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Abstract of Thesis/Dissertation

Applicant

Doctoral Program in Animal and Food Hygiene

Graduate School of Animal Husbandry

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 16160002

Name of Applicant: Pan Zhifei

Signature of Applicant: Pan Zhi-fei

Title : Assessment of anaerobic digestate of dairy manure as biofertilizer: Environmental risk and potential in suppressing plant disease

(乳牛ふん尿の嫌気性消化液の肥料としての評価：環境リスク及び土壌病害の抑制可能性)

Abstract

Recent years, there is increasing worldwide interest in technology producing renewable energy sources as a result of global warming and the increasing consumption of fossil fuels. Livestock wastes, such as manure, present a potential source of various hazards to human life and the environment, and production has increased sharply as the development of livestock industry. Therefore, anaerobic digestion (AD) of livestock manure seems to be a promising method to treat large amount of livestock manure and produce biogas, which is a renewable energy source. Together with biogas production, AD process also produces a liquid residue called digestate, which is considered a valuable fertilizer consisting of partially degraded organic matter, microbial biomass and inorganic compounds. Many studies have been conducted to investigate the fertilizer property of digestate. However, there is concern about the environmental risk of digestate application as livestock manure contains various pathogenic bacteria. Furthermore, the widespread use of antibiotics in livestock has increased the frequency of antibiotic resistant bacteria (ARB) in livestock wastes. On the other hand, some antagonistic bacteria in digestate may extend the utilization of digestate. Therefore, this PhD thesis was focused on two main objectives: to investigate the survival of pathogenic bacteria with and without antibiotic resistance during anaerobic digestion at different

temperatures and to detect antagonistic activity of bacteria in digestate.

In chapter 1, the effect of digestion temperature on survival of pathogenic and cefazolin resistant bacteria in dairy manure was investigated in batch reactors. Lab-scale batch anaerobic digestions were conducted under mesophilic (37 °C), thermophilic (55 °C) and hyper-thermophilic (65 °C) temperatures. Results showed that *Enterococcus*, *Salmonella*, and *Acinetobacter* with and without cefazolin resistance were eliminated by AD treatment under each temperature, however, *E.coli* and *Campylobacter* were detected in digestates. Among cefazolin resistant bacteria, only *Campylobacter* was survived under mesophilic temperature. These results indicated that AD process under high temperature could effectively reduce pathogenic and cefazolin resistant bacteria in dairy manure. However, *Campylobacter* was tolerant to AD treatment, appropriate management practices should be implemented to minimize the sanitary risks of bacterial transmission when applying digestate to agricultural field.

In chapter 2, the survival of pathogenic bacteria with and without cefazolin resistance was investigated in semi-continuous stirred-tank reactors. Two laboratory scale anaerobic reactors digesting dairy manure were conducted at mesophilic and thermophilic temperature respectively. Results showed that pathogenic and cefazolin resistant bacteria were reduced by AD and significantly reduced in thermophilic anaerobic digestion. However, more pathogenic bacteria residues were found in digestates from continuous reactors than those from batch reactors. On the other hand, antagonistic bacteria (*Bacillus* and *Pseudomonas*) were detected at higher loads in digestates than in feedstock. *Bacillus* loads in mesophilic and thermophilic digestates were 2.68×10^6 and 0.43×10^6 CFU/g DM, and *Pseudomonas* loads were 0.83×10^4 CFU/g DM in MAD and 7.53×10^4 CFU/g DM, respectively. These results showed anaerobic digestion is effective on pathogenic bacterial reduction and increased antagonistic bacteria, which may expend biological component potential of digestate to suppress soil-borne plant diseases caused by phytopathogen.

In the last chapter, potential of antagonistic activities of anaerobic digestate against phytopathogens were investigated by detecting the antagonistic activities of *Bacillus* and *Pseudomonas* in anaerobic digestates. *Bacillus* suppressed growth of phytopathogens, while *Pseudomonas* did not show any antagonistic activities. In addition, the populations of antagonistic *Bacillus* were much higher in mesophilic digestate than that in thermophilic digestate, and the

highest population was 2.53×10^5 CFU/g DM against *Cercospora beticola*. These results indicated that *Bacillus* was an effective antagonistic bacterium in digestate against phytopathogens. Furthermore, two selected isolates, B11 (*Bacillus subtilis*) and B59 (*Bacillus licheniformis*), were applied in field experiments and showed significant reduction in percent infection of potato late blight (*Phytophthora infestans*). These results demonstrate the benefits of digestate in suppressing soil-borne plant diseases caused by antagonistic bacteria.

The results from this PhD thesis show that (1) operating temperature of AD process is the major determinant factor that affects the survival of pathogenic bacteria with and without antibiotic resistance, and higher temperature showed higher reduction rates. Although hyper-thermophilic digestion showed highest reduction of pathogenic bacteria, high energy input and unstable process stability may be the problem. Some pathogenic bacteria were detected in mesophilic digestate, therefore, appropriate management practices, such as sterilization, are recommended to minimize the sanitary risks of bacterial transfer to agricultural land from the application of mesophilic digestate. (2) Anaerobic batch reactor showed higher bacteria reduction efficiency than continuous reactor. (3) Anaerobic digestion had increased population densities of *Bacillus* and *Pseudomonas* in digestates compared with dairy manure. It appeared that the suitable temperatures and available nutrients in digesters stimulated their growth. *Bacillus* and *Pseudomonas* are involved in acidogenesis stage of anaerobic digestion process. Further research is recommended to ascertain the role of temperature and nutrients on the growth of *Bacillus* and *Pseudomonas* in the anaerobic digester. (4) *Bacillus* was an effective antagonistic bacterium in digestate against phytopathogens. Further research is recommended to ascertain the antagonistic activity of *Pseudomonas* in digestates against other phytopathogens and to ascertain the appropriate application of anaerobic digestate.