UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Microbial community analysis using next-generation sequencing and bioinformatics tools to better understand biological waste and wastewater treatment

by Quynh Anh Nguyen

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of Professor Duc Long Nghiem & Professor Huu Hao Ngo

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, **Quynh Anh Nguyen** declare that this thesis, is submitted in fulfilment of the requirements for the award of **Doctor of Philosophy**, in the **School of Civil and Environmental Engineering** at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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LIST OF PUBLICATIONS

1. **Nguyen QA**, Vu HP, McDonald JA, Nguyen LN, Leusch FDL, Neale PA, et al. Chiral Inversion of 2-Arylpropionic Acid Enantiomers under Anaerobic Conditions. Environmental Science & Technology. 2022;56(12):8197-208.
2. **Nguyen AQ**, Nguyen LN, Johir MAH, Ngo HH, Nghiem LD. Linking endogenous decay and sludge bulking in the microbial community to membrane fouling at sub-critical flux. Journal of Membrane Science Letters. 2022;2(1):100023.
3. **Nguyen AQ**, Nguyen LN, Xu Z, Luo W, Nghiem LD. New insights to the difference in microbial composition and interspecies interactions between fouling layer and mixed liquor in a membrane bioreactor. Journal of Membrane Science. 2022;643:120034.
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5. **Nguyen AQ**, Vu HP, Nguyen LN, Wang Q, Djordjevic SP, Donner E, et al. Monitoring antibiotic resistance genes in wastewater treatment: Current strategies and future challenges. Science of The Total Environment. 2021;783:146964.
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LIST OF ACHIEVEMENTS

1. Winner of 2021 Women in Engineering and IT HDR Award.
2. UTS Vice-Chancellor's Conference Fund 2021.
3. Honourable Mention in *National Innovation Game – Northern Territory Series – Territorians discovering opportunity in Renewable Energy* - team effort (2021).
4. Polish NAWA PROM International Scholarship exchange of PhD candidates (2020).
5. Research Training Program and UTS Faculty of Engineering and IT Scholarship (2018 – current).
6. 1st prize poster presentation at *2nd International Conference on Green Technologies for Sustainable Water* (2019).
7. Polish NAWA PROM PhD students exchange scholarship recipient (2019).
8. Best oral presentation award at *Technology and Innovation Research Showcase* (2018).
9. Awarded travel bursary at the Winter School in Mathematical and Computational Biology (2018).

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LIST OF ABBREVIATIONS

2-APA	2-arylpropionic acid
ACN	Acetonitrile
AD	Anaerobic digestion
ANCOM	Analysis of composition of microbiomes
ASV	Amplicon sequent variant
BMP	Biochemical methane potential
BOD	Biological oxygen demand
CAS	Conventional activated sludge
CEs	Collision energies
COD	Chemical oxygen demand
CS	Corn silage
DCM	Dichloromethane
DO	Dissolved oxygen
EF	Enantiomeric fraction
EPS	Extracellular polymeric substance
EROD	Ethoxyresorufin-O-deethylase
F/M	Food-to-microorganism
FISH	Fluorescence in situ hybridization
GH	Glycoside hydrolase
HLB	Hydrophilic/lipophilic balance
HRT	Hydraulic retention time

ISTD	Internal standard
LCBM	Lignocellulosic biomass
LH	Lucerne hay
LOQ	Limits of quantification
LR	Loading rate
MBR	Membrane bioreactor
MENA	Molecular ecological network analysis
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MRM	Multiple reaction monitoring
N.D.	Not determinable
NA	Not applicable
ND	Not detected
NGS	Next-generation sequencing
NSAID	Non-steroidal anti-inflammatory drug
NSW	New South Wales
OH	Oaten hay
ORP	Oxidation-reduction potential
PcoA	Principal coordinate analysis
PEA	Phenylethylamine
PL	Polysaccharide lyases
PN/PS	Protein/polysaccharide
PVDF	Polyvinylidene difluoride
QIIME	Quantitative insights into microbial ecology
qPCR	Quantitative polymerase chain reaction
RMT	Random matrix theory

RUSITEC	Rumen simulation technique apparatus
sCOD	Soluble chemical oxygen demand
SIP	Stable-isotope probing
SMP	Soluble microbial product
SPE	Solid-phase extraction
SRT	Solids retention time
TEA	Triethylamine
TMP	Transmembrane pressure
TOA	Total organic acids
TOC	Total organic carbon
TS	Total solids
TVFA	Total volatile fatty acid
UF	Ultrafiltration
UPGMA	Unweighted pair group method with arithmetic mean
VFA	Volatile fatty acid
VS	Volatile solid
VSS	Volatile suspended solids
WS	Wheat straw
WWTP	Wastewater treatment plant

ABSTRACT

Waste/wastewater treatment often rely on microbes and biotransformation for removing contaminants and environmental restoration. Insights into the microbial communities associated with these processes can help develop better operational strategies. Three common environmental engineering processes were investigated in this thesis to demonstrate the application of next-generation sequencing and bioinformatics tools to elucidate the link between microbial community and process performance.

The first process was membrane fouling in membrane bioreactors (MBRs). Nutritional deficiency led to endogenous decay and sludge bulking, which in turn triggered membrane fouling under sub-critical flux. The mixed liquor and fouling layer possessed similar microbial composition. The most dominant filamentous order *Thiotrichales* (>60%) positively correlated with fouling severity. Under high-flux conditions, MBR biofilm and mixed liquor possessed different microbial structures. Low-abundance taxa (<1%) such as *Victivallales* and *Blastocatellia* 11-24 drove the divergence between the two communities. These taxa also played key roles in fouling development and positively correlated with fouling indicators. Knowledge of MBR fouling-associated microbial taxa can help improve fouling control strategies, reduce the cost of membrane cleaning and energy consumption, enhance MBR application and increase the treated water quality.

The second process was lignocellulosic biomass (LCBM) valorisation using rumen microbes. Biomethane potential analysis showed that rumen microbes can produce four times more volatile fatty acids (VFA) than anaerobic sludge. However, VFA accumulation led to pH drop which in turn resulted in process inhibition, suggesting the need for continuous extraction of VFA from the system. A novel rumen-MBR was evaluated, showing continuous VFA production at 438 mg VFA/g substrate. Acetic and propionic acids accounted for >80% of the total VFA produced. Most of the produced VFA (73 ± 15%) was continuously extracted by an ultrafiltration membrane. Shifts in dominant rumen microbes during operation did not impact VFA yield. This work provides an important foundation for the development of a sustainable pathway for producing renewable chemicals in a circular economy.

The third process was chiral inversion of 2-arylpropionic acids (2-APAs) in biological waste and wastewater treatment. Despite possessing highly similar chemical structures, eleven 2-APAs exhibited diverse and distinctive chiral inversion behaviours. Both unidirectional and bidirectional chiral inversions of 2-APAs were observed under aerobic and anaerobic conditions. Potential microbes involved in chiral inversion, including *Candidatus_Microthrix*, *Rhodococcus*, *Mycobacterium*, *Gordonia,* and *Sphingobium*, are aerobic or facultative anaerobic bacteria. This

is the first study to report chiral inversion behaviours of a comprehensive suite of 2-APAs during biological treatment.