# Effect of herbaceous biomass and food waste addition in anaerobic digestion of sewage sludge



# 博　　士　　論　　文

**Effect of herbaceous biomass and food waste addition in anaerobic digestion of sewage sludge**

下水汚泥の嫌気性消化における草本系バイオマスおよび食品廃棄物の混合効果

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# Chaper 1. Introduction

## 1.1. Background

### 1.1.1. Current situation of biomass

Fossil fuels, which are hydrocarbons or derivative thereof, were formed from dead plants and animals millions of years ago. Common fossil fuels include coal, fuel oil and natural gas. The utilization of fossil fuels has greatly promoted the development of large-scale industries. Since fossil fuels cannot be reproduced, the current exploiting and consuming situation will result in a depletion of fossil fuels. In addition, the burning of fossil fuels is considered to be the largest source of emission of carbon dioxide, which is one of the greenhouse gases, and thus has been considered to contribute to the global warming. In order to achieve a sustainable society, it has become necessary to find some alternative fuels that has less impact on the environment. Utilization of biomass is considered to be promising as one of the resources.

Biomass, a concept that represents the amount (mass) of biological resources (bio), generally refers to renewable organic matters of biological origin. Since the first oil crisis in 1974, researches on the utilization of biomass as energy, such as bioethanol, which is made of sugar cane and used as fuel, have been undertaken in many countries including Japan. The annual generation and utilization situation of biomass has been shown in **Table 1.1** [1]. As it is shown in the figure, there are numerous kinds of biomass, such as sewage sludge, food waste, and non-edible parts of agricultural crops. The utilization situations of each kind of biomass differ largely. Recently, in addition to energy, biological resources other than fossil fuels are also used as industrial raw materials to produce such as bio-plastic and plant fibers. There are many advantages of biomass utilization, for example, biomass products are abundant and renewable; biomass can be used to produce not only energy but also many other kinds of products as mentioned in the previous contents; many kinds of waste such as raw waste and animal excreta can be treated and turned into energy. Although incineration of biomass releases carbon dioxide into the atmosphere, it also captures carbon dioxide through photosynthesis during its growth, thus to use biomass as alternative resource of fossil fuels could greatly contribute to the reduction of greenhouse gas emission. In addition, the great abundance of biomass could also provide continuous resource for the new bio or energy industries; and as compared with the power generation of solar or wind, less cost is needed. However, on the other hand, the energy density of biomass is relatively low compared to fossil fuels, and the wide and separated generation increase

the collection and transportation cost. In addition, since most of herbaceous biomass is generated seasonally, continuous supply is remained to be resolved.

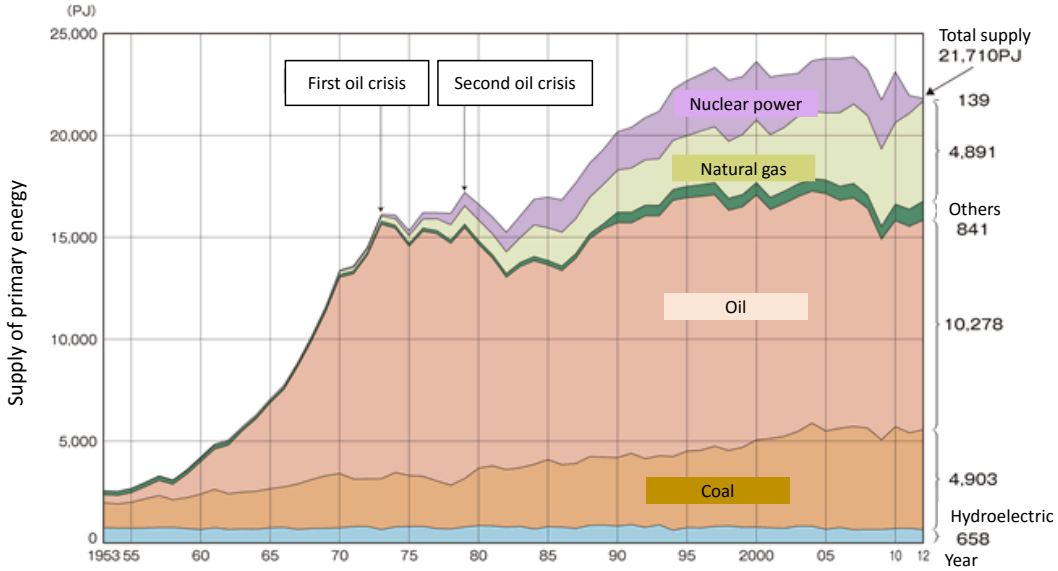
The importance of biomass is drawing more and more attention in the world, and in January 2009, 75 countries signed onto New Clean Energy Agency, and the International Renewable Energy Agency (IRENA) has been launched. IRENA aims at the promotion and dissemination of the utilization of renewable energy across the globe. The objective renewable energy includes bio-energy, geothermal, ocean energy, solar energy, hydroelectric power and wind power. The main activities include the analysis of current situation of renewable energy; assistance of the developing countries; promotion of research network construction; ensuring the development, organization and accessibility of existing information in a usable format. IRENA is the only organization that both developed and developing countries participate for renewable energy. Due to the establishment of IRENA, it can be expected to achieve the transition of many countries to a sustainable energy future.

The Supply of primary energy in Japan was shown in **Figure 1.1** [2], and the primary supply of Japan and global was shown in **Figure 1.2.** [3]The data showed that with the economic growth in Japan, the primary energy supply kept increasing, and it has become a nearly flat state since 1995. It also indicated that the supply rate of primary energy mainly depends on the oil and nuclear power, while the utilization of biomass as energy was low. However, due to the warm and rainy climate conditions in Japan, a considerable abundance of biomass can be expected. In order to achieve goals of global warming prevention, recycling-oriented society, strategic industrial development and rural areas activation, Ministry of Agricultural, Forestry and Fisheries (MAFF), and many other related organizations or local governments cooperated and developed specific initiatives and action plans on the promotion of biomass utilization, as a result, ‘Biomass Nippon Strategy’ was approved by the Cabinet in December 2002. The outline includes providing basic national strategy to realize sustainable society with the full utilization of biomass, and beginning to create Biomass Town in 2004, which aims at promoting the utilization of biomass in local town, a goal of the utilization of 90% (carbon basis) of waste biomass and 40% of unused biomass. [4] In March 2006, based on the previous biomass utilization situation, as well as the establishment of ‘Kyoto Protocol Target Achievement Plan’, ‘Biomass Nippon Strategy’ was revised aiming at Fortifying Biomass Town creating and use of biomass energy, including full-scale introduction of domestic bio-fuel, promotion of utilization of unused biomass such as forest residues. In addition, Japan has become a member of IRENA at the second meeting of Steering Preparatory Committee which was held in Sharm El

Sheikh of Egypt on 29 July 2009. As a member of IRENA, it could be expected of the promotion of the development and dissemination of renewable energy, as well as strengthening the international competitiveness of related industries in Japan. The Great EAST Japan Earthquake on March 11, 2011 led to the review of the nuclear energy use and furtherly accelerated the widespread of biomass utilization in Japan.

**Table 1.1** Annual generation and utilization of biomass in Japan (2009) [5]

| | Annual generation (million tons) | Utilization situation |
|---|---|---|
| Livestock excreta | 87 | Compost (90%) |
| Sewage sludge | 79 | Construction materials, compost (75%) |
| Black liqour | 70 | Enegy (100%) |
| Waste paper | 36 | Raw materials, energy (60%) |
| Waste food | 19 | Fertilizer, fodder (25%) |
| Sawmill residues | 4.3 | Papermaking materials, energy (95%) |
| Construction waste wood | 4.7 | Papermaking materials, livestock dressing (70%) |
| non-edible parts of crops | 14 | Fertilizer, fodder, livestock dressing (30%) |
| Forestry residues | 8 | Papermaking materials (1%) |




※1 PJ（=$10^{16}$ J) is equivalent to the heat of 25,800 kl oil

**Figure 1.1** Primary energy supply in Japan. [2]

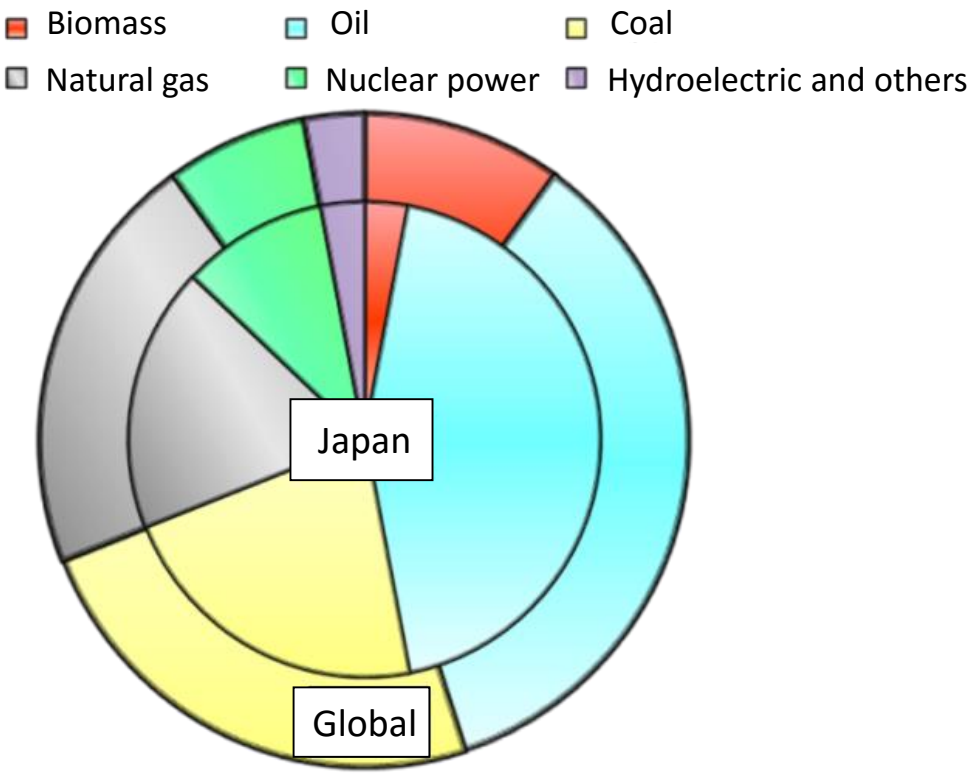



**Figure 1.2** Primary energy supply in Japan and global (2007). [3]

### 1.1.2. Utilization methods of biomass as energy

As shown in **Figure 1.2** [3], the utilization rate of biomass as energy in Japan is relatively low. There are several different utilization methods of biomass as energy in Japan as was shown in **Table 1.2**. [4] Anaerobic treatment methods such as ethanol fermentation and methane fermentation could contribute to saving treatment cost as well as energy, since different from aerobic treatment, oxygen is not required for the microbial activities during the treatment process. [6] Ethanol fermentation, as described in **Table 1.2**, has been extensively studied in the United States. In Japan, with the selling of gasoline E3 containing 3% of bio-ethanol in Osaka, and ethyl tertiary butyl ether (ETBE) containing 7% of bio-gasoline that made from bio-ethanol, the utilization rate of biomass is increasing [7]. On the other hand, through methane fermentation, the biodegradable organic matters containing in the waste biomass can be decomposed and energy can be recovered as methane gas. Reactors with large volumes could also act as cushion tank, and easy maintenance and operation could be possible. The digested residues are normally easy to be composted, and it is also excellent in terms of epidemiological safety against virus pathogenic bacteria. In addition, the amount of waste biomass could be reduced significantly after digestion, thus small-scale incineration facilities to treat the digested residues are sufficient. [8] Digested sludge, one of the residues of methane fermentation, is considered as stable organics, and can be degradable for decades if stored at appropriate conditions (as acid soils that water has been removed). Therefore, as charcoal fixation of carol reef, it is possible to fix carbons in the form of carbon dioxide that contained in the final residues of methane fermentation, by the use of appropriate land. [6] From the above points, methane fermentation has a great significance from the point of view of economic, as well as the contribution to global environmental conservation. However, unlike ethanol that has been widely developed, there is no existing methane fermentation facility where agricultural biomass is used as raw material for methane fermentation. However, as researches on methane fermentation that use sewage sludge or residues from ethanol fermentation process have been conducted, it is expected of effective energy recovery system from agricultural waste.

**Table 1.2** Overview of commercialized conversion technologies. [5]

| Technology | Overview | Applicable biomass | Products |
|---|---|---|---|
| Composting | Decomposition of biomass using aerobic micro-organisms to produce fertilizer which is useful for crop growth. | Livestock waste, food waste | Fertilizer, soil conditioner |
| Methane fermentation | Decomposition of biomass using anaerobic micro-organisms to produce methane gas. | Livestock waste, food waste | Electricity, heat (gas engine electricity generation) |
| Carbonization | Heating biomass in a low oxygen content to produce charcoal through thermal decomposition. | Wood, livestock waste, food waste | Fertilizer, soil conditioner, fuel |
| Animal feed production | Production of animal feed by drying or liquefying biomass. | Food waste | Animal feed |
| Biodiesel fuel production | Esterification of cooking oil waste to produce light oil substitute. | Cooking oil waste, energy crop | Diesel engine fuel |
| Ethanol fermentation | Fermentation of sugars and starches ,and distillation and dehydration of unrefined ethanol. | Sugars, starches | Bioethanol (gasoline substitute) |
| Direct combustion | Direct combustion of biomass to generate heat or electricity using a steam turbine. | Woody biomass, agricultural waste, poultry manure | Electricity, heat (steam turbine electricity generation) |
| Solid fuel production | Conversion technology to produce solid fuels such as pellets and refuse-derived fuel (RDF). | Wood waste, food waste | Solid fuels |

### 1.1.3. Utilization situation of biomass

The utilization situation of several typical kinds of biomass, sewage sludge, food waste and agricultural waste are introduced below.

#### 1.1.3.1 Sewage sludge

Sewage treatment is a process to move contaminants in wastewater or sewage from household, factory, economically or naturally. A basic aerobic sewage treatment process, conventional activated sludge process is shown in **Figure 1.3**. [9] In the sewage treatment process, a large amount of sludge is being generated, accounting for about 0.3%-0.5% of total sewage amount. Untreated sludge contains large amounts of toxic and hazardous substances, including parasite ovum, pathogenic microorganisms, bacteria, synthetic organic compounds and heavy metals, etc.; useful substances such as plant nutrients (nitrogen, phosphorus, potassium), organic matters and water, etc.. Therefore, promptly treatment and disposal of sludge is necessary to achieve: (1) the normal operation of sewage treatment plants and treatment effects; (2) proper disposal or utilization of toxic and hazardous substances; (3) stable processing of organics that are easy to corrupt and stink; (4) comprehensive utilization of organic substances. In short, the aim of sludge treatment is to achieve recycling, reduction and stabilization and utilization of sludge. Options for sludge treatment include stabilization, thickening, dewatering, drying and incineration. The excess sewage sludge is thickened and dewatered before final disposal. Typical treatment and disposal of sewage sludge are shown in **Figure 1.4**. [9]

In the treatment process of sludge, through anaerobic digestion, organic matters in sludge can be used by anaerobic microorganisms to convert to biogas, of which main content is methane and can be reused as gaseous fuel. Normally the utilization of produced methane in anaerobic digestion is used in wastewater treatment facilities. After dewatering, incineration or melting, the water content is sludge is decreased and sludge volume can be greatly reduced. Based on the sludge properties, the products of dewatering, incineration or melting can be used as fertilizer or construction materials.

In Japan, the annual generation of sewage sludge, of which water content is about 97%, is about 75 million tons, accounting for 30% of the total biomass generation. The generation and recycle ratios of sewage sludge is shown in **Figure 1.5**. [10] The inorganic matters in sewage sludge contain Si, Ca, Al, etc. and can be used as construction material, the usage as which accounts for over a half of the total sewage utilization. However, in the total solids of sludge, about 80% is organic matters, thus

sewage sludge is considered as a useful biomass resource. As it was shown in **Figure 1.6** [10], about 13.0% of the organics are used for generating biogas, 0.7% for sludge fuel and 9.7% are used for agricultural applications. The unused organics as biomass (utilization as construction material is excluded) accounts for about 76.6%, and the current utilization of organics as biomass is only 23.4%. [10] Therefore, in order to use the organic waste efficiently, and to achieve the goal of sound sewer management and business, it is necessary to take measures to promote the utilization of sewage sludge as energy and improve the biomass in the future.

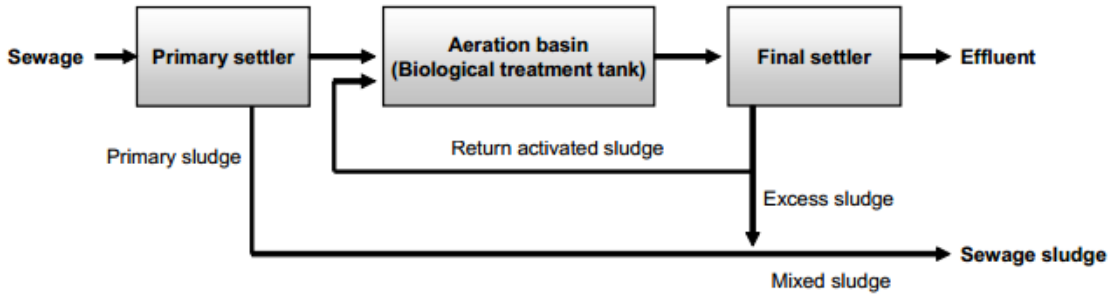



**Figure 1.3** Flow of conventional activated sludge process. [9]

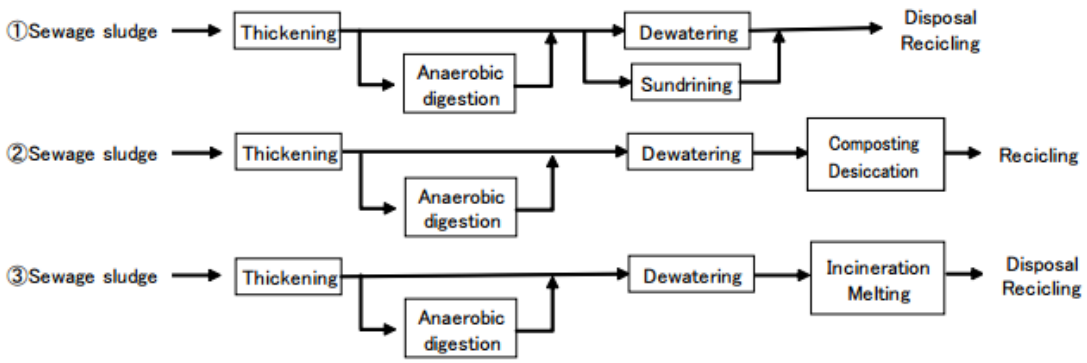



**Figure 1.4** Typical sludge treatment process flow [9]

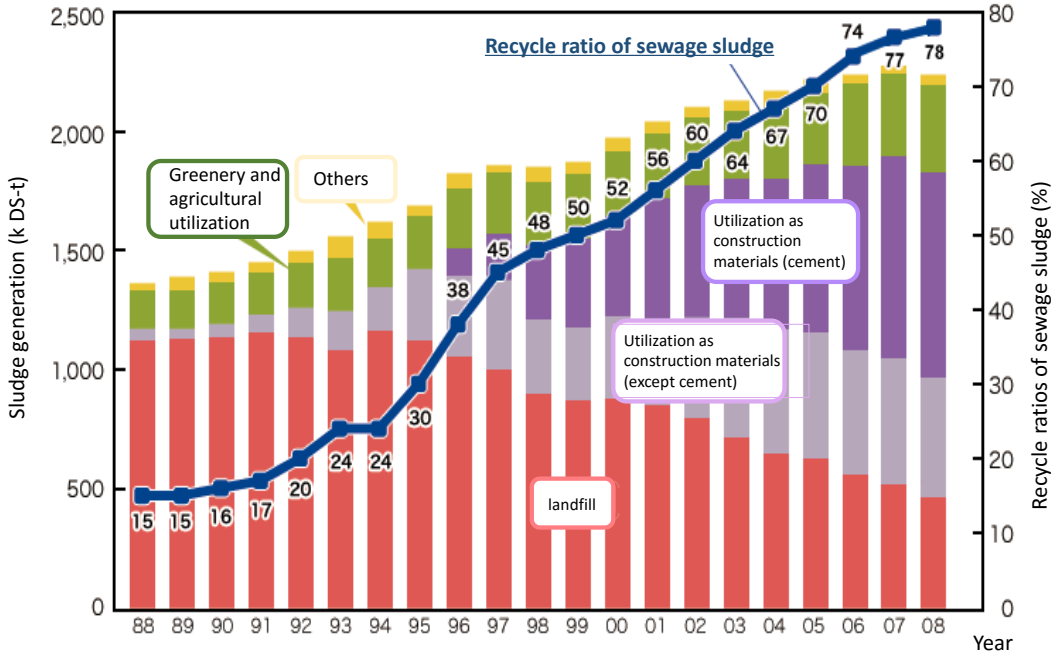



**Figure 1.5** Generation of sewage sludge (solid waste) and the variation of recycle ratios of sewage. [10]

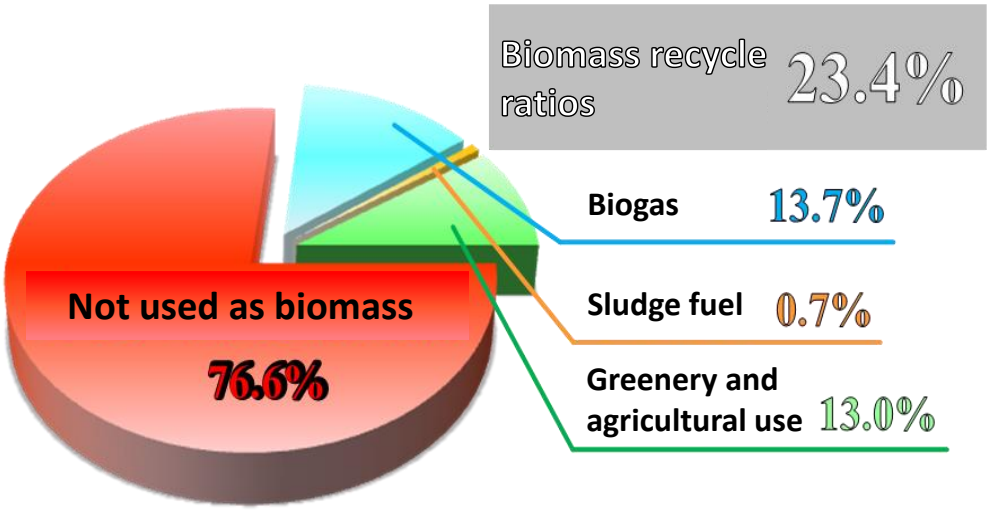



**Figure 1.6** Utilization situation of sewage sludge. [10]

#### 1.1.3.2 Food waste

According to the Food Recycling Law in Japan, food waste refers to the excess food generated after the use for food, or those discarded without being used for food; or the food substance which is not able to meet the standards of food that generated or discarded in the food manufacturing, processing or cooking process. [11] In Japan, approximately 20 million tons of food waste is generated from the food-related fields every year. The total food waste generation and reuse efficiency were of food waste generated in the food-related fields was shown in **Figure 1.7**; the total recycling implementation ratios of recycling food resources of the entire food-related fields was shown in **Table 1.3**; reuse implementation ratios of food waste of the food-related fields of which the generations were less than 100 tons was shown in **Table 1.4**. [12] Food Recycling Law has been revised in 2007, and since 2008, in addition to the surveys of reuse implementation ratios of recycling food resources of the food-related fields of which the generation was over 100 tons, the situations of that below 100 tons also have been surveyed and included in the annual report, and thus the situation of food waste in the entire food-related fields has been investigated and understood. According to **Figure 1.7** [12], it showed that from 2008 to 2010, the total generation of food waste was decreasing, and the reuse efficiency showed as slight increasing but kept stable. And according to **Table 1.3 and Table 1.4** [12], despite the fact that up to 82% of the total reuse implementation ratios were relatively high, that traded as feed accounted for more than half of the total utilization. Excluding the utilization as feed, that there was approximately 7.86 million tons of manufacturing food waste, and about half of that (about 4.5 million tons) was treated by incineration or landfill, the utilization as energy have not been implemented. In addition, in the fields of which the generation was below 100 tons, the total generation of food waste was approximately 2.33 million tons, however, only 12% of which has been reused and most of the rest has been treated by incineration or landfill.

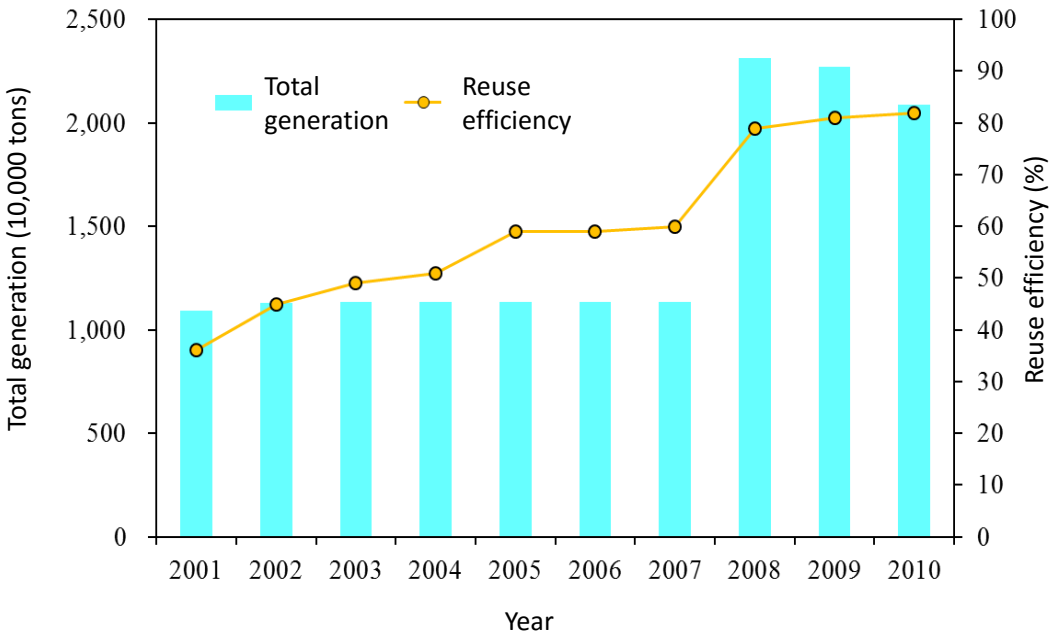



**Figure 1.7** Total generation and reuse efficiency of food waste in the food-related fields. [12]

**Table 1.3** Total recycling implementation ratios of food waste of the entire food-related fields. [12]

| Industries | Annual generation (10,000 tons) | Recycle implementation ratios (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Generation prevention | Reuse | Specific useage | | | | Heat recovery | Reduction |
| | | | | | Feed | Fertilizer | Methane | Others | | |
| Food manufacturing | 1715 | 94 | 10 | 71 | 77 | 16 | 4 | 3 | 3 | 11 |
| Food wholesale | 22 | 52 | 9 | 43 | 36 | 48 | 1 | 15 | 0 | 1 |
| Food retailing | 119 | 37 | 8 | 29 | 46 | 32 | 4 | 18 | 0 | 1 |
| Restaurant industry | 229 | 17 | 4 | 10 | 33 | 41 | 3 | 23 | 0 | 2 |
| Total | 2086 | 82 | 9 | 62 | 76 | 17 | 3 | 4 | 2 | 9 |

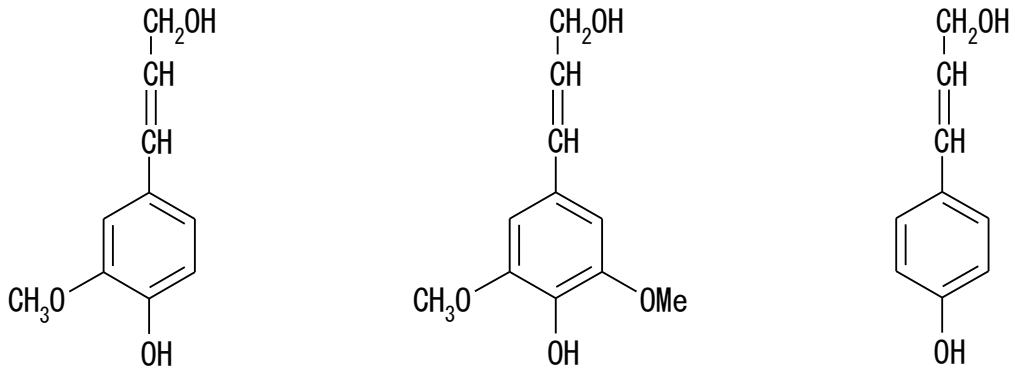
**Table 1.4** Total recycling implementation ratios of food waste of the food-related fields of generation below 100 tons. [12]

| Industries | Annual generation (10,000 tons) | Recycle implementation ratios (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Generation prevention | Reuse | Specific useage | | | | Heat recovery | Reduction |
| | | | | | Feed | Fertilizer | Methane | Others | | |
| Food manufacturing | 27 | 50 | 0 | 45 | 31 | 50 | 2 | 17 | - | 2 |
| Food wholesale | 10 | 36 | 0 | 30 | 16 | 55 | - | 29 | - | 0 |
| Food retailing | 29 | 20 | 1 | 16 | 34 | 26 | 0 | 40 | - | 1 |
| Restaurant industry | 167 | 6 | 2 | 5 | 50 | 13 | 0 | 37 | - | 2 |
| Total | 233 | 14 | 2 | 12 | 36 | 35 | 1 | 28 | - | 2 |

#### 1.1.3.3 Herbaceous biomass

Herbaceous biomass is a kind of resource that carbon contents are mainly contained and has high potential of energy. However, as compared to some kinds of waste biomass such as sewage sludge, that the main organic contents in herbaceous biomass are lignin, and with strong lignocellulose structure, the biodegradability is usually poor and thus the utilization of herbaceous biomass has not been proceed yet. Lignin has a branching of many three-dimensional network structure produced by dehydrogenation polymerization of 4-hydroxy-cinnamyl alcohol with poor water-solubility. Therefore, the structure between cells are strong because the cell walls and pores between cells are filled with lignin, which is more hydrophobic than cellulose or hemicellulose. In addition, the structure of lignin is biologically stable, which is resistant to biodegrade, because of the carbon-carbon bond or the carbon-oxygen-carbon bond has given wood a strong resistance to rot. Guay acyl propane, main structure of lignin backbone of softwood or primitive land plants (1), acyl propane structure and syringyl propane structure, main structure contents of hardwood (2), and Guay acyl propane, consisting of syringyl propane structure 4-hydroxyphenyl propane structure, the main contents of grasses, were shown in **Figure 1.8**. The figure of the structures showed that with the evolution of plants, that structural units of lignin is becoming complicated. [13, 14]

As shown in **Table 1.1**, that effective utilization of non-edible parts of crops, herbaceous biomass, as well as forestry residues, has not been progressed yet. As a practical case, the current utilization of biomass, which is a typical kind of herbaceous biomass, is described as below. The domestic generation of rice straw, is approximately 9 million tons per year. The ratio of the incinerated-treatment decreased as compared to the previous situation; however, as shown in **Figure 1.9** [15], approximately 76% of the generated rice straw are treated by mixed with soil. By mixed with soil, the organic contents in rice straw are degraded under anaerobic condition, through the degradation process, a large amount of methane gas, as well as nitrogen monoxide are generated and emitted into atmosphere, which will exacerbate the greenhouse effect, because methane and nitrogen monoxide are also greenhouse gases. Due to the reasons described above, more effective utilization if biomass is of great significance.




(1) (2) (3)

(1) Coniferyl alcohol (Guay acyl propane structure)

(2) Sinapyl alcohol (Syringyl propane structure)

(3) p-Hydroxycinnamic alcohol (4-hydroxy-phenyl propane structure)

**Figure 1.8** The primary structure of the lignin. [13, 14]

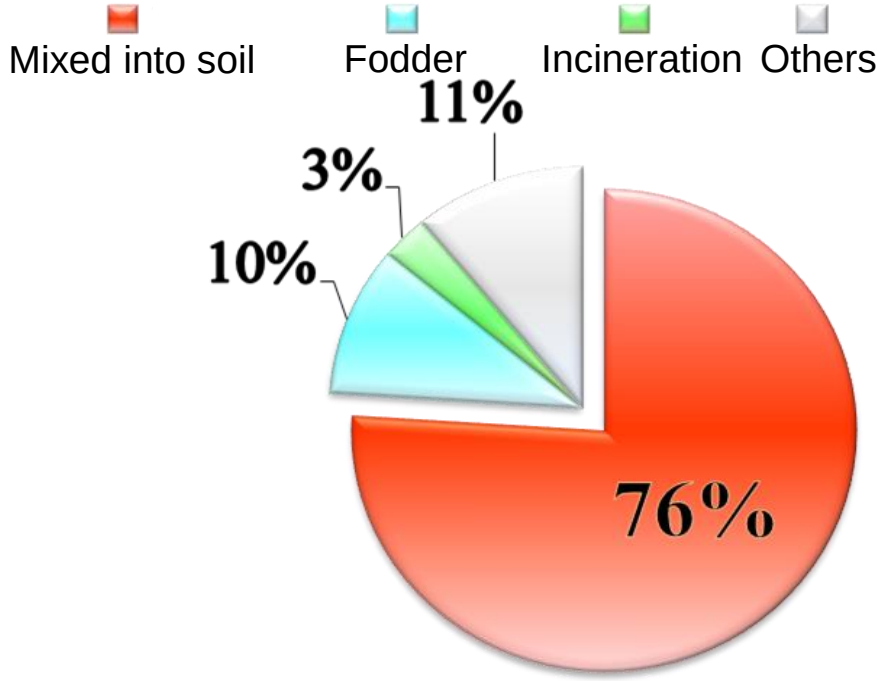



**Figure 1.9** Utilization situation of rice straw (2006). [15]

### 1.1.4. Methane fermentation

Methane fermentation is a process that under anaerobic conditions, by the activities of microorganisms that mainly consist methanogenic bacteria, organics are degraded and biogas, of which methane gas contains about 60-70%, will be generated. Through methane fermentation, organic waste such as sewage sludge, kitchen garbage, livestock waste, etc. can be utilized as energy resources. With this characteristics superior to other energy production processes, fermentation has a great significance for treatment of organic wastes, as well as the preservation of the global environment. [16]

Methane fermentation is the consequence of a series of metabolic interactions among various groups of microorganisms. The degradation of organic matters in fermentation process can be divided into four processes, as shown in **Figure 1.10**. [16, 17]

1) Hydrolysis

In this process, polymeric materials such as lipids, proteins, and carbohydrates are degraded into soluble monomers such as amino acid, glucose or higher fatty acids, which are then consumed by microbes.

2) Acidogenesis

Products of hydrolysis process are degraded into volatile fatty acids such as butyric acid, propionic acid, formic acid and acetic acid, etc. or alcohols.

3) Acetogenesis

In this process, butyric acid, propionic acid and other fatty acids more than $C_3$ are degraded into acetic acid or hydrogen.

4) Methanogenesis

In this process, acetic acid and hydrogen are degraded and methane and carbon dioxide are produced.

Therefore, microorganisms involving in the methane fermentation process mainly include, (a) Fermentative bacteria involving in hydrolysis and acid fermentation; (b) acetogenic bacterial involving in degradation of fatty acids such as propionate acid, butyric acid, etc.; (c) acetogen bacteria involving in acetic acid production and (d) methanogen involving in methane production.

To look the current situation of methane fermentation in Japan [18], that in Hokkaido,

methane fermentation is widely used due to the large amounts of livestock waste generation; generation of kitchen garbage is increasing continuously, but most methane fermentation are applied for treating sewage sludge. Among 2100 sewage treatment facilities in Japan, methane fermentation are applied in about 308 facilities. However, there are approximately 6,000 methane fermentation facilities existing in Germany, and in some cities where the temperature in winter is usually as low as -20 °C, such as Dalian in China, where it is quite difficult to conduct methane fermentation, about 100 thousand household-scale of fermentation facilities are being applied.

However, in recent years, based on the methane fermentation of excess sludge, addition of other biomass in the sludge digestion process to conduct co-digestion has drawing attentions all over the world. Target biomass include kitchen garbage, food waste, agroforestry waste, animal excreta, etc. By co-fermentation of sludge and waste biomass, it is possible to achieve more effective utilization of biomass, reduction of treatment cost, increasing in biogas yield and reduction of carbon dioxide emission, as well as building low carbon and resource recycling-based society. Especially in some rural areas, where biomass abundance is quite large, effective conduction of the co-fermentation can be expected. In Suzu city in Ishikawa prefecture, “Suzu Biomass Energy Promotion Plan” were approached, a comprehensive biomass methane fermentation facility was started and in this plant, five kinds of biomass including wastewater sludge, kitchen garbage, human waste, Jokaso sludge and agriculture discharged sludge are added in the sewage sludge digestion process. This facility was started in 1997 and has been running smoothly. It is estimated that the centralized treatment of biomass has cut down the treatment cost for 43 million yen per year [19], compared to the cost that biomass was treated separately in local town. In addition, according to the planning target of this facility since 1997, by the year of 2025, the total cost will be reduced by 72% through centralized treatment, compared to the treatment cost of separate treatment of these biomass. [20] Most of the current anaerobic digestion of sludge (methane fermentation) are conducted under relatively low concentration (average 3%), thus large scale of plants are needed, meaning high construction and operating cost; meanwhile, the thickened sludge generation is quite a lot, leading to limiting treatment capacity. To promote co-digestion of sludge and mixed biomass under high concentration at small scale that are applicable in rural areas is expected in the future. In addition, it is also highly expected that the digester in the sewage treatment plant could accept a large amount of herbaceous biomass, through which the C/N ratio can be adjusted to a suitable rage for the growth of organism involved in methane production, which has been widely studied.

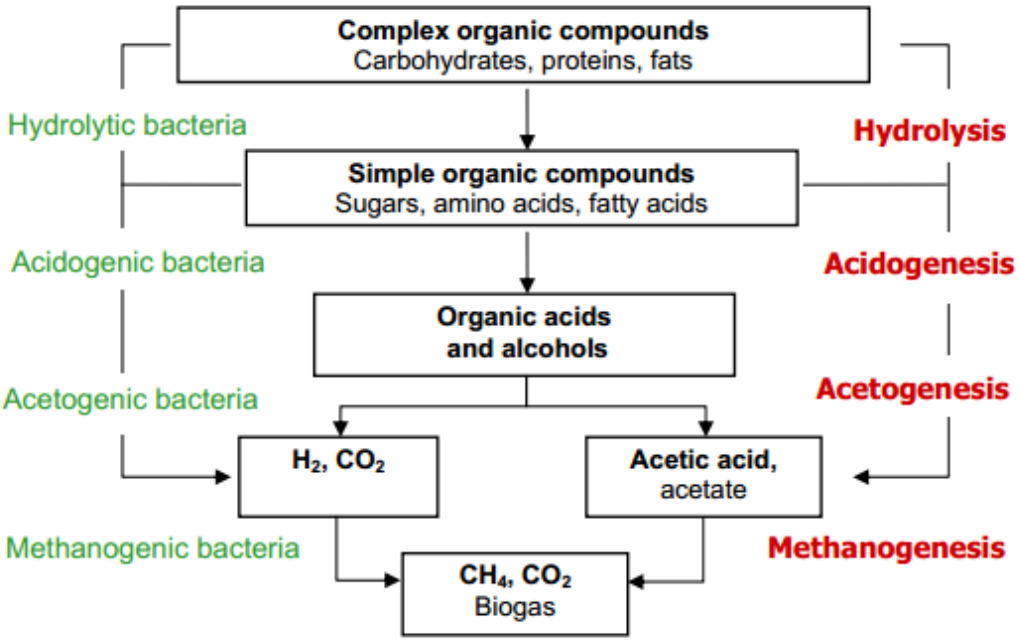



**Figure 1.10** Methane fermentation process. [16, 17]

## 1.2. Purposes

In the present study, the co-digestion of sewage sludge generated from small-scale sewage treatment facilities, and other kinds of waste biomass generated in local town, was proposed. The study focused on the methane gas recovery of several kinds of biomasses, the digester performance of co-digestion, and the variation of dissolved organic compositions and microbial community in the anaerobic digestion, with the addition of biomass.

In Chapter 2, high solid anaerobic digestion of sewage sludge generated from oxidation ditch process, which is widely applied in small-scale sewage treatment facilities, and waste fried tofu which is generated from a tofu manufacturing plant, was conducted under thermophilic condition. The substrates concentrations were increased gradually. The biogas production, digester performance under different organic loading, and the variation of microbial community were studied.

In Chapter 3, a co-digestion of sewage sludge generated from conventional activated sludge process and rice straw, which is a typical kind of biomass generated in Ishikawa, was conducted. The biogas production of rice straw, and the dewaterability of digested sludge was evaluated, to study the effect of rice straw addition in the sludge digestion process.

In Chapter 4, a co-digestion of sewage sludge and rice straw was conducted, and the variation of dissolved organic matters (DOM) in the supernatant of digested sludge with the addition of rice straw, was studied.

If the food waste and rice straw, which has a great abundance in the area where agriculture is well developed, could be indicated as effective energy resource for methane fermentation, it is possible to establish proper methane fermentation system that is suitable for the rural areas, which is called as Satochi-Satoyama in Japanese. The establishment of methane fermentation system suitable for rural areas is of great significance for the promotion of building a low-carbon and recycling-oriented society.

# Chapter 2. Methane Recovery and Microbial Community Analysis of a High Solid Thermophilic Co-digestion of Sewage Sludge and Waste Fried Tofu

## 2.1. Introduction

The generation of sewage sludge is increasing rapidly around the world, and there is a great need for effective methods to treat the existing and future accumulations of sewage sludge. In recent years, 220 million tons (dry weight) of sewage sludge are being generated every year in Japan. [21] Approximately 80% of the sludge solids are organic contents, and only 23.9% are used as biomass. [22] Anaerobic digestion (AD) is suggested as an effective method for treating sewage sludge, by which the organic contents can be biodegraded and methane gas can be produced and used as energy. [23, 24] AD has thus been widely applied in many wastewater treatment plants (WWTPs) throughout the world. In recent years, study on AD has not been limited to a certain biomass, the co-digestion of several kinds of organic waste, such as food waste, animal manure, agricultural waste, etc. are also widely studied. [25-29] Compared to digestion of single substrate, co-digestion of two or more substrates is drawing much attention because it is possible to solve the problems of single substrate, for example, low organic contents that leads to low biogas production, high nitrogen contents that may cause ammonia inhibition, high concentration of heavy metals, variation of seasonally generated biomass, etc.. [30] However, despite the widely application of AD, in many small-scale WWTPs (daily mean flow<10,000$m^3$), the utilization of organics in sewage sludge has not yet been preceded effectively. In Japan, the number of total municipal WWTPs is 2100, and 1500 are small scales, accounting for approximately 70% of the total. [21] In those plants, around 1000 are using oxidation ditch (OD) process for treating wastewater, accounting for approximately 50% of the total. OD process is widely applied in small-scale WWTPs due to some advantages over conventional process, such as its lower requirement of operational skills, and the stability against the variation of influent loading or temperature. [31] However, in the WWTPs using OD process in Japan, AD is rarely used for sludge treatment except for only one facility located in Suzu, Ishikawa. This is because that the hydraulic retention time of aeration tank (normally 24-36 hours) is relatively long. [32] In addition, as shown in figure 1, compared to conventional activated sludge process, OD process does not consist of a primary sedimentation tank prior to aerobic reactor, thus the biodegradability of the excess sludge from an OD process is relatively poor. In addition, since the generation of the sludge is low in a single facility, taking into consideration of the implementation and operation cost, it is not benefit

effective to promote AD in each facility. However, the generation of sludge in those plants cannot be ignored and needs to be resolved. If AD could be applied in these WWTPs, the sludge could be treated efficiently and methane gas recovery could be possible.

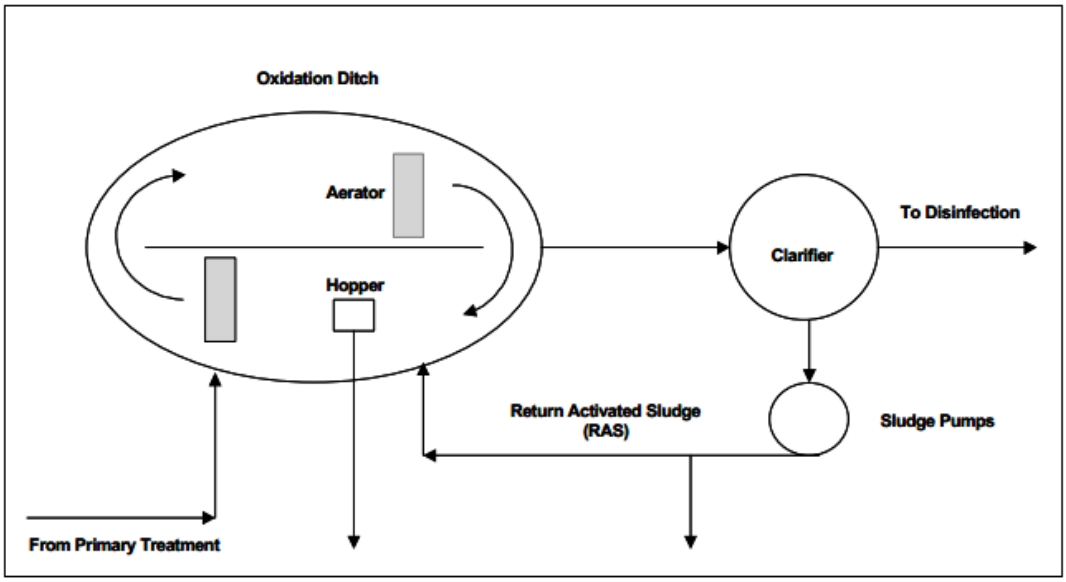



**Figure 2.1** Oxidation ditch process flow. [33]

A previous study of the authors' showed that in Ishikawa Prefecture in Japan, there are a large amount of OD sludge and waste fried tofu, a typical organic waste in local town, being generated every year and efficient treatment is needed. [34] Fried tofu contains high content of protein and oil, as estimated by Li (2005) that the theoretical methane production potential of fat and protein are 0.998 L/g and 0.52 L/g, respectively [35], and thus high methane recovery from fried tofu could be expected. If the waste fried tofu could be used in a nearby WWTP for co-digesting with sludge, it might be possible to recover methane gas and treat the organic waste effectively. Therefore, in the authors' previous study, a co-digestion experiment of OD sludge and waste fried tofu was conducted at mesophilic temperature [34], and the results showed that the fried tofu addition contributed greatly to the methane gas production; in addition, stable digestions were obtained at substrate concentration of 100 g/L for sludge digestion and 101.5 g/L for co-digestion. As it was reported that thermophilic digestion has some advantages over mesophilic digestion, including higher digestion rate, greater conversion of organics to biogas, as well as destruction of pathogenic microorganisms. [36-39] Therefore, to improve the digestion efficiency, a co-digestion of OD sludge and fried tofu was conducted at thermophilic temperature in the present study and the digesting performance was evaluated. Furthermore, since the methane fermentation is the consequence of a series of metabolic interactions among at least four physiologically different microbial

groups (trophic groups), hydrolyzing bacteria, fermenting bacteria, acetogenic bacteria and two types (i.e., acetoclastic and hydrogenotrophic) of methanogenic archaea [40], thus it is necessary to study the microbial community structure. The purpose of this study was 1) to investigate the maximum organic loading rate (OLR) for stable digestion of both mono-digestion of sludge and co-digestion; 2) to study the effect on the digestion process due to the addition of waste fried tofu at thermophilic temperature. In addition, the microbial community structures were analyzed by performing polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technology, to study the effects on microbial community of the addition of fried tofu.

## 2.2. Materials and methods

### 2.2.1. Preparation of raw materials

The feed sludge used in this study was dewatered sludge (**Figure 2.2**) produced by the Kashima Chubu sewage treatment plant in Nakanoto-cho, Ishikawa, Japan. In this plant, sewage is treated using an OD process and the produced sludge is dewatered by a screw press. For the present study, inoculum sludge was taken from a thermophilic anaerobic digester that treats sewage sludge at the Daishojigawa sewage treatment plant in Ishikawa, Japan. Waste fried tofu was collected from a fried tofu manufacturing plant located in Nakanoto-cho. Two types of fried tofu (**Figure 2.3**) were used as substrates: dry fried tofu and raw fried tofu. Prior to use (<15 days), the tofu samples was cut into 30–40mm segments with scissors. The sludge and dry fried tofu were stored at 4°C, and the raw fried tofu was stored at −4°C to prevent spoilage. The characteristics of the inoculum and feed substrates are shown in **Table 2.1**.

**Table 2.1** Characteristics of the inoculum, feed sludge and fried tofu

| Parameter | Inoculum | Feed sludge | Dry fried tofu | Raw fried tofu |
|---|---|---|---|---|
| TS (% w/w) | 1 | 85.0 | 91.0 | 55.0 |
| VS (% TS) | 74.0 | 84.5 | 90.0 | 90.4 |
| C (% TS) | - | 44.3 | 48.5 | 65.3 |
| H (% TS) | - | 6.4 | 6.3 | 9.8 |
| N (% TS) | - | 8.0 | 4.6 | 5.5 |
| C/N ratio | - | 5.6 | 10.5 | 11.9 |

**Figure 2.2** Dewatered oxidation ditch sludge


**Figure 2.3** Waste fried tofu used in the continuous digestion experiment (1: dry fried tofu, 2: cut dry fried tofu 3: raw fried tofu, 4: cut raw fried tofu)

### 2.2.2. Reactor operation

Two lab-scale anaerobic reactors, each with a working volume of 3 L, as shown in **Figure 2.4** and **Figure 2.5**, were used in the experiment. The reactors were set in a thermostatic chamber and the temperature was maintained at 55°C using a temperature control system. Run 1 was set as a control group, and the feed substrate was OD sludge. In Run 2, a mixture of fried tofu and OD sludge with a total solid (TS) ratio of 0.45 (dry weight basis) was used as the feed substrate. Substrate concentration was adjusted by diluting with distilled water.

In the acclimation period, the digestion was operated at the sludge retention time (SRT) 25d for 25 days, and then SRT 15d for 42 days. The substrate concentration of the feed substrates was 30 g/L in Run 1 and 43.5 g/L in Run 2. After the acclimation, the digestion was operated under the conditions shown in **Table 2.2.** In Run 1, the OD sludge was diluted to 30 g/L and fed to the reactor (Period I). After 20 days, the Substrate concentration was increased to 50 g/L (period II) and the reactor was operated for 73 days. During period III, the Substrate concentration was increased to 70 g/L and the reactor was operated for 97 days.

In Run 2, a mixture of waste fried tofu and sludge was fed as the substrates. The concentrations of sludge were the same as that in Run 1, and the mixing ratio of fried tofu to sludge was 0.45:1. In period IV, the Substrate concentration of Run 1 was increased to 100 g/L, whereas in Run 2, the Substrate concentration was decreased to the same as that used in period II (72.5 g/L). Period IV was operated for 97 days.

During Period III, a sharp decrease in biogas production was observed in Run 2. To study whether the suppression to methane fermentation was due to the high organic load, 30 ml of the digested sludge from the co-digester was added into a 120 ml-syringe and digested for 5 days, the biogas production was evaluated.

**Figure 2.4** Reactors used in lab-scale co-digestion experiment

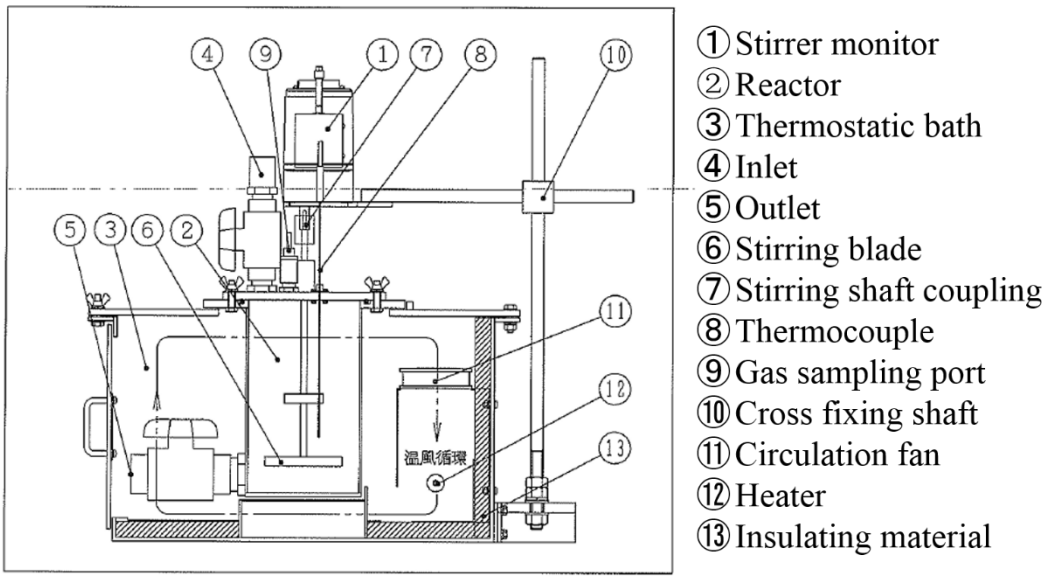



**Figure 2.5** Experiment reactor design

### 2.2.3. Analytical methods

Biogas generated in the digesters were collected continuously, biogas yield was measured regularly at room temperature by wet gas meter (Shinagawa Corp., Japan); biogas composition was analyzed by a gas chromatography (GC-8TCD, Shimadzu, Japan). Analysis of the digested sludge was conducted weekly. Total solids (TS) and volatile solids (VS) were measured according to standard methods (APHA, 2005). pH of the sludge samples was measured with a pH meter (LAQUAF-71, Horiba, Japan). Sludge samples were centrifuged at 10,000 rpm for 30 min, and the supernatants were filtered using a membrane filter (0.2μm) and then diluted with pure water for analysis. Concentrations of dissolved organic carbons (DOCs) and dissolved total nitrogen (DTN) were measured using a TOC/TN analyzer (TOC-V, Shimadzu, Japan). Ammonium concentrations of filtered samples were quantified with the use of an ion chromatograph (HIC-SP, Shimadzu), and concentrations of volatile fatty acids (VFAs) were measured using the ion chromatograph post-column pH-buffered electro-conductivity method (HPLC Organic Acid Analysis System, Shimadzu).

### 2.2.4. Microbial community analysis

DNA samples of seed sludge and digested sludge were extracted at the steady state of each of the four operating periods. Sludge samples were centrifuged at 10,000 rpm for 20 min, and DNA samples were extracted from the precipitated sludge using a Power Soil DNA kit (MoBio Laboratories, CA) and stored at −20°C for further processing.

Bacterial and archaeal 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the universal primers shown in **Table 2.3**. [41, 42] For the bacterial analysis, primer 2 and primer 3 were used, and PCR and DGGE were conducted as described by Muyzer et al. (1993). [41] For the Archaea analysis, PCR and DGGE were conducted according to Øvreås et al. (1997). [42] Since the PCR amplification of DNA samples from sludge is relatively difficult, a nested PCR amplification was first performed using primers PREA46f and PREA1100r. The first amplification PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega, USA) and then used as templates in the second amplification using primers PARCH 340f and PARCH 519r. In this work, the second PCR amplification was performed using a touchdown protocol of 94°C for 10 min, followed by 18 cycles of 1 min at 94°C, 1 min at 64°C (decreasing in each two cycles by 1°C), and 2 min at 72°C, followed by another 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. The final elongation step was 10 min at 72°C.

In the DGGE process, we used an 8% polyacrylamide gel and 15%–55% denaturant at 200V for 3 h to identify the bacterial community, and the denaturant concentration for archaeal was 40%–60%. After electrophoresis, the gels were stained for 30 min with SYBR Green I nucleic acid gel stain (1:10,000 dilution, Life Technology, Tokyo) and photographed on a UV transilluminator (UVP) with a digital camera (DMC-LX5, Panasonic, Tokyo). DNA samples eluted from the DGGE samples were used as a template for reamplification. Reamplification products were purified and used to perform a sequence reaction using an ABI PRISM 3100 Genetic Analyzer (Applied Bio-systems Japan, Tokyo), and then decoded by the genetic research facility at the Kanazawa University Advanced Science Research Center (Cancer Institute). The sequences obtained were searched with the programs BLAST and FASTA at the DNA Data Bank of Japan (DDBJ) (http://www.ddbj.nig.ac.jp), and the related species were searched.

**Table 2.2** Operating conditions of continuous digestion experiment

| Parameter | Run 1 | | | | Run 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Period I | Period II | Period III | Period IV | Period I | Period II | Period III | Period IV |
| Sludge TS (g/L) | 30 | 50 | 70 | 100 | 30 | 50 | 70 | 50 |
| Fried tofu TS (g/L) | - | - | - | - | 13.5 | 22.5 | 31.5 | 22.5 |
| OLR ($kg/m^3$-VS/d) | 2.5 | 4.2 | 5.9 | 8.5 | 3.8 | 6.3 | 8.8 | 6.3 |
| Operation time (d) | 1-20 | 21-93 | 94-191 | 192-288 | 1-20 | 21-93 | 94-191 | 192-288 |

**Table 2.3** Sequences of primers used in the PCR-DGGE analysis

| Primer | Target | Sequence | Reference |
|---|---|---|---|
| Primer 2 | Bacteria, V3 region | 5'ATTCCGCGGCTGCTGG | Muyzer et al. 1993 |
| Primer 3 | Bacteria, V3 region | 5'CCTACGGGAGGCAGCAG | Muyzer et al. 1993 |
| PREA 46f | Archaea | 5'(C/T)TAAGCCATGC(G/A)AGT | Øvreås et al. 1997 |
| PREA 1100r | Archaea | 5'(T/C)GGGTCTCGCTCGTT(G/A)CC | Øvreås et al. 1997 |
| PARCH340f | Archaea, V3 region | 5'CCCTACGGGG(C/T)GCA(G/C)CAG | Øvreås et al. 1997 |
| PARCH519r | Archaea, V3 region | 5'TTACCGCGGC(G/T)GCTG | Øvreås et al. 1997 |

## 2.3. Results and discussion

### 2.3.1. Operating performance of sludge digestion (Run 1)

The thermophilic digestion experiment was performed for 288 days. The cumulative biogas production, concentrations of DOC, VFAs, DTN, $NH_4^+$-N, TS and VS of Run 1 are shown in **Figure 2.6**, and the results of methane yields and VS removal rates are summarized in **Table 2.4**. The biogas collection was failed during day 61-112, 150-200 and 261-289 in Run 1; and during day 47-73 and 261-289 in Run 2, due to the leak problem of gas bags. VS removal rates were calculated according to the Eqs. (1), which was described by Hidaka et al. (2013). [36]

$$\text{VS removal rate} = \frac{VS_{theo} - VS_{out}}{VS_{theo}} \quad (1),$$

where $VS_{theo}$ refers to the theoretical VS of the digested sludge calculated assuming that the reactor was at complete mixing condition and the VS of the substrates are not removed. In addition, VS removal rates during period I was not calculated due to the short SRT.

In the sludge digestion (Run 1), in periods I and II, of which OLR were 2.5 and 4.2 kg VS/$m^3$/d, TS and VS were degraded stably, and average VS removal rate of period II was 31%. Stable generation of biogas was achieved, with methane yields of 0.08 and 0.05 L/g TS, respectively. pH was not controlled and maintained stably during the periods and ammonia nitrogen concentrations were below 1050 mg/L. In addition, no VFAs were detected, indicating that the methane fermentation was conducted stably in periods I and II. In period III, substrate concentration was increased to 70 g/L, and OLR was increased to 5.9 kg VS/$m^3$/d. Concentrations of DOC, DTN increased, and acetate and propionate were detected from day 114, with concentrations up to 470 and 330 mg/L detected, respectively. However, accumulation of VFAs disappeared after day 177. The VS removal rate and methane yield were the same as those of period II, and sludge digestion was considered stable with OLR up to 5.9 kg VS/$m^3$/d. In Period IV, when the substrate concentration was increased to 100 g/L, and OLR was 8.5 kg VS/$m^3$/d, the generation of biogas almost stopped, and VS removal rate decreased to 27%. At the initial stage of period IV, a dramatic increase in DOC was observed. Accumulations of VFAs were also observed, and concentrations up to 2280 mg-C/L of acetate, 1070 mg-C/L of propionate were detected. Meanwhile, decrease in pH was observed. Although it is well known that pH variation generally has an effect on the methanogens activity, pH values during period IV varied slightly around 7.5, which was suggested as suitable range for methanogens. In addition, concentrations of TN and $NH_4^+$-N also showed an obvious increase with the

increase of substrate concentration. $NH_4^+$-N concentrations up to 1890 mg/L were detected in period III, and sharply increased to 3760 mg/L in period IV. It is known that ammonia nitrogen can provide nutrient as well as partial alkalinity for methane fermentation; however, it is also widely indicated as a strong inhibitor to methanogens when the concentration excesses some certain values. In thermophilic digestion process, the inhibition concentration ammonia nitrogen was reported as 2500 mg/L [43], the high ammonia concentration in period IV was considered to contribute strongly to the inhibition to methane production. The operating performance showed that at thermophilic temperature, stable operation of OD sludge could be possible when the substrate TS was below 70 g/L.

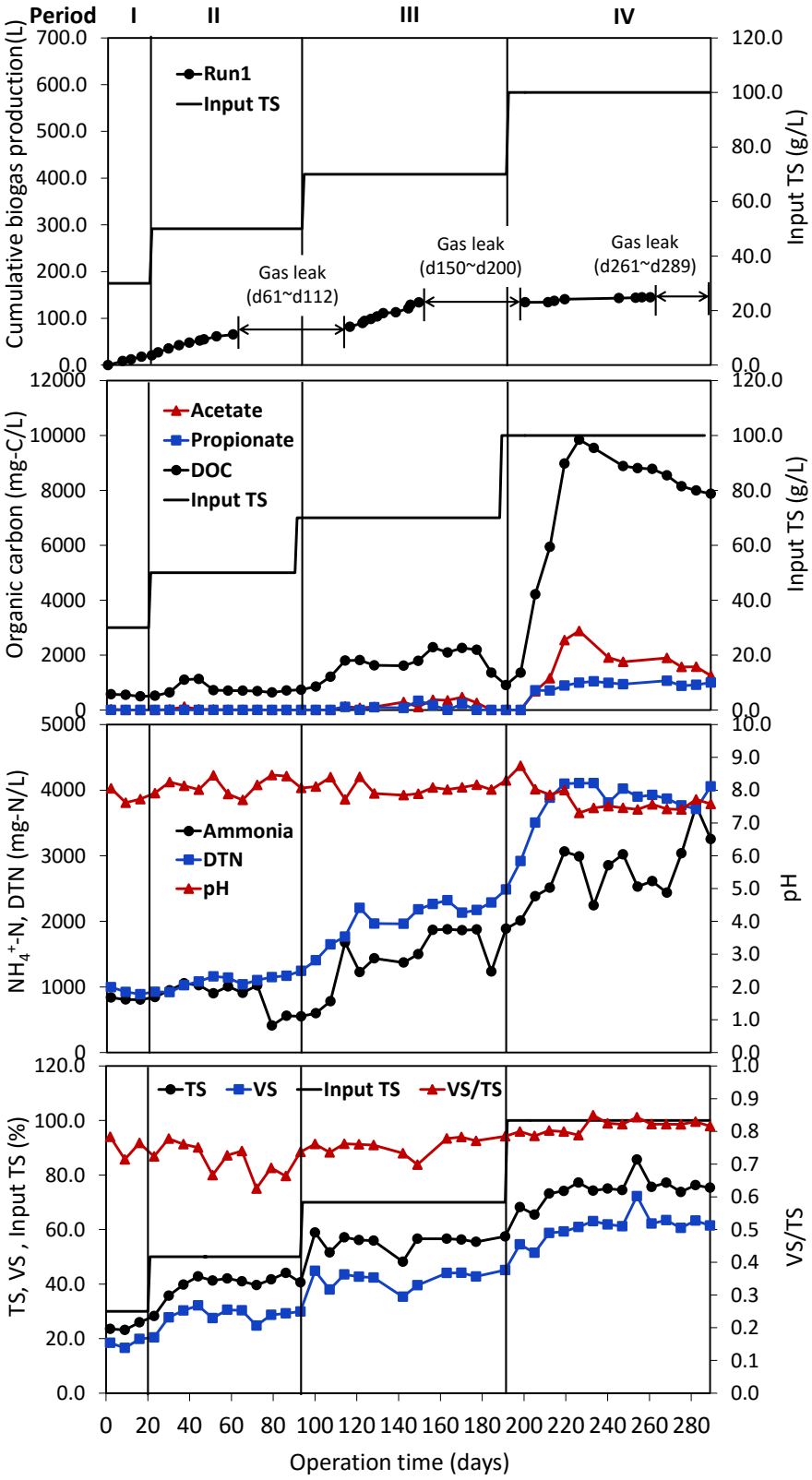



**Figure 2.6** Cumulative biogas production, variation of input TS, organic carbons, ammonium, DTN, and TS and VS concentrations of the digested sludge of sludge digester (Run 1)

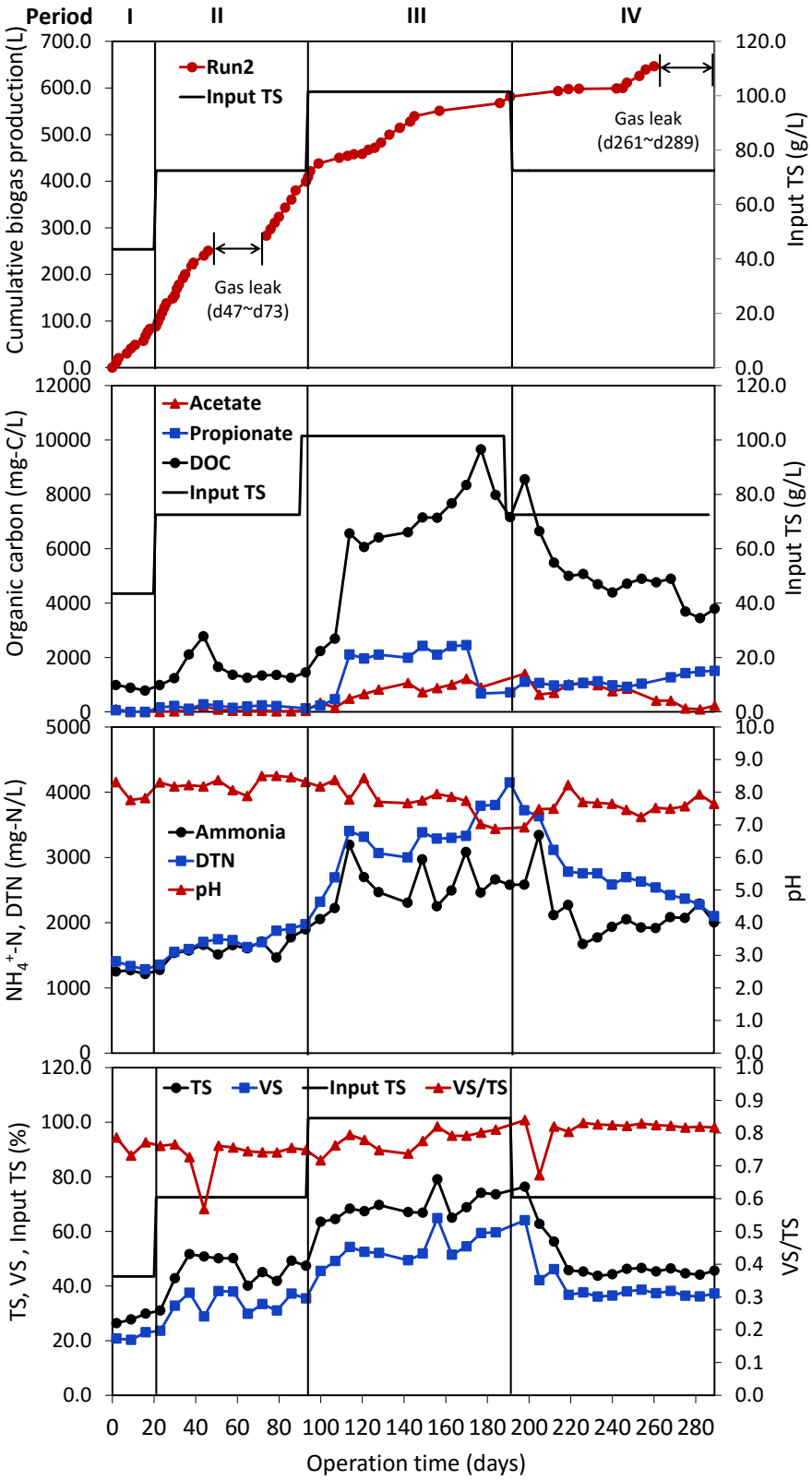



**Figure 2.7** Cumulative biogas production, variation of input TS, organic carbons, ammonium, DTN, and TS and VS concentrations of the digested sludge of sludge digester (Run 2)

**Table 2.4** Results of $CH_4$ production rate, $CH_4$ yields and content, TS and VS removal ratios of Run 1 (sludge digestion) and Run 2 (co-digestion) in each period.

| | Operation period | $CH_4$ production rate (L/d) | $CH_4$ yield (L/g-TS) | $CH_4$ yield of tofu mixture (L/g-TS) | Average $CH_4$ content (%V/V) | VS removal ratio of total input (%) | VS removal ratio of tofu mixture (%) |
|---|---|---|---|---|---|---|---|
| Run1 | Period I | 0.70 | 0.08 | - | 67 | - | - |
| | Period II | 0.81 | 0.05 | - | 63 | 31 | - |
| | Period III | 0.98 | 0.05 | - | 59 | 31 | - |
| | Period IV | 0.10 | 0.00 | - | 54 | 27 | - |
| Run2 | Period I | 2.87 | 0.22 | 0.54 | 65 | - | - |
| | Period II | 3.87 | 0.18 | 0.45 | 59 | 46 | 64 |
| | Period III | 1.53 | 0.05 | 0.06 | 50 | 38 | 58 |
| | Period IV | 0.03 | 0.00 | - | 57 | 43 | - |

※$CH_4$ production rates and yields were calculated excluding the data of gas leak periods. (d61-112, 150-200, 261-289 of Run 1; d47-73, 261-289 of Run 2)

VS removal ratios of Run1 and Run 2 were not calculated due to the short operation time.

VS removal ratio of tofu mixture in period IV was not calculated due to the different TS of input in Run 1 and Run 2

### 2.3.2. Operating performance of co-digestion (Run 2)

The cumulative biogas production, concentrations of DOC, VFAs, DTN, $NH_4^+$-N, TS and VS of Run 2 are shown in **Figure 2.7**, and the results of methane yields and VS removal rates are summarized in Table 4. VS removal rates of fried tofu mixture were calculated assuming that the degradation of sludge in Run 2 was the same as that of Run 1. In period I, biogas was generated stably; methane yield of total input substrates was 0.22 L/g TS, and approximately 0.54L methane was generated from per gram of tofu mixture, and this result was close to the methane production potential, 0.55L/g TS that obtained in our previous study. [34] Methane fermentation was suggested to be conducted stably without accumulation of VFAs or ammonia inhibition. In period II, the substrate TS was increased to 72.5 g/L, and OLR was 6.3 kg VS/$m^3$/d. Concentrations between 22-84 mg/L of acetate, and 160-280 mg/L of propionate were detected, and $NH_4^+$-N concentrations between 1270-1900 mg/L were detected. Methane yields of total input substrates and tofu mixture were 0.18 and 0.45 L/g TS, which decreased by 18.2% and 16.7%, respectively, as compared to those of Period I. Reduction of methane production and accumulation of VFAs indicated that slight inhibition occurred during this period. However, as estimated by Li (2005) [35], that the theoretical methane yields of raw garbage, cow slurry and municipal waste were 0.51, 0.54 and 0.47 L/g VS, respectively. Taking into consideration that the practical biogas production is normally less than the theoretical one, the fried tofu could be considered as good biomass resource with a methane yield of 0.45 L/g TS (0.52 L/g VS). In period III, when the substrate TS was increased to 101.5 g/L (OLR was 8.8 kg VS/$m^3$/d), a rapid increase in DOC was observed, and high concentrations of acetate (340-1220 mg/L) and propionate (460-2450 mg/L) were detected. During the same period, decrease in pH values was observed. Meanwhile, a dramatic increase in $NH_4^+$-N concentration was observed, with concentrations up to 4150 mg/L were detected. Since fried tofu contains high content of protein, the degradation of which was considered to contribute greatly to the high ammonia concentration in Run 2. Biogas generation almost stopped after day 145, and average methane yield of this period was 0.03 L/g TS. Meanwhile, methane gas production of tofu mixture was -0.01 L/g TS, indicating that the methane generation was suppressed in the period. To study whether the suppression was caused due to the over load, the digested sludge was extracted and digested for another five days in a syringe, and the obtained methane yield of the digested sludge was approximately 0.05 L/g TS, which was much lower than those of period I and II. The result indicated that ammonia was the main inhibitor during period III. In period IV, to study whether methane fermentation could be recovered, substrate

TS was decreased to the same as in period II decrease in DOC, VFAs, TN and $NH_4^+$-N concentrations were observed; and increase in methane content and VS removal rates were also obtained. However, compared with the digester performance of Period II, higher concentrations of $NH_4^+$-N (1930-3300 mg/L), acetate (90-1400 mg/L) and propionate (920-1510 mg/L) were detected, and VS removal rate of 43% was lower than that of period II. In addition, recovery of biogas production was not observed. It is indicated that by decreasing the substrate concentration, inhibition to methane fermentation was mitigated slightly, but complete recovery was not obtained.

On the other hand, compare the co-digestion with the sludge digestion, during the stable operating periods I and II, the addition of fried tofu significantly increased the biogas production and VS removal efficiency. The methane yields of total substrates increased by 175% and 260% in periods I and II, respectively; and the VS removal rates of total substrates in period II increased by 44%. In addition, the methane yields of tofu mixture during stable periods were 6.8-9 folds of those of OD sludge, and thus waste fried tofu was considered as a good bioresource for methane recovery.

The ammonia accumulation was considered as the main inhibitor to the methane fermentation in the present study, therefore, the authors assumed a threshold values of ammonia nitrogen and calculated the addition amount of fried tofu. As shown in **Table 2.5**, the average $NH_4^+$-N concentrations of both runs were calculated according to the data of period III, and the attribution to ammonia generation of tofu mixture was calculated by excluding the $NH_4^+$-N that generated from sludge, which was considered the same as the concentration of Run 1, and the amount of tofu mixture was calculated. The results showed that assuming the threshold value of $NH_4^+$-N was 2000 mg/L, by decreasing the addition of tofu mixture to 11.6 g/L might mitigate the ammonia inhibition. However, decreasing the tofu addition might lead to the issue of the inefficient treatment of waste fried tofu, which means the mixing ratio based on the wastes abundance in local town was preferred. Adjusting C/N ratio of the substrates was widely suggested as effective on the control of ammonia inhibition. According to **Table 2.1**, the C/N ratio of OD sludge was higher than that of fried tofu; in addition, Figure 1 showed that high concentrations of $NH_4^+$-N (1230-1890 mg/L) were also detected in period III of sludge digestion, therefore in order to adjust the C/N ratio to a suitable range for anaerobic digestion, adding other kinds of waste biomass with higher C/N ratio, for example, agricultural waste, was preferred.

**Table 2.5** Contribution to ammonia production of sludge and fried tofu, and the addition of fried tofu to decrease the ammonia generation

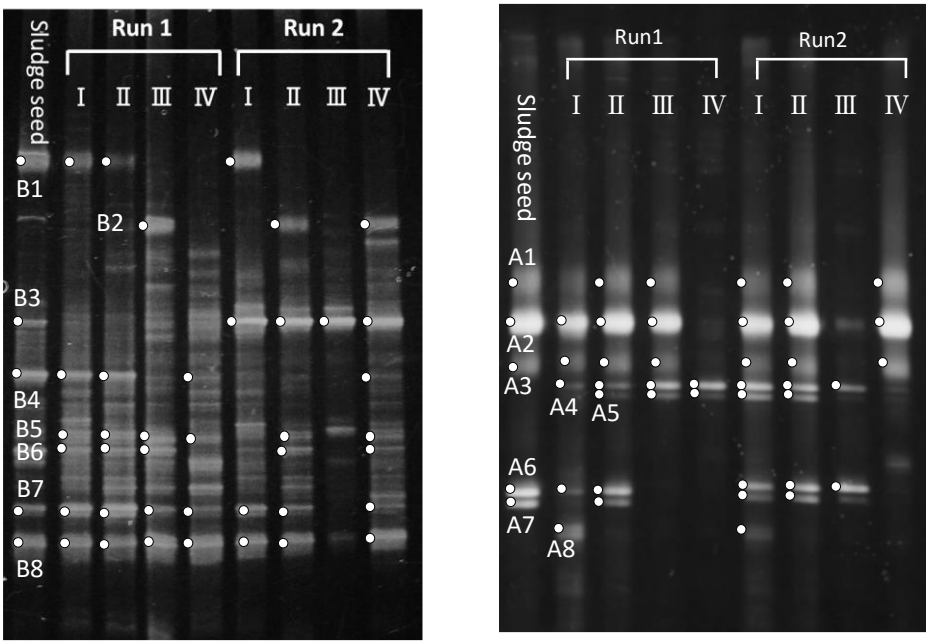
| $NH_4^+$-N con. (mg/L) | Period III | Assumed threshhold |
|---|---|---|
| Generated from sludge+tofu (Run 2) | 2651 | 2000 |
| Generated from sludge (Run 1) | 1620 | 1620 |
| Generated from tofu (Run 2- Run 1) | 1031 | 380 |
| Tofu addition (g/L) | 31.5 | 11.6 |

### 2.3.3. Comparison of the thermophilic and mesophilic digestion

The authors compared the operating performances of the thermophilic digestion and the mesophilic digestion that conducted in the previous study [34], and the comparison results were summarized in **Table 2.6**. Methane yields of total substrates and tofu mixture, and VS removal rates were calculated in the period with maximum substrate TS. The results showed that for the sludge digestion, although the maximum substrate TS was not increased, since the sludge retention time was shortened remarkably (10 days at thermophilic and 25 days at mesophilic temperature), the maximum OLR for stable performance was increased by approximately 73.5%; while VS removal rates and methane production were close with that in mesophilic digestion. On the other hand, in the co-digestion, maximum OLR was increased by 80% at thermophilic temperature, however, VS removal efficiency and methane production was decreased.

**Table 2.6.** Performance comparison of the digestion at thermophilic and mesophilic temperature.

| | Present study (55°C) | | Previous study (35°C) (Togari et al., 2013) | |
|---|---|---|---|---|
| Substrates | Run 1 | Run 2 | Sludge | Sludge+Tofu |
| Max OLR (kg-VS/$m^3$/d) | 5.9 | 6.3 | 3.4 | 3.5 |
| Max substrate TS (g/L) | 70.0 | 71.5 | 100.0 | 101.5 |
| $CH_4$ yield (L/g-TS) | 0.05 | - | 0.06 | - |
| $CH_4$ yield of tofu (L/g-TS) | - | 0.45 | - | 0.51 |
| VS removal ratio (%) | 31 | 45 | 31 | 50 |




(a) Bacteria profiles (b) Achaea profiles

**Figure 2.8** DGGE profiles of 16S rRNA gene fragments from samples in Run 1 and Run 2 during the stable phase of each period.

### 2.3.4. Microbial community structure change during digestion

The DGGE profile of bacteria was shown in **Figure 2.8 (a).** In the bacteria DGGE profile, a total of eight obviously visual bands (B1-8) were detected, and the banding patterns showed that most bands detected during period I and II in Run 1 were similar to those of seed sludge, and some bands (B1 and B4) disappeared when the substrate concentration was increased; however, no significant change was observed with the change of organic loading rate. In period IV, methane fermentation was inhibited due to the high ammonia concentration, but no obvious change of detected bands was observed, indicating that the bacteria was resistant to the inhibition. On the other hand, the bacteria banding patterns of Run 2 showed that most bands detected in Run 2 were detectable in Run 1; however, with addition of fried tofu, Band B3 was significantly strengthened; meanwhile, Band B4 disappeared. In the co-digester, most bands disappeared in period III, and then detected in period IV. The results revealed that the bacteria involving in the hydrolysis process was inhibited at OLR of 8.8 kg VS/$m^3$/d; and when the substrate TS was decreased, inhibition to bacteria was recovered.

The archaea DGGE profile was shown in **Figure 2.8 (b)**, and a total of eight visual bands were detected. The banding patterns showed that the addition of fried tofu had little effect on archaea community structure. On the other hand, the bands of archaea in Run 1 were the same during period I to III, which was in agreement with the result

that anaerobic digestion of OD sludge was stable when substrate concentration was below 70 g/L. However, most bands detectable in period I to III disappeared in period IV, and this was considered to be caused by the inhibition to methanogens. As shown in **Table 2.7**, Bands A1, A2 and A3 were close (99%) to *Methanosarcina thermophile*, which is an extremely anaerobic thermophilic archaeal and uses acetate to produce methane. [44] Bands A4 and A5 were close (98%) to *Methanothermobacter thermautotrophicus*, which uses $H_2$ and $CO_2$ to produce methane [45], and Bands A6 and A7 were close to *Methanothermobacter crinale*, which uses only $H_2$ to produce methane [46]. On the other hand, bands A4 and A5 showed contrary results between Run 1 and Run 2. In Run 1, the bands were still detectable at high organic load of Run 1, indicating its resistance to inhibition; however, the results in Run 2 showed that the activity of the archaeal was inhibited and failed to recover at high organic load. The information about the sensitivity to ammonia concentrations of aceticlastic and hydrogenotrophic methanogens in literatures was conflicting [47], and in the present study, *Methanothermobacter thermautotrophicus*, which is a hydrogenotrophic methanogens, showed relatively higher tolerance to the inhibition, and this result was in accordance with the study of Wiegant and Zeeman (1986) [48], which indicated that ammonia has stronger inhibition to the formation of methane from $H_2$ and $CO_2$. Bands A6 and A7 disappeared in Run 1 due to the inhibition; however, it showed the same change as bands A4 and A5 in Run 2. The results of microbial community analysis showed that in the sludge digestion process, bacteria was resistant to the inhibition while most methanogens were inhibited due to the high concentration of ammonia; in the co-digestion process, activity of both bacteria and archaea was affected by the inhibition; in addition, the fried tofu addition showed an effect on the bacteria community structure, while little effect on archaea community structure was observed.

**Table 2.7** Closet sequences recovered from DGGE bands of archaea

| Band name | Accession No. | Closest sequence | Similarity (%) |
|---|---|---|---|
| A1, A2, A3 | NR118372 | Methanosarcina thermophila | 99 |
| A4, A5 | NR074260 | Methanothermobacter thermautotrophicus | 98 |
| A6, A7 | NR117968 | Methanothermobacter crinale | 97 |
| A8 | NR102903 | Methanosaeta concilii | 96 |

## 2.4. Conclusions

A high solid co-digestion of sewage sludge from oxidation ditch and waste fried tofu was conducted at thermophilic temperature, and the performance of sludge digestion and co-digestion was evaluated. In sludge digestion, the operation was stable at substrate concentrations below 70 g/L (OLR 5.9 kg VS/$m^3$/d), and methane yield of OD sludge was approximately 0.05 L/g-TS. In the co-digestion, the digester was conducted stably at substrate concentrations below 72.5 g/L (OLR 6.3 kg VS/$m^3$/d), and methane yield of tofu mixture was 0.45 L/g-TS. The fried tofu addition contributed greatly to the methane gas production. Microbial community analysis showed that the addition of fried tofu had significant effect on the bacteria community, while little effect on archaea community.

# Chapter 3. Improvement of dewatering characteristics by co-digestion of rice straw with sewage sludge

## 3.1 Introduction

In wastewater treatment process, a large amount of sewage sludge is generated. Since untreated sludge contains toxic and hazardous substances including pathogenic microorganisms, synthetic organic compounds and heavy metals, etc., there is a great need for effective methods to treat the existing and future accumulations of sewage sludge. Meanwhile, sludge rich in valuable substances such as nutrients including nitrogen and phosphorus, as well as organic matters, thus sludge is considered an effective bio-resource. Anaerobic digestion (AD), in which biodegradable organic matters can be converted to biogas that mainly contains methane, is considered a cost-effective energy and material recovery process for organic wastes including sewage sludge and has been widely applied in many wastewater treatment plants (WWTPs). [29] In recent years, study on anaerobic digestion is not limited to a certain kind of substrate, but a combination of two or more substrates, because the anaerobic co-digestion (AcoD) of several kinds of biomass has more advantages over single digestion. [49] However, despite the advantages of AD such as energy recovery and reduction of the overall mass of final residue, it was reported that the sludge dewaterability was deteriorated after digestion. [50] The addition of biomass in the digester may further affect the dewaterability of digested residues. Togari et al. [34] has conducted the co-digestion experiment of sewage sludge and waste fried tofu, and reported that the dewaterability of digested sludge was deteriorated due to the addition of waste fried tofu. It is well known that usually by dewatering sludge with water content of 96%-98% up to water content of 80%, the sludge volume can be reduced to 1/5 to 1/10. The dewatering process is closely related to transportation or further treatment such as drying or incineration, as well as the expense because in most cases sludge disposal cost is depending on the volume. Since co-digestion is widely studied and co-digestion of several kinds of biomass has been proposed in some WWTPs, [51] and the dewatering process plays a crucial role in the volume reduction and handling properties of sludge. [52, 53] In addition, addition of biomass in the digester could also contribute to the increasing of final residues, which is undesirable in the sewer plant. It is therefore necessary to study the impact on sludge dewaterability of the biomass addition in the digestion process.

On the other hand, an investigation of biomass utilization in Japan revealed that the abundance of inedible agricultural residues is around 5 million tons (carbon basis) per

year, and approximately 70% has not been utilized efficiently. [54] Rice straw, which is a typical agricultural waste, contains high carbon contents and is considered useful bioresources. Rice straw has been widely studied as a co-substrate for anaerobic digestion and the co-digestion has been reported to be feasible by controlling operating conditions. [28, 55-56] However, there are few reports on the dewaterability of digested residues. Since rice straw contains mainly lignocellulose, it can be expected to improve the dewaterability of digested residues according to Örmeci and Vesilind [57]. Komatsu et al. has conducted a co-digestion experiment of sewage sludge and rice straw and reported that methane production was increased and water content of dewatered sludge was decreased. [58] Nakakihara et al. has also reported that the dewaterabiliity of digested residues was improved by adding rice straw in the digester. [59] However, the dewatering experiments were conducted at laboratory-scale and the sludge dewaterability was evaluated mainly by capillary suction time (CST) and/or the water content of dewatered sludge. The CST and water content of dewatered sludge are commonly used for evaluating dewaterability; however, the measurements are limited for quantitative prediction. [60] It is therefore necessary to study the actual dewatering behaviour at larger scale and discuss in more details. In the present study, a co-digestion experiment of sludge and rice straw was conducted at laboratory scale, and the dewaterability of digested sludge was evaluated using belt presses that are widely applied in WWTPs. The aim of the present study was to evaluate the impact on dewatering characteristics of digested sludge due to the addition of rice straw in the digestion process.

### 3.2 Materials and methods

#### 3.2.1 Feedstock preparation

Sewage sludge (mixture of primary and secondary sludge) collected from Johoku wastewater treatment plant (Kanazawa, Japan) was used as feed substrate in the experiments. In the wastewater treatment plant, conventional activated sludge process is applied and the daily dewatered sludge (moisture: 77.9%) generation is approximately 27 $m^3$. Total solids (TS) of feed sludge was approximately 15000 mg/L and was thickened to 30000 mg/L before use. Digested sludge was collected from the mesophilic digester in the same WWTP and used as inoculum, the TS of digested sludge was approximately 10000 mg/L. Rice straw was collected in local region and was pre-treated by a softening machine which has been reported by Nakade et al.. [61] The VTS (VS/TS) of pre-treated rice straw was 0.89, and the particle size of rice straw samples was approximately 2-3 mm. Sewage sludge was collected once a week and

stored at 4°C; pretreated rice straw was dried at 105°C for over 24h and stored in a drying chamber before use.

### 3.2.2 Batch experiment to study the pretreatment efficiency of rice straw

A batch experiment was conducted to evaluate the methane production potential of raw sludge. Syringes with working volume of 100 mL were used as reactors **(Figure 3.1**). 20 mL of digested sludge were used as inoculum, and 10 mL raw sludge of which TS were adjusted to 30,000 mg/L were added. Digestion was operated for 30 days at 35 °C, and the volume of produced biogas were measured by the scale line of syringes. Methane production potential of rice straw and efficiency of pre-treatment was also obtained by the batch experiment, in which 0.3g-dry weight of rice straw was mixed to the 30 mL of digested sludge in the syringe. Biogas production was also measured.


**Figure 3.1** Syringes used as digesters in the batch experiment

### 3.2.3 Operation of continuous digestion experiment of sewage sludge and pretreated rice straw

Two plastic tanks with each working volume of 10L were used as reactors. Mesophilically (35°C) digested sludge, collected from the digester in the same WWTP with sewage sludge, was used as inoculum in the experiment. During the operation periods, substrate feeding and withdrawal was conducted once per day on week days to adjust the sludge retention time (SRT) to 25 days. Acting as control, Run 1 was fed with sewage sludge alone, while Run 2 was fed with the mixture of sludge and pre-treated rice straw. The mixing ratio of sewage sludge to rice straw was 1:0.5 (dry weight basis). Biogas was collected in a 20 L Aluminium Bags (GL Sciences Inc., Japan). The reactors were set in a thermostatic chamber at 35°C.

### 3.2.4 Analytical methods

Total Solids (TS) and Volatile Solids (VS) of digested sludge were measured according to Standard Methods (APHA-AWWA-WEF, 1998). pH of sludge samples was

measured with a pH meter (LAQUA F-71, Horiba, Japan). Digested sludge samples were centrifuged at 10000 rpm for 15 min, and supernatants were filtered with a 0.2μm membrane filter for further analysis. Concentrations of dissolved organic carbon (DOC) and total nitrogen (TN) of the filtered supernatants were measured using a TOC/TN analyzer (TOC-V, Shimadzu, Japan). Ammonia concentrations were quantified using an ion chromatograph (HIC-SP, Shimadzu), and concentrations of volatile fatty acids (VFAs) and bicarbonate were measured with an ion chromatograph post-column pH-buffered electro-conductivity method (HPLC Organic Acid Analysis System, Shimadzu). Generated biogas volume was measured using wet gas meter (Shinagawa, Japan), and methane content was determined using a gas chromatography (GC-8A, Shimadzu, Japan).

### 3.2.5 Properties analysis of digestion sludge and dewatering experiment

After 129 days of operation, to evaluate the dewatering characteristics of digested sludge, a dewatering experiment was conducted. The analysis of pH, alkalinity, TS, VS, crude fibre content, anion level, crude protein content, contents of fibrous materials (100 mesh and 200 mesh), conductivity and capillary suction time (CST) were conducted by Ishigaki Maintenance CO., LTD., Japan. The device used for CST measurement was shown in **Figure 3.2**. Before the dewatering experiment, a coagulant selection test was carried out to select the optimal coagulants. Five kinds of cationic polymer coagulants were used in the test, including ICD-3832 (Coagulant A), ICG-1811 (Coagulant B), IZ-89AK3 (Coagulant C), ICK-3229B (Coagulant D) and ICK-3202SV (Coagulant E) (Ishigaki Maintenance CO., LTD., Japan). Coagulants A was liquid while Coagulants B, C, D and E were powders. Each coagulant was diluted to 0.2% (w/v) before use and added gradually and mixed with 200 mL of digested sludge, until no further increase of sludge flocs were observed, the TS ratio of added coagulant to sludge was determined as optimal dose ratio. After mixed well with coagulant, sludge was poured into a Buchner funnel and filtered by gravity filtration test device (**Figure 3.3**); the filtrate volume was recorded at the time of 10s, 30s, and 60s. Filtrated sludge was sandwiched between filter fabrics and dewatered at 0.1 MPa for 1 min by the device shown in **Figure 3.4**. The diameter, thickness, and mass of sludge cakes were measured and detachability was evaluated. The dewaterability was evaluated mainly by the CST, filterability (through rate during gravity filtration) and water content of sludge cakes; coagulants were selected according to both the dewatering performances and coagulant dose ratio. Based on the results, two of the coagulants that showed better dewatering properties and cost-efficient were selected in the test for the further dewatering experiment. Each selected coagulant was mixed with digested

sludge based on the optimum dose ratio respectively, then sludge was filtered and dewatered using a belt press (**Figure 3.5**), of which filter fabric tension was 6 N/mm and filter fabric speed was 1m/min. Specific filtration rates (dry weight of dewatered sludge in unit time and unit filter fabric length), water contents and thickness of sludge cakes were measured. Furthermore, taking into account that the addition of biomass in the digestion process could contribute to the amount increasing in dewatered residues, which is undesirable in the WWTP, we simulated the amount of dewatered sludge generated under the operating condition of digestion and dewatering experiment of this study. In the case of digesting $1m^3$ of raw sludge, assuming that the volume change due to rice straw and coagulant addition is negligible, the amount of dewatered sludge *m* can be calculated as Eqs (2),

$$M = \frac{TS*v}{1-c} \qquad (2)$$

Where *TS* [g/L] is the TS of digested sludge; *v* [$m^3$] is the assumed total volume of sludge, i.e., $1m^3$; *c* [%] is the water content of dewatered sludge.

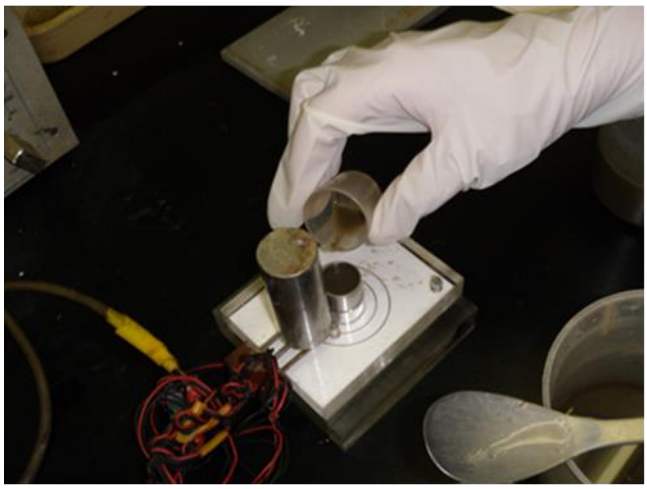


**Figure 3.2** Device used for CST measurement

**Figure 3.3** Simple gravity filtration measuring device

**Figure 3.4** Simple dewatering tester used in the coagulant selection test

**Figure 3.5** Belt press used in the dewatering experiment

### 3.3 Results and discussion

#### 3.3.1 Improvement on biogas production of pretreatment

The accumulative methane gas production is shown in **Figure 3.6**. $CH_4$ yield of raw sludge was 0.28 L/g-TS, and the production potential of un-treated rice straw and softened rice straw were 0.19 and 0.22 L/g-TS, respectively. Therefore expressed softening -treated rice straw was used in the continuous experiment.

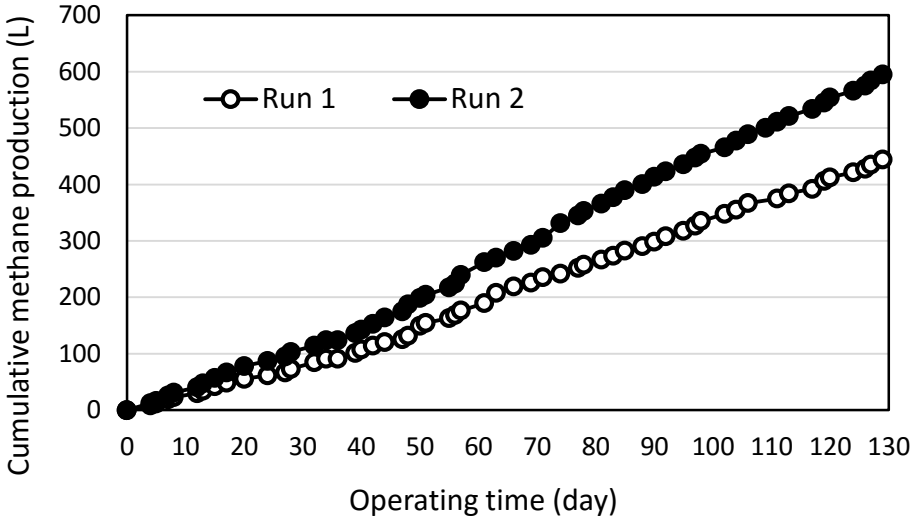



**Figure 3.6** Cumulative methane production in Run 1 (sludge digestion) and Run 2 (co-digestion of sludge and rice straw) in the continuous experiment.

#### 3.3.2 Operating performance of continuous digestion experiment

The digestion performance was evaluated based primarily on the pH, TS, VS, volatile fatty acids (VFAs), ammonia-nitrogen, methane yields and contents. During 129 days of operation, biogas was generated stably in both digesters as shown in **Figure 3.1**. The pH and DOC variation of digested sludge are shown in **Figure 3.2**, and the TS, VS of input substrates and output of digested sludge is shown in **Figure 3.3**. The optimal values of pH in methane fermentation vary between 6.5~7.5, [62] and in this study, pH values varied between 6.9~7.3 in Run 1 and 7.0~7.3 in Run 2, which was suggested optimal range for methane production bacteria. After day 19, DOC concentrations of 90-180 mg-C/L was detected in Run 1, and 90~190 mg-C/L detected in Run 2. VFAs were quantified in this study, and only acetic acid and propionic acid were detected. In the initial operating periods (day 1-27), no VFAs were detected in both Runs. In Run 1, from day 34, low concentrations of acetic acid and propionic acid were sometimes detected, with concentrations below 10 mg-C/L. This result was suggested to be caused by the slight variation of the raw material collected from the

WWTP. In Run 2, no organic acids were detected during all operating periods. The addition of rice straw was suggested to have positive effect on the stable operation of digestion. According to Figure 3, the TS and VS of digested sludge in Run 1 increased slightly during day 41 to day 48, but kept stable after day 48, suggesting the organic material was degraded stably during operation. In Run 2, the TS and VS of digested sludge increased slightly from day 76 to day 97, but it was relatively stable during all operating periods. These results suggested that the digestion was conducted stably in both runs without organic acids accumulation or ammonium inhibition.

The biogas production and VS removal efficiency results are summarized in **Table 3.1**. In the experiment, $CH_4$ production increased by 27% by the addition of rice straw. Methane yield of total input substrate were 0.34 L/g-VS of Run 1 and 0.29 L/g-VS of Run 2; assuming that the methane yield of sewage sludge in Run 2 was the same as those in Run 1, approximately 0.18 L of methane was estimated to be produced from one gram of dry rice straw (TS base). This result was lower compared to the result of Nakakihara et al., [59] which was 0.26 L/g (TS base). Since rice straw was collected and stored for long period use, the degradation of rice straw during storage period was suggested to be the cause of low methane production in this study.

**Table 3.1** Summary of methane production rate, average $CH_4$ content, $CH_4$ yield of sludge and rice straw and VS removal ratio of Run 1 (sludge digestion) and Run 2 (co-digestion of sludge and rice straw) in the continuous experiment.

| | Run 1 | Run 2 |
|---|---|---|
| $CH_4$ production rate (L/day) | 3.7 | 4.7 |
| Average $CH_4$ content (%) | 58 | 56 |
| $CH_4$ yield of total substrates (L/g-VS) | 0.34 | 0.29 |
| $CH_4$ yield of rice straw (L/g-VS) | - | 0.18 |
| TS removal ratio (%) | 62 | 56 |
| VS removal ratio (%) | 75 | 66 |

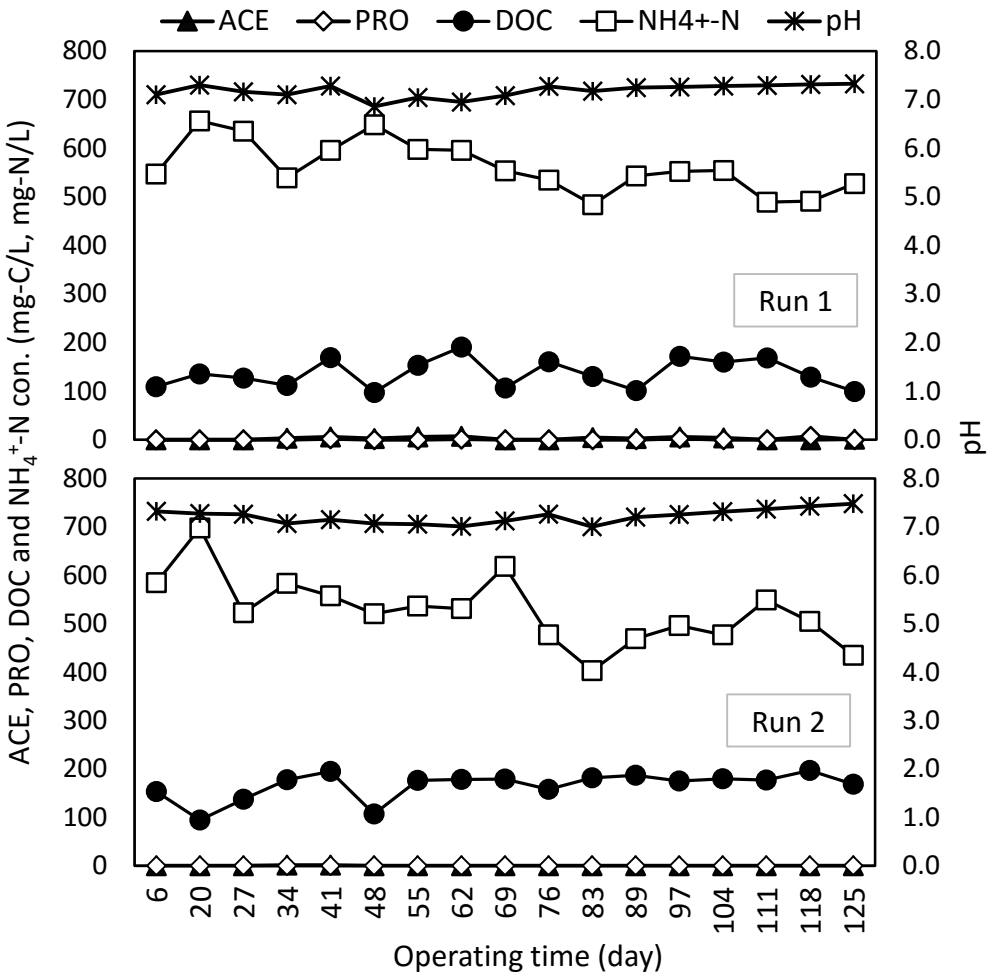



**Figure 3.2** Concentrations of acetic acid (ACE), propionic acid (PRO), dissolved organic carbons (DOC), ammonia-nitrogen ($NH_4^+$-N) and pH values in Run 1 (sludge digestion) and Run 2 (co-digestion of sludge and rice straw) in the continuous experiment.

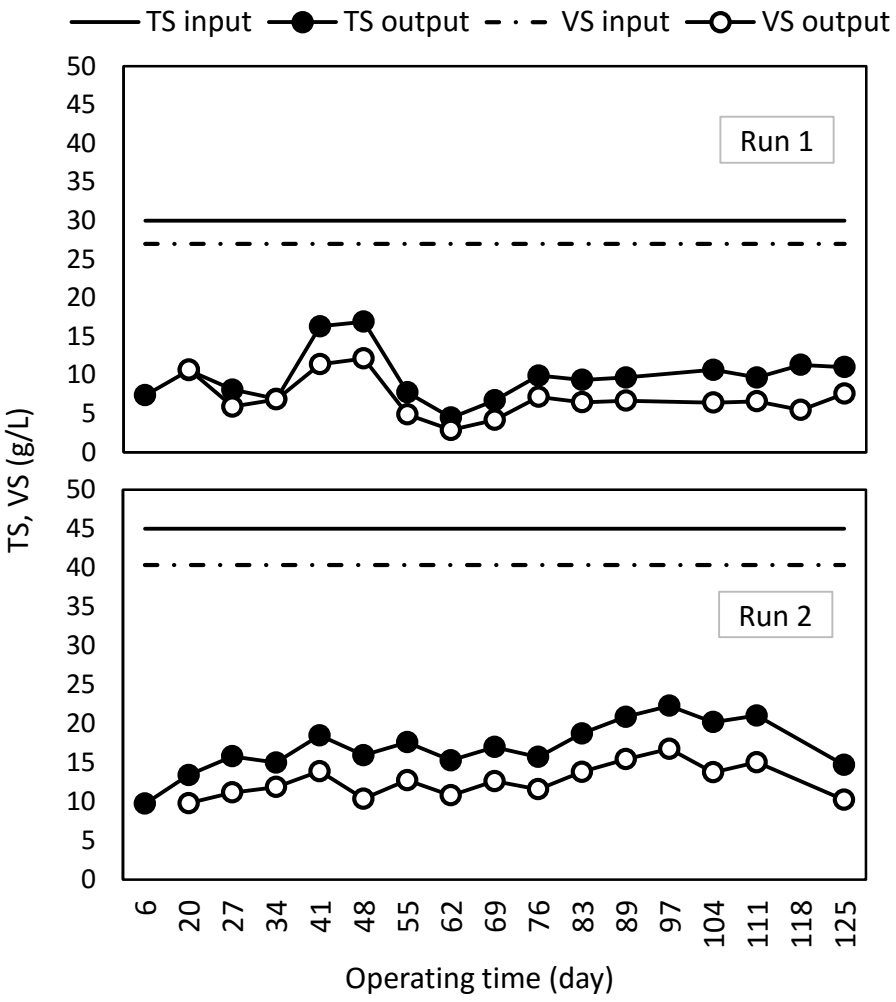



**Figure 3.3** Input total solids (TS) and volatile solids (VS), output TS and VS of Run 1 (sludge digestion) and Run 2 (co-digestion of sludge and rice straw) in the continuous experiment.

### 3.2.3 Evaluation on dewaterability of digested sludge in dewatering experiment

The analysis results of digested sludge properties are shown in **Table 3.2**. By addition of rice straw, TS and fibrous materials contents of digested sludge shows significant increasing. No significant difference of M alkalinity and anion degree, of which higher value shows higher coagulant dose ratio, was suggested. Normalized CST was calculated by dividing CST by the initial Substrate concentration according to Yu et al.. [53] The normalized CST of Run 2 was 6.6 s L/g-TS, and the measurement of Run 1 was failed to conduct. In our previous study, the CST measurement of the sludge digested under the same condition showed that the normalized CST of digested sludge was 33.9 s L/g-TS; and according to the CST and TS results of Nakakihara et al., [59] the normalized CST value of digested sludge under the same operating condition was

43.5 s L/g-TS. Since larger normalized CST values suggested worse dewaterability, [63] it could be suggested that the dewaterability of co-digested sludge was much better than that of digested sludge. Besides, the fibrous material (100 and 200 meshes sieves) contents in the co-digested sludge were greater than digested sludge of Run 1 by 410% and 223%, respectively, which also demonstrated that the dewaterability was better in the co-digested sludge; and the result agreed with result of the CST measurement.

**Table 3.2** Properties of digested sludge of Run 1 (sludge digestion) and Run 2 (co-digestion of sludge and rice straw) in the continuous experiment.

| Parameters | Run 1 | Run2 |
|---|---|---|
| pH | 7.1 | 6.9 |
| M alkalinity (mg-$CaCO_3$/L) | 1900 | 2000 |
| Anion degree ( mEq/g-TS) | 0.6 | 0.4 |
| SS (mg/L) | 8200 | 17400 |
| TS ( mg/L) | 9900 | 20400 |
| VTS (%) | 68.7 | 71.5 |
| Crude protein (%/TS) | 28 | 21 |
| Crude fiber (%/SS) | 6.4 | 30.3 |
| Fibrous material (100 mesh)(%/SS) | 6.9 | 35.2 |
| Fibrous material (200 mesh)(%/SS) | 12.3 | 39.8 |
| Normalized CST (s･L/g-TS) | — | 6.6 |

In the coagulants selection test, coagulants were decided mainly based on the types of coagulants, coagulant dose ratio and water content of sludge cakes. Optimum coagulant dose ratio and water contents of sludge cakes are described in **Figure 3.4**. By using Coagulant C, water contents of sludge cakes were the lowest in both runs, however, the coagulant dose ratio was the highest, which is not cost-efficient and thus was not selected. By using Coagulant B, the coagulant dose ratio was close to coagulant A, however, no difference of water content was observed between Run 1 and Run 2, Coagulant B was therefore not selected. Among Coagulant A, D and E, there was no significant difference in water contents of sludge cakes. Considering Coagulant A was liquid type, while D and E were powders, to study the dewatering performance using different coagulant types, Coagulant A was selected. In addition, Coagulant E was selected based on the lower coagulant dose ratio and water content of sludge cake compared to Coagulant D.

In the dewatering experiment, Coagulant A and Coagulant E were diluted to 0.2 w.t % and then added into 3 L of sludge respectively and mixed uniformly. When no more flocs increased, digested sludge was filtered by gravity, and then dewatered by a belt

press. The results of dewatering experiment are described in **Table 3.3**. The specific filtration rate (SFR: dry weight of dewatered sludge in unit time and unit filter fabric length) and water content of sludge cakes are main factors to characterize the dewatering performances in the WWTP. In the case of using Coagulant A, the SFR of Run 1 and Run 2 were 93.8 and 170 kg-TS/mh, respectively. By the addition of rice straw, the SFR was increased by 81.2%, and water content of sludge cakes decreased by 8.2%. In the case of using Coagulant E, the SFR increased by 174.2% and water content decreased by 13.4%, respectively. Since higher filtration rate indicates higher capacity for sludge dewatering, and in this study, by the addition of rice straw, the filtration rate was increased significantly with using either coagulant. The sludge disposal cost is normally based on the volume, and lower water content of sludge cakes indicates smaller sludge volume, which could make further treatment or disposal easier, it is therefore important to minimize the generated sludge amount, namely, to lower the water content of dewatered sludge in a WWTP. In the case of using belt press, the water content of dewatered sludge is usually 75%-80%, and in the present study, the water contents of dewatered sludge in Run 2 were 69.0% and 70.8%, respectively, which was remarkably improved compared with that of Run 1. In addition, these results also indicated that the liquid coagulant (Coagulant A) was more effective for dewatering digested sludge than the addition of powder coagulant (Coagulant E). The simulated calculation of dewatered sludge amount under the operating and dewatering condition in the present study showed that in the case of using Coagulant A, the generated sludge amount of single digestion and co-digestion would be 45.0 and 63.3 kg, respectively; in the case of using Coagulant E, the results were 61.4 and 67.2, respectively; by the addition of rice straw, the generated sludge amount would increase by 40.6% and 9.6%, respectively. However, since the input TS of substrates in the co-digester was 50% higher than the single digester in the initial state, the increasing in generated dewatered sludge is less than the added amount of input rice straw. It can be expected that if more effective coagulant can be utilized, despite the fact of TS increasing due to biomass addition, the generated amount of dewatered sludge will not increase significantly.

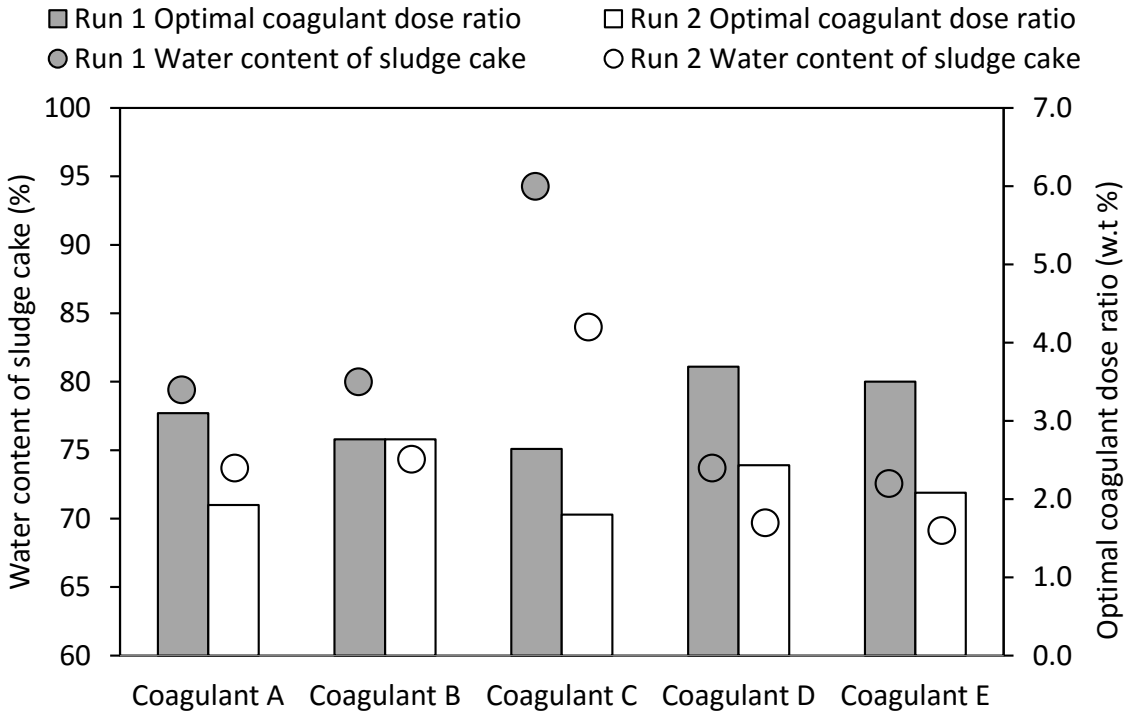



**Figure 3.4** Optimal addition ratio of each coagulant and water contents of sludge cake in the coagulant selection test. (Run 1, sludge digestion; Run 2, co-digestion of sludge and rice straw).

**Table 3.3** Unit mass, thickness and water contents of dewatered sludge cakes of Run 1 (sludge digestion) and Run 2 (co-digestion of sludge and rice straw), filtration rate of belt filter in the dewatering experiment.

| Runs | Coagulant | Unit mass ($g/0.01\ m^2$) | Thickness (mm) | Water content (%) | Specific filtration rate (kg DS/mh) |
|---|---|---|---|---|---|
| Run 1 | Coagulant A | 47.4 | 7 | 75.2 | 93.8 |
| Run 2 | | 62.9 | 10 | 69 | 170 |
| Run 1 | Coagulant B | 41.2 | 5 | 81.8 | 55.1 |
| Run 2 | | 61.1 | 9 | 70.8 | 151.3 |

### 3.4 Conclusions

A continuous co-digestion experiment of sewage sludge and softened rice straw was conducted and dewaterability was evaluated by a dewatering experiment using belt press. Digestion performance was stable during the whole operating period, and methane production of sewage sludge and rice straw were 0.34 and 0.18 L/g-VS respectively. By the addition of rice straw, fibrous materials increased and normalized CST decreased remarkably, indicating that the dewaterability was improved. After dewatered by a belt press, when coagulant A was used, the water content of dewatered

sludge cakes decreased by 8.2% and specific filtration rate increased by 81.2% due to rice straw addition; when coagulant E was used, the water content of dewatered sludge decreased by 13.4% and filtration rate increased by 174.6%. It revealed that dewaterability of digested sludge was improved significantly by the addition of rice straw.

# Chapter 4. Variation of dissolved organic compositions in the mesophilic anaerobic digestion of sewage sludge with the addition of rice straw

## 4.1 Introduction

Compared to mono-digestion that widely used in many wastewater treatment plants (WWTPs), anaerobic co-digestion is now drawing much attention because by mixing two or more waste together, it is possible to overcome some drawbacks of single digestion, i.e., low organic loadings or high N contents. [49] Herbaceous biomass such as rice straw and husk are suggested to be useful bio-resources and it was reported that by adding rice straw to sludge digestion process, biogas production was increased and dewaterability of the digested sludge was improved. [64]

In our previous studies, the methane gas production was increased remarkably due to the addition of waste fried tofu or rice straw. In addition, the evaluation of dewaterability of digested sludge was suggested to be improved by the addition of rice straw in the digestion process.

Recently, in certain large-scale methane fermentation facilities located in Kobe and Osaka, it has been proceeded to recover raw garbage or food waste and co-digest with sewage sludge, and co-digestion of sewage sludge and several kinds of waste biomass is expected to be progressed in the future. If operation technologies of small-scale methane fermentation as described in the present study could be developed, it is considered that co-digestion of several kinds of biomass could also be promoted in the small-scale facilities. On the other hand, waste biomasses other than raw garbage, such as the coffee residues used in Kurobe, and herbaceous biomass that used in the pilot-plant experiment of Nagaoka, are also highly expected to be utilized in the methane fermentation facilities. Previous studies about organic dissolved matters in the digested sludge have reported that the addition of some kinds of biomass such as raw garbage and fried tofu, significantly contributed to the increasing of ammonia, which could cause inhibition to methane fermentation. Studies on ammonia inhibition in the digestion process and measurements to prevent inhibition have been widely reported. However, there are few studies focusing on the variation on the dissolved organic matters of digested sludge by the addition of biomass in sludge digestion process. However, by the addition of waste biomass, it is concerned that it might bring adverse effect on a wastewater treatment process that receives dehydration filtrate. Therefore, it is necessary to study the impact on the organic compositions of supernatant and microbial community by the addition of biomass. In this chapter, the effects of co-

digestion on organic compositions of digested supernatant were examined in a laboratory scale of co-digester.

## 4.2 Materials and methods

### 4.2.1 Preparation of inoculum and substrates

Mesophilic anaerobic digested sludge collected from a digester in Johoku Wastewater Treatment Plant in Kanazawa, Ishikawa was used as inoculum in the present experiment. The TS of digested sludge was approximately 10000 mg/L. In the wastewater treatment plant, conventional activated sludge process is applied and the daily dewatered sludge (moisture: 77.9%) generation is approximately 27 $m^3$. Sewage sludge (mixture of primary and secondary sludge) collected from the same plant was used as feed substrate. The total solids (TS) of sewage sludge was approximately 15000 mg/L, and the sludge was centrifuged to adjust the TS to approximately 30000 mg/L before fed to the digester. Rice straw collected from rice fields in Komatsu city, Ishikawa was used as co-substrate. The rice straw was pre-treated by a softening compress machine. The VTS (VS/TS) of pre-treated rice straw was 0.89, and the particle size of rice straw samples was approximately 2-3 mm. Sewage sludge was collected once a week and stored at 4°C; pre-treated rice straw was dried at 105°C for over 24h and stored in a drying chamber before use. The contents of C, H, N was measured by the combustion method using a CHN Corder MT-5 (Yanako, Tokyo, Japan).The characteristics of the inoculum and feed substrates are shown in **Table 4.1.**

**Table 4.1** Characteristics of inoculum and substrates

| Parameter | Inoculum | Sewage sludge | Rice straw |
|---|---|---|---|
| TS (% w/w) | 1.0 | 15.0 | 99.5 |
| VS (% TS) | 0.71 | 0.91 | 0.89 |
| C (% TS) | - | 45.1 | 39.9 |
| H (% TS) | - | 6.8 | 5.6 |
| N (% TS) | - | 5.4 | 0.6 |
| C/N ratio | - | 8.4 | 70.0 |

### 4.2.2 Operation of continuous digestion experiment

Three digesters with each working volume of 1 L shown in **Figure 4.1** were used as reactors. Two single digesters acting as control groups and a co-digester were operated in this experiment. Substrates of single digesters were sewage sludge (Run 1) and rice straw (Run 2), and the mixture of sludge and rice straw was fed to the co-digester (Run 3). The mixing ratio of sewage sludge to rice straw was 1:0.5 (dry weight basis). The operation was conducted at 35°C, and Sludge retention time (SRT) was 25d. Input total solids (TS) of sludge was adjusted to 30 g/L by concentration and TS rice straw was adjusted to 15 g/L by dilution water. In Run 2, to provide inorganic salts and trace elements for the growth of microbial, the dilution water was made by diluting the stock solution by 1000-fold, of which the compositions were shown in **Table 4.2.** The extraction and substrates feeding was performed once a day on weekdays. Produced biogas was collected in a gas bag in a 10 L Aluminium Bags (GL Sciences Inc., Japan).


**Figure 4.1** Reactors used in the continuous experiment

**Table 4.2** Inorganic salts and trace elements compositions of stock solution

| Substrates | Concentration (mg/L) |
|---|---|
| $NaHCO_3$ | 71 |
| KCl | 174 |
| $CaCl_2$ | 51 |
| $KH_2PO_4$ | 91 |
| $MgCl_2 \cdot 6H_2O$ | 130 |
| $FeSO_4 \cdot 7H_2O$ | 9.15 |
| $ZnSO_4 \cdot 7H_2O$ | 0.43 |
| $CoCl_2$ | 0.13 |
| $MnCl_2 \cdot 4H_2O$ | 0.99 |
| $CuSO_4 \cdot 5H_2O$ | 0.25 |
| $NaMoO_4 \cdot 2H_2O$ | 0.22 |
| $NiCl_2 \cdot 2H_2O$ | 0.27 |
| $Na_2SeO_4$ | 0.12 |
| $H_3BO_3$ | 0.011 |

### 4.2.3 Analytical methods

Total Solids (TS) and Volatile Solids (VS) of digested sludge were measured according to Standard Methods (APHA-AWWA-WEF, 1998). pH of sludge samples was measured with a pH meter (LAQUA F-71, Horiba, Japan). Digested sludge samples were centrifuged at 10000 rpm for 30 min, and supernatants were filtered with a 0.2μm membrane filter for further analysis. Concentrations of dissolved organic carbon (DOC) and dissolved total nitrogen (TN) of the filtered supernatants were measured using a TOC/TN analyzer (TOC-V, Shimadzu, Japan). Ammonia concentrations were quantified using an ion chromatograph (HIC-SP, Shimadzu), and concentrations of volatile fatty acids (VFAs) and bicarbonate were measured with an ion chromatograph post-column pH-buffered electro-conductivity method (HPLC Organic Acid Analysis System, Shimadzu). Generated biogas volume was measured using wet gas meter (Shinagawa, Japan), and methane content was determined using a gas chromatography (GC-8A, Shimadzu, Japan).

### 4.2.4 Determination of proteins and humic compounds

The concentrations of proteins and humic substances were determined by Modified Lowry methods according to Frolund et. al. (1995). [65] According to the method, $CuSO_4$ reacts only with the proteins of the samples, and they measured twice of the

samples, one was with the addition of $CuSO_4$ and one was without. They found that absorbance in the protein standards increased by 20% due to the addition of $CuSO_4$, while there was no change for the humic acid standards. Consequently, they came up with equations of absorbance of protein and humic acid as following:

$$A_{total} = A_{protein} + A_{humic} \quad (3)$$

$$A_{blind} = 0.2\, A_{protein} + A_{humic} \quad (4)$$

$$A_{protein} = 1.25\, (A_{total} - A_{blind}) \quad (5)$$

$$A_{humic} = A_{blind} - 0.2\, A_{protein} \quad (6)$$

Where $A_{total}$ is the total absorbance with $CuSO_4$, $A_{blind}$ is the total absorbance without $CuSO_4$, $A_{humic}$ is the absorbance due to humic compounds, and $A_{protein}$ is the absorbance due to proteins.

In the present study, Bovine serum albumin (BSA) purchased from Sigma-Aldrich was used as standard for protein determination and humic acid purchased from Wako was used as standard for humic substances determination. The absorbance was read at 750 nm (DR 3900, HACH, Germany). The details of the procedure was described in **Supplementary 4.1**.

### 4.2.4 Measurement of carbohydrates

The concentrations of carbohydrates were measured by Phenol–Sulfuric Acid method (DuBois et al., 1956). Glucose standard solution was used to simulate carbohydrates. The measurement was performed as: 1 ml of the sample was added into a test tube, then 1 ml of 5% phenol solution and sulfuric acid (96-98%) were added. The sample and reagent were mixed by vortex and left for over 30 min at room temperature to cool down. The absorbance was read at 490 nm (DR 3900, HACH, Germany). Details of the procedure was described in **Supplementary 4.2**.

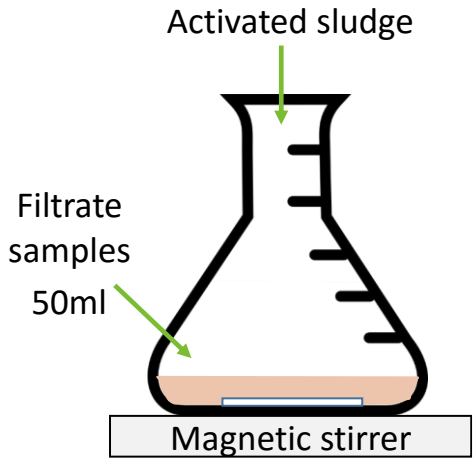
### 4.2.5 EEM fluorescence spectra

To study the variation of dissolved organic matters (DOM) species and the content of digested sludge, the samples was examined using EEM fluorescence spectra (FP-8200, Jasco, Japan). The EEM spectra were obtained with corresponding scanning emission spectra with the range of 200 nm-580 nm at 5 nm increments, and the excitation wavelength varied from 210 nm to 600 nm at 5 nm sampling intervals. The scanning speed was set at 1000 nm/min. The spectrum of pure water was read as blank.

### 4.2.6 Biodegradability evaluation batch experiment

On Day 90, a biodegradability evaluation batch experiment was conducted. Digested sludge of each reactors were centrifuged and then filtered by a 0.2 μm membrane filter to get the filtrate samples. In the batch experiment, 50 ml of filtrate samples were mixed with 5 ml activated sludge of which MLVSS was about 4000 mg/L, mixture was cultured in 100 ml Erlenmeyer flasks, and put on magnetic stirrer to maintain aerobic condition for the mixture. Schematic diagram of batch experiment reactor was shown in **Figure 4.2**. Samples were taken at 0, 6, 24 and 48 h and filtered through 0.2μm membrane filter. Concentrations of COD, proteins, humic matters, VFAs and carbohydrates were measured as described in previous contents (materials and methods). EEM fluorescence spectra of samples which were diluted by 20-fold were obtained.

**Figure 4.2** Schematic diagram of biodegradability evaluation batch experiment



### 4.3 Results and discussion

#### 4.3.1 Digester performance

Digesters were operated for 94 days, the cumulative biogas production was shown in **Figure 4.3**. Biogas were generated stably in Run 1 (sludge digestion) and Run 3 (co-digestion of sludge and rice straw). Biogas generation of the digester of rice straw was relatively low. The figure also showed that in the present study, the co-digestion generated more methane than the addition of those generated in both single digesters, wh**ich means that 1+1>2 was achieved in the biogas production.**

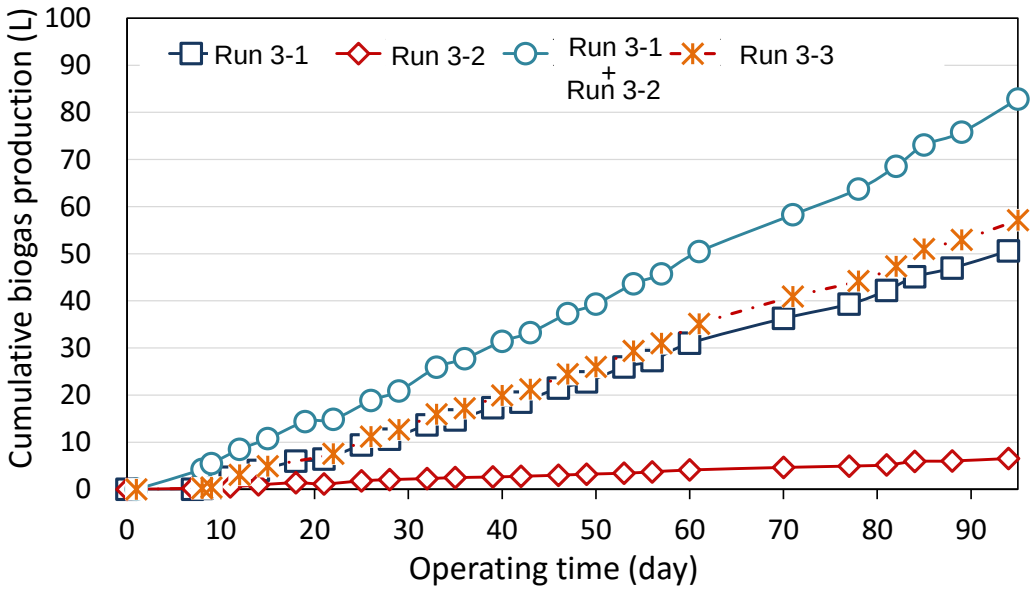



**Figure 4.3** Cumulative biogas production of the continuous experiment.

Volatile fatty acids (VFAs) were quantified in the study, and only acetate and propionate were detected. Variations of VFAs and pH of the continuous experiment were shown in **Figure 4.4**. pH was not controlled in Run 1 or Run 3, while in Run 2, it was adjusted after day 72. In Run 1, approximately 22 mg-C/L of acetate was detected on day 72, however, it was degraded then. While in Run 3, 108 mg-C/L of propionate was detected on day 44, and no acetate or propionate was detected during other periods. pH values of Run 1 and Run 3 were stable, maintaining between 6.9-7.0 in Run 1 and 6.8-7.0 in Run 3, which was suitable range for methanogens. The results of VFAs and pH indicated that digestions in Run 1 and Run 3 were conducted stably. However, in Run 2, approximately 13-257 mg-C/L of total VFAs were detected in all periods. During the same periods, pH values were detected between 6.1-6.4. Since nitrogen content in rice straw is relatively low, to provide nitrogen source for microorganism growth, ammonia was added in the Run 2. And after day 72, pH was adjusted in Run 2, and slight increase in pH and decrease in VFAs were observed,

however, no obvious increase in biogas was observed.

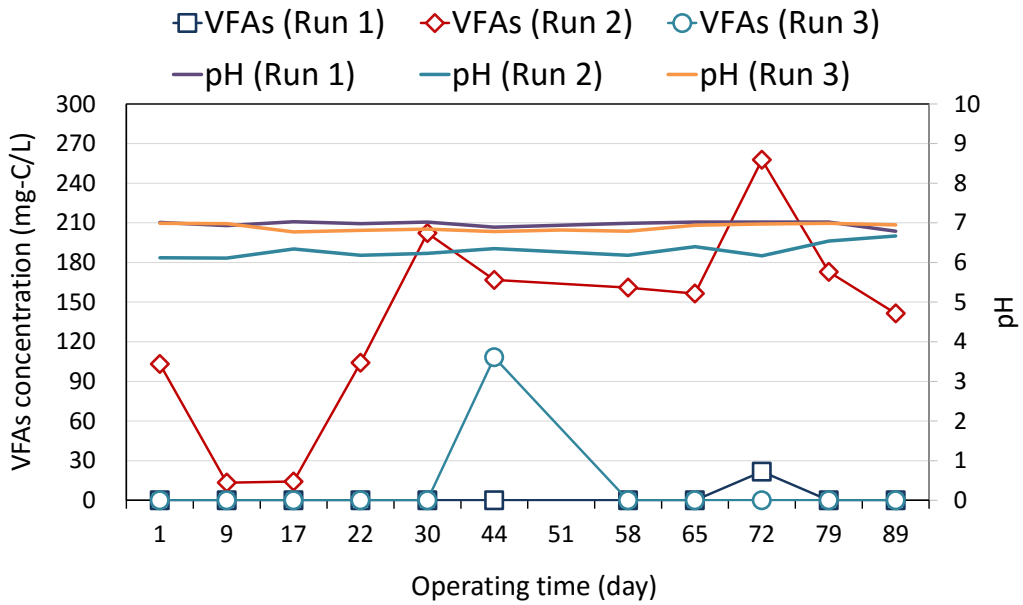



**Figure 4.4** Variations of VFAs and pH values of reactors in the continuous digestion.

To evaluate the degradation efficiency of organics in the substrates, VS and COD were analysed, and the VS and COD removal efficiencies were shown in **Figure 4.5 and Figure 4.6, respectively**. VS and COD removal efficiency were calculated as following equations:

$$\text{VS removal efficiency (\%)} = \frac{VS_{in} - VS_{out}}{VS_{in}} \quad (7)$$

$$\text{COD removal efficiency (\%)} = \frac{COD_{in} - COD_{out}}{COD_{in}} \quad (8)$$

VS removal efficiencies after 25 days varied from 64.0% to 70.7% in Run 1, 36.8% to 43.0% in Run 2 and 62.3% to 66.3% in Run 3. VS were removed in all reactors. And the variations of COD removal efficiencies showed the same change as those of VS. Organic matters were degraded stably in all reactors. In Run 2, despite an approximately 40% organics removal efficiency, low biogas generation and VFAs accumulation indicated that methane production process could be the main reason of the low biogas production in the mono-digestion of rice straw.

Results of methane gas production and organic removal efficiencies were summarized in Table 4.2. Methane conversion rates were calculated as following equation:

$$CH_4\ conversion\ rate\ of\ total\ COD\ (\%) = \frac{\mathrm{CH_4}\ production\ rate}{COD_{in} * 0.35} \times 100 \quad (5)$$

Where $COD_{in}$ is the daily input COD of each reactor; 0.35 refers to the methane yield (NL) of 1 g of COD. [66]

Methane yield of rice straw was calculated by supposing that the methane yield of sludge in Run 3 was the same as that in Run 1. In the present study, approximately 0.27 L of methane gas was generated from 1 g of rice straw, and this result was the same as that reported by Nakakihara et al. (2014). [59] VS and COD removal efficiencies in Run 1 and Run 3 were stably during all periods, with efficiencies over 60%; and methane conversion rates of COD in Run 1 and Run 3 also were stable, with values over 64%. However, TS, VS and methane conversion rate in Run 2 were relatively poor, suggesting that effective degradation of rice straw by single digestion was not achieved.

**Table 4.2** Summary of continuous experiment

| | Run1 | Run2 | Run3 |
|---|---|---|---|
| $CH_4$ production rate (L/day) | 0.36 | 0.04 | 0.52 |
| Average $CH_4$ content (%) | 57 | 54.7 | 55.3 |
| $CH_4$ yield of total substrates (L/g-TS) | 0.3 | 0.07 | 0.35 |
| $CH_4$ yield of rice straw (L/g-TS) | - | 0.07 | 0.27 |
| Total TS removal efficiency (%) | 58.6 | 29 | 54.3 |
| Total VS removal efficiency (%) | 67.4 | 40.1 | 64.4 |
| Total COD removal efficiency (%) | 63.9 | 40.4 | 61.5 |
| $CH_4$ conversion rate of total COD (%) | 64.9 | 16.1 | 64.3 |

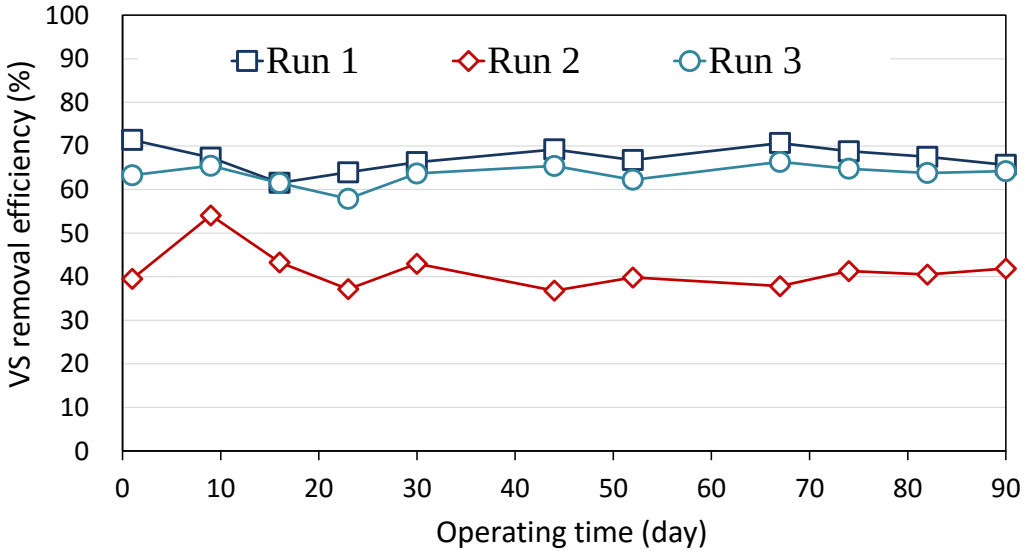



**Figure 4.5** VS removal efficiencies of the continuous digestion.

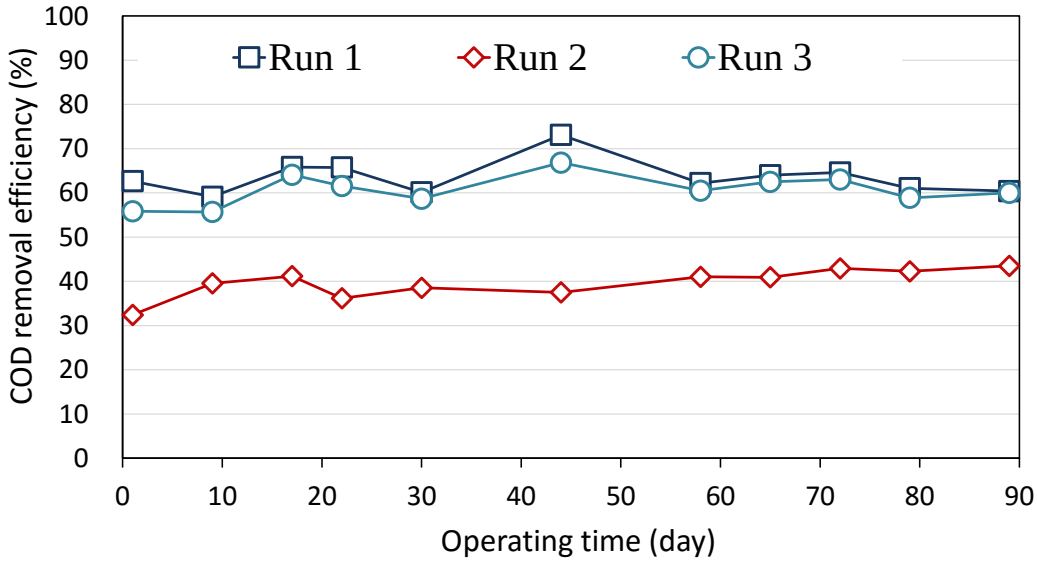



**Figure 4.6** COD removal efficiencies of the continuous digestion

### 4.3.2 Compositions of dissolved organic matters

Average concentrations of TOC, TN, proteins, humic matters and carbohydrates of each reactor were shown in **Figure 4.7**. It is indicated that the addition of rice straw contribute significantly to the increase in TOC, humic matters and carbohydrates. TOC of Run 2 was the highest in all reactors, indicating that efficient removal of organic matters dissolved from rice straw was not achieved. High concentrations of humic matters in Run 2 and Run 3 indicated that the degradation of rice straw increased humic matters significantly. In addition, it was found that concentrations of all the dissolved organic matters detected and TN showed the same result: Run 1+Run 2>Run 3. This result showed that some organic matters dissolved from rice straw that could not be degraded in single digestion were degraded in the co-digestion. One reason was suggested as that by mixing sewage sludge and rice straw, C/N ratio of the mixture could be adjusted to a relatively proper range for methane production bacteria. In the present study, C/N ratios of sludge and rice straw were 8.4 and 70.0, respectively. The mixing ratio of rice straw to sludge was 0.5/1.0 (TS basis), and it can be calculated than the C/N ratio of mixture was 11.4, which was relatively suitable for methane fermentation compared as the single substrate. In addition, despite the addition of inorganic salts and trace elements in Run 2, sludge was suggested to contain some other kinds of elements, which might improve the microbial activity. These factors were reported as the reasons that co-digestion showed positive interactions of the substrates. [49]

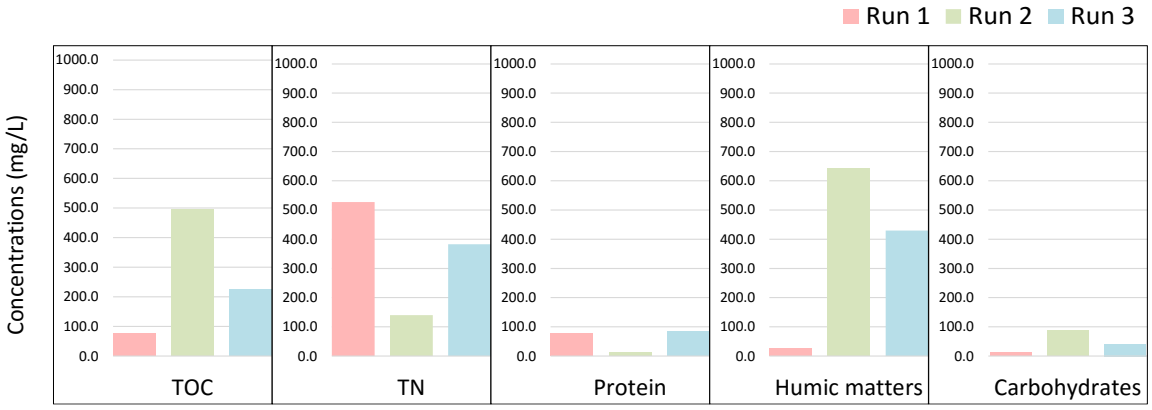



**Figure 4.7** Concentrations of TOC, TN, proteins, humic matters and carbohydrates.

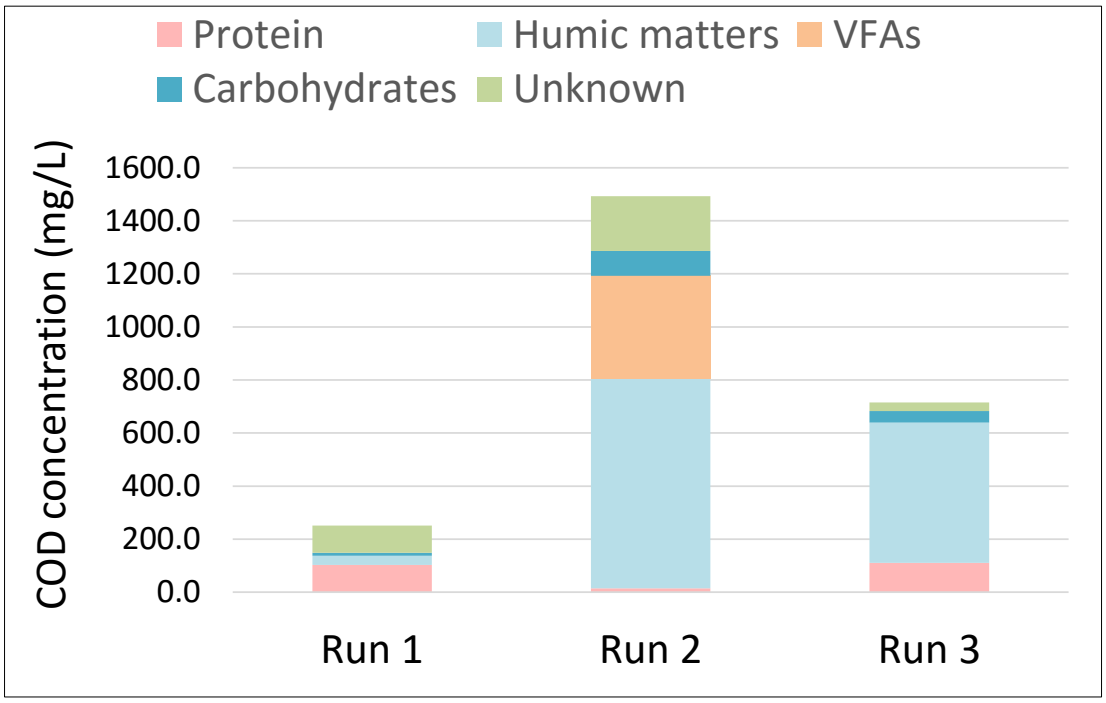



**Figure 4.8** Compositions of dissolved organic matters in digested sludge in the continuous digestion experiment.

To further understand the organic compositions in the reactors, the concentrations of organic matters were summarized in **Figure 4.8**. The results showed that proteins were the main compositions of dissolved organic matters (DOM) in Run 1, while humic matters accounted for over 50% of those in Run 2 and Run 3. In addition, it was indicated that the degradation of humic matters and some unknown DOM were improved by co-digestion. However, humic matters were increased obviously due to rice straw addition.

### 4.3.3 EEM fluorescence spectra

To distribute the biodegradable and non-biodegradable materials in the dissolved organic matters, EEM fluorescence spectra was used in the present study. The spectra of Run 1, Run 2, and Run 3 were shown in **Figure 4.9, 4.10** and **4.11**, respectively. Peak A was located in Ex/Em of 240-250 / 425 nm. Peaks B, C, D were located in Ex/Em of 270/300-305 nm, 220/300 nm, 280/425 nm, respectively. Peaks E, F and G were located in Ex/Em of 275/370-375 nm, 375/425 nm, and 275/425 nm, respectively. According to Chen. et al. [67], Peak A belong to fulvic acid-like substances, Peak B belong to the soluble microbial by-product-like substances (tyrosine- & protein-like substances), Peak C belong to aromatic protein, Peak D belong to humic acid-like substances, Peak E belong to soluble microbial by-product-like substances (tryptophan & protein-like substances), Peak F is related to hydrophobic humic acids, and Peak G belong to humic acid-like substances. Substances corresponding to each peak and the assumed biodegradability of the substances are described in **Table 4.3** according to Yu et. al.. [68] One and three peaks appeared in Run 1 and Run 2 revealed that the degradation of rice straw caused a peak of humic acid-like substances. Compared to Run 1, Run 3 had additional peaks of soluble microbial by-product and humic acid-like organics. The detection of the additional peaks were suggested to be generated by the degradation of rice straw. In addition, the specific intensities of soluble microbial by-product-like substances and tyrosine like-protein in Run 3 were stronger than Run 1, indicating that the rice straw addition caused the enrichment of the fluorescence materials.

**Table 4.3** Substances corresponding to each peak and assumed biodegradability of the substances of digested sludge.

| | Peak | Substances | Assumed Biodegradability | Ex/Em wavelengths (nm) |
|---|---|---|---|---|
| Run 1 | A | fulvic acid-like substances | Low | 240-250 / 425 |
| Run 2 | B | soluble microbial by-product (tyrosine-& protein-like substances) | High | 270/300-305 |
| | C | Tyrosine-like protein | High | 220/300 |
| | D | humic acid-like substances | Low | 280/425 |
| Run 3 | E | soluble microbial by-product (tryptophan- & protein-like substances) | high | 275/370-375 |
| | F | hydrophobic humic acids | Low | 375/425 |
| | G | humic acid-like substances | Low | 275/425 |

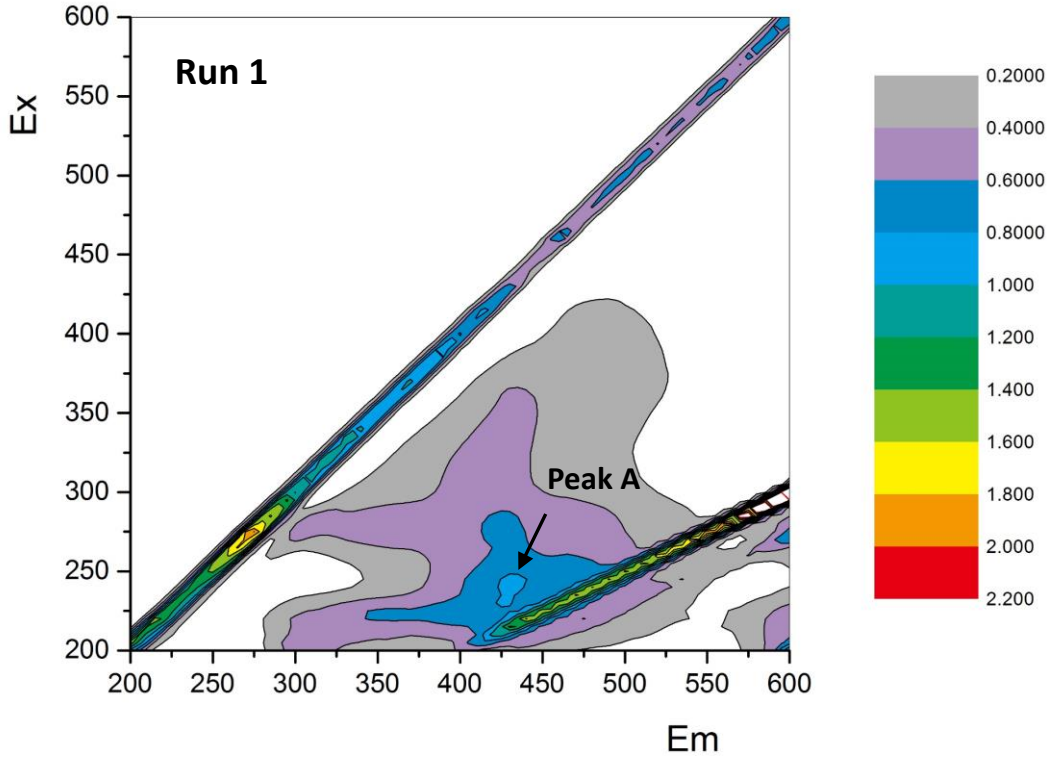



**Figure 4.9** Fluorescent EEMs of digested sludge samples of Run 1 (sludge digester)

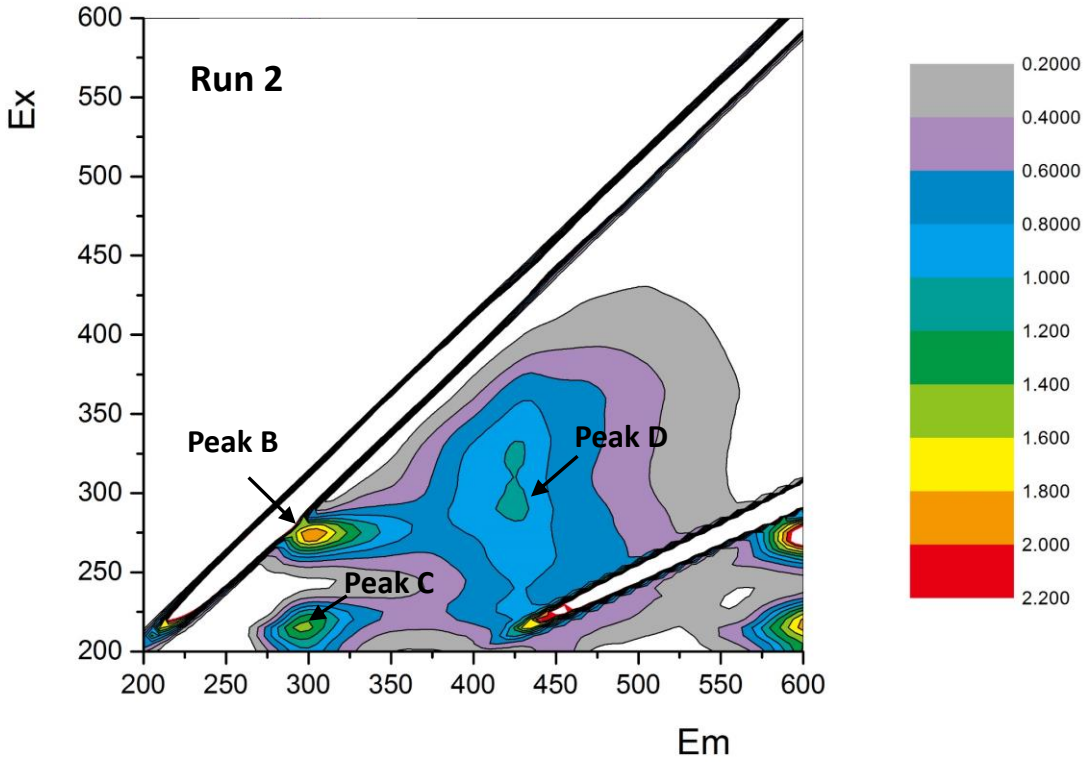



**Figure 4.10** Fluorescent EEMs of digested sludge samples of Run 2 (rice straw digester)

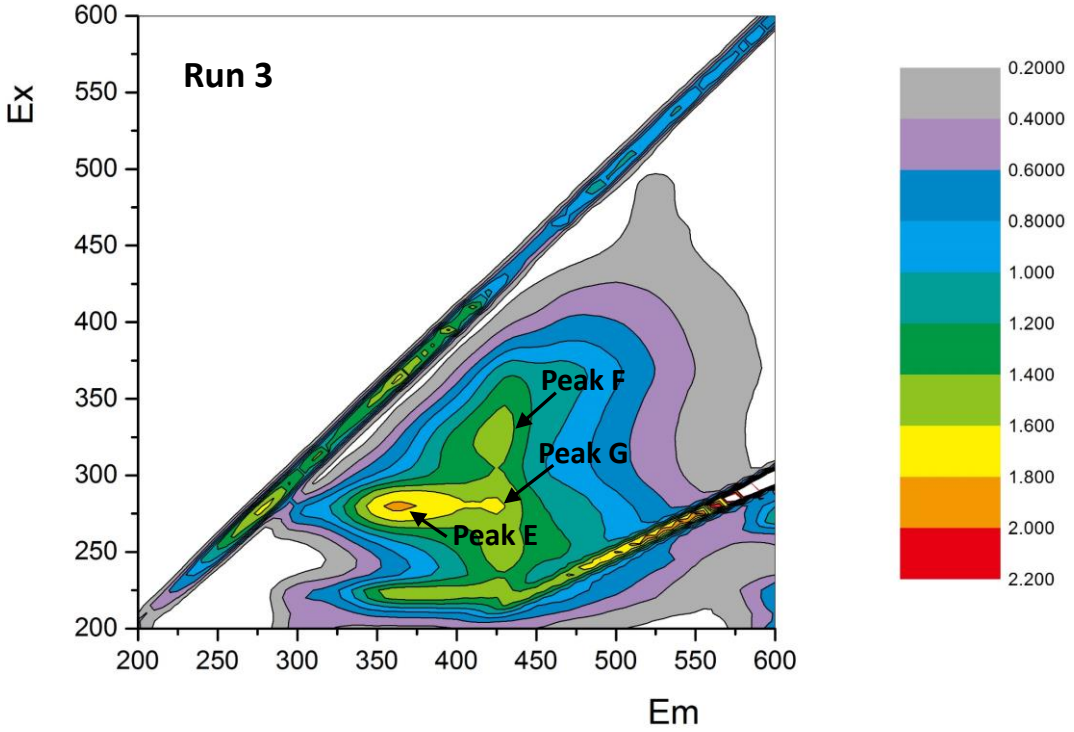



**Figure 4.11** Fluorescent EEMs of digested sludge samples of Run 3 (co-digester of sludge and rice straw)

### 4.3.4 Biodegradability evaluation batch experiment

#### 4.3.4.1 Run 1 (sludge digestion)

To evaluate the biodegradability of dissolved organic matters in digested sludge under anaerobic condition, a batch experiment was conducted, and the compositions and variations of COD and fluorescent EEMs of samples were measured. The COD variation and fluorescent EEMs of Run 1 were shown in **Figure 4.12** and **Figure 4.13**, respectively; and the variation ratios (%) of total COD and each organic component were shown in **Table 4.4**. **Figure 4.12** showed that no obvious change in total COD was observed in Run 1, and humic matters decreased significantly, while carbohydrates and proteins maintained relatively stable. After 6 h of cultivation, humic matters decreased by 63.4%, while COD decreased slightly (1.7%). The increase in unknown organics was suggested to be caused due to the degradation of humic matters. After 24 h, no significant change in the organics were observed. After 48 h, approximately 47.8% of carbohydrates were degraded, and unknown organics decreased as compared to that of 6 h.

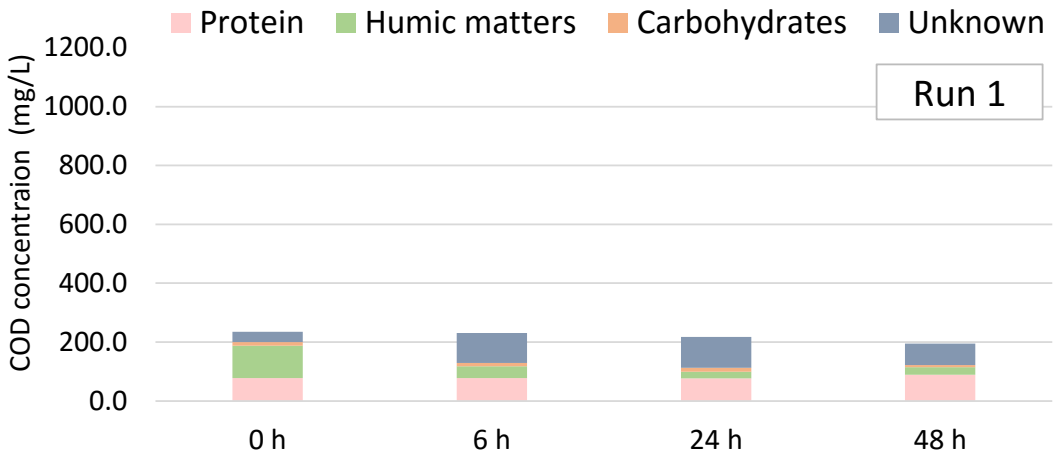



**Figure 4.12** Variations of COD of Run 1 in the biodegradability evaluation experiment

**Table 4.4** Variation ratios (%) of total COD and each organic component at each period of Run 1 in the biodegradability evaluation batch experiment. ※ negative values refer to decrease, and positive values refer to increase of organic matters.

| Run 1 | 6 h | 24 h | 48 h |
|---|---|---|---|
| COD | -1.7 | -7.7 | -17.0 |
| Proteins | 0.0 | -1.6 | 14.4 |
| Humic substances | -63.4 | -79.2 | -76.3 |
| Carbohydrates | -9.3 | 1.9 | -47.8 |
| Unknown | 192.3 | 201.6 | 111.3 |

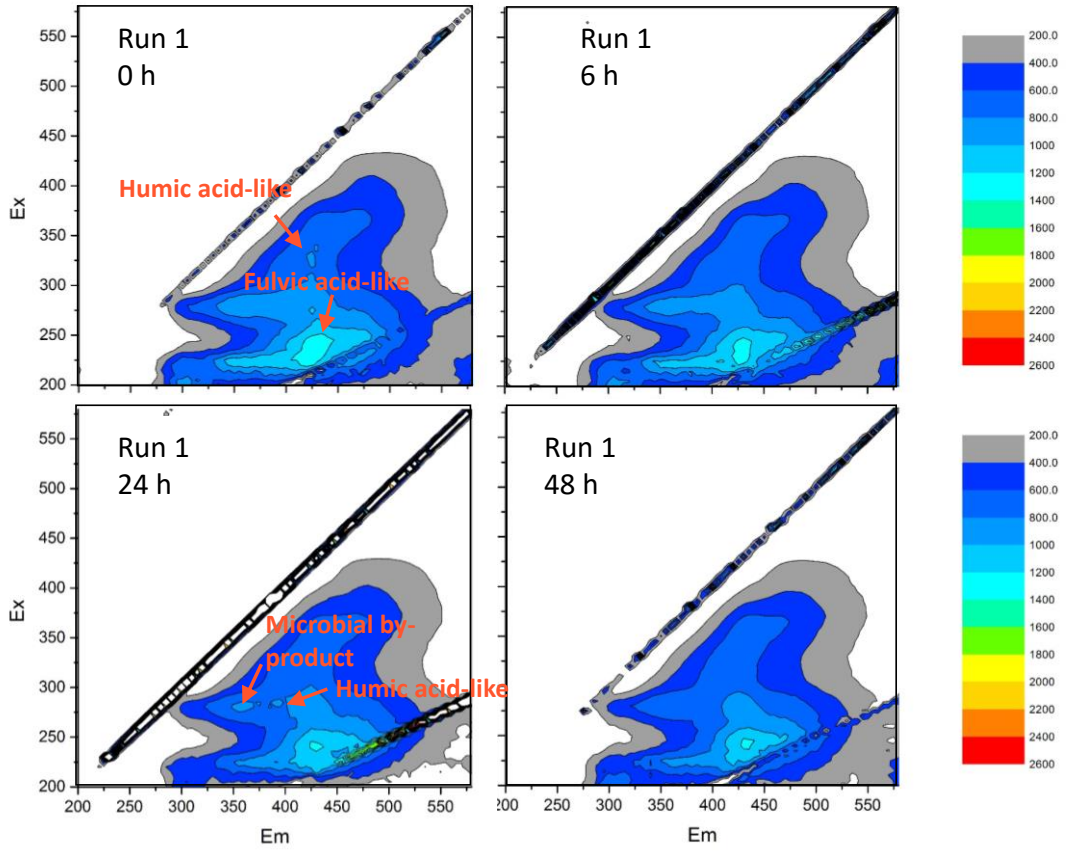



**Figure 4.13** Variation of fluorescense EEMs of Run 1 in the biodegradability experiment

The fluorescent EEMs showed that dominant fluorescent substances in Run 1 were fulvic acid-like organics. After 6h, no obvious change in the fluorescent substances was observed. After 24h, microbial by-products and fulvic acid-like substances decreased. However, after 48 h, fluorescent substances (such fulvic like-organics) were still detected, indicating that aerobic treatment could not remove all the fluorescent substances produced in the anaerobic digestion process.

The Results of biodegradability evaluation batch experiment revealed that by aerobic treatment, up to 63.4% of the humic matters produced in the anaerobic digestion process could be removed after 6 h, however, removal of COD and fluorescent substances were not obtained. After 24 and 48 h, removal rates of total COD were less than 20%, although some fluorescent substances (microbial by-product organics) was degraded, some fluorescent substances (fulvic acid-like substances) were still detectable.

#### 4.3.4.2 Run 2 (rice straw digestion)

The COD variation and fluorescent EEMs of Run 2 were shown in **Figure 4.14** and **Figure 4.15**, respectively; and the variation ratios (%) of total COD and each organic component were shown in **Table 4.5**. **Figure 4.14** showed that no obvious change in total COD and organic compositions after 6 h were observed in Run 2, and after 24h, DOC decreased by half. Degraded COD in Run 2 were mainly unknown compositions, most of which were supposed as VFAs, as shown in the COD compositions of continuous experiment (Figure 4.8). Table 4.5 showed that approximately 40% of total COD of was removed after 24h. Removed COD was considered as humic matters, carbohydrates and unknown organics (VFAs were suggested as main contents). As compared to Run 1, that humic matters produced from rice straw has less biodegradability due to the removal rates less than 30%.

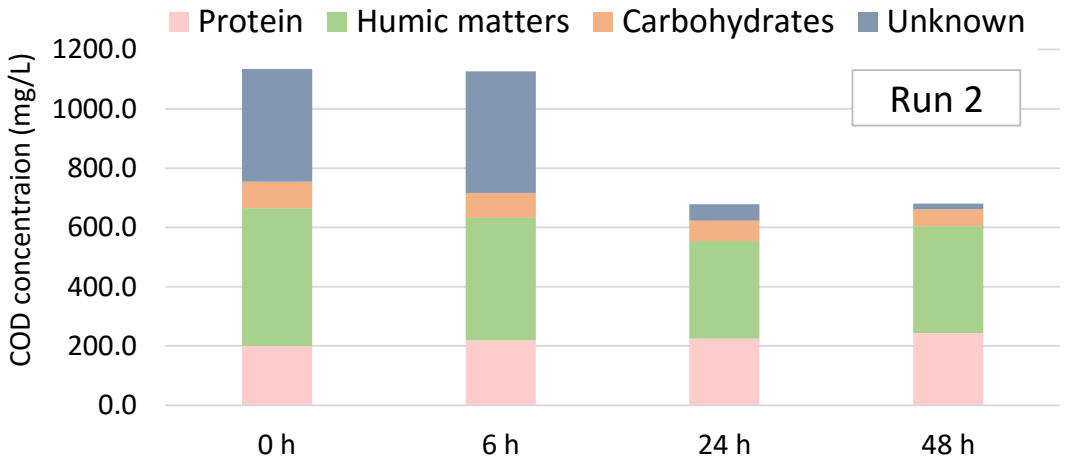



**Figure 4.14** Variations of COD of Run 2 in the biodegradability evaluation experiment

**Table 4.5** Variation ratios (%) of total COD and each organic component at each period of Run 2 in the biodegradability evaluation batch experiment. ※ negative values refer to decrease, and positive values refer to increase of organic matters.

| Run 2 | 6 h | 24 h | 48 h |
|---|---|---|---|
| COD | -0.8 | -40.3 | -40.1 |
| Proteins | 9.4 | 12.5 | 21.8 |
| Humic substances | -11.1 | -29.3 | -22.4 |
| Carbohydrates | -5.3 | -22.9 | -37.6 |
| Unknown | 7.6 | -85.5 | -94.8 |

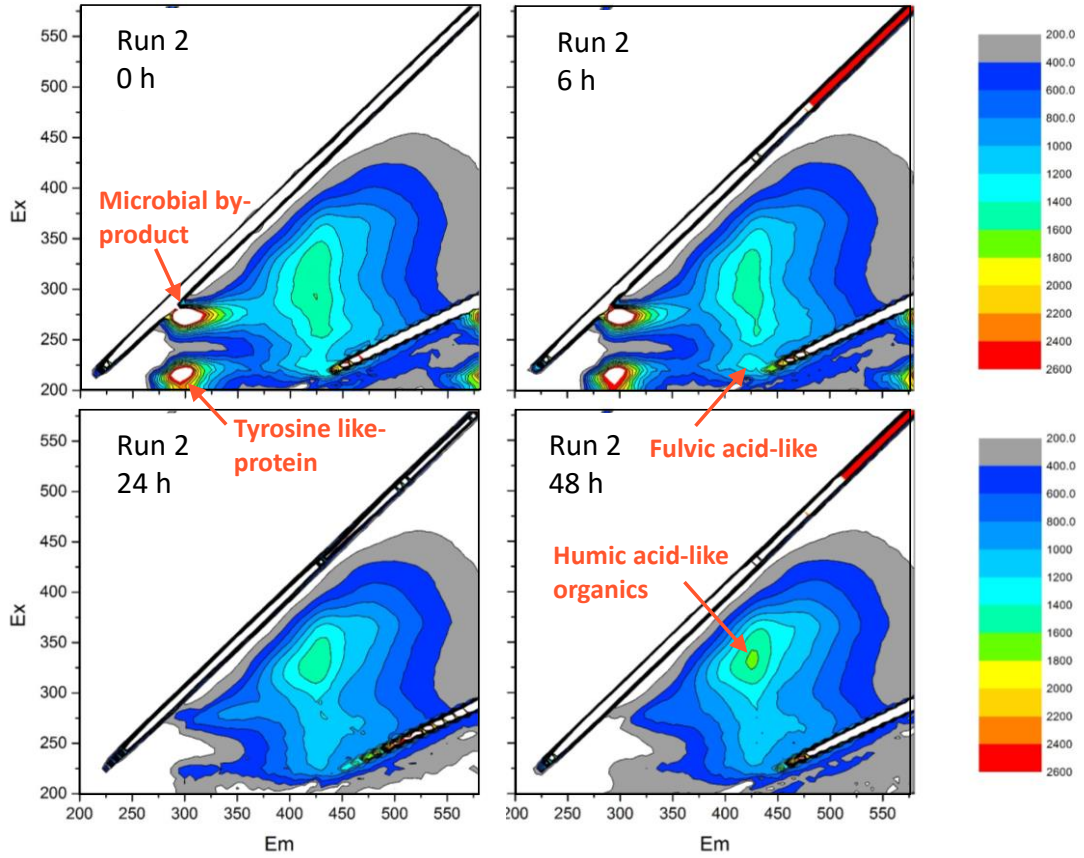



**Figure 4.15** Variation of fluorescense EEMs of Run 2 in the biodegradability experiment

The fluorescent EEMs showed that dominant fluorescent substances in Run 2 were microbial by-product, tyrosine-like proteins and humic acid-like organics. After 6h, no obvious change in the fluorescent substances was observed. After 24h, peaks of microbial by-products, tyrosine-like proteins and fulvic acid-like substances disappeared, indicating that microbial by-products and tyrosine like-proteins, as well as fulvic acid-like substances were degraded. Peaks of humic acid-like organic were detected in all treatment process, indicating that humic acid-like organics that produced in in the anaerobic digestion of rice straw could not be removed through aerobic treatment.

The Results of biodegradability evaluation batch experiment revealed that by aerobic treatment, less than 10% of the organic matters produced in the anaerobic digestion process could be removed after 6 h. approximately 40% of the total COD could be removed after 24h. Humic matters produced from rice straw were degraded by 29.3% after 24h, however, the biodegradability was lower as compared to that of Run 1. Microbial by-products and fulvic-acid like substances were removed, however, fluorescent humic matters were still detectable after 48 h of aerobic treatment.

#### 4.3.4.3 Run 3 (co-digestion of sludge and rice straw)

The COD variation and fluorescent EEMs of Run 3 were shown in **Figure 4.15** and **Figure 4.16**, respectively; and the variation ratios (%) of total COD and each organic component were shown in **Table 4.6**. **Figure 4.15** showed that despite the slight variations of each organic compositions, no significant variations in total COD were observed in the aerobic treatment process. Table 4.6 showed that after 6h, the main organics that was degraded were humic matters, with a degradation rate of 22.1%. As compared to Run 1 (sludge digestion), contents of humic matters in Run 3 increased remarkably (Figure 4.8), but the removal rates of humic matters in Run 3 was much lower, indicating that the biodegradability of humic substances produced from rice straw degradation was poor than that generated from sludge digestion. Carbohydrates were degraded by 32.5% after 48h, but the removal of total COD was 1.7%, indicating that the organic matters generated in the co-digestion of sludge and rice straw was resistant to biodegrade under aerobic condition.

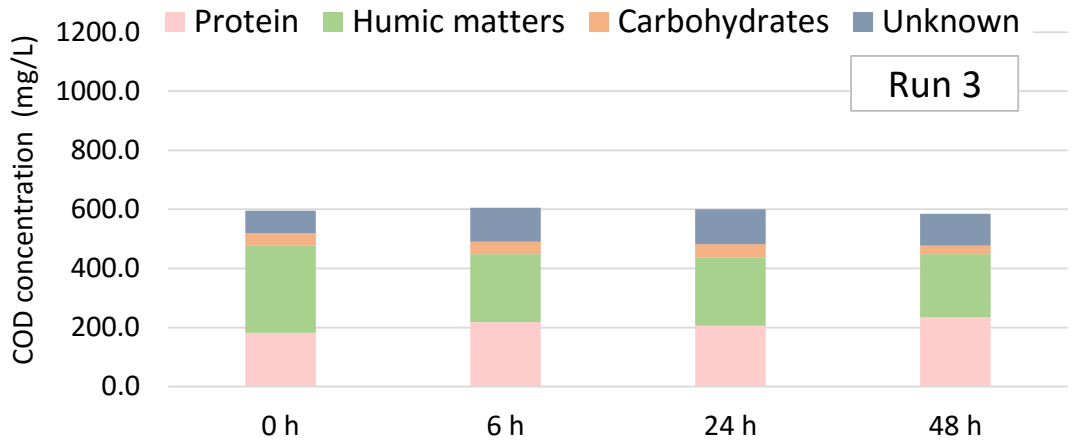



**Figure 4.16** Variations of COD of Run 3 in the biodegradability evaluation experiment

**Table 4.6** Variation ratios (%) of total COD and each organic component at each period of Run 3 in the biodegradability evaluation batch experiment. ※ negative values refer to decrease, and positive values refer to increase of organic matters.

| Run 3 | 6 h | 24 h | 48 h |
|---|---|---|---|
| COD | 1.8 | 0.8 | -1.7 |
| Proteins | 19.8 | 13.0 | 28.7 |
| Humic substances | -22.1 | -21.9 | -27.6 |
| Carbohydrates | -1.1 | 7.0 | -32.5 |
| Unknown | 53.9 | 56.8 | 43.7 |

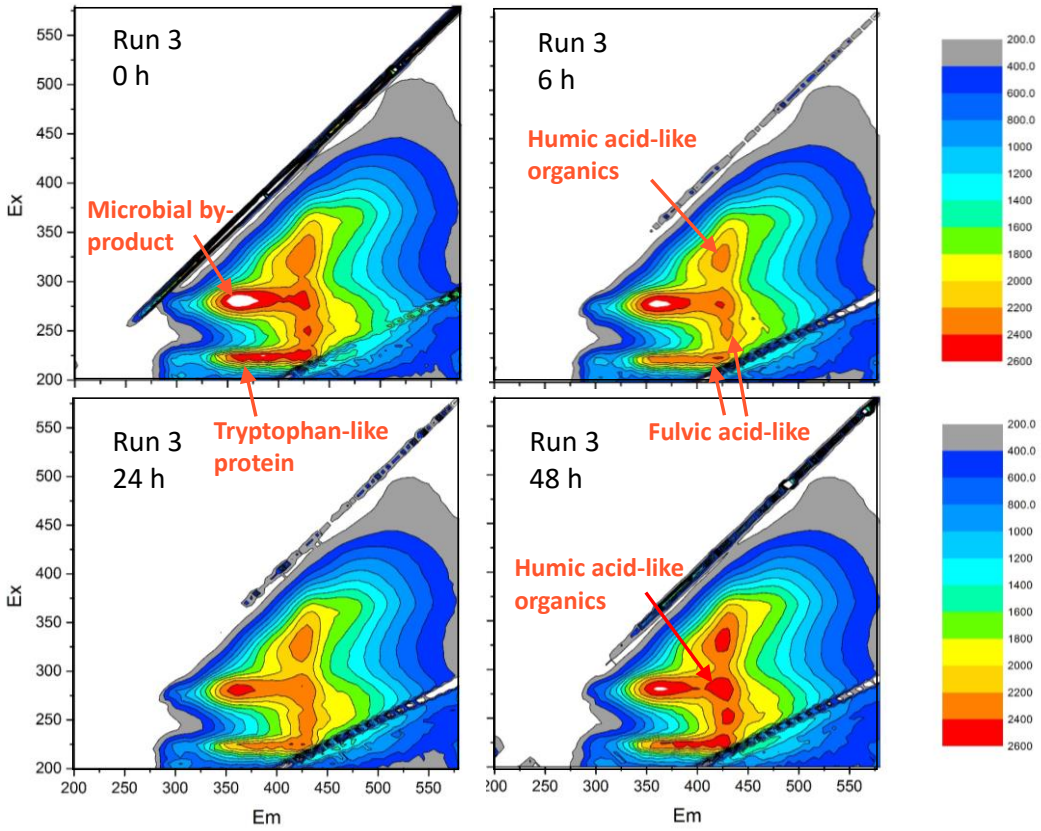



**Figure 4.17** Variation of fluorescense EEMs of Run 3 in the biodegradability experiment

As shown in Figure 4.17, fluorescent substances detected in Run 3 were microbial by-products, tryptophan-like proteins, humic acid-like organics and fulvic acid-like substances. After 6h aerobic treatment, intensities of the peaks corresponding to the detected fluorescent substances decreased slightly, however, all peaks observed at initial sample were also detected after 6h. After 24h, intensities of the fluorescent organics decreased significantly as compared to initial sample, however, after 48h, stronger intensities of the peaks observed in former phases were detected, and one of the reason might be that the enrichment of the fluorescent substances were caused by the degradation of the microbial.

The Results of biodegradability evaluation of Run 3 revealed that by aerobic treatment, the total organic matters produced in the anaerobic digestion process were resistant to be removed. Humic matters produced from rice straw were degraded by approximately 22% after 6 and 24h, however, the biodegradability was lower as compared to that generated from sludge digestion. Microbial by-products and Tryptophan-like proteins are considered as with relatively high biodegradability, however, fluorescent substances generated from anaerobic digestion process were not removed.

## 4.4 Conclusions

The co-digestion of sludge and rice straw indicated that degradation of organic matters dissolved from rice straw was improved through co-digestion. The addition of rice straw caused significant increase in humic contents in the dissolved organic matters, which were less biodegradable compared to that generated from sludge digestion. Biodegradability evaluation of DOM in the digested sludge showed that little organics were removed in all reactors after 6h of aerobic treatment. Rice straw addition caused the enrichment of some fluorescent substances (microbial by-product and humic acid-like organics), which were not degraded under aerobic condition.

# Chapter 5 Conclusions

## 5.1 Conclusions

In chapter 2, the impact on methane gas production, digestion performance and microbial community by the addition of waste fried tofu in sludge digestion process was studied. In addition, to achieve higher treatment efficiency, the effect of OLR on digestion performance was also evaluated. The results revealed that under thermophilic temperature, digestion was sludge was conducted stably at substrate concentrations below 70 g/L (OLR 5.9 kg VS/$m^3$/d), and methane yield of OD sludge was approximately 0.05 L/g-TS. When the substrate concentration was increased to 100 g/L (OLR 8.5 kg VS/$m^3$/d), high concentrations of ammonia were detected, and methane fermentation was suggested to be inhibited. The co-digestion of sludge and fried tofu was conducted stably wat substrate concentrations below 72.5 g/L (OLR 6.3 kg VS/$m^3$/d), and approximately 0.45 L/g-TS of methane gas was suggested to be generated from 1 g of tofu mixture. By further increase the substrate concentrations to 101.5 g/L (OLR 8.8 VS/$m^3$/d), concentrations up to 4150 mg-N/L was detected, meanwhile, accumulation of VFAs was observed, suggesting that methane fermentation was inhibited. By decreasing substrate concentrations, decrease in ammonia, VFAs concentrations were observed, and VS removal rates increased. However, little biogas was recovered, indicating that by decreasing the substrate concentration, inhibition to methane fermentation was mitigated slightly, but complete recovery was not obtained. Results of gas generation showed that fried tofu addition contributed greatly to the methane gas production. Microbial community analysis showed that by increasing substrates concentrations gradually, little effects on bacteria community was recognized, while during the period that ammonia inhibition was suggested, most DGGE bands of bacteria and archaea detected in previous phases disappeared, indicating that high ammonia concentrations showed strong inhibition to the activities of methanogens. In addition, fried tofu addition had significant effect on the bacteria community, while little effect on archaea community. Dominant archaea in both reactors were *Methanosarcina thermophile (99%)*, which use acetate to generate methane.

In Chapter 3, co-digestion of sewage sludge and rice straw was conducted using 10 L reactors to enhance the dewaterability of digested sludge by the addition of rice straw. Anaerobic digestion was conducted stably during the whole operating periods, and specific methane yields of sewage sludge and rice straw were 0.34 and 0.18 L/g-VS respectively. The contents of fibrous materials in digested sludge increased

significantly, and normalized CST decreased remarkably, indicating that the dewterability was improved due to the addition of rice straw. Two kinds of coagulants that showed better performance in the coagulant selection test were used, and the dewaterablity of digested sludge was evaluated by a belt filter press. When coagulant A was used, the water content of dewatered sludge cakes decreased by 8.2% and specific filtration rate increased by 81.2% due to rice straw addition; when coagulant E was used, the water content of dewatered sludge decreased by 13.4% and filtration rate increased by 174.6%. The results revealed that dewaterability of digested sludge was improved significantly by the addition of rice straw. The simulated calculation of dewatered sludge volume under the operating and dewatering condition in the present study showed that in the case of using Coagulant A and E, generated dewatered sludge increased by 40.6% and 9.6%, respectively, due to rice straw addition. However, since the input TS of substrates in the co-digester was 50% higher than the single digester in the initial state, the increasing in generated dewatered sludge is less than the added amount of input rice straw. It can be expected that if more effective coagulant can be utilized, despite the fact of TS increasing due to biomass addition, the generated amount of dewatered sludge will not increase significantly. Rice straw was considered as good biomass resource for both methane recovery and improvement of dewaterability of digested sludge.

In Chapter 4, co-digestion of sewage sludge and rice straw was continued using 1 L of reactors, to study the variation of dissolved organic matters (DOM) in the digested sludge by the addition of rice straw. The results revealed that degradation of organic matters dissolved from rice straw was improved through digestion with sludge. Rice straw addition showed a strong impact on the increasing in humic contents, which were less biodegradable as compared to that generated from sludge. Biodegradability evaluation of DOM in the digested sludge showed that little organics were removed after 6h of aerobic treatment in all reactors. Rice straw addition caused the enrichment of some fluorescent substances (microbial by-product and humic acid-like organics), which were not degraded under aerobic condition.

### 5.2 Future prospects

Waste fried tofu was considered as good biomass resource for the high biodegradability and methane production potential. However, low C/N ratios of tofu mixture caused ammonia inhibition which prevented the further increase of substrate concentrations. On the other hand, rice straw was proved that it was also a good biomass resource for methane fermentation due to methane gas recovery and the

improvement of dewaterability of digested sludge. Since rice straw contains high content of organic carbons, and with a relatively high C/N, rice straw is suggested to be effective for C/N ratio adjustment, which was reported widely as a method to mitigate ammonia inhibition. It can be expected to conduct the thermophilic digestion of sewage sludge, fried tofu and rice straw, as well as other possible waste biomass generated in local town. Measurement to control inhibitors, especially ammonia inhibition, should be emphasized. In addition, waste biomass addition was indicated to have impact on microbial community structures variation, further analysis on microbial community during the digestion process should be conducted.

On the other hand, rice straw addition was indicated to cause the increasing of humic substances in the DOM of digested sludge, as well as the enrichment of some fluorescent substances, in the lab-scale experiment. It is necessary to evaluate the effect of biomass addition on DOM compositions of digested sludge, based on the evaluation of samples from WWTPs, as well as the potential effect of organics generated due to biomass addition. In addition, it is also necessary to propose improvement measures to remove the organics with poor biodegradability that are generated from waste biomass in addition to sewage sludge.

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Tingting Gu

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# Supplementary 1

## Determination of protein and humic substances

### 1. Preparation of reagents

✧ Reagent A: 2% w/v $Na_2CO_3$ in 0.1N NaOH

✧ Reagent B: 1% w/v $CuSO_4$

✧ Reagent C: 2% w/v $Na_2$・tartrate

Reagent A,B and C ⇒ Preparation in advance (stored at 4℃)

### 2. Experimental manual

① Mix: 49 ml Reagent A + 1 ml Reagent B+ 1 ml Reagent C

※Mix slowly and prepare before use in order to avoid precipitation.

② Addition of 2.5 ml of the wastewater/effluent sample in a test tube.

③ Addition of 2.5 ml of Reagent A or Reagent Mix, mixing by vortex and left to rest for 10 min

④ Preparation of Reagent E: Folin-Ciocalteau reagent.

Phenol reagent : ultrapure water＝1：1

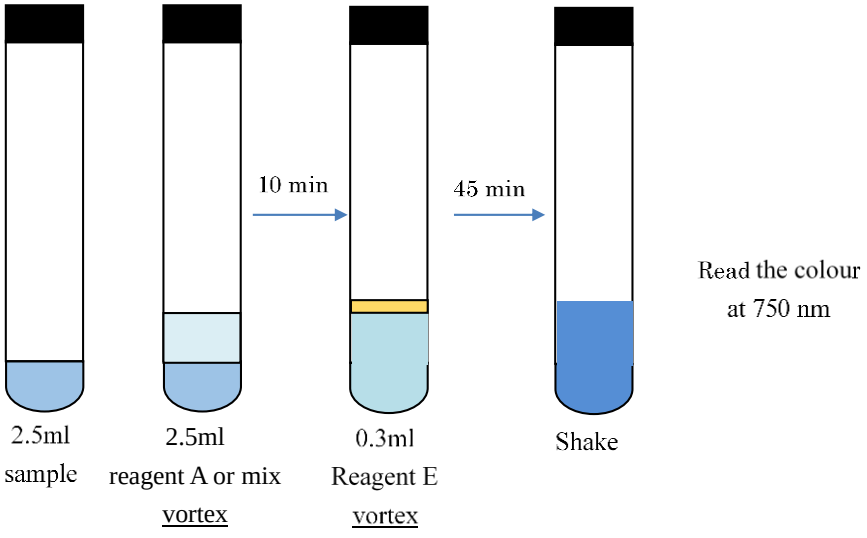
※Because the diluted reagent is unstable, prepare 1X Folin-Ciocalteu Reagent on the same day of use.

### 3. Absorbance determination and concentration calculation

① With the spectrophotometer set to 750nm, zero the instrument on a cuvette filled only with water.

② Subsequently, measure the absorbance of all the samples.

※Before the start of the experiment, turn on the spectrophotometer and allow the spectrophotometer to warm-up for at least 30 minutes before using it.



**Calculation**

$A_{total} = A_{protein} + A_{humic}$ (1)

$A_{blind} = 0.2\ A_{protein} + A_{humic}$ (2)

And the equations can be transformed to:

$A_{protein} = 1.25\ (A_{total} - A_{blind})$ (3)

$A_{humic} = A_{blind} - 0.2\ A_{protein}$ (4)

Where $A_{total}$ is the total absorbance with $CuSO_4$, $A_{blind}$ is the total absorbance without CuSO4, $A_{humic}$ is the absorbance due to humic compounds, and $A_{protein}$ is the absorbance due to proteins.

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## Supplementary 2

**Phenol–Sulfuric Acid method (DuBois et al., 1956)**

**PRINCIPLE**

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490 nm.

**MATERIALS**

Phenol 5%: Redistilled phenol (50g) dissolved in water and diluted to 1L.

Sulfuric acid 96% reagent grade.

Standard glucose: Stock—100 mg in 100 mL of water.

**PROCEDURE**

1. Add 1 mL of standard solution and sample to each tube.
2. Set a blank with 1 mL of water.
3. Add 1 mL of phenol solution to each tube.
4. Add 5 mL of 96% sulphuric acid to each tube and shake well.
5. After 10 min shake the contents in the tubes and place in a water bath at 25–30°C for 20 min.
6. Read the colour at 490 nm.
7. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.