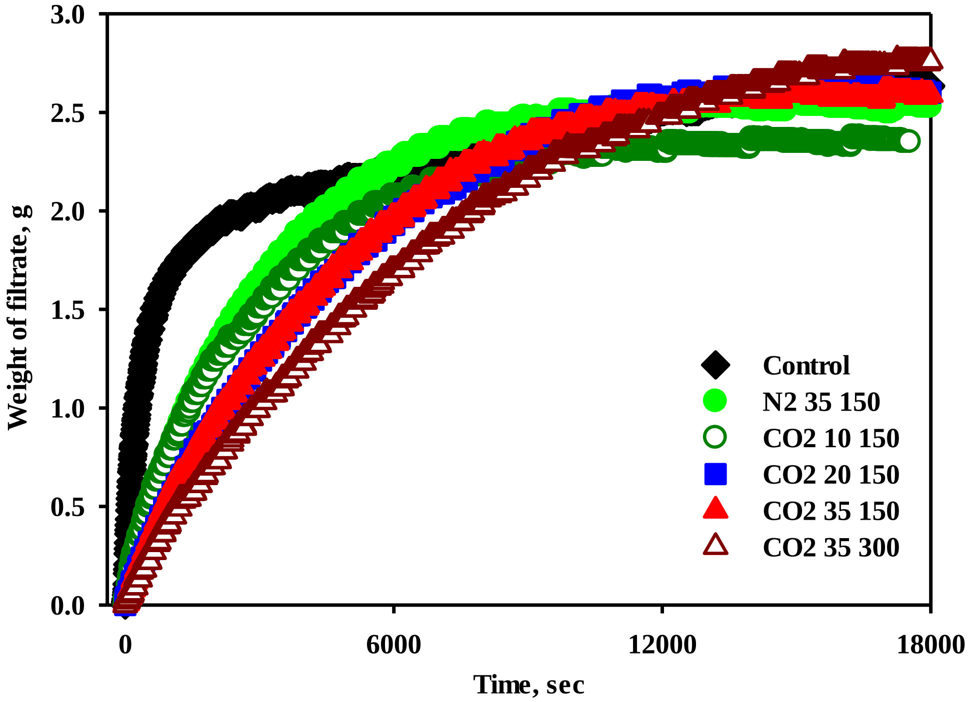


Figure 28. The weight of filtrate change with time through the filtering test with 0.2 μm filter

Filtering test using 0.2 μm filter was done to measure dewatering effect with smaller pore size. 5 g of sludge was filtered using 0.2 μm filter and the weight of filtrate was recorded with time.

In Figure. 28, the filtrates were separated at different rate with time. The filtration rate was different, and it shows the same trend like filtration using 0.45 μm filter. the filtrate of class I (Control) were separated most easily. And, the filtrate of class II (N2 35 150) was separated slower than of class I. the filtrates of class III (CO2 20 150) and class IV (CO2 35 150) were separated much slower. This result is also due to viscosity increase and pore blocking.

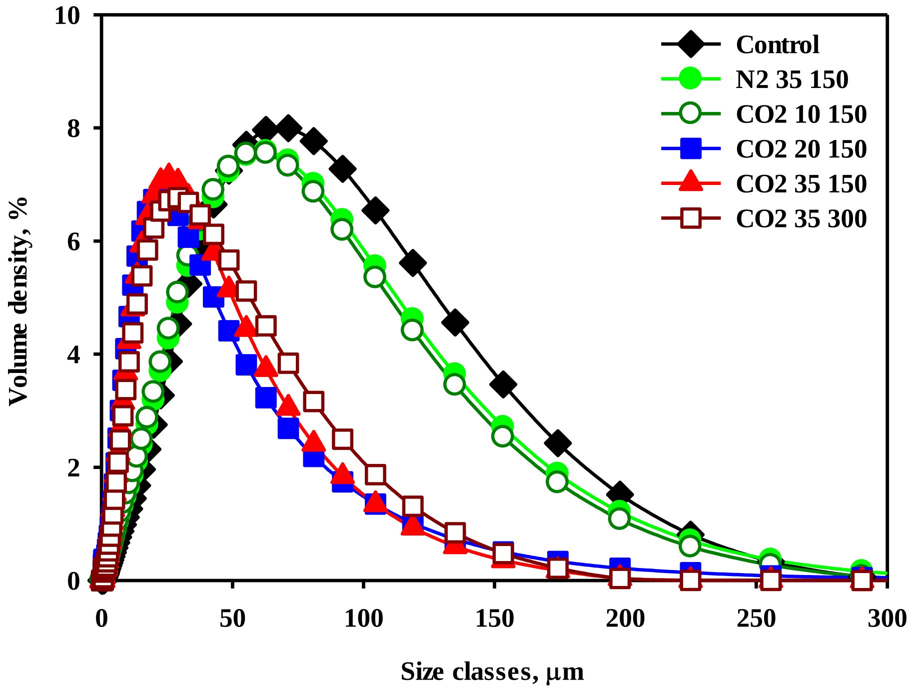
Y. Liu, et al. (2003)[22] was found out the relation between the amount of soluble organic materials and sludge dewatering effect with respect to filter-based dewatering test. If the amount of soluble organic materials increases to a certain amount, the organic materials makes sludge more flocculated and sludge dewatering effect increase. However, it there is much more amount of soluble organic materials, sludge becomes more hydrophilic and sludge dewatering effect decrease. Therefore, the dewatering measurement based on filter has limitation to represent sludge dewatering effect.

4.5 Understanding of dewatering test based on filtration

Sludge dewatering test based on filter can be affected by sludge viscosity and pore blocking. Therefore, sludge dewatering test based on filter cannot represent the dewatering effect all range of sludge. Through CST and filtering test, all treatments, including stirring, pressure shift, CO_2 dissolution, QFGD, and CO_2 liquefaction made CST increase and filtration rate slower. However, sludge settlement was significantly improved by QFGD. To understand sludge dewatering effect, sludge particle size distribution and resistance test were done.

4.5.1 Particle size distribution

Particle size distribution was measured in μm and nm range. In μm range particle size distribution measurement, sludge was measured by particle sizer. And, in nm range particle size distribution measurement, after sludge was centrifuged (5000 g, 30min, 20 ℃), the supernatant was filtered using 0.8 μm syringe filter. sludge particles whose size is smaller than 0.8 μm in the filtrate was used to measure size distribution in nm range.



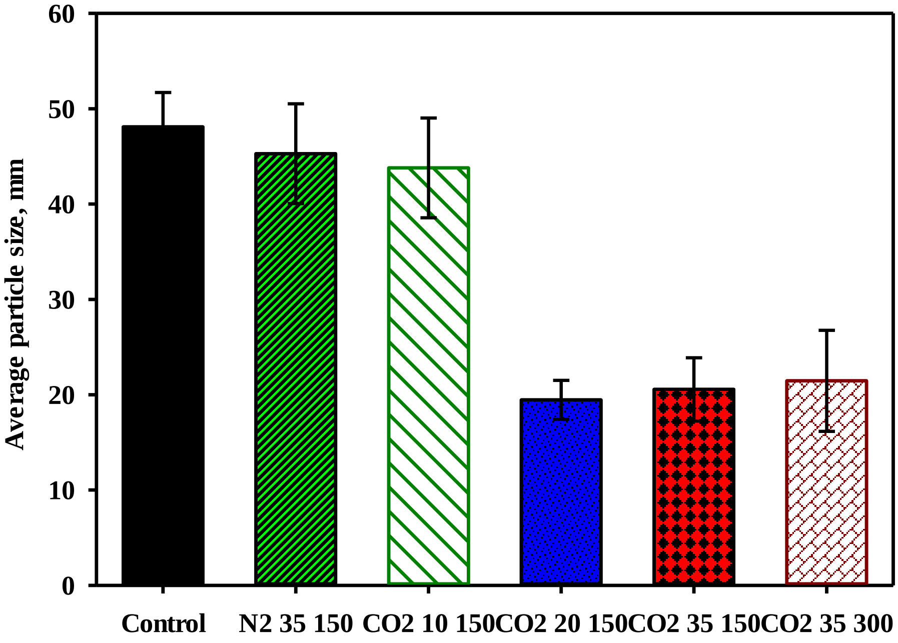
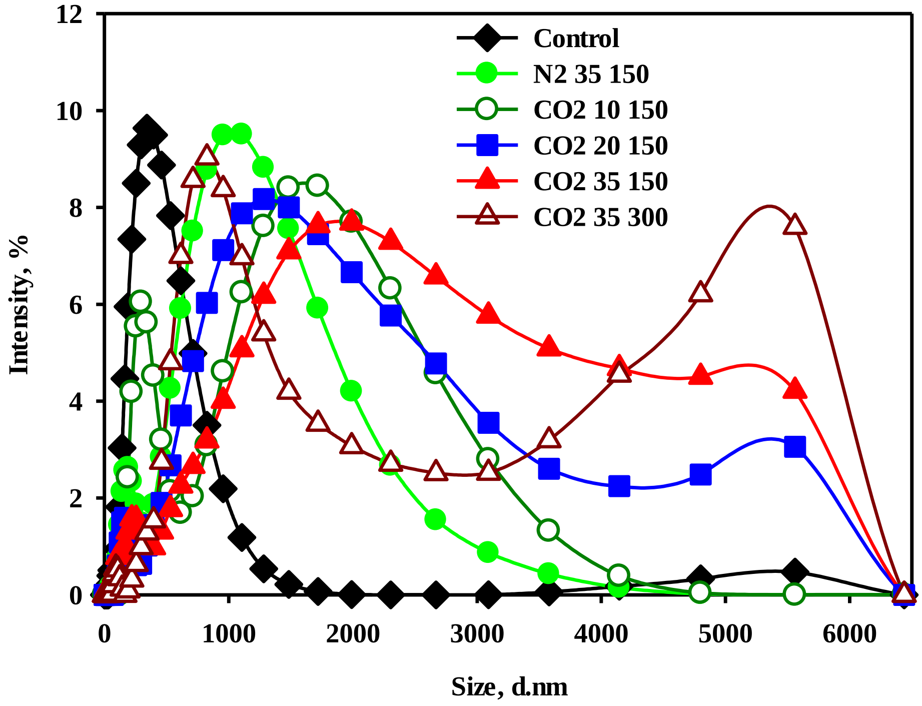
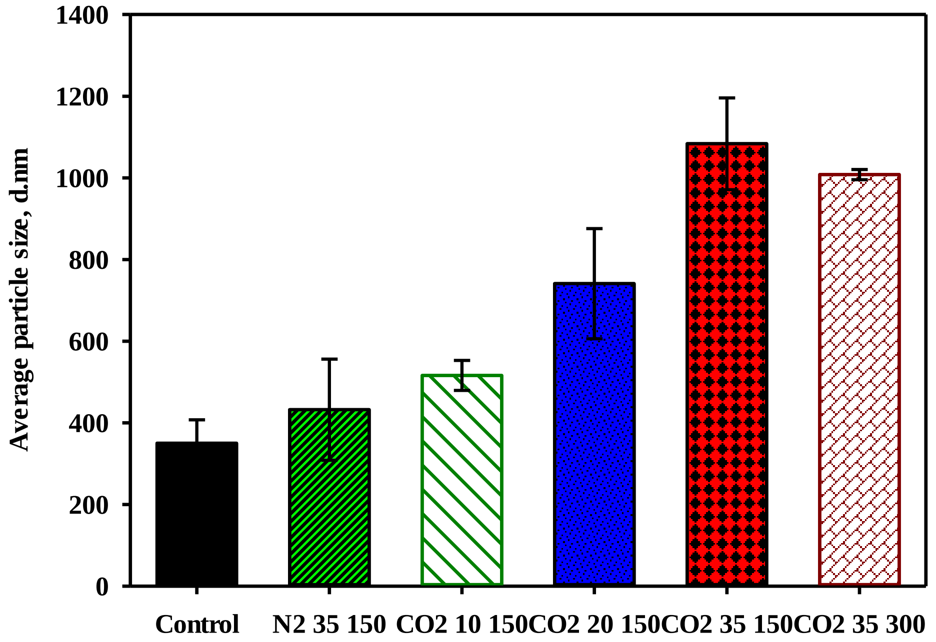


Figure 29. Particle size distribution (above) and average particle size (below) of sludge in μm range

Figure. 29 showed the sludge particle size distribution in μm range. Class I (control) sample had particle size distribution in largest size range and average particle size was 55.5 μm. Class II (N2 35 150 and CO2 10 150) samples had a particle size distribution in smaller size range than class I and average particle size was 49 μm at N2 35 150 and 47.5 μm at CO2 10 150. This result was because 3 treatments including stirring, pressure shift, and CO_2 dissolution made sludge flocs disintegrated. Class III (CO2 20 150) sample had a particle size distribution in significantly smaller size range than class II and average particle size was 20.9 μm. And, Class IV (CO2 35 150 and CO2 35 300) samples had a particle size distribution in similar size range with class III and average particle size was 22.9 μm at CO2 35 150 and 25.2 μm at CO2 35 300. This means QFGD caused significant sludge disintegration and CO_2 liquefaction did not significantly effect on particle size change. Therefore, through the sludge particle size distribution in μm range, it was found out that QFGD makes sludge flocs disintegrated significantly.


Control
N2 35 150
CO2 10 150
CO2 20 150
CO2 35 150
CO2 35 300
Intensity, %
0
2
4
6
8
10
12
0
1000
2000
3000
4000
5000
6000
Size, d.nm


Average particle size, d.nm
0
200
400
600
800
1000
1200
1400
Control
N2 35 150
CO2 10 150
CO2 20 150
CO2 35 150
CO2 35 300

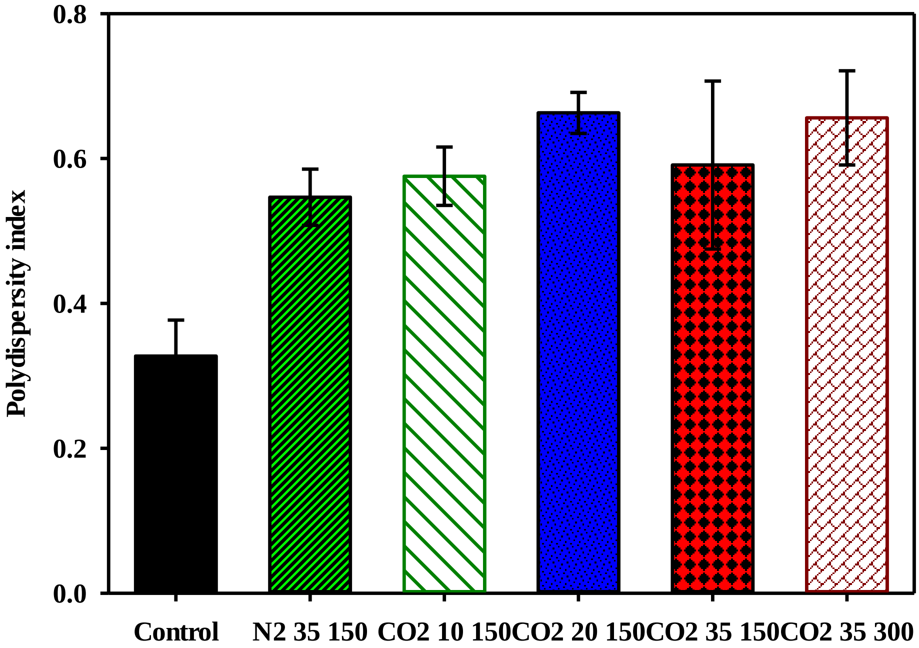


Figure 30. Particle size distribution, average particle size, and Polydispersity of sludge particles filtered 0.8 μm filter

To measure the particle size distribution in particles smaller than 0.8 μm, the particle size distribution in nm range was measured. Figure 30. Showed particle size distribution, average particle size, and Polydispersity of sludge samples. Polydispersity (PDI) means the degree of graph dispersity. If PDI increases, more various size of particles presents in sample. If PDI decreases, less various size of particles presents in sample.

Sludge in class I (Control) has only a single peak in less than 1000 nm range. Sludge in class II (N2 35 150 and CO2 10 150) had two peaks including small peak in less than 1000 nm range and large peak in less than 4000 nm range. Sludge in class III (CO2 20 150) has three peaks including the smallest peak in less than 1000 nm, largest peak in less than 4000 nm, and the other peak in larger than 4000 nm. Sludge in class IV (CO2 35 150 and CO2 35 300) had tree peaks including the smallest peak in less than 1000 nm, the largest peak in less than 4000 nm, and the other peak in larger than 4000 nm. In class I, there was one peak in less than 1000 nm range. This peak can be considered due to originally present fine particles. In class II, there was two peaks. One peak in less than 1000 nm can be considered due to originally present fine particles. And, the other peak can be considered due to fine particles produced by sludge disintegration. Considering the particle size distribution in μm range, as sludge was disintegrated by stirring, pressure shift, and CO_2 dissolution, sludge can be divided into fine particles in a nm range. By this disintegration, sludge in class II can have 2 peaks in a nm range particle size distribution. In class III, there were three peaks. One peaks in less than 1000 nm can be considered due to originally present fine particles and the remaining two peaks can be considered due to fine particles produced by stirring, pressure shift, CO_2 dissolution, and QFGD. In class IV, there were three peaks. One peaks in less than 1000 nm can be considered due to originally present fine particles and the remaining two peaks can be considered due to fine particles produced by stirring, pressure shift, CO_2 dissolution, QFGD, and CO_2 liquefaction. However, there was no difference between the particle size distributions of class III and IV, CO_2 liquefaction did not influence on sludge disintegration.

The average particle size of class I was the smallest, 282.2 nm and its PDI was 0.315. The average particle sizes of class II (N2 35 150 and CO2 10 150) were 519.8 nm in N2 35 150 and 542.3 nm in CO2 10 150. Their PDI were 0.574 in N2 35 150 and 0.604 in CO2 10 150. As fine particles were produced by sludge disintegration caused by stirring, pressure shift, and CO_2 dissolution, average particle sizes in class II was larger than in class I and PDIs also increased. The average particle size of class III and IV were 836.4 nm in CO2 20 150, 1004 nm in CO2 35 150, and 999.4 nm in CO2 35 300. Their PDIs were 0.643 in CO2 20 150, 0.673 in CO2 35 150, and 0.61 in CO2 35 300. The average particle sizes in class III and IV was larger than in class I and II due to sludge disintegration caused by stirring, pressure shift, CO_2 dissolution, and QFGD in class III, and stirring, pressure shift, CO_2 dissolution, QFGD, and CO_2 liquefaction in class IV. As more sludge flocs were disintegrated by QFGD or CO_2 liquefaction and more fine particles present in sample, the average particle size and PDI increased than class I and II.

Therefore, through particle size distribution analysis, although stirring, pressure shift, CO_2 dissolution can disintegrate sludge flocs, QFGD can disintegrate more sludge flocs and produce the large amounts of fine particles with much more diverse size.

4.1.1 Resistance test

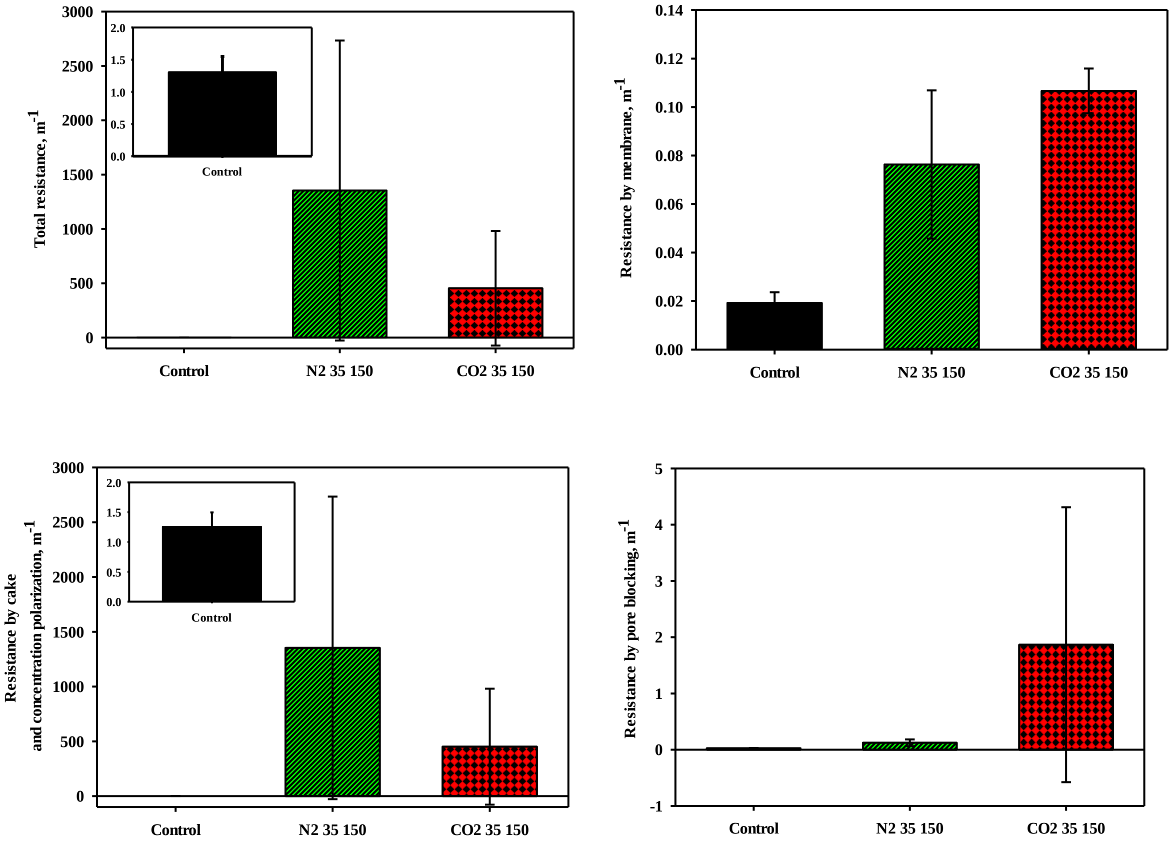


Figure 31. Resistance from sludge on the 0.2 μm filter during filtering test (Feed: DI water, pressure: 1(±0.2) bar N_2 gas)

To find out the major factor causing resistance to sludge filtering test, several types of resistance composing total resistance were compared numerically. Through sludge was filtered, and washed on the filter, sludge cake was covered on surface of filter and sludge particles were embedded in filter pore. DI filtration was employed to obtain resistances. The resistance calculated from DI filtration with pure filter was considered as the resistance due to membrane. The resistance calculated from DI filtration with sludge cake-covered filter was considered as the resistance due to sludge cake and pore blocking. The resistance calculated from DI filtration with the washed filter was considered as the resistance due to pore blocking. The resistance due to sludge cake can be obtained by subtracting the resistance due to pore blocking from the resistance due to sludge cake and pore blocking. Total resistance was same as the resistance due to sludge cake and pore blocking.

In Figure 31, each resistance in class I (Control), II (N2 35 150) and IV (CO2 35 150) were compared separately. Total resistance in control was very small than other classes. Total resistance in class II was significantly larger than in class IV. As the resistance by membrane had some difference among classes, it was negligible. The resistance by sludge cake was almost similar with total resistance. This means that sludge cake made in class II can more inhibit filtrate from passing through the cake than in class III. As the released organic materials in class II makes the binding between sludge and water stronger, the filtrate becomes difficult to release from sludge flocs. The resistance by pore blocking in class I was the smallest. The resistance by pore blocking in class IV had significantly higher than in class II. this was because through the treatment in class IV, more sludge flocs were disintegrated, and more fine particles were formed. These fine particles were embedded during filtering test and inhibit the filtrate from flowing through the filter pore. Therefore, in class II and IV, the major reason why the sludge dewatering effect decrease was sludge cake. As the pore blocking by fine particles less affected on sludge resistance than sludge cake, the resistance in class IV was relatively more affected by pore blocking than in class II.

4.6 The effect of QFGD on disinfection

As mechanical dewatering methods, such as centrifuge, filter press, and filtering test remove free water, stronger methods to attack sludge flocs can improve sludge dewatering effect. However, much stronger dewatering methods which can make sludge microbial release water is essential to improve sludge dewatering effect. In conventional freezing method, ice formation could make microbial release water by three mechanisms: Express, Elute, and rupture. During freezing process, ice could be formed outside and inside microbial cell. When ice is formed outside microbial cell, sludge microbial cells flocculate by the exclusion of ice and release water in cell. When ice is formed on the surface of cell, the ice absorbs water to form ice continuously. As the result, as water is extracted from microbial cell, microbial cell is dewatered. When ice is formed inside cell, the inner volume of cell increases. When the inner volume increases to fatal volume with ice growth, the cell membrane becomes ruptured and water in cell is released. As the quick-freezing occurs, 3 mechanisms of freezing method on microbial cell can apply on QFGD. In this test, the disinfection effect of QFGD on sludge was tested.

Each sample were stained by live/ dead *Baclight*™ bacterial staining kit. In the kit, there are 2 dyes, green-colored SYTO 9 staining the cell with intact membrane and red-colored PI staining the cell with damaged cell. The stained sludge was observed through CLSM. CLSM can observe fluorescence stained particles in 3D image. Stained particles were counted by the software. Particles with higher intensity than 346W were sorted and each particle were divided into the particles with size less than 1.24 μm and counted.

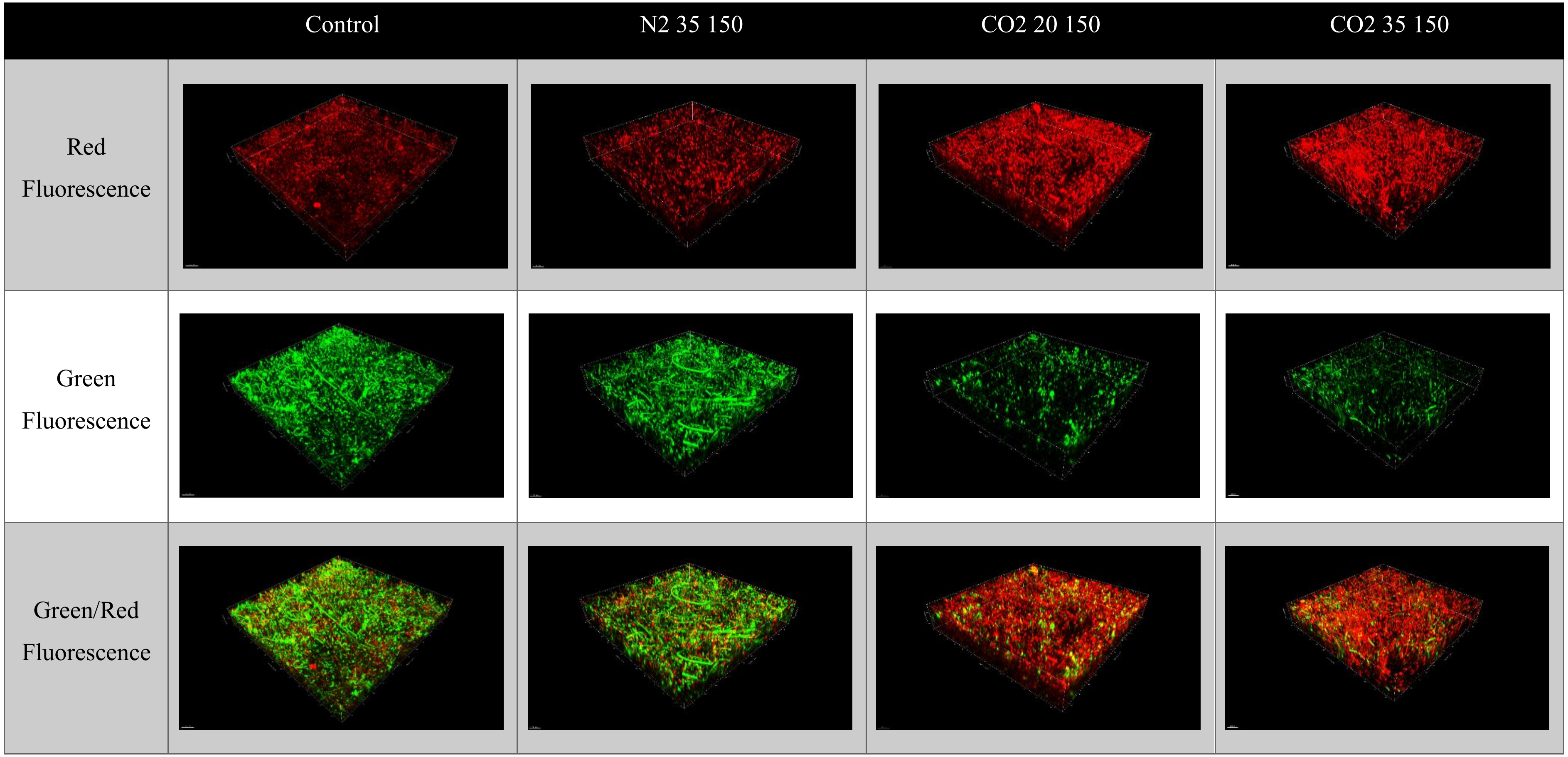


Figure 32. CLSM Images of sludge stained by Live/dead *Baclight*™ bacterial staining kit

Figure 32 showed the CLSM image of sludge. In class I (control), as green color was more intense than red color, intact cell presented more than damaged cell. And, as there were red-colored particles, there was some damaged-microbial cell in the class I sample. In class II (N2 35 150), as green color was more intense than red color, intact cell presented more than damaged cell. And, as there were red-colored particles, there was some damaged-microbial cell in the class II sample. Therefore, stirring, pressure shift, and CO_2 dissolution seem not to damage microbial cell in sludge. In class III (CO2 20 150), as red color was more intense than green color, intact cell presented less than damaged cell. And, as there were green-colored particles, there was some intact microbial cell in the class III sample. This means that QFGD method cannot make all microbial cells damaged entirely. In class IV (CO2 35 150), as red color was more intense than green color, intact cell presented less than damaged cell. And, as there were green-colored particles, there was some intact microbial cell in the class IV sample. This means that QFGD with CO_2 liquefaction method cannot make all microbial cells damaged entirely. In the CLSM image with only green-colored particles, as class increases, the intensity of green decreased. In the CLSM image with only red-colored particles, as class decreases, the intensity of red increased. Especially, in class III and IV, green intensity significantly decreased than class I and II. Red intensity in class III and IV significantly increased than in class I and II. Therefore, it was found that QFGD has the disinfection effect.

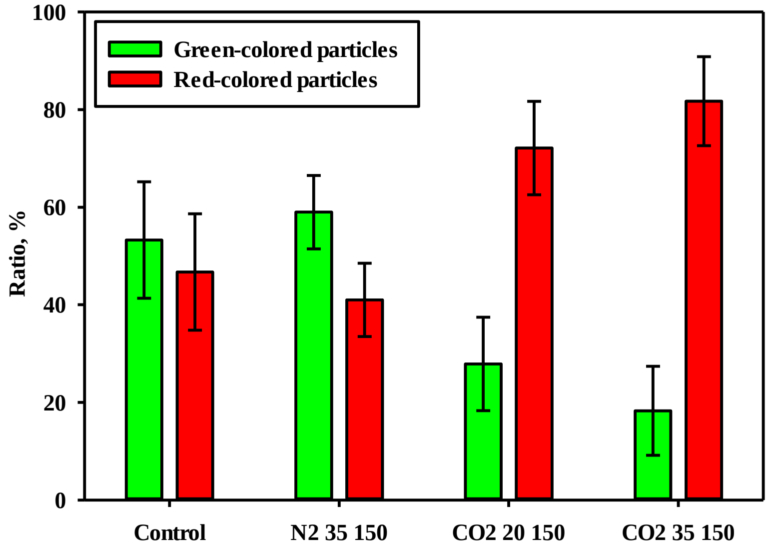


Figure 33. Ratio of green and red–colored particles in sludge through CLSM imaging analysis with Live/Dead *Baclight*™ bacterial staining kit

To compare the ratio of intact or damaged microbial cell numerically, stained microbial cell were counted. In Figure 33, Although control sample seems to have more intact microbial cell than damaged microbial cell in image, there were 55 % of intact microbial cell and 45 % of damaged microbial cell in control sample. Although class II sample seems to have more intact microbial cell than damaged microbial cell in image, there were 60 % of intact microbial cell and 40 % of damaged microbial cell in class II sample. Although class III sample seems to have more damaged microbial cell than intact microbial cell in image, there were 28 % of intact microbial cell and 72 % of damaged microbial cell in class III sample. Although class IV sample seems to have more intact microbial cell than damaged microbial cell in image, there were 18 % of intact microbial cell and 82 % of damaged microbial cell in class IV sample. Therefore, cell viability decreased from 60 % to 28 % through QFGD.

In class I sludge, the ratio of damaged microbial cell was almost 50 %. This might be caused by sample sampling, storing, staining, and measuring steps. As there was a little difference between the ratios of class I and II, stirring during reaction, pressure shift, and CO_2 dissolution could not affect cell viability. However, as there was a significant increase of damaged cell ratio in class III, QFGD could makes sludge viability decrease and this represented disinfection effect of QFGD. In class IV, as the ratio was similar with in class III, there was no effect of CO_2 liquefaction on sludge disinfection.

The structure of gas hydrates is similar with of ice and CO_2 can dissolved inside and outside microbial cell. The gas hydrates treatment has been used as the alternatives of the conventional freezing method. Therefore, this disinfection result can be understood by 3 mechanisms of the conventional freezing method.

Mechanism I	When ice forms outside cells, high concentrated solution layer is formed around cell due to ice structure. Then water inside cell moves expels from cell for osmotic gradient and cell becomes dehydrated.
Mechanism II	When ice forms on the surface of cells, ice absorbs water in cell for its growth. Then cell becomes dehydrated.
Mechanism III	when ice forms inside cell, inner cell volume becomes suddenly increases and cell membranes becomes ruptured if the volume becomes over fatal level. Then, cell releases inner water.

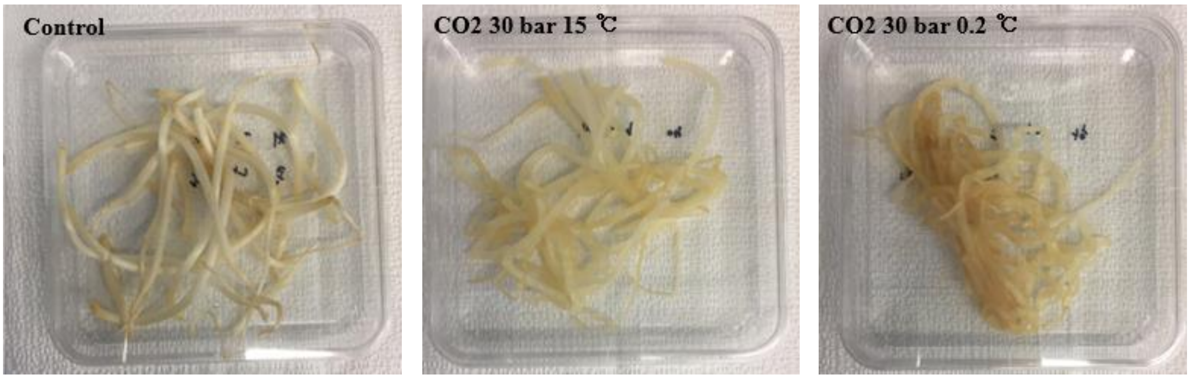
In addition to disinfection effect, cell rupture through the QFGD also induces liquid released from cell. Therefore, QFGD could improve dewatering efficiency and disinfection effect through cell rupture.

5. Conclusion

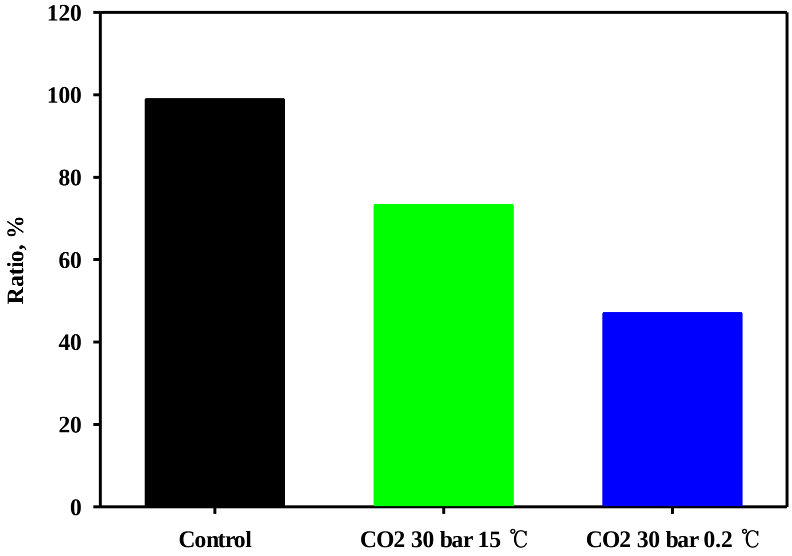
In this study, the effect of QFGD on sludge dewatering and cell rupture was investigated for improve sludge treatment. CO_2 gas hydrates were formed in the condition at 0.2(±0.1) ℃ temperature and over 20 bar pressure. Sludge sample was also frozen in that condition. In over 35 bar pressure condition at 0.2 ℃ temperature, CO_2 liquefaction occurred. Therefore, the samples were divided into 4 classes: Class I (Control), Class II (N2 35 150 and CO2 10 150), Class III (CO2 20 150), and Class IV (CO2 35 150 and CO2 35 300). As the microbial cell was ruptured by QFGD, organic materials were released from cell and made the concentration of soluble organic materials increase. And, the cell rupture induced surface charge more negative from -13.10 mV to -16.63 mV by the released organic materials. It also induced in the supernatant that conductivity increased from 10 mS/cm to 14 mS/cm and osmotic pressure increased from 122.8 mmol/kg to 162.3 mmol/kg. In rheology, as inner structure, including microbial cell, was already broken down, the area of hysteresis loop and viscosity decreased. As the inner substance, originally positioning in cell, was released by QFGD, the density of sludge increased, and coagulation could make more compact solid. As the result, the settled volume became reduced, which makes treatment loading alleviated, and the supernatant had more organic compound, which has an advantage to organic source for cultivation. Dewatering test based on filter showed sludge dewatering effect becomes poor as the ruptured microbial cell was changed into more compact sludge cake and more fine particles can block the filter pore. The resistance by sludge cake was the major factor which decreased sludge dewatering effect based on filter than pore blocking. As cell rupture, induced by QFGD, decreased the ratio of intact cells from 50 % to 28 %, QFGD had the disinfection effect on sludge. Therefore, QFGD method makes sludge treatment more efficient by reducing the treatment loading of sludge and disinfecting microbial in sludge.

6. Supplementary data

6.1 Preliminary test 1 – Soybean sprout



Supplementary data 1. Dewatered soybean sprouts after each treatment (Mass of sample: 400 g, Dewatering was done by a centrifugal dehydrator (1600 rpm, 5 min), Each treatment methods was same as the previous QFGD process.)

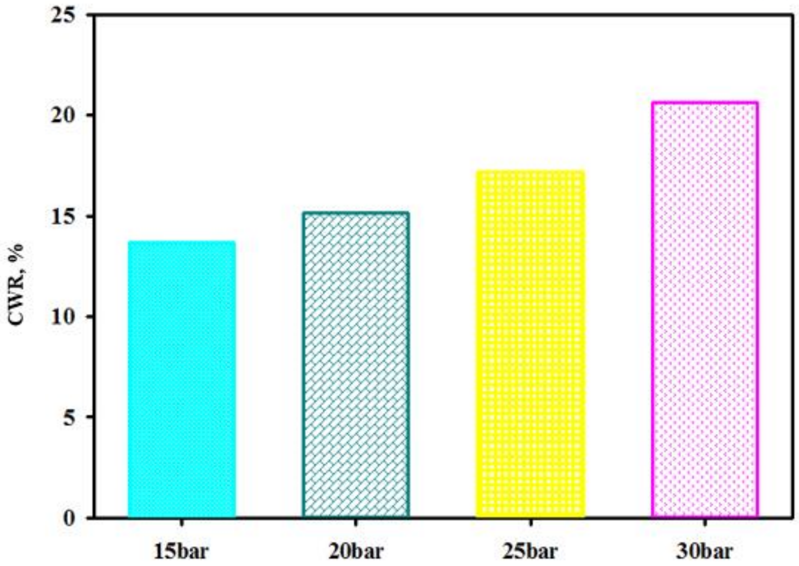


Supplementary data 2. The dewatered weight ratio of treated soybean sprout and control

Soybean sprout was treated by each treatment. The process of CO2 30 bar 15 ℃ was QFGD process which CO_2 was used as guest molecules in 30 bar pressure and 15 ℃ temperature. The process of CO2 30 bar 0.2 ℃ was the QFGD process which CO_2 was used as guest molecules in 30 bar pressure and 0.2 ℃ temperature. In the naked observation, only CO2 30 bar 0.2 ℃ had gas hydrates formation following quick freezing. In the other hand, CO2 30 bar 15 ℃ had no change in the naked observation. After treated soybean sprouts were dewatered, CO2 30 bar 15 ℃ had more shrink than control and CO2 30 bar 0.2 ℃ had more shrink than CO2 30 bar 15 ℃. In the dewatered weight ratio of treated soybean sprout and control, every treatment showed the dewatered weight reduction. CO2 30 bar 0.2 ℃ showed more decrease than CO2 30 bar 15 ℃. As soybean sprout, containing water, showed some shrink by both 2 treatments, the dewatered weight reduction was caused by water release. Therefore, QFGD made more water released from soybean sprout than pressure shift and CO_2 dissolution.

6.1 Preliminary test 2 – Raw sludge

Supplementary data 3. Image of frozen raw sludge after QFGD (Condition: CO_2 (Purity 99.999 % from Korea SEM) as guest molecules, 30 bar as the reaction pressure, 0.2 ℃ as the stabilized temperature, 4 h as the reaction time)



Supplementary data 4. Centrifuge weight reduction(CWR) of raw sludge with different treatment (Stabilized temperature = 0.2(±0.1) ℃, Reaction time = 4 h, Guest molecules = Carbon dioxide (Purity 99.999 % from Korea SEM))

Raw sludge was treated by QFGD to check the possibility of its sludge application. Like Supplementary data 3, through QFGD, raw sludge was frozen with distinct parts. And, as reaction pressure increased from 15 bar to 30 bar, centrifuge weight reduction (CWR) slightly increased. CWR means the weight difference of centrifuged pellet through treatment. As the centrifuged solid weight can be considered as disposed sludge weight, it found that QFGD improves the efficiency of sludge treatment.

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