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学　位　論　文　要　旨

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論文題目：Evaluation of hygienic properties of residues after anaerobic digestion (digestate) of dairy cow manure estimated from biological analysis.
（生物学的分析に基づく乳牛ふん尿嫌気発酵消化液の衛生評価）

要旨

To determine the effects of anaerobic digestion treatment on the survival of weed seeds in dairy cow manure, weed seeds (*Rumex obtusifolius* L.) were mixed into dairy cow manure, and the seed germination rates and percentage of dormant seeds after mesophilic (35 °C) and thermophilic (55 °C) anaerobic digestion processes were investigated in Chapter 1. The survival rates of *Rumex obtusifolius* L. seeds heat-treated at 35 °C and 55 °C were 75.5% and 0%, respectively, compared to 81.6% and 0% in anaerobic digestion processes at the same temperatures. The survival rates at 35 °C treatment were similar between the anaerobic digestion and heat treatments, but the percentage of seeds in primary and secondary dormancy was higher in the anaerobic digestion treatment compared to heat treatment. Although digestate have a high value as liquid fertilizer, mesophilic digestate should be treated with caution owing to the presence of dormant weed seeds.

In Chapter 2, the elimination of pathogenic bacteria in livestock manure during anaerobic diegstion in a full-scale biogas plant was investigated using livestock manure as a raw material. Manure was collected from reception pits, digester tanks, and digestate storage tanks of four biogas plants with mesophilic (38 °C) and thermophilic (55 °C) fermenters currently in operation in Hokkaido, Japan. *Coli-aerogenes* and *Enterococcus* in manure were cultured to analyze the survival rates after the digestion process. As a result, no *Coli-aerogenes* and *Enterococcus* were detected in either manure in the fermenter or digestate after anaerobic digestion in the thermophilic biogas plant. In the mesophilic biogas plant, although the number of *Coli-aerogenes* and *Enterococcus* decreased with digestion, *Coli-aerogenes* and *Enterococcus* in manure were found in the fermenter and digestate after anaerobic digestion. The mean decimation reduction time (T_{90}) of *Coli-aerogenes* and *Enterococcus* in mesophilic digestion were 13.3 and 16.7 days, respectively.

Large quantities of antimicrobial agents have been used in dairy farming to treat and prevent livestock diseases. There has been a global problem of the resulting emergence of antimicrobial-resistant bacteria in livestock manure. Cefazolin (CEZ) is

the antibiotic most commonly used in treating dairy mastitis, and CEZ-resistant (CEZ-R) bacteria are detected in manure of dairy cows administered with the antimicrobial. The study in Chapter 3, the effects of thermophilic anaerobic digestion on the survival of CEZ-R and CEZ-susceptible (CEZ-S) bacteria and substrate-specific extended-spectrum β-lactamase (ESBL)-producing bacteria (*E. coli*, *Klebsiella/Enterobacter/Citrobacter*, *Proteus*, *Pseudomonas*, and *Acinetobacter*) in dairy cow manure was examined. As a result, the number of CEZ-R bacteria was reduced to 0.93% by thermophilic anaerobic digestion, including a decrease in the number of *E. coli*, other coliforms, and other bacteria to below the limit of detection, 0.27%, and 1.05%, respectively. In contrast, there was a slight increase in ESBL-producing *Pseudomonas*. These results suggested that the risk of spreading cephazolin-resistant bacteria was reduced in the thermophilic anaerobic digestion process of livestock manure, but some antimicrobial-resistant bacteria remained.

The study in Chapter 4 also focused on the analysis of CEZ-R *Pseudomonas* spp. because there was a slight increase in ESBL-producing *Pseudomonas* spp. during thermophilic anaerobic digestion. Due to the mixture of pathogenic and plant growth-promoting species in *Pseudomonas* spp., *P. aeruginosa*, *P. fluorescens*, and other pseudomonads were classified and quantitatively analyzed using commercial PASA medium and newly prepared CAP medium (commercial Cetrimide medium to which L-arginine and phenol red were added to classify species according to differences in color development).

The evidence for these classifications was performed by PCR confirmation. *Pseudomonas* spp. decreased after both mesophilic and thermophilic anaerobic digestion, indicating that anaerobic digestion at thermophilic was more effective than at mesophilic during the anaerobic digestion process. *P. aeruginosa* was not detected in either manure collected or digestate after the mesophilic and thermophilic anaerobic digestion process. In contrast, CEZ-R *Pseudomonas* in mesophilic digestate increased relative to manure before the digestion process. *P. fluorescens*, which was present in manure and all CEZ-R, was no longer detected after mesophilic or thermophilic anaerobic digestion. Fluorescent pseudomonads, other than *P. aeruginosa* and *P. fluorescens*, increased after the anaerobic digestion process, and most of them were CEZ-S. In addition, an increase in the mesophilic anaerobic digestion process was observed in CEZ-R *Pseudomonas* spp. other than fluorescent pseudomonads. The percentage of bacterial counts suggested that most CEZ-R *Pseudomonas* spp. increased by the mesophilic anaerobic digestion process were other than fluorescent pseudomonads. Thus, due to the increase of CEZ-R *Pseudomonas* spp. other than fluorescent pseudomonads in mesophilic anaerobic digestion, the risk of spread of resistant bacteria to the environment should be considered when using digestate as liquid fertilizer.

To use livestock manure safely and effectively, microbial risks should be avoided, and it is important to understand the characteristics of treatment methods. Although

many pathogens are reduced by mesophilic and thermophilic treatment, considering the effectiveness of pathogen reduction, the anaerobic digestion process of livestock manure should be conducted at high temperatures. However, there is a need for more energy to maintain the biogas plant at higher temperatures and larger volume digester tanks to increase the throughput and extend HRT. Therefore, when constructing a biogas plant, it is important to understand the properties of livestock manure and organic waste (secondary raw material) to be mixed and treated and select an appropriate treatment method considering the balance between the profit to be gained from digestate, construction costs, and energy input. Because it is difficult to easily change an existing biogas plant once a system has been established, it is possible to obtain safer digestate by adding attached equipment, such as sterilization tanks. Some biogas plants install a sterilization tank to obtain safe digestate and treat digestate at a high temperature of 70 °C for about 1 h. In some foreign countries, including the European Union, the United Kingdom, the United States of America, and People's Republic of China, hygiene standards for microorganisms in digestate have been established, and sterilization of digestate is mandatory in the EU and other countries.

Anaerobic digestion is a technology that produces renewable energy through effective processing of livestock manure. Furthermore, digestate is widely recognized as a good fertilizer and is epidemiologically safe. This study presented the possibility of anaerobic digestion to reduce the risks as spreading weed seeds, pathogens, antimicrobial-resistant bacteria, antimicrobial-resistant genes to dairy and agricultural field, and to enhance the number of bacteria which promote crop growth. I hope that this study may contribute to society by resolving issues with effective use of resources, reducing methane and CO_2 emissions, and livestock manure disposal issues.

備考　1　論文題目が英語の場合には，（　）書きで和訳を付す。

2　博士論文については，日本語の場合１８００～２２００字，英語の場合１０００～１４００語とする。修士論文については，それ以下でもかまわない。

3　図表は，要旨には記載しないこととする。

4　枚数は１枚を超えても差し支えない。