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DISSERTATION OUTLINE

**DYNAMICS OF THE MICROBIOME AND
PREDICTED BIOCHEMICAL PATHWAYS IN
METHANE FERMENTATION IN RESPONSE
TO ORGANIC LOAD SHOCKS**

A dissertation by

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DISSERTATION OUTLINE

CHAPTER 1 – Introduction

This chapter introduced Anaerobic Digestion (AD), the challenge imposed by organic loading rate (OLR) fluctuations, and how the study intended to address the problem. AD is a process wherein a consortium of microorganisms converts an organic material to methane and carbon dioxide as end products. One of the key process parameters influencing the performance of anaerobic digesters is the OLR. It is primarily dictated by the waste treatment demand which may fluctuate considerably, especially for practical-scale installations. However, fluctuations in OLR cause shocks to the AD microbiome leading to unstable operations due to resulting imbalances in the biochemical processes occurring within AD. Handling these OLR fluctuations would require a digester with a large volume which is especially impractical for community-scale applications, limiting the potential of AD in advancing a circular economy. As such, there is a need to tackle the issue from the anaerobic digester microbiome level and find a way to train the microbiome to enhance its capability and flexibility in handling high organic load shocks, eliminating the need for excessively large digesters. Leveraging on bioinformatics, this study aims to enhance the resilience of AD microbiome itself against repeated OLR shocks.

CHAPTER 2 – Effect of one-time organic load shock on the short-term response of anaerobic digester microbiome

This chapter covers the first part of the study which elucidated the differences in short-term microbial community response to organic load shocks with difference in complexity of the acclimatization substrate used for the inoculum. By directly comparing the effect of acclimatizing using a simple substrate and a complex substrate not only on the macroscopic anaerobic digester performance but also on the microbial community and biochemical pathway level, it was attempted to elucidate how the dominance of certain microbial groups are relevant to the biochemical changes within the AD system, and ultimately, the resulting methane productivity. The response of the AD microbiome to OL shocks differed depending on the complexity of the sludge acclimatization substrate. Microbial diversity decreased with higher load for sludge acclimatized to a simple substrate. Correspondingly, methane yield decreased. Microbial diversity remained stable despite high loads for sludge acclimatized with complex substrate in which the methane yield remained the same across varying OL. AD sludge acclimatized to a complex substrate has higher resilience against organic load shock making it more favorable. Moreover, the contribution of microbial communities to major substrate utilization pathways was even across different organic loads when the inoculum was acclimatized to a more complex substrate indicating expanded functionality of the microbiome.

CHAPTER 3 – Effect of frequency of organic load shock on anaerobic digester microbial community progression

This chapter covers the second part of the study which tackled the anaerobic digester performance, microbial community progression, and biochemical pathway dynamics in the anaerobic digester inoculated with the microbiome acclimatized to a complex substrate, in response to repeated high organic load shocks at different frequencies. It was found that less frequent organic load shocks impose higher impact to the microbiome. This led to the breakdown of the microbiome network structure necessary to maintain stable anaerobic digestion performance, eventually resulting to loss of methane productivity. The network structure breakdown and the corresponding loss of methane production suggest shift in microbiome activity towards production of other products that are not contributing to net methane production.

CHAPTER 4 – Effect of acclimatization organic loading rate to the resilience of anaerobic digester against repeated organic load shocks

This chapter covers the third part of the study which compared the differences in the anaerobic digester performance, microbial community progression, and biochemical pathway dynamics between anaerobic digesters acclimatized using low and high OLR that is subjected to repeated impactful high organic load shocks. It was found that the anaerobic digester microbiome sustained its methane productivity even after repeated OLR shocks in the digester inoculated with high-load acclimatized seed. It was inferred

that acclimatizing at high OLR increased the resilience of the microbiome against high organic load shocks which was achieved through stronger interactions that were established within the microbiome network. This maintained the microbial activity towards execution of biochemical pathways leading to methane production. The findings suggest that anaerobic digester microbiome attains higher resilience against high organic load shocks by acclimatization using a high OLR.

CHAPTER 5 – Conclusions and Recommendations

The final chapter discusses the general conclusions and recommendations. The results of this study point towards a proposed acclimatization strategy aiming to obtain an AD microbiome that would be resilient against repeated high organic load shocks. It is proposed that acclimatizing the microbiome using a targeted complex substrate and a high organic loading rate would produce a highly robust AD system. The system would allow reduction of the reactor size while maintaining flexibility in handling high organic load shocks due to varying loads and diversity of feedstock. These propositions further promote circular economy by proliferating small-scale AD for use in communities.