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Abstract of Thesis/Dissertation

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Title : Assessment of fertilizer properties of digestate from anaerobic digestion of dairy manure

(乳牛ふん尿の嫌気消化液の肥料特性評価)

Abstract

The large amount of livestock manure and slurry produced from livestock husbandry has a potential hazard source to the environment and public health, if they are improperly managed or treated. Treatment of these organic wastes in biogas plants (BGPs) with a biochemical technology; anaerobic digestion (AD), is considered the most suitable disposal because it recycles organic wastes, produces renewable energy, reduces greenhouse gas (GHG), and provides valuable bio-fertilizers. Recently, BGPs receive much attention. As a result, a lot of BGPs have been installed worldwide. With the development of BGPs, the amount of anaerobic digestate (digested residue after AD process) also increased sharply. The sustainability of full scale BGPs depends highly on the appropriate disposal of anaerobic digestate.

In Hokkaido, Japan, 330 BGPs are now in operation and anaerobic digestate from these BGPs is mostly used as a fertilizer for agricultural field. Anaerobic digestate contains large of plant nutrients, especially in inorganic plant-available forms, which could be used to reduce the consumption of mineral fertilizers. Generally, AD process can be conducted under mesophilic or thermophilic temperatures. Mesophilic digestion requires lower energy cost with a higher stability process, while thermophilic digestion leads to more rapid digestion and a higher reduction rate of

pathogen. However, little is known about their effects on the fertilizer properties of digestate.

Agricultural application of anaerobic digestate has caused public concern in recent years due to the risk related to transportation of pathogenic bacteria and heavy metals to the environment. In addition, the effect of microorganisms in organic fertilizers has recently attracted attention, especially for the plant growth promoting effects of *Bacillus* and *Pseudomonas* species, which have been widely researched. Plant growth promoting bacteria (PGPB) can occupy the rhizosphere of many plant species and have beneficial effects on plant growth directly by assisting in nutrients acquisition or providing phytohormones, or indirectly decreasing inhibitory effects of various fungal pathogens. However, anaerobic digestate is a host to numerous PGPB and little attention has been focused on the isolation and characterization of PGPB from anaerobic digestate.

Therefore, this PhD thesis was focused on two main objectives: to investigate fertilizer properties of mesophilic and thermophilic digestates from livestock manure for plant nutrient contents, a special attention was given to plant growth promoting bacteria (PGPB); and to evaluate the environmental risks related to pathogenic bacteria and heavy metal contents.

In Chapter 1, mesophilic and thermophilic digestates from laboratory scale anaerobic digesters were collected for the analysis of plant nutrients, which were N and NH_4^+-N, P (P_2O_5), K (K_2O), Ca (CaO), and Mg (MgO). For environmental risks, pathogenic bacteria (*Salmonella*, *Campylobacter*, *Escherichia coli,* and *Enterococcus*) and heavy metals (Mn, Zn, Cu, and Ni) were investigated. The results show that the two digestates contained similar amount of plant nutrients, while thermophilic digestate had higher NH_4^+-N content (12.2 g/kg) than that of mesophilic digestate (9.8 g/kg). The contents of pathogenic bacteria and heavy metals were analyzed to determine their environmental risk. The reduction rates of pathogenic bacteria were above 90% in the thermophilic digestate, and the maximum rate was 99.7% for *E. coli*, which was higher than that in mesophilic digestate (a minimum of 73.2% for *Campylobacter* and maximum of 96.9% for *E. coli*), which indicates that thermophilic digestate has a lower risk to the environment. Lower levels of heavy metals were detected in digestates from dairy manure than those in other feedstocks.

In Chapter 2, plant growth promoting *Bacillus* and *Pseudomonas* were isolated from mesophilic and thermophilic digestates and characterized. Three different plant growth promoting activities, which are phosphate solubilization ability, siderophore production and phytohormone production, as well as antifungal activity were selected and 200 bacteria were isolated from each

digestate. The isolated bacteria, based on plant growth promoting traits, were selected and inoculated with common wheat seeds to evaluate their plant growth promoting activities. The results showed that *Bacillus* in dairy manure increased significantly after anaerobic digestion. Twenty-five bacterial isolates from mesophilic digestate and 12 bacterial isolates from thermophilic digestate showed positive plant growth promoting traits or antifungal activity. In plant growth promoting assay, all isolates significantly promoted growth of wheat seedlings. Seedlings stem length was increased from 28.5% to 38.6% by bacteria inoculation. In addition, bacteria inoculation increased seedlings stem weight from 113.3% to 214.2% and root weight from 108.6% to 207.2% as compared to un-inoculated control.

Chapter 3 was focused on the bacterial load (plant growth promoting bacteria and pathogenic bacteria) in anaerobic digestates from two full scale biogas plants (BGPs) in Hokkaido. Anaerobic digestate samples were collected from feedstock tank, fermentation tank, sterilization tank and storage tank at Mikage biogas plant. In Shikaoi biogas plant, anaerobic digestate samples were only collected from feedstock tank and storage tank. The results showed that *Bacillus* in feedstock decreased after anaerobic digestion in full scale BGPs, which was different from the results of chapter 2. Furthermore, pathogenic bacteria, except *Campylobacter*, were eliminated. These results indicated that there was a difference in bacteria reduction rate between laboratory scale and full scale anaerobic digestion. However, *Bacillus* was detected at a high level in two digestates from BGPs, which indicates that digestates may be a potential bio-fertilizer. On the other hand, *Campylobacter* residue was detected after both laboratory scale and full scale anaerobic digestion, which was considered a possible source of environmental contamination.

The results from this PhD thesis show that (1) operating temperature of AD process is the major determinant factor that affects the fertilizer properties of anaerobic digestate. High temperature leads to high contents of inorganic plant-available nutrients (NH_4^+-N) and high reduction rate of pathogenic bacteria after AD process. However, higher cultivable bacteria and higher percent of PGPB were observed in mesophilic digestate than that in thermophilic digestate. (2) Anaerobic digestate is a large reservoir of bacteria capable of promoting plant growth. These bacteria were able to colonize the rhizosphere with digestate application and could increase the availability of nutrients for plant and decrease disease symptoms, which make digestate an effective biofertilizer. (3) The heavy metals in anaerobic digestate are likely to show an increased risk to the environment. In this

study, the heavy metal concentrations of digestates were lower than in other feedstock, but not decreased. Therefore, it is imperative to remove these heavy metals before the application of anaerobic digestate, especially when the feedstock used, such as sewage sludge, contains high contents of heavy metal. (4) In full scale biogas pants, all detected bacteria were reduced to undetectable level expect *Bacillus* and *Campylobacter*. The presence of *Bacillus* also makes anaerobic digestate a potential bio-fertilizer. However, *Campylobacter* residue is considered a possible source of environmental contamination.