



New Approaches to Control Antibiotic-Resistant Bacteria in Anaerobic Digestion Processes for Sustainable Conversion of Dairy Cattle Manure

有, 靖儀

(Degree)

博士 (農学)

(Date of Degree)

2025-03-25

(Date of Publication)

2027-03-25

(Resource Type)

doctoral thesis

(Report Number)

甲第9223号

(URL)

<https://hdl.handle.net/20.500.14094/0100496504>

※ 当コンテンツは神戸大学の学術成果です。無断複製・不正使用等を禁じます。著作権法で認められている範囲内で、適切にご利用ください。



博士論文内容の要旨

氏 名 _____ 有 靖儀 _____

専攻・講座 _____ 食料共生システム学専攻・生産環境工学講座 _____

論文題目（外国語の場合は，その和訳を併記すること。）

New Approaches to Control Antibiotic-Resistant Bacteria in
Anaerobic Digestion Processes for Sustainable Conversion of
Dairy Cattle Manure

乳牛ふん尿の持続可能な変換のための嫌気性消化における
抗生物質耐性菌制御への新しいアプローチ

指導教員 _____ 井原 一高 _____

1. Introduction

The reliance on antibiotics in animal husbandry to maintain livestock health and promote growth has resulted in significant environmental challenges. A substantial proportion of antibiotics given to animals are excreted unmetabolized, leading to antibiotic residues in manure. These residues promote the development and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) in the environment, threatening both animal and human health.

Anaerobic digestion (AD), a sustainable method for manure treatment and biogas production, has been widely adopted. However, when manure containing antibiotic residues is used as a substrate, AD can inadvertently propagate antibiotic resistance, with ARB and ARGs potentially reentering soil and water ecosystems through digestate as organic fertilizer. Given the global recognition of antibiotic resistance as a critical environmental threat, optimizing AD to mitigate the transmission of ARB and ARGs is imperative. Biochar, a cost-effective and environmentally friendly material, has garnered attention for its potential to enhance AD performance. Its abundant functional groups, high porosity, and large surface area contribute to stabilizing AD processes and regulating microbial communities. Exploring the mechanisms by which biochar improves AD efficiency while controlling ARB and ARGs is crucial for developing comprehensive strategies to ensure the safety and sustainability of biomass-derived products.

2. Previous Researches

2.1 Antibiotic

Antibiotics have been essential in medicine and veterinary treatment, effectively controlling bacterial infections by targeting pathogen reduction. However, their overuse, especially in agriculture, has raised significant concerns. Global antibiotic consumption in crops and livestock reached 63,151 tons in 2010, projected to rise by 67% by 2030, with Asia seeing a 46% increase. This widespread availability and efficacy have led to misuse, threatening their long-term effectiveness. Antibiotics are categorized by their spectrum of action (broad- vs. narrow-spectrum) and mechanisms of activity: inhibiting bacterial cell wall synthesis (e.g., *β -lactam*), disrupting cell membranes (e.g., polymyxin), inhibiting protein synthesis (e.g., tetracycline), and blocking DNA replication and transcription (e.g., quinolones). These mechanisms highlight their effectiveness in treating infections but also stress the need for prudent use to prevent resistance.

Cefazolin (CEZ), a first-generation β -lactam cephalosporin, is widely used to treat bacterial

(Name: 有 婧仪 NO. 2)

infections due to its efficacy against Gram-positive and some Gram-negative bacteria, with enhanced stability against β -lactamases. In dairy farming, CEZ is commonly used to treat mastitis, a disease affecting milk quality and quantity caused by bacteria like *Staphylococcus aureus* and *Escherichia coli*. The extensive use of CEZ for mastitis treatment contributes to its overuse in the industry. 17–90% of antibiotics administered to livestock are excreted in manure, with CEZ residues detected in milk and manure. These residues, resistant to degradation, raise concerns about milk safety, environmental contamination, and the persistence of pharmacologically active compounds during manure storage. While CEZ is prevalent, tetracyclines like oxytetracycline show higher residual concentrations in cattle manure, highlighting broader environmental risks in livestock systems.

2.2 Antibiotic-resistance spreading

Antibiotic resistance is a critical global threat, causing millions of deaths annually and projected to exceed 10 million deaths per year by 2050 if unchecked. The extensive use of antibiotics, particularly in agriculture, has made animal manure a reservoir of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), fostering horizontal gene transfer and the proliferation of antibiotic-resistant bacteria (ARB). β -lactam resistance genes are especially prevalent, with multidrug-resistant strains compromising antimicrobial treatments for humans and livestock. This issue, driven by residual antibiotics and the evolution of ARGs, presents a global public health challenge and environmental crisis, categorized as "combined contaminants". Addressing this escalating problem requires urgent action to mitigate the environmental selection pressure exerted by antibiotics.

2.3 Anaerobic Digestion

Anaerobic digestion (AD) is an eco-friendly technology for manure treatment, reducing pollution while producing renewable energy through biogas. The process involves hydrolysis, acidogenesis, acetogenesis, and methanogenesis, driven by key microbes like acidogenic bacteria and methanogens. Hydrolysis breaks down complex molecules into simpler compounds, which acidogenic bacteria convert into volatile fatty acids (VFAs) during acidogenesis. VFAs are further transformed into acetate and hydrogen in acetogenesis, providing substrates for methanogens to produce methane in the final stage. AD can be single-stage, combining all phases in one reactor, or two-stage, separating the acidogenic and methanogenic phases to optimize conditions. The methanogenic phase requires a higher pH than the acidogenic phase, and pH fluctuations affect VFA levels and microbial activity. Cefazolin residues can inhibit hydrogen- and acid-producing bacteria, reducing VFA and hydrogen production. This study employs a two-phase AD approach to mitigate these effects and enhance process efficiency.

The use of manure containing antibiotic residues as AD feedstock introduces challenges,

(Name: 有 婧仪 NO. 3)

particularly regarding ARBs and ARGs, which may persist in digestate and pose environmental and health risks when used as fertilizer. While AD can mitigate ARGs, its efficiency depends on system configurations and operational conditions. In single-stage AD, all biochemical processes occur in one reactor, leading to fluctuating conditions. Mesophilic AD (35°C) achieves moderate ARB and ARG removal was lower than thermophilic AD (55°C) and temperature-phased AD. However, high COD levels in single-stage systems exacerbate VFA accumulation, impairing microbial activity. Two-stage AD separates acidogenic and methanogenic phases, optimizing conditions for antibiotic degradation and system performance. This configuration reduces antibiotic inhibition on microbes and enhances ARG removal, though ARG concentrations may rebound in later stages.

2.4 Biochar Additive in anaerobic digestion

Biochar (BC), derived from agricultural residues such as straw, bamboo, and olive pruning, offers a cost-effective and sustainable solution in AD processes. Its stable properties enhance AD performance by promoting methanogenesis and stabilizing microbial communities via mechanisms like DIET. Studies show BC addition can significantly boost biogas and methane production. BC also effectively reduces ARG abundance in AD systems through adsorption, bacterial community modulation, and prevention of horizontal gene transfer (HGT). It optimizes environmental conditions, decreasing ARG proliferation. Research highlights BC's potential, with reductions in ARGs such as *tet W* and *bla*_{CTX-M} significantly. These findings underscore BC's promise to enhance AD efficiency while mitigating ARG dissemination, contributing to sustainable waste management and environmental protection.

3. Survival of antibiotic-resistant bacteria during mesophilic single- and two-stage anaerobic digestion with cefazolin-spiked dairy cow manure

AD offers a sustainable and eco-friendly method for treating raw manure while generating organic fertilizer. However, using manure containing antibiotics as substrates could lead to the dissemination of ARB and ARGs in AD reactors, potentially posing risks of human infection through the application of organic fertilizers. Consequently, addressing these critical concerns requires the elimination of ARB from contaminated animal manure. Numerous studies have demonstrated the potential of AD in reducing ARG abundance in manures. However, in single-stage anaerobic digestion AD, both the acidogenic phase (AP) and methanogenic phase (MP) occur in the same reactor. This might lead to interactions between microorganisms from the two phases, and since the optimal environmental conditions for each phase differ, the single reaction conditions in single-stage AD may limit the efficiency of both phases. In contrast, two-stage AD separates AP and MP into different reactors, demonstrating higher gas production

(Name: 有 婧仪 NO. 4)

performance, better control of ARGs, and greater stability, especially in the presence of antibiotic residues. This improvement was possibly due to the promotion of hydrogen-producing bacteria, which are effective in degrading diverse metabolites and limiting the activities of pathogenic strains. Limited studies have explored the impact of two-stage AD on ARGs. The operation of two-stage AD was modified in previous studies, and the impact of the acidogenic and methanogenic phases on the dynamics of ARB remains insufficiently understood. There is also limited information available to demonstrate the effect of CEZ on ARB survival during single- and two-stage AD processes. It is important to note that while much of the research on antibiotic resistance has focused on ARGs, the existence of ARB could provide more intuitive evidence for evaluating antibiotic-resistant potential hosts and microbial structures from a macroscopic perspective. Furthermore, it remains unclear whether single-stage AD and two-stage AD have varying implications for ARB enrichment. This study primarily focuses on using CEZ-contaminated cattle manure as an AD substrate to examine the effects of single-stage AD and the acidogenic and methanogenic phases of two-stage AD on the quantity and removal of ARB. Comparing single- and two-stage AD systems, we assessed ARB removal rates, biogas yield, volatile fatty acid concentration, and microbial populations. The results revealed that in the two-stage AD system, the MP exhibited the highest removal rates of CEZ-resistant (CEZ-r) and oxytetracycline-resistant (OTC-r) bacteria at 9.2–16.0 % and 68.9–72.4 %, respectively. However, the MP had a higher proportion of ARB among the total culturable bacteria (23.8–30.1%) compared to the AP and single-stage AD. CEZ negatively impacted ARB removal rates and biogas production in both systems, with a more pronounced effect in single-stage AD. Biogas yield in the two-stage AD ranged from 337.7–385.6 mL/g VS, which was 11.0–25.8% higher than that of the single-stage AD, regardless of CEZ addition. The reduction in biogas production due to CEZ was primarily attributed to the suppression of VFA production. As for microbial populations, the changes in the percentages of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were likely related to the variations in ARB numbers, and the abundance of *Methanosaetaceae* significantly influenced biogas production.

4. The impact of biochar characteristics on single-stage ad performance and ARB survival

While AD can potentially reduce ARG abundance and mitigate environmental pollution, its effectiveness is contingent upon operational conditions and microbial structures. The stability of AD relies on the harmonious interaction among diverse bacteria and archaea. Yet, these microbial communities can be susceptible to disruption from adverse environmental conditions and exposure to toxic substances. The presence of antibiotic residues in AD substrates can lead to ARG spread and impact process efficiency and stability. Transmission of ARG in the

(Name: 有 婧仪 NO. 5)

environment typically occurs through potential host bacteria capable of carrying ARG. The results of our previous indicated that although the use of two-phase AD is more effective in controlling ARG than single-phase AD, there was still a risk of ARB rebound in the MP phase. Therefore, the addition of additives to the AD process to further explore ARB control methods is warranted. BC's rich functional groups and high porosity structure contribute to the enhanced removal of ARGs in AD processes. This enhancement occurs through several mechanisms: adsorption and elimination of antibiotics, manipulation of bacterial communities and MGEs to prevent HGT, direct elimination of ARGs via oxidation by persistent free radicals (PFRs), reduction of co-selection pressure, and optimization of environmental factors to decrease the overall abundance of ARB. Recent studies have explored the effects of BC on ARGs in various waste treatment processes, suggesting that BC can interact with and control the proliferation and dissemination of ARGs in the environment. The findings underscore the promising role of biochar in effectively managing ARGs in AD systems. Moreover, while the mechanisms underlying the reduction of ARGs by biochar are not fully elucidated, studies suggest that factors such as substrate raw material, BC particle size, and BC dosages play significant roles. Despite numerous studies investigating the relationship between BC and potential ARG host bacteria as well as methanogens, there remains a critical gap in understanding the interplay between ARG host bacteria and ARB. To address this gap, our study adopts a comprehensive approach by investigating two prevalent types of biochar, Bamboo BC and Olive BC, at varying dosages. This approach aims to uncover the complex interactions among biochar characteristics, microbial behavior, ARB dynamics, and AD performance. Findings reveal significant reductions in CEZ-r across all reactors, ranging from 12.9 % to 76.5 %. Both Bamboo and Olive BC increased CEZ-r removal by 3.1–5.9 times compared to the control. Additionally, BC supplementation prevented the rise in CEZ-r percentage within the total bacteria count observed in the control reactor. Bamboo BC outperformed Olive BC in enhancing biogas yield, with Bamboo-15 and Bamboo-30 showing significant increases of 43.2 % and 48.0 %, respectively, compared to the control. Adding BC in AD regulates ARB by decreasing potential ARG hosts and impeding the transmission of resistance. It also enhances biogas production by improving the efficiency of methanogenic bacteria and optimizing the methanogenic pathway. This research provides insights into how BC can be used to enhance AD performance and mitigate ARB proliferation, offering a sustainable approach to waste management and energy production.

5. Effects of biochar on the survival of ARB during the two-stage anaerobic digestion with CEZ-spiked dairy raw manure

(Name: 有 婧仪 NO. 6)

BC possesses excellent physical and chemical properties, including a high surface area, porosity, rich content of surface functional groups, and favorable electrochemical characteristics. These properties allow biochar to stabilize anaerobic systems, adsorb and neutralize inhibitors, and effectively suppress ARGs and ARB. The previous studies found that biochar addition significantly reduced the abundance of several resistance genes, including *intI* 1, *par* C, *tet* X, *tet* W, *bla*_{CTX-M}, *bla*_{TEM}, *aac*(6')-Ib-cr, and *erm* B. In our previous study, we also found that adding 15 g/L bamboo biochar in single-stage mesophilic AD effectively controlled CEZ-r. Although

these studies have examined the propagation of antibiotic resistance in the AD process from various perspectives and highlighted the advantages of biochar as an additive for controlling ARGs, significant gaps remain in understanding how biochar affects ARB survival during both the AP and MP under the combined stress of antibiotic residues and high chemical oxygen demand (COD) load. Specifically, it is unclear whether biochar can inhibit the spread of antibiotic resistance between acid-producing bacteria while preserving their acidogenesis in the presence of antibiotics. Additionally, there is limited knowledge about its ability to block HGT among microorganisms while promoting DIET. To address these gaps, this study investigates the interplay between microbial communities, antibiotic resistance, and biogas production in AD systems, focusing on AP and MP under the influence of high organics load, CEZ, and biochar. High organics addition represented by glucose addition at 10 g/L significantly suppressed CEZ-r but phase-dependently influenced, in the control reactor increasing the CEZ-r proportion from 0.30% in AP to 36.3% in MP. CEZ residues elevated CEZ-r by 2.5% and 9.3% compared to the control during AP at 0 and 5 g/L glucose. Although AP suppressed CEZ-r to 0.23% in the CEZ-added reactor at 10 g/L glucose, MP rebounded CEZ-r to 8.3%. Biochar addition reduced CEZ-r to below 1.0% at 5 and 10 g/L glucose in both phases, while it increased CEZ-r by 2.5% in MP compared with AP at 0 g/L glucose. CEZ residues also inhibited biogas production by reducing *Methanosaetaceae* abundance and impairing acetic acid conversion, causing 9.1%, 28.1%, and 12.1% yield reductions at 0, 5, and 10 g/L glucose compared to their control. Biochar alleviated CEZ's negative impact, increasing *Methanosaetaceae* abundance by 1.0% and 6.6% at 0 and 10 g/L glucose, respectively compared to the CEZ-added reactor. This was potentially due to enhanced direct interspecies electron transfer facilitated by biochar. These findings emphasize the critical roles of organic load and biochar in modulating ARB dynamics and mitigating CEZ's adverse effects on methane production through microbial community adjustments.

6. Conclusion

(Name: 有 婧仪 NO. 7)

This research contributes to the development of enhanced AD systems that are not only efficient in organic waste treatment and renewable energy production but also effective in mitigating the environmental risks posed by antibiotic resistance. It also provides a highly integrative and novel insight into the control of antibiotic resistance in AD systems.

The addition of CEZ negatively affected AD systems by reducing both the removal efficiency of ARB and biogas production, primarily through its influence on VFA dynamics. However, the two-stage AD system demonstrated a higher overall biogas yield compared to the single-stage system, regardless of CEZ addition. Among the phases, the MP exhibited superior ARB removal compared to the AP and single-stage AD. Nevertheless, the high percentage of ARBs observed in the TCB during the MP highlights a potential risk, particularly under CEZ stress.

To enhance ARB removal, several strategies are proposed, including comprehensive studies on multiple antibiotics in dairy farming, long-term AD studies under various conditions, and the analysis of ARGs alongside ARBs. Additionally, modifying AD systems through pretreatment, additives, and optimized reactor conditions can improve ARB and ARG removal efficiency, ensuring sustainable waste treatment and reducing antibiotic resistance risks.

The potential of BC as a sustainable additive to mitigate ARB proliferation and improve organic waste decomposition and biogas production. Bamboo BC emerged as a superior agent compared to Olive BC, particularly in reducing ARB numbers and enhancing biogas production. Notably, a lower concentration of Bamboo BC (15 g/L) was more effective than a higher concentration (30 g/L), underscoring the importance of dosage optimization. Bamboo BC significantly improved microbial interactions, such as DIET, enriching the biogas production pathway. In contrast, the efficiency of Olive BC was limited, likely due to particle stacking caused by its larger size.

The phase-specific effects of glucose and CEZ on ARB dynamics. During the AP, glucose concentrations suppressed ARB survival, particularly at 10 g/L, while ARB proliferation occurred during the MP. Biochar addition played a critical role in stabilizing microbial communities, mitigating the adverse effects of CEZ on methane production, and supporting substrate decomposition. The protective properties of biochar enhanced microbial resilience under pharmaceutical stress, reduced the abundance of CEZ-resistant bacteria, and improved substrate decomposition and SCOD removal efficiency.

This study provides foundational insights into the complex interactions between microbial communities, antibiotic resistance, and biogas production in AD systems. It underscores the potential of biochar to enhance system stability, optimize substrate decomposition, and mitigate antibiotic resistance risks. Future work should focus on refining biochar dosages, evaluating

(Name: 有 婧仪 NO. 8)

their long-term impacts, and advancing integrated waste treatment strategies to achieve a more sustainable and environmentally resilient approach to organic waste management.

氏名	有 靖儀		
論文 題目	New Approaches to Control Antibiotic-Resistant Bacteria in Anaerobic Digestion Processes for Sustainable Conversion of Dairy Cattle Manure (乳牛ふん尿の持続可能な変換のための嫌気性消化における抗生物質耐性菌制御への新しいアプローチ)		
審査 委員	区 分	職 名	氏 名
	主 査	教授	井原 一高
	副 査	教授	伊藤 博通
	副 査	教授	竹中 慎治
	副 査	助教	吉田 弦
	副 査		
要 旨			
畜産や酪農における抗生物質の使用は、家畜の疾病管理や成長促進に寄与する一方で、環境に深刻な影響を及ぼしている。動物に投与された抗生物質の多くは完全に代謝されることなく排泄され、ふん尿中に抗生物質残留物として蓄積される。抗生物質の残留は、抗生物質耐性菌（ARB）や抗生物質耐性遺伝子（ARG）の出現および拡散を助長し、家畜およびヒトの健康を脅かす可能性がある。 嫌気性消化（AD）は、ふん尿の資源化とバイオガス生産のための持続可能な技術として広く用いられている。しかしながら、抗生物質残留物を含むふん尿を投入材料とした場合、排出される消化液を通じて ARB や ARG が土壌や水域に再び害を及ぼすリスクが指摘されている。抗生物質耐性が深刻な環境問題として世界的に認識されている現状を踏まえると、AD の機能向上が求められる。本研究では、乳牛ふん尿を対象とし、嫌気性消化における薬剤耐性菌の消長の解明と、添加剤としてバイオ炭の可能性について検討を行った。 本論文は、1 章から 6 章の 6 つの章で構成されている。1 章は緒論、2 章は研究背景と既往の研究について示されている。 3 章では、牛乳房炎の治療薬として用いられるセファゾリン（CEZ）を添加した乳牛ふん尿を用いた一相式および二相式嫌気性消化における抗生物質耐性菌の消長について論じている。先行研究において、嫌気性消化が家畜ふん尿中の ARG の減少に寄与する可能性が報告されている。一相式嫌気性消化では、酸生成相（AP）とメタン生成相（MP）の両方の過程が同一のリアクタ内で行われるため、両相の微生物が相互に作用する。しかし、各相の微生物にとって適切な環境条件は異なることが知られている。これに対して、酸生成相とメタン生成相を別々のリアクタで行う二相式嫌気性消化は、酸生成とメタン生成の条件を個別に設定できる。本章では、セファゾリンを添加した乳牛ふん尿を用いた回分式嫌気性消化における ARB の消長を調査するため、一相式と二相式の嫌気性消化を比較した。二相式嫌気性消化では、メタン生成相において CEZ 耐性菌（CEZ-r）およびオキシテトラサイクリン耐性菌（OTC-r）の除去率が高く、それぞれ 9～16 %および 69～72 %であった。しかしメタン生成相では、培養可能な全細菌のうち ARB 菌の割合が、酸生成相および一相式嫌気性消化と比べ、24～30 %高くなった。CEZ は、ARB 除去率とバイオガス生産に悪影響を及ぼしたが、一相式嫌気性消化においてその影響がより顕著であった。二相式嫌気性消化におけるバイオガス収率は CEZ の添加に影響せず、一相式と比べ 10～20 %高くなった。CEZ 添加によるバイオガス生成量の減少は、主に揮発性脂肪酸（VFA）生産の抑制に起因したことが考えられた。 4章では、バイオ炭の添加が一相式嫌気性消化における抗生物質耐性菌の消長に与える影響について論じている。バイオ炭（BC）は、その豊富な官能基と高い多孔性構造が特徴である。近年、嫌気性消化において微生物群集間における電子伝達を促進させる添加剤として、多くの報告がなされている。 本章では、乳牛ふん尿を用いた嫌気性消化において、バイオ炭の添加が抗生物質耐性菌の消長とバ			

氏名	有 靖儀
イオガス生成に及ぼす影響を検討した。粒径の異なる竹生物炭もしくはオリーブ生物炭を、15 g/L と 30 g/L の条件で中温嫌気性消化に添加し、バイオガス生成量、有機物代謝、抗生物質耐性菌消長および微生物の消長を解析した。その結果、すべてのリアクタで CEZ 耐性菌 (CEZ-r) が 12.9 % から 76.5 % の範囲で減少した。竹生物炭もしくはオリーブ生物炭の添加は、対照区と比較して CEZ-r 除去率を 3.1 ～ 5.9 倍増加させた。さらに、生物炭の添加により、対照リアクタにおいて観察された総細菌数に対する CEZ-r の割合の上昇が抑制された。竹生物炭はオリーブ生物炭よりもバイオガス収率を向上させ、竹生物炭を 15 g/L もしくは 30 g/L 添加した場合、対照区と比べそれぞれ 43.2 %、48.0 % の増加を示した。嫌気性消化に生物炭を添加することによって、潜在的な ARG を減少させ ARG の伝播を阻害することにより、ARB が制御可能であることが示された。同時に、メタン生成の効率向上も可能であることが明らかになった。得られた知見は、生物炭添加が嫌気性消化の機能強化と ARB 増殖抑制への寄与を示すものであり、持続可能な家畜ふん尿管理とエネルギー生産へのアプローチを提供するものである。 5章では、CEZを投入した乳牛ふん尿を用いた二相式嫌気性消化における抗生物質耐性菌の生存に対するバイオ炭の影響について論じている。前章において、ADプロセスにおける抗生物質耐性の伝播を多角的に検討し、ARG制御のための添加物としてのバイオ炭の利点を示している。しかし、抗生物質の残留と高い有機物負荷という複合的ストレス下で、バイオ炭が酸生成およびメタン生成の両相におけるARBの生存にどのように影響するかは十分に解明されていない。特に、バイオ炭の添加が抗生物質が存在する中で酸生成を維持しつつ、酸生成細菌間で抗生物質耐性の拡大を抑制できるかは不明である。本章では、高有機物負荷、セファゾリン添加そしてバイオ炭添加の条件下において、酸生成相とメタン生成相における微生物群集、抗生物質耐性そしてバイオガス生産の相互作用について検討した。10 g/Lのグルコース添加による高有機物条件下において、CEZ-rは抑制された。対照区と比較すると、CEZ-rの割合は酸性相では0.30 %であったが、メタン生成では36.3%に増加した。CEZが残留する場合、グルコース添加なし、もしくは5 g/L添加した酸生成相において、対照区と比較するとCEZ-rは2.5 %、9.3 %それぞれ増加した。グルコースを10 g/Lに設定したCEZ添加リアクタでは、酸性相においてCEZ-rは0.23 %に抑制されたが、メタン生成相では8.3 %となった。バイオ炭を添加し、5 g/Lもしくは10 g/Lのグルコースを添加したところ、酸生成相およびメタン生成相においてCEZ-rは1.0%未満に低下した。CEZの残留は<i>Methanosaetaceae</i>の存在量を減少させ、酢酸への変換が阻害されることによってバイオガス生成量が減少したと考えられた。バイオ炭の添加は、CEZの影響を緩和することが明らかになった。これは、バイオ炭によって促進された直接的な種間電子伝達によるものと考えられた。 6 章では、総合考察を行っている。本研究は、抗生物質の残留を想定した家畜ふん尿の嫌気性消化プロセスにおける、微生物群集、抗生物質耐性菌およびバイオガス生産の複雑な相互作用に関する知見を提供するものであり、とくにバイオ炭の添加は嫌気性消化プロセスの安定性を向上させるとともに抗生物質耐性リスクを軽減できることを明らかにした。 本研究で得られた成果は、家畜ふん尿処理と再生可能エネルギーの生産を目指した嫌気性消化において、抗生物質耐性による環境リスクを軽減する上で不可欠なプロセスの機能強化を実現し、抗生物質耐性制御への新しいアプローチを切り拓くものである。よって、学位申請者の有 靖儀は、博士（農学）の学位を得る資格があると認める。	