THESIS

Improving the economic performance of anaerobic digestion by integrating lactic acid recovery into two-stage food waste digestion

Christopher Heinz Bühlmann

Bachelor of Engineering Hons
(Chemical and Metallurgical)

Thesis submitted for the fulfilment of the requirements for the degree of

Doctor of Philosophy

Discipline of Engineering and Energy

College of Science, Health, Engineering and Education

Murdoch University

Murdoch, Western Australia, Australia

(2022)

DECLARATION

I declare that this thesis is my own account of my research and contains as its main content, work which has not previously been submitted for a degree at any tertiary education institution.

Christopher Heinz Buhlmann

ACKNOWLEDGMENTS

The completion of this doctoral thesis would not have been possible without the valuable support of all the people around me. I would like to express my utmost appreciation to my supervisory team, Prof Parisa A. Bahri, Dr Bede S. Mickan, and Dr Stephan Tait. Your guidance, support, and professional advice, throughout the duration of this research project has been invaluable and I am extremely grateful for all your assistance. I could not have asked for a better supervisory team to guide me through my PhD.

I would like to take this opportunity to thank Murdoch University for awarding me the scholarship and providing me technical support throughout my PhD project. Furthermore, I would also like to thank Richgro for providing valuable funding assistance for analyses throughout this project which were necessary to complete the research. I would also like to thank Geoff Richards, Tim Richards, Maria Sevo, Tony Doherty, and the rest of the Richgro team for providing support throughout the project.

To my friends I thank you for all the times I needed advice or time to recharge. You were all there when I needed you and I am truly thankful. I would like to give a special thanks to Bryce Albertani for providing me with all the caffeine I could possibly need to complete this thesis, and for making sure I gave myself time away from my computer screen. I appreciate all the visits and distractions during the long days and nights at Uni.

I would like to give special thanks to my lovely girlfriend Kirstie Horton, who supported me through my journey and made sure I squeezed in holiday and reading time whenever I could and made sure I took time to relax.

Finally, to my family, for their unwavering love and support throughout my PhD. Without their unyielding support, I would never have made it to this point, and I am eternally grateful.

STATEMENT OF CONTRIBUTION

The content in this thesis was developed by the Candidate with advice from their supervisory panel.

The following individuals contributed to the thesis.

Contributor	Christopher H. Buhlmann	Parisa A. Bahri	Bede S. Mickan	Stephan Tait	David Parlevliet
Overall Contribution (%)	70	10	9	9	2
Concept Development (%)	85	5	5	5	-
Data Collection (%)	100	-	-	-	-
Data Analyses (%)	85	5	5	5	-
Drafting of Chapters (%)	85	5	5	5	-
Review and edit chapters (%)	76	8	8	8	-
Review and edit thesis (%)	80	15	-	-	5
Oversight and administration of PhD progress (%)	-	80	-	-	20

Contribution indicates the total involvement the student and other contributors (supervisors etc.) has had in the creation of the thesis.

By signing this document, the Candidate and Principal Supervisor acknowledge that the above information is accurate and has been agreed to by all other contributors.

Candidate **Principal Supervisor**

ABSTRACT

The global production of food waste (FW) is of significant economic and environmental concern, having been estimated to produce 8% of globally produced anthropogenic greenhouse gas emissions and result in the loss of nearly USD1 trillion each year. Consequently, the correct disposal and recovery of value from FWs is a global challenge and responsibility. Anaerobic digestion (AD) is a capable technology which can recycle FW to produce renewable energy and recover nutrients. However high capital, operational, and management costs and low value of biogas and digestate lead to questionable economic benefit. As a result, the AD technology heavily relies on subsidies and policy incentives for feasibility. Integration of lactic acid (LA) production technologies into AD could convert the low-value process into a high-value LA-AD biorefinery, reducing reliance on government support

This study aims to address the above by exploring the integration of LA production to AD within the FW context. This involved detailed investigations into the production of LA from FWs, including within the commercial FW context, and integration of FW fermentation for LA into existing AD facilities. Accordingly, following optimisation of LA fermentation, and exploring the feasibility of recovering LA along with its impact on downstream AD, multiple integration scenarios were proposed detailing the potential economic benefits from integrating LA production into FW AD.

The assessment of LA production within the FW context was first explored. For this, LA production within a pre-fermenter at a commercial two-stage FW AD facility was monitored, exploring the impact of environmental conditions, feedstock composition, and operational procedures on LA production performance and stability. Results showed standard operation of the pre-fermenter, favoured the formation of LA leading to LA being the dominant organic acid produced from fermentation. Furthermore, standard operation of the AD facility led to the selective dominance of *Lactobacillus*, a bacterium commonly associated with LA production. While LA production fluctuated over the monitoring period, the LA concentration was surprisingly stable, especially considering the variation in process variables (pH, temperature, retention time, feed rate, and feed composition). Even so, it was outlined that there would be significant opportunity to

improve LA production performance, and consequently, economic performance by targeted process optimisation and control.

Optimisation of LA fermentation showed the commercially adapted inoculum was capable of high LA yields and selectivities. In addition, the results showed optimal conditions promoted the growth of *Lactobacillus*, while alternative flanking microorganisms were inhibited. Moreover, optimisation effectively eliminated the conversion of LA to butyrate, allowing the sustained accumulation of LA. Further study of the commercial inoculum showed LA production could be effectively enhanced by supplementing FW with a simple carbohydrate (sucrose) and implementing partial digestate recirculation. While digestate enhanced LA production, it also increased microbial diversity which promoted the production of alternate organic acids. However, the effects of digestate could be effectively controlled through sucrose addition, which promoted the growth of *Lactobacillus* and inhibited the growth of the flanking community.

Following optimisation of fermentation, the feasibility of recovering LA from complex fermentation media and its impact on downstream AD performance was explored. While real commercial broth reduced LA uptake, compared to pure LA solutions, LA was effectively recovered from highly complex fermentation media. Moreover, LA recovery only led to a minor reduction in methane production following the AD of the solid and liquid extraction residues. In this respect, LA production could outweigh the loss in methane production in terms of relative value, indicating the LA-AD biorefinery concept could be commercially attractive. A technoeconomic assessment indeed showed the benefit of integrating LA production into two-stage FW AD, yielding a highly profitable scenario. Furthermore, while integration scenarios were most profitable, Greenfield LA-AD biorefinery scenarios showed significantly higher profitability estimates compared to sole FW AD.

Finally, the insight achieved into different aspects of the LA-AD biorefinery led to a series of recommendations for future research in the context of the FW biorefinery concept.

NOTE ON THE THESIS FORMATTING

This thesis is presented as a thesis by publication. It has been divided into nine chapters, three of which contain published work while the other chapters are in late draft form and will be submitted for publication shortly after the submission of the thesis. As some of the thesis consists of independent published work, some repetition of the literature was unavoidable. Prior to each chapter, a short description outlining the contents of that chapter and its role in the research is presented. To maintain consistency between chapters, each published chapter has been formatted to that of the thesis. Table, figure, and equation labels have been renumbered to allow for easy cross reference between chapters if needed with the first number denoting the chapter number, followed by the table number for that chapter (e.g. Table 2.4). The reference style for each chapter has been adjusted to the Chicago style, regardless of which journal the paper was published in, to maintain consistency within the thesis. An individual reference list has been provided for each chapter, which are located at the end of each individual chapter. The supplementary data for each chapter has been added to the end of the thesis as appendices.

LIST OF PUBLICATIONS BY CANDIDATE

Published Journal Papers

Bühlmann, C.H., Mickan, B.S., Tait, S., Renton, M., Bahri, P.A., 2021. Lactic acid from mixed food wastes at a commercial biogas facility: Effect of feedstock and process conditions. *Journal of Cleaner Production* 284, 125243. (Impact Factor: 11.072 (2021), 1st Quartile) – Chapter 3

Bühlmann, C.H., Mickan, B.S., Tait, S., Bahri, P.A., Developing a food waste biorefinery: lactic acid extraction using anionic resin and impacts on downstream biogas production, *Chemical Engineering Journal*, 2021, 133243. (Impact Factor: 16.744 (2021), 1st Quartile) – Chapter 6

Bühlmann, C.H., Mickan, B.S., Tait, S., Batstone, D.J., Mercer, G.D., Bahri, P.A., 2022. Lactic acid from mixed food waste fermentation using an adapted inoculum: Influence of pH and temperature regulation on yield and product spectrum. Journal of Cleaner Production, 133716. (Impact Factor: 11.072 (2022), 1st Quartile) – Chapter 4

Papers In Late Draft Form

Bühlmann, C.H., Mickan, B.S., Tait, S., Bahri, P.A, Lactic acid production from two-stage anaerobic digestion: An examination of applying the biorefinery concept to anaerobic digestion. – Chapter 2

Bühlmann, C.H., Mickan, B.S., Tait, S., Batstone, D., Bahri, P.A, Lactic acid production from food waste at an anaerobic digestion biorefinery: Effect of digestate recirculation and sucrose supplementation. – Chapter 5

Bühlmann, C.H., Mickan, B.S., Tait, S., Fogarty, J.J., Bahri, P.A, Techno-economic feasibility of integrating lactic acid recovery into existing food waste anaerobic digestion facilities. – Chapter 7

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AD	Anaerobic Digestion
BF	Brownfield
BMP	Biomethane Potential
CBA	Cost Benefit Analysis
CCR	Carbon Catabolite Repression
CNF	Conventional Fermentation
COD	Chemical Oxygen Demand
FDP	Fructose 1,6-DiPhosphate
FVW	Fruit and Vegetable Waste
FW	Food Waste
GF	Greenfield
HRT	Hydraulic Retention Time
IRR	Internal Rate of Return
KW	Kitchen Waste
LA	Lactic Acid
LAB	Lactic Acid Bacteria
LDH	Lactate DeHydrogenase
MLS	Mainline Scenario
N	Nitrogen
NPV	Net Present Value
OLR	Organic Loading Rate
OP	Optical Purity
PICURSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
PLA	Poly-Lactic Acid
Response Surface Methodology	RSM
ROI	Return on Investment
SDS	Side-stream Scenario
SDT	Sucrose and Digestate experimental Treatment
TS	Total Solids
United States Dollar	USD
Volatile Fatty Acid	VFA
VS	Volatile Solids
WFS1	Waste Feedstock No. 1
wt	Weight
GRAS	Generally Regarded As Safe

CHAPTER 1 INTRODUCTION

1.1 PROJECT BACKGROUND (IN BRIEF)

The project background and problem statement has been outlined in more detail in the introduction section of Chapter 2 (Literature Review).

Food waste (FW), which is defined as any solid or liquid food intended for human consumption lost along the food supply and consumption chain, is produced in high volumes and tends to grow with population (ARCADIS, 2019; Wolka & Melaku, 2015). The current rate of global FW production has been estimated at 1.3-1.6 billion tonnes per annum which is associated with an economic loss of nearly one trillion USD every year and the production of 8% of globally produced anthropogenic greenhouse gas emissions (Demichelis et al., 2018; FAO, 2021; WBA, 2018). While countries have devised strategies to reduce FW production (ARCADIS, 2019; UN, 2019), the generation of FW along the supply chain is inevitable and treatment methods should aim to reclaim value from this waste stream (Slorach et al., 2019).

Anaerobic digestion (AD) is a mature technology capable of recycling FWs for the production of bioenergy and recovery of nutrients (Edwards et al., 2015). The complex biological process which governs AD is composed of four primary steps, namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Depending on the AD design, these processes may be conducted within a single vessel, or in a two-stage design separating hydrolysis and acidogenesis from acetogenesis and methanogenesis. Compared to the single stage design, two-stage systems have been associated with improved process stability and higher methane yields owing to their capacity to conduct the biological processes at their optimal conditions (Bo & Pin-jing, 2014; De Gioannis et al., 2017). Two-stage AD can play a vital role in renewable energy production and redistribution of nutrients while simultaneously reducing the environmental impacts associated with FW disposal (Chapter 2).

Traditional two-stage AD facilities have two traditional financial benefits, biogas or power generation, and fertiliser production. However, due to high construction, operational, and managemental costs, waste treatment with AD can often lead to questionable economic benefit without support from subsidies or policy incentives

(Bastidas-Oyanedel & Schmidt, 2018; Kim et al., 2016). Consequently, the adoption rate of the AD technology varies significantly from country to country where government support differs (Chapter 2). Some AD operations may charge a gate-fee to provide a third revenue source and reduce the reliance on government support. However, this creates uncertainty within AD operations which rely on gate-fees as a major revenue source as many biowastes are suitable for composting, incineration, or animal feed production, creating a competitive market which may undercut AD gate-fees (Edwards et al., 2015). AD may alternatively be considered for utilisation within a larger integrated FW biorefinery, producing various high-value end products, such as lactic acid (LA), from biomass.

LA is a highly versatile building block chemical with a variety of uses within the food, pharmaceutical, and cosmetic industries, with recent applications including the production of biodegradable plastics (Lin & Wang, 2007). Recent literature has shown LA production technologies boost the economic viability of AD and reduce the reliance on subsidy support (Bastidas-Oyanedel & Schmidt, 2018; Demichelis et al., 2018; Kim et al., 2016). Whilst these findings are promising, they considered the development of new biorefinery solutions and didn't consider integration into existing AD infrastructure. Utilisation of existing AD capital would result in significant capital savings associated with an LA-AD biorefinery. However, it is unknown how the standard operation of a commercial AD facility may influence LA production. Various factors, such as fermentation pH, temperature, feed composition, and retention time may not be subject to control, likely naturally fluctuating with surrounding environmental conditions and feed composition. Carbohydrates are the main substrate for LA production, making FW a promising substrate for LA production (Demichelis et al., 2017; Kim et al., 2016). However, FW likely won't be the sole substrate of an AD facility, and it unknown how varying feed composition may impact LA production. Furthermore, pH depression during LA production is a major barrier to high LA yields and productivities due to the inhibitory effects imposed by the highly acidic conditions (Alves de Oliveira et al., 2018). Optimisation and control of environmental conditions for LA fermentation would likely result in significant improvements to the fermentation process, though the LA production capability of an existing microbial community within the pre-fermenter is unknown. Furthermore, while LA production has been suggested to aide AD economics, it is important to also consider the downstream effects of LA recovery on AD. While valuable,

LA is a substrate for methane formation and its removal will logically reduce the organic load to the digesters. The overall impact of LA recovery on AD has not been explored in literature and is a crucial factor to consider when integrating LA recovery. These questions surrounding the integration of LA production within AD led to the current research project to investigate the feasibility of integrating LA production into existing commercial AD systems aiming to improve process economics.

1.2 RESEARCH QUESTIONS

1.2.1 Lactic acid production at a commercial anaerobic digestion facility

How does the operation of a commercial two-stage anaerobic digestion facility impact lactic acid fermentation? – Chapter 3

Efficient LA production requires the strict control of environmental conditions to ensure optimal and consistent production of LA. However, the pre-fermentation stage within two-stage AD may function with limited control, allowing factors such as pH, temperature, hydraulic retention time, and organic loading rate to vary naturally with changing feedstock composition and surrounding environmental conditions. Understanding how natural variation in the complex composition of the waste streams and operating conditions may influence LA production is pivotal to integrating LA production into a mixed FW AD facility.

1.2.2 Lactic acid recovery and the impact on downstream methane yield

Can lactic acid be recovered from real fermentation broth and what are the effects on downstream anaerobic digestion performance? – Chapter 6

Adsorption in a promising method for LA recovery and has generally been explored for synthetic broths, broths produced from relatively pure feedstocks, and simple experimental FW broths, but has yet to be explored for a commercial mixed FW broth. The mixed fermented FW broth would resemble the complex composition of future FW biorefineries and it is important to explore the capability adsorption to recover LA from these complex mixtures. Furthermore, while LA can provide an economic benefit to AD, LA is a carbon source for methane formation and its extraction will logically impact the downstream methane yield. Determining the impact of LA recovery on the downstream methane yield can provide valuable data to determine the overall economic impact of LA production on AD economics.

1.2.3 Lactic acid production capability of industrial inoculum

What is the lactic acid production capacity of the commercially produced anaerobic fermentation inoculum? Can lactic acid production be enhanced with carbohydrate and nitrogen addition? – Chapter 4 and 5

Following monitoring of the industrial pre-fermenter, LA was identified as a major product produced during the uncontrolled fermentation of the commercial FW mixture. It was identified that the limited control of fermentation conditions likely inhibited further LA fermentation of the mixed FW, restricting the LA production potential. The operational pH and temperature are fundamental to LA fermentation, directly controlling the LA yield and production rate. It is important to optimise and control these process variables to maximise LA production and improve process economics.

Following the optimisation of temperature and pH, it was found the industrial inoculum was capable of yields similar to those reported in literature. Due to the limited ability of LA bacteria to secret enzymes to break down complex polysaccharides to simple sugars, FW is naturally limited in its LA production potential. Various reports in literature have aimed to improve LA production by incorporating various chemical, physical, or enzymatic pre-treatment methods. However, these methods are costly and may require the use of complex unit operations. Alternatively, the addition of a simple sugar, such as sucrose, could increase the carbohydrate content of the FW and improve LA fermentation. Furthermore, the supplementation of nitrogen as been associated with improved LA yields from FW fermentation. FW digestate naturally contains elevated ammonium levels and may be suitable as a nitrogen source for LA production while also containing various other nutrients to aide LA production.

1.2.4 Technoeconomic feasibility

What is the technoeconomic feasibility of integrating lactic acid fermentation and production into existing two-stage anaerobic digestion? – Chapter 7

Following supplementation of FW with sucrose and assessment of digestate recirculation on fermentation performance, it was found that LA production improved with both sucrose and digestate addition. The optimal supplementation rate of sucrose and digestate were utilised, along with the optimal environmental conditions to conduct a simple

technoeconomic assessment to determine the economic feasibility of integrating LA production into AD.

1.2.5 Research objective

The AD of FWs for renewable energy production and recovery of nutrients is currently not economically viable without government support. This is in large part due to the high production and operation costs in combination with the low economic value of traditional AD products (biogas and digestate). Boosting the economic performance of AD through the integration of LA production technologies has been explored for new FW AD solutions but has yet to be considered for existing facilities. Monitoring the performance of and industrial AD pre-fermenter, optimising the conditions for LA production, characterising LA recovery via adsorption with real fermentation broth, and exploring the impact of LA recovery on downstream methane formation would provide valuable data for predicating the economic feasibility of integrating LA production into AD

With this is mind, the aim of this thesis was to answer the researcher questions put forward in Section 1.2, and the integration of FW pre-fermentation for LA production is proposed. The results of this thesis are used, together with the results of literature, to conduct a simple technoeconomic assessment to estimate the economic benefits of LA production within existing AD facilities. It should be noted that a high-level approach was taken when conducting the technoeconomic assessment with capital and operational costs extrapolated from literature. A more detailed and accurate technoeconomic assessment is required to gain a more detailed understanding of the impacts of LA production within existing AD facilities, however, the assessment within this thesis provides an initial outline on the feasibility and economic benefits provided from LA production.

1.3 ORGANISATION OF THE THESIS

The following thesis is presented as a Thesis by Publication. The thesis itself has been broken down into eight chapters, some with no publications and others containing a single publication. A foreword accompanies each chapter, outlining the significance and objective of the work, and, where necessary, outlines how it relates to the previous chapter. As most of the thesis consists of independent published work, some repetition of the literature was unavoidable, particularly within the introduction sections of each chapter. To maintain consistency within the thesis, table, figure, and equation labels have

been renumbered to allow for easy cross reference between chapters if needed with the first number denoting the chapter number, followed by the table number for that chapter (e.g. Table 2.4 indicates Table 4 in Chapter 2). The text and headings have been formatted to be consistent throughout the thesis. Tables and figures have been placed to give the desired flow and structure and may not be presented in the same location or format (for flow and aesthetics purposes) as they are found in their respective publications. The reference style for each chapter has been adjusted to the Chicago style regardless of which journal the paper was published in to maintain consistency within the thesis.

Every other aspect of the published work has been maintained as it was when published which includes, but not limited to, section, figure, table, and equation order. Any supplementary material published along with chapters has been included at the end of the thesis as appendices and are reference within the text by first stating the relevant appendix and then the table or figure number (e.g. Fig. B5 references Figure 5 in Appendix B).

1.4 REFERENCES

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

ARCADIS. 2019. National Food Waste Baseline.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

Bo, Z., Pin-jing, H. 2014. Performance assessment of two-stage anaerobic digestion of kitchen wastes. Environmental Technology, 35(10), 1277-1285.

De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R., Spiga, D. 2017. Energy recovery from one- and two-stage anaerobic digestion of food waste. Waste Management, 68, 595-602.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Edwards, J., Othman, M., Burn, S. 2015. A review of policy drivers and barriers for the use of anaerobic digestion in Europe, the United States and Australia. Renewable and Sustainable Energy Reviews, 52, 815-828.

FAO. 2021. FAO calls for action against food loss, waste in Asia-Pacific. in: The Nation - Thailand, Knowledge Bylanes.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Lin, H.-T., Wang, F.-S. 2007. Optimal design of an integrated fermentation process for lactic acid production. AIChE Journal, 53(2), 449-459.

Slorach, P.C., Jeswani, H.K., Cuéllar-Franca, R., Azapagic, A. 2019. Environmental sustainability of anaerobic digestion of household food waste. Journal of environmental management, 236, 798-814.

UN. 2019. Resolution Adopted by the General Assembly on 25 September 2015, (Eds.) J. Walker, A. Pekmezovic, G. Walker, John Wiley & Sons, Ltd. Chichester, UK, pp. 333-374.

WBA. 2018. Global Food Waste Management: An Implementation Guide For Cities.

Wolka, K., Melaku, B. 2015. Exploring selected plant nutrient in compost prepared from food waste and cattle manure and its effect on soil properties and maize yield at Wondo Genet, Ethiopia. Environmental Systems Research, 4(1), 9.

CHAPTER 2 LITERATURE REVIEW

Foreword

The first step in developing the LA-AD biorefinery is an in-depth review on the current literature relevant to the topic of study. The primary aim of this review chapter was to determine, firstly, the current state of LA fermentation and how it has been applied to process FWs; secondly, to determine the major factors which influence LA production and recovery from FWs; and thirdly, to understand the current literature surrounding the integration of LA production into AD facilities and the key knowledge gaps to address via the thesis research.

2.1 INTRODUCTION:

Food waste (FW) is a relentless problem facing the modern world. Recent estimates have valued the annual 1.3 billion tonnes of globally produced FW at nearly 1 trillion US dollars (Braguglia et al., 2018) and associated 8% of global anthropogenic greenhouse gas emissions with its production (WBA, 2018). In an effort to reduce the production of FW, the United Nations have set a goal to halve the production of FW per-capita at the consumer and retail levels by 2030 (UN, 2019). Furthermore, the sustainable treatment of FW falls within multiple United Nations Sustainable Development Goals (UNSDGs), including the creation of sustainable production and consumption patterns, and taking action against climate change (UN, 2022). Consequently, the development of sustainable technologies for the recovery of value from this waste stream is becoming a focus point for modern research efforts.

Anaerobic digestion (AD) is a mature technology widely utilised for processing various organic wastes, including FW. In commercial applications, AD is commonly applied for processing large volumes of organic waste for the production of renewable energy (Comparetti et al., 2013). More recently, researchers have been investigating the applicability of AD on different waste streams, including lignocellulosic biomass, FW, sewage sludge, and animal manure (Carlsson et al., 2012; Mata-Alvarez et al., 2014; Sawatdeenarunat et al., 2015). In reference to the UNSDGs, the application of AD for FW recycling acts on numerous sustainability goals, including the promotion of sustainable agriculture (Goal 2), production of clean energy (Goal 6), development of sustainable cities (Goal 11), ensuring sustainable production and consumption patterns (Goal 12), and combats climate change (Goal 13). However, the low economic value of the standard AD outputs (i.e. biogas and digestate) and high cost of investment, management, and operation limit the feasibility of AD to farm-scale applications unless financial incentives are provided by governments (Gebrezgabher et al., 2010; Massaro et al., 2015). Consequently, there has been a growing body of literature aiming to value-add to the AD technology with additional biorefinery technologies to recover additional high-value products (Chen et al., 2018; Choi et al., 2017; López et al., 2018).

Similar to oil refineries, biorefineries can produce a variety of fuels, chemicals, and materials, but utilise biomass as the raw material instead of fossil-derived resources (Figure 2.). AD has the potential to produce a variety of high value biomaterials,

biochemicals, and biofuels (Bastidas-Oyanedel & Schmidt, 2018; Moraes et al., 2014; Sawatdeenarunat et al., 2016) with lactic acid (LA) being of particular interest in recent research (Demichelis et al., 2018; Demichelis et al., 2017; Kim et al., 2016). LA is a high-value chemical utilised in a variety of industries, including the food and pharmaceutical industries, with modern applications aiming to produce biodegradable plastics (i.e. polylactic acid (PLA)) (Demichelis et al., 2017). Recent market estimates have forecast significant growth in the demand for LA over the coming years due to its applicability in multiple industries and the role it plays in the production of PLA (Alves de Oliveira et al., 2018; Biddy et al., 2016; Castro-Aguirre et al., 2016; Nester, 2018).

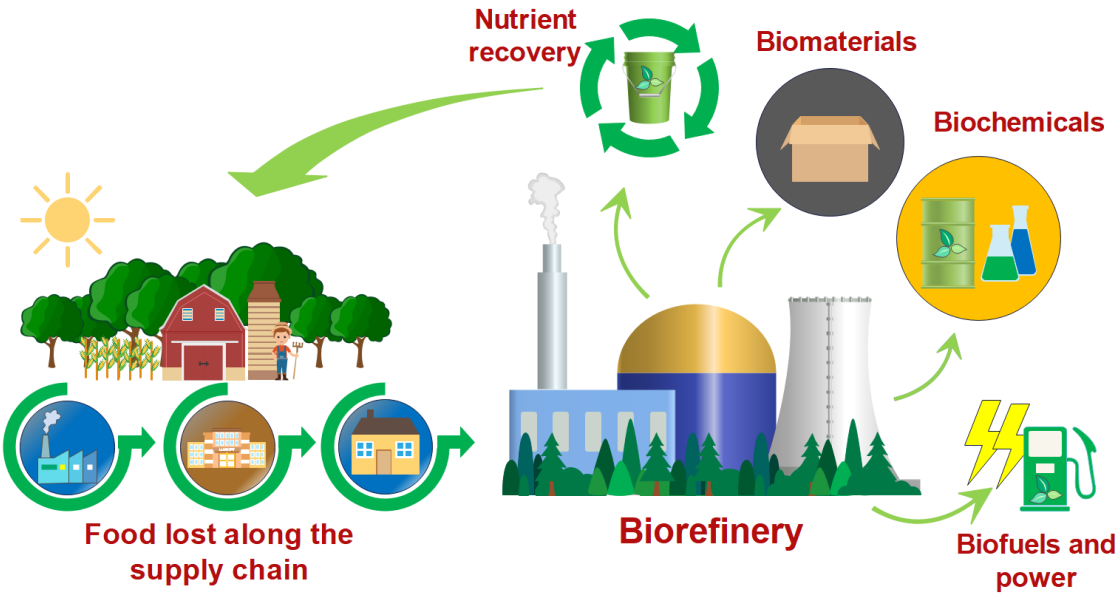


Figure 2.1: The food waste biorefinery concept utilising food waste produced throughout the supply chain for the production of valuable bio-products (Based on Mongkhonsiri et al. (2020)).

Recent key literature has explored the integration of LA production within an AD facility design, aiming to generate an advanced FW biorefinery (Bastidas-Oyanedel & Schmidt, 2018; Demichelis et al., 2017; Kim et al., 2016). The two processes can be closely integrated to synergistically improve their performance. For example, AD can provide essential power and heat required to meet the energy needs of LA fermentation and downstream separation and recovery, while the residues from LA recovery can be utilised within AD for biogas production (Figure 2.2). Furthermore, LA fermentation can improve the economic performance of the AD technology, possibly even eliminating the need for subsidy support (Bastidas-Oyanedel & Schmidt, 2018). However, challenges still exist for the development of future LA-AD biorefineries, including those related to LA fermentation of wastes and its integration into AD. With this in mind, the following

review explores the potential of integrating the LA fermentation and recovery into AD for improved profitability through an increased high value revenue stream. The FW AD context is briefly introduced from an industrial process perspective. The current status of LA fermentation and production is then explored along with an examination of current key challenges. Finally, important research prospects are outlined to identify critical areas that require investigation, which led to the research objectives addressed by the thesis investigations.

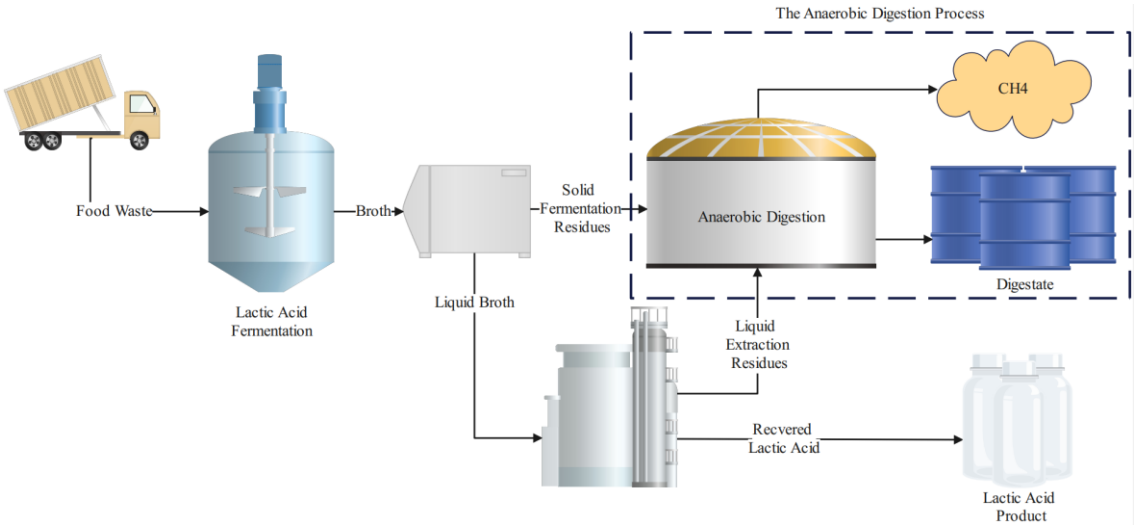


Figure 2.2: Example of the integrated food waste LA-AD biorefinery

2.2 ANAEROBIC DIGESTION

AD is a complex multi-stage biological process which sequentially breaks down organic material to its basic components (e.g. CO_2 and CH_4). A variety of high value metabolites are generated during this process which, if recovered, could boost the economic feasibility of AD technology. AD has a long research history related to industrial applications, dating as far back as the 17th century when Van Helmont noted that decaying organic material produced flammable gases (Abbasi et al., 2012). Following over 300 years of development, and with the advancement of modern technology (including process control) it is possible to utilise the AD for organic waste management (including food waste) at large scale (Edwards et al., 2015). AD harnesses naturally occurring biological processes occurring in the absence of oxygen (i.e. anaerobic), which can be classified into four fundamental progressive stages, namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each decomposing organic waste (such as FW) into progressively simpler metabolites (Melville et al., 2014; Sawatdeenarunat et al., 2015). If AD is allowed to progress to completion, the ultimate product of digestion is biogas, a

mixture of mostly CO_2 and methane. Modern AD projects are generally developed to utilise one of two design modes (Fig. 2.3) 1) Single-stage designs conduct all the above biological processes within a single vessel being fed with organic waste and producing biogas, and 2) two-stage designs aiming to split (stage 1) hydrolysis and acidogenesis from (stage 2) acetogenesis and methanogenesis, by incorporating a pre-fermentation vessel for stage 1 with a short hydraulic retention time, prior to a much larger digester with much longer hydraulic retention time for the stage 2 digestion. Several modern AD facilities incorporate two-stage designs which allow stage 1 biological processes to be separately optimised from the stage 2 biological processes, yielding improved performance and stability (Aslanzadeh et al., 2014; Nasr et al., 2012; Schievano et al., 2014).

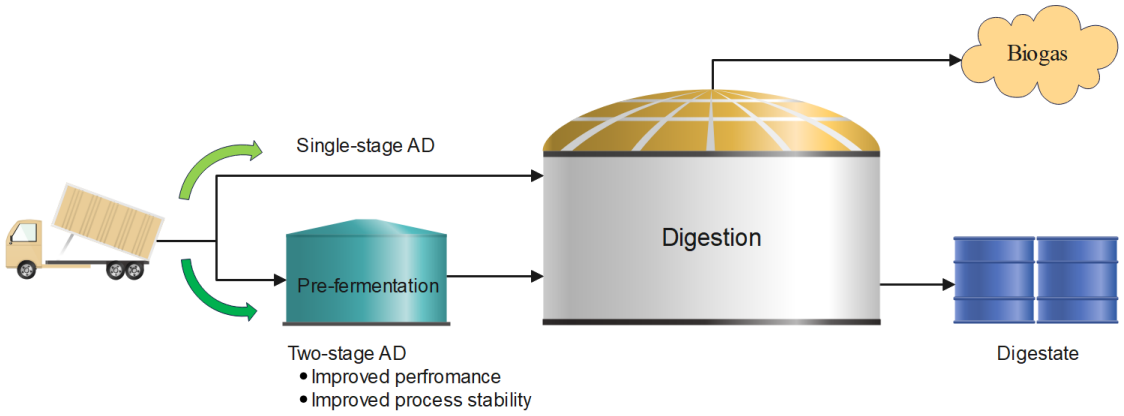


Figure 2.3: Comparison of single- and two-stage AD designs.

2.2.1 Current status, challenges and opportunities for AD

The development of new AD projects within developed and developing countries vary with local waste feedstock availability and the needs for biogas energy. Small-scale AD units are the most common in developing countries such as Bangladesh, India, and Nepal, with the biogas primarily utilised for cooking purposes (Zaman & Reynolds, 2015). The residual solids following digestion are typically used for the fertilisation of crop soil and as fish feed in aquaculture (Sawatdeenarunat et al., 2016). In contrast, developed countries utilise AD for processing large volumes of organic waste while utilising the biogas for the production of power or, increasingly, compressed biomethane (Comparetti et al., 2013; Zhao et al., 2010).

A number of significant challenges complicate development of commercial AD projects within developed countries, such as authorisation to connect to the electrical grid,

feedstock type and availability, physical construction, plant reliability, and the removal, management and costs of the liquid by-product, digestate (Insight, 2016). These challenges are primarily related to technical feasibility or acquiring approval from approvals bodies. However, as a single digester can cost up to US$ 600 per annual ton of capacity (RWI, 2013), financing can also be a considerable challenge. Moreover, digestate can also be a major cost burden to a commercial AD facility (Section 2.2.1.1). Consequently, the success of commercial AD projects has heavily relied on subsidies (Gebrezgabher et al., 2010), limiting uptake of AD technology to date, because benefits and support provided by governmental policies can differ both internationally and nationally. For example, Europe is currently the pioneer of AD technology with 18,943 biogas plants in operation by the end of 2019 (EBA, 2021). However, the extensive implementation of AD around Europe is heavily driven by the financial subsidy policies provided by European governments to renewable energy producers and environmental regulations being strict but well developed, providing certainty to new projects (Linville et al., 2015; Vasco-Correa et al., 2018). In contrast, while the US has introduced the Renewable Portfolio Standard to provide financial incentives for AD, the subsidies provided are relatively poor, favour wind and solar over biomass-based technologies such as AD, and also compete with existing subsidies for fossil-fuel energy sources (Edwards et al., 2015). Consequently, due to the limited financial support from governments, the financial viability of AD has been brought into question in the US, and as a result the adoption of AD technology has been significantly slower, with approximately 2,200 AD plants currently in operation (Simet & Fletcher, 2017).

Australia is an example of virtually no uptake of the AD technology, having 242 known AD projects in operation, predominantly consisting of municipal sewage sludge digesters and landfill gas facilities (Tait et al., 2021). While government programs have aided the development of some AD projects for FW and abattoir waste processing (ReNu, 2017), the lack of incentives within Australia to promote the development of the technology limit its widespread adaption. However, several policies do aim to support the development of new AD installations such as the Emission Reduction Fund (ERF) and the Renewable Energy Target (RET) scheme which aim to provide an additional revenue stream for facilities by generating and selling Australia Carbon Credit Units, and Small-Scale Technology Certificates (STC) or Large-Scale Generation Certificates (LGC), depending on the size of the AD installation (STC<100kW<LGC) (Carlu et al., 2019).

However, several barriers still harm the development of AD in Australia. ENEA, in collaboration with various Australian energy agencies and the Australian government, outlined several regulatory deficiencies which currently hinder the development of AD within Australia including; the absence of any national target for biogas production, financial uncertainties around power exports, and lack of uniform landfill levies among states (Carlu et al., 2019) which creates revenue uncertainty for new and existing AD projects.

2.2.1.1 Digestate

The liquid by-product produced from AD is commonly referred to as 'digestate' and, depending on the process and feedstock, can contain particulates in the wide range of 3.5-13% (Plana & Noche, 2016). Generally, this solid fraction is composed of undigested material, such as lignin, while the liquid fraction contains mobile nutrients such as available nitrogen in the form of NH_4-N (approx. 1000-5000 $ppm_{NH4\text{-}N}$ depending on the feedstock composition (Akhiar, 2017; Coelho et al., 2018; Häfner et al., 2022; Teglia et al., 2011). Depending on the substrate composition, hydraulic retention time of the AD process, and process design (e.g. single- or two-stage), the characterisation of the solid and liquid fractions will vary.

While there are potential uses for AD digestate (i.e. for soil amendment or displacing synthetic fertilisers), digestate is generally seen as a cost-burden due to its bulky nature, dilute nutrient content (as compared to chemical fertilisers), and costs associated with its storage, transport, and land application (Turnley et al., 2016). Digestate can be used for soil amendment, however, the relatively low ammonium concentrations (as compared to synthetic fertilisers) results in a relatively low fertiliser value, leading to the need to handle high volumes for land application, and the presence of chemical and biological contaminants may exacerbate risk associated with its utilisation (Nkoa, 2014; Vaneeckhaute et al., 2017). Standards, such as British standard "PAS110:2014", which is seen as the baseline quality specification for digestate, aim to ensure digestate is safe and reliable for land application (WRAP, 2017). Even so, the use of digestate within agriculture has shown its benefit, improving plant growth (Mickan et al., 2022) and producing crop yields similar to urea following soil injection (Riva et al., 2016; Zilio et al., 2021). Furthermore, researchers have explored alternative uses for digestate, such as growing microalgae (Ayre et al., 2017) and producing bioethanol (Sambusiti et al., 2016),

with some researchers showing digestate could be a promising nutrient supplement and/or process water resource within the biotechnology industry to aid fermentation processes (Ujor et al., 2020; Wang et al., 2021; Zhang et al., 2019).

2.2.2 Lactic acid as a potential by-product from anaerobic digestion

LA is an intermediate produced during the pre-fermentation stage of two-stage AD and has been the focus of a few recent studies (Bastidas-Oyanedel & Schmidt, 2018; Chenebault et al., 2022; Demichelis et al., 2018; Kim et al., 2016). LA ($C_3H_6O_3$) is a three-carbon organic acid which has two primary commercial production methods, 1) chemical synthesis from petrochemicals, and 2) via microbial fermentation (Figure 2.44). The chemical synthesis of LA is expensive and produces a mixture of D-LA and L-LA (the two stereoisomers of LA), while for many applications only L-LA is desired (Komesu et al., 2017c; Papagianni, 2012). On the other hand, the production of LA via biological routes can overcome these high costs while also reducing the cost of the raw material, product impurities, and dependence on other industries (Pal et al., 2009). Consequently, 90 % of LA production at commercial scale is conducted via the biological route (Lee, 2015).

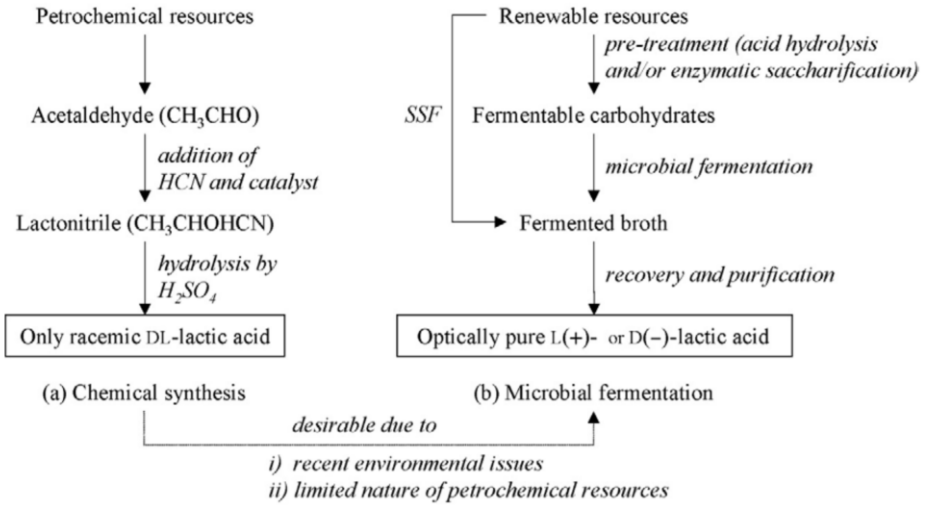


Figure 2.4: Chemical and biological synthesis of lactic acid (Adopted from (Wee et al., 2006))

LA is generally sold as an 88 wt% solution and recent predictions suggest there will be significant growth in the LA market to 1,960 kton by 2024/2025 with a market value of USD 9.8 billion (Alves de Oliveira et al., 2018; Presswire, 2018). The price of LA generally follows the price of starch and sugar feedstocks, and recently has reached around 3.0-4.0 $USD \cdot kg_{88wt\%\ LA}^{-1}$ (Alves de Oliveira et al., 2018). It is expected that PLA (marketed as a compostable bioplastic) will play a significant role in the expansion of the

LA market with forecasts estimating by 2025, 50% of all globally produced LA will be utilised for PLA production (Carcus, 2012; Djukić-Vuković et al., 2019), likely somewhat driven by the positive public sentiment of compostable plastics. The rapid growth of the LA market, high value of LA, and results of recent literature (Demichelis et al., 2017; Kim et al., 2016) suggest the development of the LA-AD biorefinery is a promising and potentially highly profitable method to improve the economic performance of AD.

LA is primarily produced through fermentation with lactic acid bacteria (LAB) (Juturu & Wu, 2016; Karp et al., 2011; Mazzoli et al., 2014) utilising feedstocks consisting of pure sugars and nutrient-rich supplements (Abdel-Rahman et al., 2013; Hofvendahl & Hahn–Hägerdal, 2000). However, as LA is a bulk chemical, the cost of the substrates heavily influences its price (Reddy et al., 2016), resulting in the current commercial method inflating the LA price and limiting its application for PLA production (Van Wouwe et al., 2016). Alternatively, waste streams have the potential to reduce LA production expenses due to low feedstock costs (Kwan et al., 2018; Novik et al., 2017), or provide a potential additional revenue source if gate-fees can be charged. Furthermore, some waste streams, such as FW, contain nutrients that are essential for the growth of LAB and production of LA (Kim et al., 2003a), reducing or potentially eliminating the need for nutrient supplements.

A large body of literature exists exploring LA fermentation and several reviews summarising the literature have been produced (Alves de Oliveira et al., 2018; Eş et al., 2018; Ghaffar et al., 2014). Fundamental research utilising waste streams for LA fermentation (Table 2.1) has identified that LA accumulation is promoted at low pH and short hydraulic retention time (HRT) with the separation of hydrolysis and fermentation leading to further improved productivity and yield (Demichelis et al., 2017). However, conditions that promote LA production are generally avoided within AD, likely due to its much lower pKa (~3.8) compared to other organic acids (acetic (4.76), propionic (4.87) and butyric acids (4.82)), and the inhibitory effects LA, and its degradation product (propionic acid), have on methanogenesis (Bo et al., 2007; Gu et al., 2018; Tang et al., 2016). Consequently, as research generally aims to optimise biogas production (Khan et al., 2016), it is typically identified as a metabolic by-product that needs to be avoided.

Table 2.1: Summary of key research achievements from some researchers regarding the production of LA from fermentation in two-stage AD

Feed	Operation mode	Aim	Key Findings	Reference
Simulated FW	Batch and semi-continuous LBR[a]	Reduce LA production from FW fermentation	• Acidic conditions promote (3.5-4.5) *Lactobacillus* (A key microorganism in LA production) • Short HRTs promote high LA concentrations • Batch fermentation promoted LA accumulation • Organic acid production is promoted at slightly acidic pH (5-6)	(Gu et al., 2018)
Simulated FVW[b]	Continuous	Operate the fermenter at pH 4.0 for LA production for its subsequent utilisation for methane production	• Low pH (4.0) promoted *Lactobacillus* • Low hydrogen partial pressure promoted LA degradation	(Wu et al., 2016)
FW	Semi-continuous	Co-produce LA and Biogas	• LA is predominantly produced at higher temperatures (>50^{o}C) • Short HRTs (~1 day) promote LA production • 47 kg of LA and 54 m^3 can be produced which has more than twice the economic value than that of conventional AD	(Kim et al., 2016)
KW[c]	Semi-continuous	Evaluate the influence of LA on methanogenesis	• LA is the main KW fermentation product • Propionic acid concentration in the methanogenic effluent is nearly linearly proportional to rate of LA loaded into the methane reactor • LA in the methanogenic influent should be avoided to improve two-phase AD performance	(Bo et al., 2007)
Maize silage	Semi-continuous LBR for fermentation, CSTR for digestion	Examine the interrelationship between microbial communities and process parameters	• Fermentation alternates between LA production and gas production (hydrogen) • Alternating periods led to large changes in the microbial community structure • The biogas composition followed the metabolic dynamics of the fermentation phase • Stabilisation of the fermentation phase can lead to enhanced productivity for chemical and bioenergy production • LA played a key role in the presence of instabilities • Microbial chain elongation mechanisms may have played a role in fermentation instability	(Sträuber et al., 2016)
FW	-	Increase LA and Biogas production by	• LA productivity and yield improved when separating hydrolysis and fermentation	(Demichelis et al., 2017)

Feed	Operation mode	Aim	Key Findings	Reference
		separating enzymatic hydrolysis and fermentation	• Biogas yield improved by separating hydrolysis and fermentation	
FW	Batch	Investigate the effects of organic loading rate, pH, and temperature on LA production	• Hydrolysis rate increased with pH • FW serves as an inoculum for LA fermentation • High temperatures favoured hydrolysis but reduced the rate of acidification • High OLR[d] leads to systems instability, low LA yield, low VS destruction	(Tang et al., 2016)
FW	Simulation	A techno-economic evaluation of different scenarios for LA and biogas co-production	• Conversion of FW into LA and biogas is economically feasible • Integrated biorefinery was more cost effective than sole production of either LA or biogas • Integrated biorefinery reduced waste generated, AD digester volume, minimised energy demand, and enhanced production of valuable products	(Demichelis et al., 2018)

a: Leach Bed Reactor, b: Fruit and Vegetable Waste, c: Kitchen Waste, d: Organic Loading Rate.

While the conventional approach is to optimise AD for biogas production, recent literature has aimed to recover LA following fermentation (i.e. from the first-stage reactor) in two-stage AD processes. For example, Kim et al. (2016) recovered LA following FW fermentation and reported the centrifuged post experiment residues were a suitable feed for methane production. This process was found to increase the value of AD from 27 $USD \cdot ton_{FW}^{-1}$ to 60 $USD \cdot ton_{FW}^{-1}$ (Kim et al., 2016). A similar study by Demichelis et al. (2017) assessed the potential for increasing the yield of LA and biogas by separating enzymatic hydrolysis and subsequent fermentation. This approach significantly improved the yield of both LA and biogas from FW, 0.29 -> 0.33 $g_{LA} \cdot g_{dryFW}^{-1}$ and 0.73 -> 0.9 $Nm^3 \cdot kg_{FW}^{-1}$, for LA and biogas, respectively (Demichelis et al., 2017). By applying the method outlined by Kim et al. (2016) for the economic evaluation of a combined LA and AD process (and assuming similar LA separation efficiency) to the data reported by Demichelis et al. (2017) (for simultaneous saccharification and fermentation only), the co-production of LA and biogas from FW increased the value of the AD process from 15 $USD \cdot ton_{FW}^{-1}$ to 42 $USD \cdot ton_{FW}^{-1}$. Therefore, both Kim et al. (2016) and Demichelis et al. (2017) present promising results and suggest there is potential for increasing the value of FW by 120-180% by implementing LA recovery.

2.3 THE LA-AD BIOREFINERY

The LA-AD biorefinery concept has shown strong baseline potential to revolutionise the techno-economic feasibility of FW AD facilities, converting the generally low-value process into a high-value biorefinery. However, various factors are important in establishing techno-economically feasible LA-AD biorefinery concepts. These include:

1. the biorefinery feedstock and its impact on LA fermentation and the presence of contaminants that influence downstream processing;
2. pre-treatment and its influence on the LA yield and production rate;
3. LA fermentation and metabolic pathways utilised for LA production which influence selectivity, yield, and optical purity;
4. downstream separation techniques and the capability for LA to be recovered from highly complex fermentation media, and its influence on downstream methane production, and
5. the role AD will play within the biorefinery, especially for power and heat production for LA fermentation and downstream separation and recovery, and as a method for disposal of fermentation and separation residues,

all of which will impact the performance and profitability of the biorefinery concept. Therefore, the literature context for these factors is explored in the following sections.

2.3.1 Biorefinery feedstock

The price and quality of the organic feedstock utilised for the manufacture of chemicals and fuels is fundamental to the success of a biorefinery. However, it is impossible to develop a universal rule for the choice of feedstock as availability and the type of biomass available is highly dependent on location, climate, season, socioeconomic issues, and government policies (Ghatak, 2011). Ideally, the feedstocks selected should be sustainable and have a reliable supply over the lifetime of the biorefinery (20-30+ years; (Stephen et al., 2010)), and optimally the feedstocks should aim to not displace agricultural production for food, fibre and feed. Unfortunately, the volume of available biomass is also dependent on a variety of parameters such as weather and global primary markets. Feedstock supply uncertainty can be managed by incorporating mixed feedstock design, allowing the biorefinery to receive a variety of wastes which enable it to cope with supply uncertainties over the plant lifetime.

Carbohydrates are the primary substrate for LA fermentation with simple sugars such as glucose and sucrose obtained from corn, sugarcane, and cassava commonly used substrates (Olszewska-Widdrat et al., 2020). However, while high purity sugars produce the purest LA, their use inflate fermentation production costs and disturb the food supply chain as food resources are diverted for fermentation (Hofvendahl & Hahn–Hägerdal, 2000; Mazzoli et al., 2014; Singhvi et al., 2018; Vijayakumar et al., 2008). In contrast, waste streams could significantly reduce costs associated with fermentation and eliminates competition with food industries. It is important to note however that, due to their complex composition, waste streams may introduce impurities during fermentation (Yang et al., 2013).

General guidelines can be used to pre-screen a variety of potential waste feedstocks for LA fermentation, for example, the biomass should have; 1) low cost or a competitive gate-fee, 2) low levels of contaminants, 3) high rate of production, 4) high LA yield, 5) little to no by-product formation, 6) little to no pre-treatment requirement, and 7) year round availability (Vijayakumar et al., 2008). Lignocellulose has a high carbohydrate content (55-65% (Zeng et al., 2013)) and has been shown to yield significant LA (Oonkhanond et al., 2017; Zou et al., 2021). However, these carbohydrates (cellulose and hemicellulose) are wrapped in a protective polymer (lignin) that is resistant to biological degradation, and pre-treatment is often required to improve the biodegradability of the material (Sawatdeenarunat et al., 2015). Furthermore, fermentation of lignocellulose requires the supplementation of complex nutrients, as nutrients required for cell growth, such as nitrogen, are often limited (Kjersi, 2012). Yeast extract, peptone, meat extract, corn steep liquor, and malt extract are commonly utilised as nutrient supplements (Wang et al., 2015; Yang et al., 2013), but can be expensive and will increase production costs. Alternatively, co-fermenting wastes may provide an avenue for nutrient deficiencies to be managed, similar to co-digestion for AD (Lv et al., 2021).

FW is a highly promising substrate for LA fermentation, containing many of the essential nutrients and carbon sources required for LA production (Kim et al., 2016; Pleissner et al., 2017; Tang et al., 2016). Additionally, many food products are enriched with LAB, including milk, meats, cereals, and vegetables, making it both an effective substrate and an inoculum source (Kim et al., 2016). The addition of nitrogen rich sources, such as yeast extract, and addition of nutrients, such as potassium, manganese, and ammonium,

has been shown to improve fermentation of FWs (Kim et al., 2003a; Ohkouchi & Inoue, 2006; Zhang et al., 2020b) indicating nutrient deficiencies are still present. However, studies have shown FW to LA is technically feasible without nutrient supplementation (Kim et al., 2016; Kwan et al., 2016; Pleissner et al., 2017; Tang et al., 2016).

2.3.2 Pre-treatment

Pre-treatment processes are primarily designed to improve hydrolysis of complex biomass (Yang et al., 2013). Many pre-treatment methods are available including extrusion, alkali pre-treatment, organosolv, and ammonia fibre expansion (AFEX) (Table 2.2). As the composition of wastes vary significantly, there can be no universal pre-treatment method for every feedstock. However, it has been suggested that the selected pre-treatment method should aim to, 1) avoid the need for size reduction of biomass particles, 2) preserve the hemicellulose fraction for lignocellulosic biomass, 3) reduce/remove inhibitory components and minimise their formation, 4) improve accessibility to difficult components within the biomass, 5) minimise power consumption, 6) improve the properties of the biomass surface for improved microbial interactions, 7) improve the hydrolysis rate of lipids and proteins, and 8) utilise a low cost catalyst/method for recycling of the catalyst and regeneration of lignin for co-product production (for lignocellulose) (Kumar & Sharma, 2017; Parthiba Karthikeyan et al., 2018). The majority of pre-treatment methods aim to improve the biodegradability of agricultural residues (Table 2.2) due to the presence of lignin, which is a major barrier to the enzymatic saccharification (Xu et al., 2016). However, most of the available pre-treatment methods are yet to be commercialised due to the high cost of biomass pre-treatment, and many don’t meet the requirements for commercial application (Xu et al., 2016).

Table 2.2: Summary of some of pre-treatment methods for the treatment of various feedstocks (Modified from (Capolupo & Faraco, 2016; Menon & Rao, 2012))

Pre-treatment Method	Feed stock	Sugar yield	Advantages	Disadvantages	Commercial standing
Acidic	• Corn stover, spruce, polar, and switchgrass	-	• Hydrolyses hemicellulose and cellulose and alters the lignin structure	• Hazardous, toxic, and corrosive chemicals used • High cost • Gypsum formation during neutralisation • Inhibitory by-product formation	Commercially implemented. Generally dilute sulphuric acid is utilised (Menon & Rao, 2012)
Alkali	• Lignocellulosic biomass (Corn stover, bagasse, wheat straw, rice straw, and switchgrass)	-	• Removes lignin and hemicellulose. Increases the accessible surface area	• Long residence time and irrecoverable salts produced	-[c]
Liquid hot water	• Agricultural residues (sugarcane bagasse, corn stover, wheat straw, and sunflower stalks) • MSW	80-94% reducing sugars	• Removal of cellulose making enzymes • Recovery of almost pure hemicellulose • No catalyst or other chemicals • Hydrolysis of hemicellulose • No need for size reduction • High sugar recovery • Low formation of inhibitors	• Long residence time, lower removal of lignin • High energy demand • Remaining solids will need to be processed	Demonstration plant (Zheng & Rehmann, 2014)
Organosolv	• Agricultural residues (Wheat straw, sugarcane bagasse)	Up to 60% of reducing sugars	• Hydrolysis of lignin and hemicellulose • Pure lignin removal as by-product	• A condenser is required for solvent recovery • Costly process	-[c]
Ozonolysis	• Agricultural residues (wheat straw, bagasse, and peanut and poplar sawdust)	45-90% reducing sugars	• Reduces lignin content while not producing toxic residues • Moderate reaction conditions • Efficient lignin degradation	• Large quantities of ozone are needed • Costly process	-[c]
CO_2 Explosion	• Agricultural residues (Wheat straw, sugarcane bagasse)	Up to 90% of reducing sugars	• Hemicellulose removal, cellulose decrystallization, cost-effective • Increases accessible surface area • Does not imply toxic chemical generation	• Does not modify the lignin structure • Costly process • Very high pressures required	-[c]

Pre-treatment Method	Feed stock	Sugar yield	Advantages	Disadvantages	Commercial standing
Steam Explosion	• Agricultural residues (corn stalk, wheat straw, and sugarcane) • MSW • Hardwood • Forest residues	50-70% reducing sugars	• Hemicellulose removal and alters the lignin structure • Good sugar recovery • Low cost • Less hazardous process	• Incomplete destruction of the lignin-carbohydrate complex • Partial hemicellulose degradation • Generation of inhibitor compounds	Demonstrated at commercial scale at the Masonite plants (Menon & Rao, 2012) Commercialised for ethanol production (Zheng & Rehmann, 2014)
AFEX[a]	• Agricultural residues (wheat straw, corn stover, bagasse, and rice straw) • MSW	Up to 80-90% of reducing sugars	• Removes lignin and hemicellulose • Low formation of inhibitor compounds • Moderate process conditions	• Not efficient for biomass with a high lignin content • High-cost process	-[c]
Ionic Liquids	• Agricultural residues (Wheat straw, sugarcane bagasse, peanut and poplar sawdust, and corn stover)	60-85% reducing sugars	• Dissolution of cellulose and increases the amenability to cellulase	• In early stages of development • Chemicals are expensive • Solutions are viscous and difficult to handle	-[c]
Biological	• Agricultural residues (Wheat straw, rice straw) • Soft wood	20-50% reducing sugars	• Low energy input • Moderate reactor conditions • No catalyst or chemical additives • Do not imply toxic chemical generation • Low cost	• Low hydrolysis rate • Large area required	Unlikely to commercialised (Menon & Rao, 2012)
Extrusion	• Agricultural residues (Rice straw, wheat straw, and corn stover)	50-75% reducing sugars	• Moderate temperatures • Good sugar yields • High flexibility for many process modifications • Less hazardous process	• Partial hemicellulose degradation • Generation of inhibitor compounds • Incomplete destruction of lignin-carbohydrate matrix	Systems are already commercially available (Capolupo & Faraco, 2016) Commercialised counter current extrusion reactor (Zheng & Rehmann, 2014)

a: Ammonia Fibre Explosion/Expansion, b: the method can be applied to different feed stocks, c: Commercial implementation not found

In any case, as LAB struggle to ferment substrates composing of complex polymers, pre-treatment processes have shown their benefit, for example, with pulp mill residue (de Oliveira Moraes et al., 2016), corn stover (Ahring et al., 2016), and sugarcane bagasse (Wischral et al., 2019). Though FW is inherently relatively biodegradable, having been shown to produce substantial LA from direct fermentation (e.g. 0.46 $gLA \cdot gTS^{-1}$ (Tang et al., 2016), 0.18 $gLA \cdot gTS^{-1}$ (high TS content) (Yousuf et al., 2018), and 0.42 $gLA \cdot gVS^{-1}$ (Wang et al., 2021)), application of pre-treatment methods to FW have been shown to benefit LA fermentation by improving the final LA yield (Ahmad et al., 2020; Demichelis et al., 2017; Kim et al., 2003a; Kwan et al., 2016; Pleissner et al., 2016).

2.3.3 Lactic acid fermentation

Many LA producing microorganisms exist, including bacteria, filamentous fungi, and yeast. LAB are generally utilised as they are 1) Generally Regarded As Safe (GRAS), with the exception of some pathogenic streptococci strains, 2) robust organisms already adapted to the stress of industrial processes, 3) are capable of metabolising numerous mono- and di-saccharides, 4) fast growing, and 5) produce a variety of high value metabolites (Juturu & Wu, 2016; Mazzoli et al., 2014).

Many bacteria produce LA either as a primary or secondary product, but those labelled as "LAB" are exclusively grouped in the order *Lactobacillales* which include*; Lactobacillus*, *Pediococcus*, *Carnobacterium*, *Aerococcus*, *Vagococcus*, *Enterococcus*, *Teragenococcus*, *Leuconostoc*, *Weissella*, *Streptococcus*, *Oenococcus*, and *Lactococcus* (Juturu & Wu, 2016). Bacteria outside of this group have been utilised for LA production and some are outlined in Table 2.3. Most commercialised LAB fall into the genus *Lactobacillus* as they are tolerant to acidic conditions and can be easily engineered to selectively produce LA, however, other organisms applied include *Streptococcus* and *Pediococcus* (Biddy et al., 2016; Lee, 2015).

The optimal conditions for LA production vary depending on the microorganism utilised as LAB, as these can grow in the pH range of 3.5-10 and temperature range of 5-45 ^{o}C (Abdel-Rahman et al., 2013) and at even higher temperatures in some cases (Table 2.3). However, most LA fermentation processes are conducted at a pH ranging from 5.0-6.0 and at temperatures between 35-40 ^{o}C (Table 2.3). For continuous processes, short hydraulic retention times (HRTs) are generally preferred as they tend to promote LA

accumulation (Komemoto et al., 2009; Tang et al., 2016). However, a universal value for the optimal HRT is difficult to determine as it varies depending on various factors including fermenter design, waste composition, and other operational parameters (Gu et al., 2018; Kim et al., 2003b; Tang et al., 2016). Similarly, organic loading rates (OLRs) utilised for LA production vary (Kim et al., 2012; Luongo et al., 2019; Tang et al., 2016), but high OLRs generally favour the production of LA and assist in reducing the conversion of LA to other organic acids (Bo & Pin-jing, 2014; Luongo et al., 2019).

The presence of a chiral carbon in LA can complicate fermentation as different bacteria can produce L- and D-LA (the two isomers of LA) in varying quantities depending on their production of L-lactate dehydrogenase (L-LDH) or D-LDH (Eiteman & Ramalingam, 2015; Garvie, 1980; Liu, 2003). A mixture of L- and D-LA can be troublesome as the optical purity (OP; ratio of L-LA to the total LA present) affects its applicability, particularly if it's to be used for the synthesis of PLA (OP >98%) (Gandolfi et al., 2015) as several properties are impacted by the relative quantities of L- and D-LA within the polymer blend (Eiteman & Ramalingam, 2015). Furthermore, certain industries may require a specific isomeric form, such as the food and pharmaceutical industries which generally require L-LA as D-LA in high dosages can be harmful to humans (Alves de Oliveira et al., 2018). As the food industry demands the majority of the LA produced (~85%) (Ahmad et al., 2020), L-LA is generally the target for fermentation.

Studies have shown the OP can be controlled through the manipulation of environmental conditions (Gu et al., 2014; Zhang et al., 2017), supplementation of nutrients (Zhang et al., 2020b), co-fermentation (Ma et al., 2021), or utilisation of specific LAB strains (Alexandri et al., 2019; Yuan et al., 2018). However, the response of OP with changes in fermentation conditions is not consistent between studies and may be related to the differing microbial communities which form during mixed-culture fermentation, or with different bacteria utilised. Furthermore, alternate pathways for LA production exist, such as the methylglyoxal detoxification pathway which produces a racemic mixture of LA (Mazumdar et al., 2013). The presence of these pathways or alternate LAB could reduce the OP of the LA produced and may contribute to the inconsistent results reported by various studies.

Table 2.3: Operating Conditions used for LA fermentation by some researchers (adopted from (Alves de Oliveira et al., 2018))

Dominant LAB	Product	Substrate	Temperature (ºC)	Operation mode	pH control	LA Productivity (g L^{-1} h^{-1})	Yield (g·g^{-1})
***Sporolactobacillus*sp. CASD**	D-LA	Glucose	42	Fed-batch	5.0–6.0 CaCO3	4.4	0.84
***Bacillus* sp. WL-S20**	L-LA	Glucose	45	Fed-batch	9.0 NaOH	1.04	0.993
***Bacilus coagulans*C106**	L-LA	Xylose	50	Fed batch	6.0 Ca(OH)2	4	0.95
***Lactobacillus paracasei* 7BL (GMO)**	L-LA	Glucose	37	Fed-batch	6.0 CaCO3	1.79	0.99
		Wood chips				2.25	0.96
		Rice straw				5.27	0.97
Lactobacillus rhamnosus	L-LA	Defatted rice bran	42	Fed-batch	6.25 Ca(OH)2	2.56	0.937
***Lactococcus lactis*ATCC19435**	L-LA	Jerusalem artichoke	30	Fed-batch	6.0 NaOH	–	–
***Lactobacillus casei*G-02**	L-LA	Jerusalem artichoke	40	Fed-batch (SSF)	6.5 CaCO3	4.7	0.963
***Bacillus coagulans*XZL4**	L-LA	Hemp hurds (Glucose–xylose)	50	Batch	5.5 CaCO3	–	0.900 0.840
***Bacillus coagulans*LA204**	LA	Pretreated corncob	50	Fed- batch	6.0 NaOH	1.37	0.77
				(SSF)			
***Lactobacillus agilis*LPB 56**	L-LA	Soybean vinasse	30	Batch	6.0 Ca(OH)2	0.86	0.849
***Bacillus coagulans*LA1507**	L-LA	Sweet sorghum bagasse	50	Open-fed-batch (SSF)	5.2–6.2 Ca(OH)2	1.59	0.437
***Pediococcus acidilactici* (GMO)**	LA	Detoxified corn stover (Cellulose)	45	Batch (SSF)	5.5 NaOH	1.45	0.715
***Lactobacillus plantarum* (GMO)**	D-LA	Delignified hardwood pulp	37	Batch (SSF)	6.0 NaOH	2.29	0.879
Lactobacillus paracasei	D-LA		34	Batch (SSF)		2.08	0.69

Dominant LAB	Product	Substrate	Temperature (ºC)	Operation mode	pH control	LA Productivity (g L^{-1} h^{-1})	Yield (g·g^{-1})
Lactobacillus coryniformis	L-LA	*Curcuma longa* waste	37		6.0 NH4OH	2.7	0.65
Lactobacillus pentosus	LA	Corn stover	37	Fed-batch (SSF)	6	1.92	0.66
Bacillus coagulans**JI12**	L-LA	oil palm empty fruit bunch hydrolysate	50	Batch (SSF)	6.0 Ca(OH)2	3.4	–
Lactobacillus rhamnosus**ATCC7469**	LA	Recycled paper sludge	37	Batch (SSF)	5.5 CaCO3	2.9	0.97
***Streptococcus* sp.**	LA and biogas	FW	35	Batch	6	3.38	0.33
***Streptococcus* sp.**	LA	FW	35	Batch (SSF)	6.0 NaOH	2.16	0.81
Rhizopus oryzae	L-LA	Xylo-oligosaccharides manufacturing waste	40	Batch (SSF)	5.5 CaCO3	1	0.6
Bacillus coagulans	L-LA	Coffee mucilage	52	Batch	6.0 NaOH	4.4	0.77
Bacillus coagulans	L-LA	Coffee pulp	52	Batch	6.0 NaOH	4.02	0.78
***Lactobacillus paracasei* KM2 (GMO)**	L-LA	Whole slurry of oil palm trunk	37	Batch	6.0 NH4OH	–	0.895
Geobacillus stearothermophilus**DSM 494**	L-LA	Raw potato starch	60	Batch	7.0 NaOH	1.8	0.66
Lactobacillus delbrueckii	LA, xylitol, activated carbon and phenolic acids	Brewer's spent grains (Cellulose)	37	Batch	6.0 NaOH	0.59	0.99
Lactobacillus pentosus**DSM20314**	LA Hetero-fermentation	Wheat bran	30	Batch	6.3 NaOH	0.3	0.73
***Lactobacillus delbrueckii* NBRC 3202**	D-LA	Cassava fibrous waste	37	Batch	6.5 NaOH	0.9	0.5
Lactobacillus casei**12A**	LA	De-oiled algal biomass + glucose	37	Batch	6.5	–	–

2.3.3.1 Metabolic Pathways for lactic acid production

A number of different metabolic pathways can be utilised by bacteria for LA production, however, LAB are primarily described as utilising the glycolytic pathway (Embden–Meyerhof–Parnas pathway; Fig. 2.5), or the phosphoketolase pathway for homo- or hetero-fermentation, respectively (Wang et al., 2015). Homo-fermentative LAB produce LA as their primary end product (Papagianni, 2012; Wang et al., 2015), while hetero-fermentative LAB metabolise pentose producing equimolar amounts of LA, carbon dioxide, and ethanol or acetate (Wang et al., 2015). The specific pathway utilised by LAB is determined at the family level (Holzapfel & Wood, 2014), with crucial differences between these pathways provided by the presence of key enzymes utilised in each route; fructose 1,6-diphosphate (FDP) aldolase and phosphoketolase for the glycolytic and phosphoketolase pathways, respectively (Alves de Oliveira et al., 2018). Homo-fermentative LAB are generally preferred for LA production due to high production of LA with minimal by-products.

Some LAB resemble obligatory homo-fermentative bacteria as they possess the capability to produce FDP aldolase, however, others are also capable of synthesising phosphoketolase, allowing hexose and pentose to be utilised via the above mentioned pathways (Holzapfel & Wood, 2014; Salminen et al., 2004). These LAB are known as facultative hetero-fermentative LAB. In this case, the behaviour of these LAB (i.e. homo- or hetero-fermentation) are determined by the carbon source that's available or from certain environmental factors such as nutritional, osmotic, and/or thermal stress (Alves de Oliveira et al., 2018). LA production through the Embden–Meyerhof–Parnas pathway is preferable as LA is the only product, maximising its yield on biomass. However, overall community metabolic pathways for LA production are influenced by environmental conditions, operating parameters, and feed composition (Holzapfel & Wood, 2014; Papagianni, 2012). Consequently, it is useful to monitor metabolic pathways present to identify potential undesired shifts in the pathways.

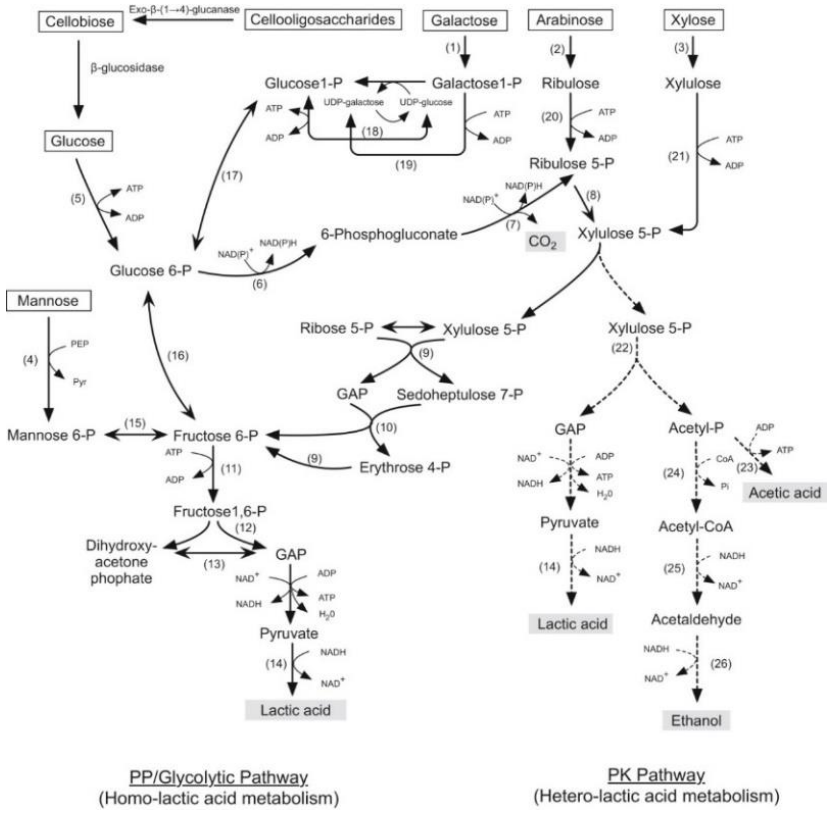


Figure 2.5: Metabolic pathways for the production of lactic acid via homo- and hetero-fermentation (Adopted from (Wang et al., 2015))

A variety of molecular methods are available for monitoring metabolic pathways during LA fermentation such as shotgun metabolomics, lipidomics, metagenomics, and predictive techniques (e.g. PICRSUt, PICRUSt2, Tax4Fun, and FaproTax). Each differs in its approach, and associated advantages and disadvantages (Table 2.4). In-silico gene inference techniques stand out compared to the other three methods as, instead of directly measuring components within sample, taxonomic compositions, which are inferred from amplicon sequencing, are used to predict microbial functional genes (Sun et al., 2020). The major advantage of in-silico techniques is its low cost compared to others which fully sequence the genome. For example, the cost of PICRUSt analyses can be 5-15 times lower than shotgun metagenomics (Mukherjee et al., 2017). However, these techniques are limited to the genomes listed in utilised databases, which are currently highly biased towards microorganisms associated with human health and associated biotechnology (Sun et al., 2020).

Table 2.4: Advantages and disadvantages of different metabolic pathway monitoring methods

Method	Techniques	Advantages	Disadvantages	References
Predictive	PICRUSt, PICRUSt2, Tax4Fun, FaproTax	• Users can customise the tool to meet the needs of their system • Predictive power will improve with time • Low cost	• Only 16S marker genes for bacteria and archaea are currently included • Quality of predictions are dependent on input data used • Biased towards human health and biotechnology	(Langille et al., 2013; Sun et al., 2020)
Metabolomics	Nuclear magnetic resonance	• Fast and highly reproducible • Minimal sample preparation • Non-destructive • Provides information on metabolite concentration and chemical structure	• Insensitivity • Magnetic fields required affect surrounding equipment • High instrument cost	(Alonso et al., 2015; Chatham & Blackband, 2001; Nagana Gowda et al., 2008; Oldiges et al., 2007; Scalbert et al., 2009)
	Mass spectrometry	• Can measure metabolites in complex bio-fluids • Can distinguish between isotopes	• Data post-processing is time intensive • Requires standards for metabolite identification • Prone to matrix effects • Can have a high cost • Requires tedious sample preparation	
Lipidomics	Raman spectroscopy	• Non-destructive • Non-invasive • Ability to obtain complex information • Obtain spectral and spatial information • No specific sample requirements	• Measurements may affect compounds within the samples • Slow • Requires advanced chemometric tools for data analysis • Had to adapt for quantitative analysis	(Jurowski et al., 2017)
	Fourier transform infrared spectroscopy	• Non-destructive • Non-invasive • Ability to obtain complex information • Obtain spectral and spatial information • No specific sample requirements	• Lower spatial resolution than Raman spectroscopy • Requires absence of water • can be slow • Requires advanced chemometric tools for data analysis	
	Nuclear magnetic resonance	• Non-destructive • large range of samples are applicable (^{31}P)[a] • Structural analysis of purified compounds (^{1}H)[a]	• Low selectivity • Sensitive to motion • High cost • Magnetic fields required affect surrounding equipment	
Shotgun Metagenomics	-	• Avoids amplification bias • Full community analysis	• High cost • Analysis of bioinformatics data is computationally intensive and complex • Provides lower taxonomic resolution than 16s rRNA data	(De Filippis et al., 2017; Langille et al., 2013; Mukherjee et al., 2017)

a: (^{31}P) Phosphorous atom is utilised, (^{1}H) hydrogen atom is utilised.

The method recommended for use in an industrial setting will primarily depend on its cost and complexity. For LA fermentation, it is expected that the microbial community will have a relatively low diversity (Kim et al., 2016; Tang et al., 2016), especially compared to communities within digesters (Wu et al., 2016). Consequently, in-silico gene inference analysis could be used as a low-cost method to monitor the metabolic activities of the biological system which could be intermittently confirmed with more complex sequencing techniques (Table 2.4) to maintain optimal LA production.

2.3.3.2 Lactic acid bacteria nutritional requirements

LAB are complex, fastidious microorganisms which require rich and complex nutrients for growth (e.g. amino acids, vitamins, carbohydrates, and minerals) with some requiring specific growth factors, such as whey, and tomato juice (Holzapfel & Wood, 2014). A variety of amino acids have been identified as essential for the growth of LAB including glutamic acid, valine, isoleucine, and leucine, which are required by nearly all LAB, while many require methionine, tryptophan, and tyrosine (Garvie, 1967; Ledesma et al., 1977). A variety of vitamins such as pantothenate, niacin and biotin and the metals, Mg^{2+}, Mn^{2+}, and Zn^{2+}, are also essential for many LAB (Holzapfel & Wood, 2014), albeit, metal ions are usually only required for enzymatic reactions (Archibald, 1986). While many nutrients have been identified as essential for many LAB, specific nutrient requirements are dependent on strain (Holzapfel & Wood, 2014).

During the fermentation of refined sugars, nutrient supplements are essential for fermentation as LAB lack many biosynthetic capabilities to synthesize nutrients for their own use (Abdel-Rahman et al., 2013; Hofvendahl & Hahn–Hägerdal, 2000). It has been hypothesised these requirements resulted from the bacteria evolving in nutrient-rich media, such as meat and milk, leading them to develop without the need for the bio-processes to synthesize these nutrients (Alves de Oliveira et al., 2018). Therefore, to make these nutrients available, yeast extract, peptone, meat extract, corn steep liquor, and malt extract are commonly utilised (Wang et al., 2015; Yang et al., 2013). As mentioned previously (Section 2.3.1), food and dairy wastes contain many of the essential nutrients required by LAB for growth and LA fermentation and may be suitable nutrient supplements for LA fermentation processes with nutrient deficiencies. Even so, literature has shown FWs may be deficient in essential nutrients, such as nitrogen for LA fermentation which limit LA production (Zhang et al., 2020a; Zhang et al., 2020b).

While literature has demonstrated the nutritional benefits of digestate within agriculture (Section 2.2.1.1), recent research has identified LA fermentation may benefit from these nutrients present within digestate. Due to the nature of the FW substrate, FW digestate naturally contains elevated ammonium concentrations (Banks et al., 2011; Buhlmann et al., 2018; Serna-Maza et al., 2015), which may be suitable for LA fermentation. Limited available research has shown the benefits of digestate on FW fermentation, improving pH stability, increasing microbial diversity, and maintaining a low oxidation reduction potential (ORP) (Wang et al., 2021), and has even been shown to be suitable as process water following pre-treatment (Zhang et al., 2019). Implementing partial digestate recirculation to LA fermentation within an LA-AD may be an effective way to promote LA production and provide an alternative use for the low-value digestate. However, additional research is required to understand the impact digestate may have on LA fermentation, the product spectrum, microbial community, and degradation pathways for LA production.

2.3.4 Challenges facing bacterial lactic acid fermentation of complex feedstocks

Although approximately 90% of the worldwide LA production is achieved through fermentation (Karp et al., 2011), the high cost of nutrient supplements and feedstocks inflate the price of the LA produced (Section 2.3.1). Waste streams can lower feedstock costs, but the fermentation of mixed sugars and introduction of various impurities creates additional challenges for LA production. Key challenges with fermentation of wastes streams to LA which need to be addressed by future LA-AD biorefineries include; substrate availability and cost, pre-treatment costs, carbon catabolite repression (CCR), microbial contamination, and low LA yield and productivity (Abdel-Rahman & Sonomoto, 2016; Hassan et al., 2019).

AD is a versatile technology, and can process a variety of wastes including FW, cardboard, grease trap residues, and fat oils (Edwards et al., 2015). The capacity for AD to receive various waste streams provides a buffer from feedstock supply uncertainties and seasonal changes in waste availability. AD facilities likely operate with some variability in feed composition and rate of feedstock receival as availabilities change with location, surrounding industries, climate, season, population density and socio-demographics, and government policies such as landfill waste diversion (Ghatak, 2011). However, there is no literature exploring the effects of dynamic feed compositions,

consistent with the operation of a commercial AD facility, on LA fermentation performance. Instead, literature exploring LA fermentation utilises a homogenous feedstock to study the effect of varying specific variables, such as process conditions (Feng et al., 2018; Tang et al., 2016; Tashiro et al., 2016) and nutrient supplements (Ye et al., 2018; Zhang et al., 2020a; Zhang et al., 2020b). As the LA yield would logically vary with changing feedstock compositions, future studies should explore the impact of varying waste compositions and identify methods to limit fluctuations in fermentation performance.

While stability in the feedstock composition may impact LA production, the maximum yield achieved and feedstock cost heavily influence the economics of fermentation (Manandhar & Shah, 2020). Even though waste streams can reduce operational costs associated with obtaining feedstocks, yields are still a challenge. Fermentation of waste to LA is primarily limited by the available carbohydrate fraction within the substrate. While carbohydrates make up a significant fraction of FW (Demichelis et al., 2017), LAB struggle to fully utilise the substrate. Pre-treatment of the FW via enzymatic, fungal, acidic, or alkali pre-treatments can effectively improve the LA yield on FWs (Ahmad et al., 2020; Demichelis et al., 2017; Kim et al., 2003a; Kwan et al., 2016; Pleissner et al., 2016), but are costly and generally produce large quantities of solid and liquid wastes which require further treatment prior to disposal (Surendra et al., 2015).

Some wastes, such as lignocellulose (following pre-treatment), can release mixed sugars which may lead to CCR (Abdel-Rahman & Sonomoto, 2016). The presence of a variety of different carbon sources may complicate fermentation as some bacteria may limit the utilisation of secondary carbon sources when a primary source is present (i.e. CCR), which is a problem for most microbial producers (Abdel-Rahman & Sonomoto, 2016; Görke & Stülke, 2008). For example, *Escherichia coli* prefer glucose over lactose as a carbon source while *Streptococcus thermophiles* prefer lactose over glucose (Brückner & Titgemeyer, 2002). These bacteria will metabolise their preferred substrate before utilising the secondary source. Mixed sugar fermentation could result in increased costs associated with separation and purification stages (Wang et al., 2015) due to lower LA yields and increased by-product formation. This behaviour can be problematic when fermenting substrates containing various carbon sources as certain sugars may require the utilisation of hetero-fermentative pathways for LA production (Figure 2.5).

Microbial contamination is also a significant risk to microbial LA production but is not widely explored for LA fermentation. AD facilities receive and utilise a variety of waste feed stocks which are primarily unsterilised. Though this does not pose a problem for AD, it can be a significant risk to the economics of LA fermentation. Many fermentation systems with optimised process conditions (e.g. pH, temperature, HRT, and OLR) tend to selectively promote LAB and LA production (Kim et al., 2016; Tang et al., 2016). However, FWs contain a variety of alternate bacteria, and are naturally enriched with many LAB (Kim et al., 2016), which may compete with target strains for substrate, reducing LA yields and selectivity. Pasteurisation is generally applied to eliminate the risk of microbial contamination, but it is costly, and many studies aim to utilise FW without pasteurisation (Feng et al., 2018; Kim et al., 2016; Tang et al., 2016; Zhang et al., 2020a; Zhang et al., 2020b). An LA-AD biorefinery could hold a distinct advantage herein this regard, producing large quantities of waste heat generated from biogas combustion which could be utilised for upstream pasteurisation. Future LA-AD biorefineries will likely utilise pasteurisation or finely tuned operational conditions to selectively promote the growth of target strains to ensure consistent LA production from fermentation.

2.3.5 Lactic acid separation and purification

The viability of biologically derived products is heavily dictated by the cost of downstream processes required to isolate the target compound (Saboe et al., 2018). However, the separation and recovery of LA is difficult due to its low vapour pressure, high affinity to water, and tendency to undergo self-esterification (Sun et al., 2006). Consequently, separation and final purification can represent up to 50% of the production costs (Komesu et al., 2017a).

Traditionally, LA is recovered via gypsum precipitation, esterification, and hydrolysis (Section 2.3.5.1). While this recovery process is effective and a proven technology, it is costly, produces large quantities of commercially non-significant gypsum waste, and requires large volumes of sulphuric acid (Alves de Oliveira et al., 2018; Jantasee et al., 2017; Komesu et al., 2017c; Singhvi et al., 2018). Consequently, research has been focused on developing and testing alternate separation and purification technologies for LA including; distillation, solvent extraction, adsorption, and membrane separation processes (reverse osmosis, ultrafiltration, and electrodialysis) (Komesu et al., 2017a).

Ideally, the separation process selected for LA recovery should be based on the efficient and economical usage of these processes, along with a consideration of their individual advantages and disadvantages (Table 2.5) (Wasewar, 2005).

Table 2.5: Advantages and disadvantages of separation processes for the recovery of lactic acid (Adopted from (Komesu et al., 2017b))

Separation Process	Advantages	Disadvantages
Precipitation	• Easily applicable in industry	• High sulphuric acid consumption • Generates large quantities of gypsum • Low product purity
Liquid-liquid extraction	• No gypsum generation • Reduced risk of thermal decomposition	• Extractant requires stripping and regeneration stages • Low product purity • Conventional extraction agents show unfavourable activity coefficients.
Membrane processes	• Great flexibility in production scale • High selectivity • High levels of purification • Potential integration with conventional fermenters	• Membranes have a high cost • Fouling of membranes • Polarization issues • Difficulties in upscaling
Molecular distillation	• Reduced risk of thermal decomposition • High purification levels • No solvents • No further purification stages needed	• Difficulties in upscaling • Requires high vacuum conditions
Reactive distillation	• Integrates reaction and separation into the same apparatus • High purification levels • Lower energy consumption	• Process is complex • specifically applied to reversible reactions in the liquid phase • Requires high reaction rates • Separation and reaction temperatures need to relatively close together • Homogeneous catalyst leads to corrosion and separation issues

2.3.5.1 Traditional process

The traditional process for the recovery of LA utilises precipitation and esterification for the commercial production of high purity LA (Fig. 2.6) (Komesu et al., 2017a; Lee, 2015). LA generally exists as a salt in the fermentation broth due to the addition of neutralising agents ($CaCO_3$, $Ca(OH)_2$, NaOH, NH_3) to maintain optimal pH (5-7) (normally $CaCO_3$, $Ca(OH)_2$) (Alves de Oliveira et al., 2018; Komesu et al., 2017b; Lee, 2015). Following fermentation, the broth may be adjusted to a pH of 10 and heated to 80 ^oC in order to solubilise the calcium lactate and coagulate proteins within the broth to simplify filtration (Lee, 2015). The broth is then filtered and re-acidified with sulphuric acid to produce LA and precipitate gypsum. The resultant mixture is then filtered,

producing a technical grade LA mixture (22-44%) (Komesu et al., 2017a). This product can be further refined to produce high purity LA, through esterification with methanol, distillation, and hydrolysis (Fig. 2.6).

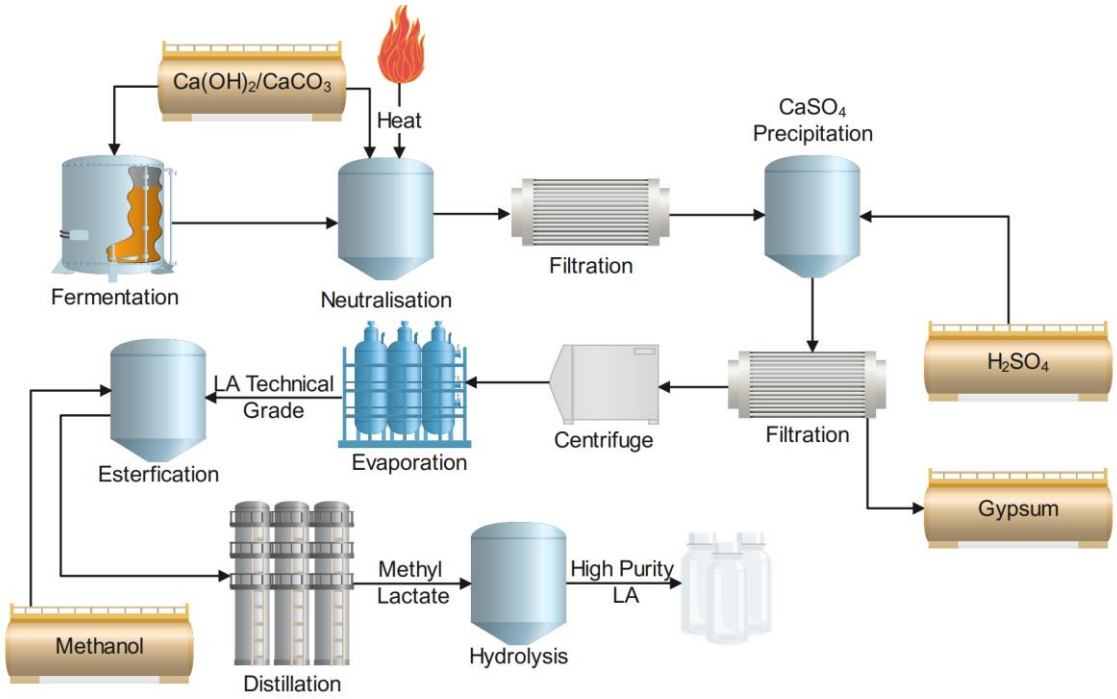


Figure 2.6: Conventional LA separation and purification processes (Modified from (Komesu et al., 2017a; Lee, 2015))

2.3.5.2 Advances in separation technologies

The current commercial method for LA separation and purification has many economic and environmental ramifications when obtaining pure LA (Section 2.3.4). Consequently, alternative methods for the recovery and purification of LA have been the focus of recent literature (Table 2.5). Recovery techniques which can be utilised *in-situ* show promise in reducing environmental impacts and reagent addition associated with fermentation and LA recovery. These technologies couple fermentation with separation for the continuous production and extraction of LA. A variety of technologies exist (Table 2.6), each with its own associated advantages and disadvantages. Two promising *in-situ* techniques include liquid-liquid extraction and ion exchange due to their high efficiency and selectivity in recovering LA from fermentation broth.

Table 2.6: Summary of advantages and disadvantages of different *in-situ* recovery techniques (Modified from (Van Hecke et al., 2014))

Recovery Technique	Operation concept	Advantages	Disadvantages	Reference
Liquid-liquid extraction	LA is dissolved in the hydrophobic solvent via a	• High efficiency and selectivity	• Solvents and impurities are toxic to microorganisms	(Gao et al., 2009; Yankov et al., 2005)

Recovery Technique	Operation concept	Advantages	Disadvantages	Reference
	reaction mechanism that varies from proton transfer to ion exchange.		• Solvent impurities may be toxic to microorganisms • Different in optimal pH for fermentation and LA extraction	
Ion exchange resins	Ion exchange resins extract dissociated LA from the aqueous phase by exchanging an anion for the dissociated lactate anion	• Improved LA productivity • Higher substrate loading • Reduced inhibition • Elimination of need for neutralising agent	• Requires elution and regeneration stages • Prone to organic fouling	(Boonmee et al., 2016; Bornak, 2012; Zhang et al., 2018)
Electrodialysis	Cation and anion exchange membranes allow the selective transfer of ions depending on their charge, allowing their selective removal into separate compartments.	• Based produced from water splitting electrodialysis can be used for pH control • LA is removed in a concentrated form	• Membrane fouling • Corrosion • High capital expenditure • High power consumption • Strict pH requirements	(Arora et al., 2007; Cassano, 2016; Li et al., 2004; Ramaswamy et al., 2013)
Electrodeionization	Electrically driven separation process, similar to electrodialysis, which incorporates ion exchange resins to provide a pathway for improved ion migration.	• High separation factor	• Nutrients from growth media may reduce separation efficiency, product purity and lead to lactate salt formation	(Arora et al., 2007; Pan et al., 2017)
Crystallization	Calcium lactate is precipitated straight from the fermentation broth without the removal of biomass or cell mass.	• Significantly improved LA productivity • High LA yield on glucose	• Requires Ca(OH)2 addition • Requires separate reactor for crystallisation due to difference in fermentation and crystallisation temperatures	(Xu & Xu, 2014)

In-situ liquid-liquid extraction is a promising method for LA recovery. A variety of solvents can be utilised including, water insoluble amines, quaternary ammonium salts, esters, or ketones. However, due to the high selectivity and efficiency of tertiary amines, as well as their poor solubility in the aqueous phase, they are also appropriate for LA extraction (Jantasee et al., 2017). A variety of tertiary amines have been tested utilising a number of different alcohols as diluents but trioctylamine in 1-octanol remains the extractant-diluent combination that provides the highest LA distribution (Krzyżaniak et al., 2013). However, it is important to note that these solvents are toxic to microorganisms (Singhvi et al., 2018), which is closely related to the hydrophobicity of the solvent, although the range of tolerable concentrations is depended by the type of microorganism (Matsumoto et al., 2004). Optimisation of the solvent concentration can reduce this toxic effect as shown by Gao et al. (2009).

Adsorption is another promising recovery technique and is widely used within industrial biotechnology as it is robust and relatively easy to operate (da Silva & Miranda, 2013). Various resins have been applied for LA recovery (Table 2.7) but weakly basic resins are generally preferred as they don't require powerful regeneration steps and have a much higher resistance to organic fouling compared to strongly basic resins (Gluszcz et al., 2004; Tung & Judson King, 1994). Several reports have explored anionic resins for LA recovery and notably Boonmee et al. (2016) and Ataei and Vasheghani-Farahani (2008) reported a 2.1-6.8 and 5 fold increase in LA productivity, respectively, when applying anion exchange to LA fermentation. Furthermore, Zhang et al. (2018) identified the cost to apply *in-situ* anion exchange for LA recovery was similar to that of $CaCO_3$ for pH control. Therefore, application of ion exchange for LA recovery can enhance the production rate of LA, allowing reduced process vessel volumes and capital costs, while simultaneously reducing the need for neutralising agents following fermentation.

While a variety of LA recovery techniques have been explored in literature (Table 2.6), they are predominantly concerned with the recovery of LA and tend not to consider implications for downstream processes. Within an integrated LA-AD biorefinery, this would not only concern further purification stages, but also downstream AD which would likely utilise the extraction residues within AD for disposal and methane generation. This would likely include the solid and liquid fraction fractions. For this the recovery method

should be carefully considered to ensure LA is selectively extracted, not only to reduce downstream costs, but also to maximise the remaining organic fraction within the residues to maximise biogas production within AD. Furthermore, the extraction method should minimise the broths exposure to toxic or inhibitory compounds which may follow the extraction residues to downstream AD and inhibit methane formation or restrict digestate use within agriculture.

Table 2.7: Summary of LA removal from via the use of anion exchange resins

Resin	Mode	Loading ($g_{LA} \cdot g_{sorbent}^{-1}$)	LA production	Notes	References
Dowex MWA-1	Synthetic solution	0.3-0.18 (pH 5-6)	-	Identified as best candidate for LA recovery	(Tung & Judson King, 1994)
Reillex 425	Synthetic solution	<0.05 (pH 5-6)	-		(Tung & Judson King, 1994)
Duolite A7	Synthetic solution	0.18-0.08 (pH 5-6)	-		(Tung & Judson King, 1994)
Amberlite IRA-910	Synthetic solution	0.3 (pH 5-6)	-	Requires pH increase to >11 for LA elution	(Tung & Judson King, 1994)
Amberlite IRA-35	Synthetic solution	0.35-0.29 (pH 5-6)	-	Identified as best candidate for LA recovery	(Tung & Judson King, 1994)
Amberlite IRA-67	*In situ* recovery	0.15 (pH 6.5)	Improved LA productivity by 2.1 to 6.8 fold compared to batch fermentation		(Boonmee et al., 2016)
335	*In situ* recovery	0.23 (pH 5.5)	Similar to conventional fermentation	Did not demonstrate any adsorption capacity for glucose	(Zhang et al., 2018)
Amberlite XAD1600 (Neutral resin)	Separation from grass silage juice	- (<3.78)	-	Undissociated LA is adsorbed onto the resin while inorganic salts and sugars were not	(Thang & Novalin, 2008)
Amberlite resin (IRA-400, Cl^-)	*In situ* recovery	-	LA productivity was 5-times higher than the conventional system		(Ataei & Vasheghani-Farahani, 2008)
Amberlite IRA 67	*In situ* recovery	80mg/ml (pH 5.0)	-		(John et al., 2008)

While only a handful of literature has examined the feasibility of utilising LA fermentation waste within AD (Demichelis et al., 2017; Dreschke et al., 2015; Kim et al., 2016), the results are promising. For example, Demichelis et al. (2017) outlined the solid

fermentation residues were appropriate for methane formation and even yielded a higher biomethane potential (BMP) than raw FWs. However, no literature has yet explored the feasibility of utilising the liquid extraction residues within AD, which may retain unfermented material or impurities produced from fermentation, such as alternative volatile fatty acids (Feng et al., 2018; Tang et al., 2016). Further work is required to understand the feasibility of utilising LA extraction residues within AD and how different recovery techniques impact downstream AD.

2.4 CONCLUDING REMARKS AND RECOMMENDATIONS

Overall, the current literature review identified challenges with integrating LA production into commercial FW AD; however, it was clear that LA production from waste streams could boost the economic performance of two-stage AD systems, providing a value-added by-product and better harnessing existing capital. FW, being composed of the primary carbohydrates and nutrients required for LA production, appears to be the most promising substrate for LA production (Section 2.3.1). Furthermore, recent literature has shown solid FW fermentation residues can be utilised within AD for methane production (Section 2.3.5.2), providing a disposal method for the fermented FWs. While these reports are promising for the LA-AD biorefinery, the commercial FW context is highly variable and complex and has yet to be fully explored, particularly in areas related to waste availability and its potential impact on LA fermentation and recovery. Furthermore, literature has yet to explore the integration into existing two-stage AD infrastructure. For this, the pre-fermenter would be converted to an LA fermenter which, to minimise start-up and operational costs, should be regulated through the control levers of pH and temperature. However, it is unknown how commercially adapted inoculum may be impacted through changing operational conditions. Although literature has identified the solid residues are suitable for methane production (Section 2.3.5.2), it is unclear how the liquid residues would influence AD which could be reused as dilution water (following LA recovery) prior to AD to reduce freshwater consumption. Furthermore, depending on the extraction method utilised, a significant portion of dissolved organic material may remain following LA recovery, which may aid methane production. However, AD is a complex and sensitive process, and it is yet not fully understood how LA recovery processes (Section 2.3.5) may influence downstream AD performance. Furthermore, the technoeconomic aspects related to utilising existing AD infrastructure have yet to be explored and may significantly impact the overall profitability of the biorefinery.

Therefore, to continue the development of the LA-AD biorefinery, it is necessary to explore the above-mentioned research areas while aiming to maximise LA production and minimise the negative impacts imposed on downstream AD. An example of a set of experimental work to further develop the LA-AD biorefinery is outlined in Fig. 2.7.

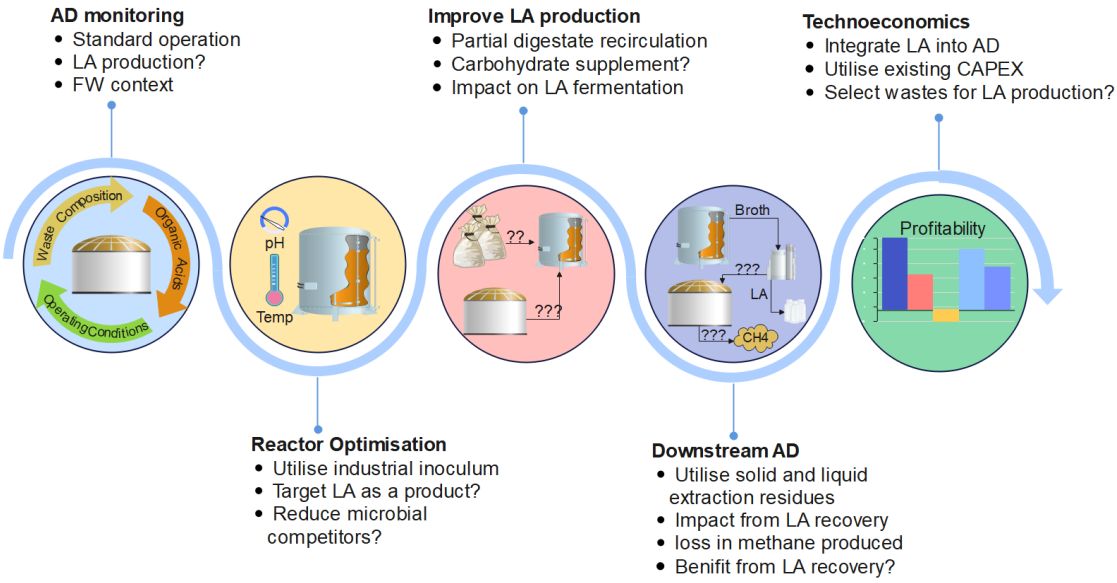


Figure 2.7: Outline of future experimental work to further develop the LA-AD FW biorefinery

Therefore, it is recommended the following areas be investigated:

- Obtain a thorough understanding of the commercial operation of FW AD facilities, based on scientific investigation, and identify how standard operation of such facilities may impact on the prospects for LA production.
- Optimisation of LA fermentation of FWs utilising the industrially produced inoculum to ensure LA can be targeted using practical process levers, such as varying process temperature and pH.
- Explore the feasibility of improving LA fermentation with digestate recirculation as a nutrient supplement, exploring impacts on LA production and community dynamics.
- Improve LA production economics by supplementing FW with a simple relatively low-value carbohydrate such as industrial sugar.

- Identify the downstream impact of LA recovery on AD, focusing on methane production.
- Explore the technoeconomic implications of integrating LA production into existing AD infrastructure, exploring alternate fermentation scenarios and Greenfield applications.

The research conducted in this thesis explores these research areas through a series of experimental work and technoeconomic modelling, with the results aiming to further the development of the LA-AD biorefinery concept.

2.5 REFERENCES

Abbasi, T., Tauseef, S.M., Abbasi, S.A. 2012. Anaerobic digestion for global warming control and energy generation—An overview. Renewable and Sustainable Energy Reviews, 16(5), 3228-3242.

Abdel-Rahman, M.A., Sonomoto, K. 2016. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. Journal of biotechnology, 236, 176-192.

Abdel-Rahman, M.A., Tashiro, Y., Sonomoto, K. 2013. Recent advances in lactic acid production by microbial fermentation processes. Biotechnology Advances, 31(6), 877-902.

Ahmad, A., Banat, F., Taher, H. 2020. A review on the lactic acid fermentation from low-cost renewable materials: Recent developments and challenges. Environmental Technology & Innovation, 20, 101138.

Ahring, B.K., Traverso, J.J., Murali, N., Srinivas, K. 2016. Continuous fermentation of clarified corn stover hydrolysate for the production of lactic acid at high yield and productivity. Biochemical Engineering Journal, 109, 162-169.

Akhiar, A. 2017. Characterization of liquid fraction of digestates after solid-liquid separation from anaerobic co-digestion plants, Université Montpellier.

Alexandri, M., Neu, A.K., Schneider, R., López-Gómez, J.P., Venus, J. 2019. Evaluation of various Bacillus coagulans isolates for the production of high purity L-lactic acid using defatted rice bran hydrolysates. International journal of food science & technology, 54(4), 1321-1329.

Alonso, A., Marsal, S., Julià, A. 2015. Analytical Methods in Untargeted Metabolomics: State of the Art in 2015. Frontiers in Bioengineering and Biotechnology, 3, 23.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

Archibald, F. 1986. Manganese: Its Acquisition by and Function in the Lactic Acid Bacteria. CRC Critical Reviews in Microbiology, 13(1), 63-109.

Arora, M.B., Hestekin, J.A., Snyder, S.W., St. Martin, E.J., Lin, Y.J., Donnelly, M.I., Millard, C.S. 2007. The Separative Bioreactor: A Continuous Separation Process for the Simultaneous Production and Direct Capture of Organic Acids. Separation Science and Technology, 42(11), 2519-2538.

Aslanzadeh, S., Rajendran, K., Taherzadeh, M.J. 2014. A comparative study between single- and two-stage anaerobic digestion processes: Effects of organic loading rate and hydraulic retention time. International Biodeterioration & Biodegradation, 95, 181-188.

Ataei, S.A., Vasheghani-Farahani, E. 2008. In situ separation of lactic acid from fermentation broth using ion exchange resins. Journal of Industrial Microbiology & Biotechnology, 35(11), 1229.

Ayre, J.M., Moheimani, N.R., Borowitzka, M.A. 2017. Growth of microalgae on undiluted anaerobic digestate of piggery effluent with high ammonium concentrations. Algal Research, 24, 218-226.

Banks, C.J., Chesshire, M., Heaven, S., Arnold, R. 2011. Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance. Bioresource Technology, 102(2), 612-620.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

Biddy, M.J., Scarlata, C., Kinchin, C. 2016. Chemicals from Biomass: A Market Assessment of Bioproducts with Near-Term Potential. ; National Renewable Energy Lab. (NREL), Golden, CO (United States). NREL/TP-5100-65509 United States 10.2172/1244312 NREL English.

Bo, Z., Pin-jing, H. 2014. Performance assessment of two-stage anaerobic digestion of kitchen wastes. Environmental Technology, 35(10), 1277-1285.

Bo, Z., Wei-min, C.A.I., Pin-jing, H.E. 2007. Influence of lactic acid on the two-phase anaerobic digestion of kitchen wastes. Journal of Environmental Sciences, 19(2), 244-249.

Boonmee, M., Cotano, O., Amnuaypanich, S., Grisadanurak, N. 2016. Improved Lactic Acid Production by In Situ Removal of Lactic Acid During Fermentation and a Proposed Scheme for Its Recovery. Arabian Journal for Science and Engineering, 41(6), 2067-2075.

Bornak, W.E. 2012. Organic Fouling of Anion Exchange Resins.

Braguglia, C.M., Gallipoli, A., Gianico, A., Pagliaccia, P. 2018. Anaerobic bioconversion of food waste into energy: A critical review. Bioresource Technology, 248, 37-56.

Brückner, R., Titgemeyer, F. 2002. Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. FEMS Microbiology Letters, 209(2), 141-148.

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum: Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

Capolupo, L., Faraco, V. 2016. Green methods of lignocellulose pretreatment for biorefinery development. Applied Microbiology and Biotechnology, 100(22), 9451-9467.

Carcus, M. 2012. Growth in PLA bioplastics: a production capacity of over 800,000 tonnes expected by 2020. in: Bio-Based News.

Carlsson, M., Lagerkvist, A., Morgan-Sagastume, F. 2012. The effects of substrate pre-treatment on anaerobic digestion systems: A review. Waste Management, 32(9), 1634-1650.

Carlu, E., Truong, T., Kundevski, M. 2019. Biogas opportunities for Australia.

Cassano, A. 2016. Integrated Membrane Processes in the Food Industry. in: Integrated Membrane Systems and Processes.

Castro-Aguirre, E., Iñiguez-Franco, F., Samsudin, H., Fang, X., Auras, R. 2016. Poly(lactic acid)—Mass production, processing, industrial applications, and end of life. Advanced Drug Delivery Reviews, 107, 333-366.

Chatham, J.C., Blackband, S.J. 2001. Nuclear Magnetic Resonance Spectroscopy and Imaging in Animal Research. ILAR Journal, 42(3), 189-208.

Chen, Y.-d., Ho, S.-H., Nagarajan, D., Ren, N.-q., Chang, J.-S. 2018. Waste biorefineries — integrating anaerobic digestion and microalgae cultivation for bioenergy production. Current Opinion in Biotechnology, 50, 101-110.

Chenebault, C., Moscoviz, R., Trably, E., Escudié, R., Percheron, B. 2022. Lactic acid production from food waste using a microbial consortium: Focus on key parameters for process upscaling and fermentation residues valorization. Bioresource technology, 354, 127230-127230.

Choi, O.K., Lee, K., Park, K.Y., Kim, J.-K., Lee, J.W. 2017. Pre-recovery of fatty acid methyl ester (FAME) and anaerobic digestion as a biorefinery route to valorizing waste activated sludge. Renewable Energy, 108, 548-554.

Coelho, J.J., Prieto, M.L., Dowling, S., Hennessy, A., Casey, I., Woodcock, T., Kennedy, N. 2018. Physical-chemical traits, phytotoxicity and pathogen detection in liquid anaerobic digestates. Waste Management, 78, 8-15.

Comparetti, A., Febo, P., Greco, C., Orlando, S. 2013. Current state and future of biogas and digestate production.

da Silva, A.H., Miranda, E.A. 2013. Adsorption/Desorption of Organic Acids onto Different Adsorbents for Their Recovery from Fermentation Broths. Journal of Chemical & Engineering Data, 58(6), 1454-1463.

De Filippis, F., Parente, E., Ercolini, D. 2017. Metagenomics insights into food fermentations. Microbial Biotechnology, 10(1), 91-102.

de Oliveira Moraes, A., Ramirez, N.I.B., Pereira, N. 2016. Evaluation of the Fermentation Potential of Pulp Mill Residue to Produce d(−)-Lactic Acid by Separate Hydrolysis and Fermentation Using Lactobacillus coryniformis subsp. torquens. Applied Biochemistry and Biotechnology, 180(8), 1574-1585.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Djukić-Vuković, A., Mladenović, D., Ivanović, J., Pejin, J., Mojović, L. 2019. Towards sustainability of lactic acid and poly-lactic acid polymers production. Renewable and Sustainable Energy Reviews, 108, 238-252.

Dreschke, G., Probst, M., Walter, A., Pümpel, T., Walde, J., Insam, H. 2015. Lactic acid and methane: Improved exploitation of biowaste potential. Bioresource technology, 176, 47-55.

EBA. 2021. EBA Statistical Report 2020 shows significant growth and potential of biomethane to decarbonise the gas sector.

Edwards, J., Othman, M., Burn, S. 2015. A review of policy drivers and barriers for the use of anaerobic digestion in Europe, the United States and Australia. Renewable and Sustainable Energy Reviews, 52, 815-828.

Eiteman, M.A., Ramalingam, S. 2015. Microbial production of lactic acid. Biotechnology Letters, 37(5), 955-972.

Eş, I., Mousavi Khaneghah, A., Barba, F.J., Saraiva, J.A., Sant'Ana, A.S., Hashemi, S.M.B. 2018. Recent advancements in lactic acid production - a review. Food Research International, 107, 763-770.

Feng, K., Li, H., Zheng, C. 2018. Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresource Technology, 270, 180-188.

Gandolfi, S., Pistone, L., Ottolina, G., Xu, P., Riva, S. 2015. Hemp hurds biorefining: A path to green l-(+)-lactic acid production. Bioresource Technology, 191, 59-65.

Gao, M.-T., Shimamura, T., Ishida, N., Nagamori, E., Takahashi, H., Umemoto, S., Omasa, T., Ohtake, H. 2009. Extractive lactic acid fermentation with tri-n-decylamine as the extractant. Enzyme and Microbial Technology, 44(5), 350-354.

Garvie, E.I. 1980. Bacterial lactate dehydrogenases. Microbiological Reviews, 44(1), 106-139.

Garvie, E.I. 1967. The growth factor and amino acid requirements of species of the genus Leuconostoc, including Leuconostoc paramesenteroides (sp.nov.) and Leuconostoc oenos. J Gen Microbiol, 48(3), 439-447.

Gebrezgabher, S.A., Meuwissen, M.P.M., Prins, B.A.M., Lansink, A.G.J.M.O. 2010. Economic analysis of anaerobic digestion—A case of Green power biogas plant in The Netherlands. NJAS - Wageningen Journal of Life Sciences, 57(2), 109-115.

Ghaffar, T., Irshad, M., Anwar, Z., Aqil, T., Zulifqar, Z., Tariq, A., Kamran, M., Ehsan, N., Mehmood, S. 2014. Recent trends in lactic acid biotechnology: A brief review on production to purification. Journal of Radiation Research and Applied Sciences, 7(2), 222-229.

Ghatak, H.R. 2011. Biorefineries from the perspective of sustainability: Feedstocks, products, and processes. Renewable and Sustainable Energy Reviews, 15(8), 4042-4052.

Gluszcz, P., Jamroz, T., Sencio, B., Ledakowicz, S. 2004. Equilibrium and dynamic investigations of organic acids adsorption onto ion-exchange resins. Bioprocess and biosystems engineering, 26(3), 185-190.

Görke, B., Stülke, J. 2008. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nature Reviews Microbiology, 6, 613.

Gu, S.-A., Jun, C., Joo, J.C., Kim, S., Lee, S.H., Kim, Y.H. 2014. Higher thermostability of l-lactate dehydrogenases is a key factor in decreasing the optical purity of d-lactic acid produced from Lactobacillus coryniformis. Enzyme and microbial technology, 58-59, 29-35.

Gu, X.Y., Liu, J.Z., Wong, J.W.C. 2018. Control of lactic acid production during hydrolysis and acidogenesis of food waste. Bioresource Technology, 247, 711-715.

Häfner, F., Hartung, J., Möller, K. 2022. Digestate Composition Affecting N Fertiliser Value and C Mineralisation. Waste and Biomass Valorization.

Hassan, S.E.-D., Abdel-Rahman, M.A., Roushdy, M.M., Azab, M.S., Gaber, M.A. 2019. Effective biorefinery approach for lactic acid production based on co-fermentation of mixed organic wastes by Enterococcus durans BP130. Biocatalysis and agricultural biotechnology, 20, 101203.

Hofvendahl, K., Hahn–Hägerdal, B. 2000. Factors affecting the fermentative lactic acid production from renewable resources1. Enzyme and Microbial Technology, 26(2), 87-107.

Holzapfel, W.H., Wood, B.J.B. 2014. Lactic Acid Bacteria : Biodiversity and Taxonomy. John Wiley & Sons, Incorporated, Hoboken, UNITED KINGDOM.

Insight, B. 2016. Challenges of building anaerobic digestion plants. in: Bioenergy Insight, Vol. 7, ad4Energy.

Jantasee, S., Kienberger, M., Mungma, N., Siebenhofer, M. 2017. Potential and assessment of lactic acid production and isolation – a review. Journal of Chemical Technology & Biotechnology, 92(12), 2885-2893.

John, R.P., Nampoothiri, K.M., Pandey, A. 2008. L(+)-Lactic acid recovery from cassava bagasse based fermented medium using anion exchange resins. Brazilian Archives of Biology and Technology, 51, 1241-1248.

Jurowski, K., Kochan, K., Walczak, J., Barańska, M., Piekoszewski, W., Buszewski, B. 2017. Analytical Techniques in Lipidomics: State of the Art. Critical Reviews in Analytical Chemistry, 47(5), 418-437.

Juturu, V., Wu, J.C. 2016. Microbial production of lactic acid: the latest development. Critical Reviews in Biotechnology, 36(6), 967-977.

Karp, S.G., Igashiyama, A.H., Siqueira, P.F., Carvalho, J.C., Vandenberghe, L.P.S., Thomaz-Soccol, V., Coral, J., Tholozan, J.-L., Pandey, A., Soccol, C.R. 2011. Application of the biorefinery concept to produce l-lactic acid from the

soybean vinasse at laboratory and pilot scale. Bioresource Technology, 102(2), 1765-1772.

Khan, M.A., Ngo, H.H., Guo, W.S., Liu, Y.W., Zhou, J.L., Zhang, J., Liang, S., Ni, B.J., Zhang, X.B., Wang, J. 2016. Comparing the value of bioproducts from different stages of anaerobic membrane bioreactors. Bioresource Technology, 214, 816-825.

Kim, D.-H., Lim, W.-T., Lee, M.-K., Kim, M.-S. 2012. Effect of temperature on continuous fermentative lactic acid (LA) production and bacterial community, and development of LA-producing UASB reactor. Bioresource Technology, 119, 355-361.

Kim, K.I., Kim, W.K., Seo, D.K., Yoo, I.S., Kim, E.K., Yoon, H.H. 2003a. Production of Lactic Acid from Food Wastes. in: Biotechnology for Fuels and Chemicals: The Twenty-Fourth Symposium, (Eds.) B.H. Davison, J.W. Lee, M. Finkelstein, J.D. McMillan, Humana Press. Totowa, NJ, pp. 637-647.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Kim, M., Gomec, C.Y., Ahn, Y., Speece, R.E. 2003b. Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. Environmental Technology, 24(9), 1183-1190.

Kjersi, C. 2012. Effects of nutrients supplementation on fermentability of lignocellulosic hydrolysates under high gravity conditions.

Komemoto, K., Lim, Y.G., Nagao, N., Onoue, Y., Niwa, C., Toda, T. 2009. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Management, 29(12), 2950-2955.

Komesu, A., Maciel, M., Filho, R. 2017a. Separation and Purification Technologies for Lactic Acid – A Brief Review.

Komesu, A., Maciel, M.R.W., Rocha de Oliveira, J.A., da Silva Martins, L.H., Maciel Filho, R. 2017b. Purification of Lactic Acid Produced by Fermentation: Focus on Non-traditional Distillation Processes. Separation & Purification Reviews, 46(3), 241-254.

Komesu, A., Oliveira, J., Martins, L., Regina Wolf Maciel, M., Filho, R. 2017c. Lactic Acid Production to Purification: A Review. BioResources, 12(2), 4364-4383.

Krzyżaniak, A., Leeman, M., Vossebeld, F., Visser, T.J., Schuur, B., de Haan, A.B. 2013. Novel extractants for the recovery of fermentation derived lactic acid. Separation and Purification Technology, 111, 82-89.

Kumar, A.K., Sharma, S. 2017. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresources and Bioprocessing, 4(1), 7.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2018. Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly(lactic acid) production. Journal of Cleaner Production, 181, 72-87.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2016. Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with Lactobacillus casei Shirota. Bioresource Technology, 217, 129-136.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology, 31(9), 814-821.

Ledesma, O.V., De Ruiz Holgado, A.P., Oliver, G., Giori, G.S.D., Raibaud, P., Galpin, J.V. 1977. A Synthetic Medium for Comparative Nutritional Studies of Lactobacilli. Journal of Applied Bacteriology, 42(1), 123-133.

Lee, H. 2015. DEVELOPMENT OF LACTIC AND SUCCINIC ACID BIOREFINERY CONFIGURATIONS FOR INTEGRATION INTO A THERMOMECHANICAL PULP MILL.

Li, H., Mustacchi, R., Knowles, C.J., Skibar, W., Sunderland, G., Dalrymple, I., Jackman, S.A. 2004. An electrokinetic bioreactor: using direct electric current for enhanced lactic acid fermentation and product recovery. Tetrahedron, 60(3), 655-661.

Linville, J.L., Shen, Y., Wu, M.M., Urgun-Demirtas, M. 2015. Current State of Anaerobic Digestion of Organic Wastes in North America. Current Sustainable/Renewable Energy Reports, 2(4), 136-144.

Liu, S.Q. 2003. Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. International Journal of Food Microbiology, 83(2), 115-131.

López, J.C., Arnáiz, E., Merchán, L., Lebrero, R., Muñoz, R. 2018. Biogas-based polyhydroxyalkanoates production by Methylocystis hirsuta: A step further in anaerobic digestion biorefineries. Chemical Engineering Journal, 333, 529-536.

Luongo, V., Policastro, G., Ghimire, A., Pirozzi, F., Fabbricino, M. 2019. Repeated-Batch Fermentation of Cheese Whey for Semi-Continuous Lactic Acid Production Using Mixed Cultures at Uncontrolled pH. Sustainability, 11(12).

Lv, Y., Chang, N., Li, Y.-Y., Liu, J. 2021. Anaerobic co-digestion of food waste with municipal solid waste leachate: A review and prospective application with more benefits. Resources, Conservation and Recycling, 174, 105832.

Ma, X., Gao, M., Li, C., Wang, N., Wang, Q., Sun, X. 2021. Effects of different lignocellulosic wastes on alleviating acidification of L-lactic acid production from food waste fermentation. Bioresource Technology, 342, 126043.

Manandhar, A., Shah, A. 2020. Techno-Economic Analysis of Bio-Based Lactic Acid Production Utilizing Corn Grain as Feedstock. Processes, 8(2), 199.

Massaro, V., Digiesi, S., Mossa, G., Ranieri, L. 2015. The sustainability of anaerobic digestion plants: a win–win strategy for public and private bodies. Journal of cleaner production, 104, 445-459.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews, 36, 412-427.

Matsumoto, M., Mochiduki, K., Kondo, K. 2004. Toxicity of ionic liquids and organic solvents to lactic acid-producing bacteria. Journal of Bioscience and Bioengineering, 98(5), 344-347.

Mazumdar, S., Blankschien, M.D., Clomburg, J.M., Gonzalez, R. 2013. Efficient synthesis of L-lactic acid from glycerol by metabolically engineered Escherichia coli. Microbial cell factories, 12(1), 7-7.

Mazzoli, R., Bosco, F., Mizrahi, I., Bayer, E.A., Pessione, E. 2014. Towards lactic acid bacteria-based biorefineries. Biotechnology Advances, 32(7), 1216-1236.

Melville, L., Weger, A., Wiesgickl, S., Franke, M. 2014. Anaerobic Digestion. in: Transformation of Biomass.

Menon, V., Rao, M. 2012. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. Progress in Energy and Combustion Science, 38(4), 522-550.

Mickan, B.S., Ren, A.-T., Buhlmann, C.H., Ghadouani, A., Solaiman, Z.M., Jenkins, S., Pang, J., Ryan, M.H. 2022. Closing the circle for urban food waste anaerobic digestion: The use of digestate and biochar on plant growth in potting soil. Journal of Cleaner Production, 347, 131071.

Mongkhonsiri, G., Charoensuppanimit, P., Anantpinijwatna, A., Gani, R., Assabumrungrat, S. 2020. Process development of sustainable biorefinery system integrated into the existing pulping process. Journal of Cleaner Production, 255, 120278.

Moraes, B.S., Junqueira, T.L., Pavanello, L.G., Cavalett, O., Mantelatto, P.E., Bonomi, A., Zaiat, M. 2014. Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: Profit or expense? Applied Energy, 113, 825-835.

Mukherjee, A., Chettri, B., Langpoklakpam, J.S., Basak, P., Prasad, A., Mukherjee, A.K., Bhattacharyya, M., Singh, A.K., Chattopadhyay, D. 2017. Bioinformatic Approaches Including Predictive Metagenomic Profiling Reveal Characteristics of Bacterial Response to Petroleum Hydrocarbon Contamination in Diverse Environments. Scientific Reports, 7(1), 1108.

Nagana Gowda, G.A., Zhang, S., Gu, H., Asiago, V., Shanaiah, N., Raftery, D. 2008. Metabolomics-Based Methods for Early Disease Diagnostics: A Review. Expert review of molecular diagnostics, 8(5), 617-633.

Nasr, N., Elbeshbishy, E., Hafez, H., Nakhla, G., Hesham El Naggar, M. 2012. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. Bioresource Technology, 111, 122-126.

Nester, R. 2018. Lactic Acid Market : Global Historical Growth (2012-2016) & Future Outlook (2017-2024) Demand Analysis & Opportunity Evaluation, Vol. 2018. Research Nester.

Nkoa, R. 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agronomy for Sustainable Development, 34(2), 473-492.

Novik, G., Meerovskaya, O., Savich, V. 2017. Waste Degradation and Utilization by Lactic Acid Bacteria: Use of Lactic Acid Bacteria in Production of Food Additives, Bioenergy and Biogas.

Ohkouchi, Y., Inoue, Y. 2006. Direct production of l(+)-lactic acid from starch and food wastes using Lactobacillus manihotivorans LMG18011. Bioresource Technology, 97(13), 1554-1562.

Oldiges, M., Lütz, S., Pflug, S., Schroer, K., Stein, N., Wiendahl, C. 2007. Metabolomics: Current state and evolving methodologies and tools.

Olszewska-Widdrat, A., Alexandri, M., López-Gómez, J.P., Schneider, R., Venus, J. 2020. Batch and Continuous Lactic Acid Fermentation Based on A Multi-Substrate Approach. Microorganisms (Basel), 8(7), 1084.

Oonkhanond, B., Jonglertjunya, W., Srimarut, N., Bunpachart, P., Tantinukul, S., Nasongkla, N., Sakdaronnarong, C. 2017. Lactic acid production from sugarcane bagasse by an integrated system of lignocellulose fractionation, saccharification, fermentation, and ex-situ nanofiltration. Journal of Environmental Chemical Engineering, 5(3), 2533-2541.

Pal, P., Sikder, J., Roy, S., Giorno, L. 2009. Process intensification in lactic acid production: A review of membrane based processes. Chemical Engineering and Processing: Process Intensification, 48(11), 1549-1559.

Pan, S.-Y., Snyder, S.W., Ma, H.-W., Lin, Y.J., Chiang, P.-C. 2017. Development of a Resin Wafer Electrodeionization Process for Impaired Water Desalination with High Energy Efficiency and Productivity. ACS Sustainable Chemistry & Engineering, 5(4), 2942-2948.

Papagianni, M. 2012. Metabolic engineering of lactic acid bacteria for the production of industrially important compounds. Computational and Structural Biotechnology Journal, 3, e201210003.

Parthiba Karthikeyan, O., Trably, E., Mehariya, S., Bernet, N., Wong, J.W.C., Carrere, H. 2018. Pretreatment of food waste for methane and hydrogen recovery: A review. Bioresource technology, 249, 1025-1039.

Plana, P.V., Noche, B. 2016. A Review Of The Current Digestate Distribution Models: Storage And Transport. WIT Transactions on Ecology and the Environment, 202, 345-357.

Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, 143, 615-623.

Pleissner, D., Neu, A.-K., Mehlmann, K., Schneider, R., Puerta-Quintero, G.I., Venus, J. 2016. Fermentative lactic acid production from coffee pulp hydrolysate using Bacillus coagulans at laboratory and pilot scales. Bioresource Technology, 218, 167-173.

Presswire, M. 2018. Global Lactic Acid Market Is Projected To Post A CAGR of 15.6% Over The Forecast Period. in: M2 Presswire.

Ramaswamy, S., Huang, H.-J., Ramarao, B.V. 2013. Separation and Purification Technologies in Biorefineries. Wiley, Somerset, UNITED KINGDOM.

Reddy, L.V., Kim, Y.-M., Yun, J.-S., Ryu, H.-W., Wee, Y.-J. 2016. l-Lactic acid production by combined utilization of agricultural bioresources as renewable and economical substrates through batch and repeated-batch fermentation of Enterococcus faecalis RKY1. Bioresource Technology, 209, 187-194.

ReNu. 2017. Market Analysis of Opportunities in Australia for Anaerobic Digestion Deployment. SM01-CC-REP-0001.

Riva, C., Orzi, V., Carozzi, M., Acutis, M., Boccasile, G., Lonati, S., Tambone, F., D'Imporzano, G., Adani, F. 2016. Short-term experiments in using digestate products as substitutes for mineral (N) fertilizer: Agronomic performance, odours, and ammonia emission impacts. The Science of the total environment, 547, 206-214.

RWI. 2013. Business Analysis of Anaerobic Digestion in the USA.

Saboe, P.O., Manker, L.P., Michener, W.E., Peterson, D.J., Brandner, D.G., Deutch, S.P., Kumar, M., Cywar, R.M., Beckham, G.T., Karp, E.M. 2018. In situ recovery of bio-based carboxylic acids. Green Chemistry, 20(8), 1791-1804.

Salminen, A., Wright, A.v., Ouwehand, A. 2004. Lactic acid bacteria: Microbiological and functional aspects. 3rd ed, New York: Marcel Dekker.

Sambusiti, C., Monlau, F., Barakat, A. 2016. Bioethanol fermentation as alternative valorization route of agricultural digestate according to a biorefinery approach. Bioresource technology, 212, 289-295.

Sawatdeenarunat, C., Nguyen, D., Surendra, K.C., Shrestha, S., Rajendran, K., Oechsner, H., Xie, L., Khanal, S.K. 2016. Anaerobic biorefinery: Current status, challenges, and opportunities. Bioresource Technology, 215, 304-313.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresource Technology, 178, 178-186.

Scalbert, A., Brennan, L., Fiehn, O., Hankemeier, T., Kristal, B.S., van Ommen, B., Pujos-Guillot, E., Verheij, E., Wishart, D., Wopereis, S. 2009. Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. Metabolomics, 5(4), 435-458.

Schievano, A., Tenca, A., Lonati, S., Manzini, E., Adani, F. 2014. Can two-stage instead of one-stage anaerobic digestion really increase energy recovery from biomass? Applied Energy, 124, 335-342.

Serna-Maza, A., Heaven, S., Banks, C.J. 2015. Biogas stripping of ammonia from fresh digestate from a food waste digester. Bioresource Technology, 190, 66-75.

Simet, A., Fletcher, K. 2017. Biogas Advances in the US. in: Biomass Magazine, BBI International.

Singhvi, M., Zendo, T., Sonomoto, K. 2018. Free lactic acid production under acidic conditions by lactic acid bacteria strains: challenges and future prospects. Applied Microbiology and Biotechnology, 102(14), 5911-5924.

Stephen, J.D., Sokhansanj, S., Bi, X., Sowlati, T., Kloeck, T., Townley-Smith, L., Stumborg, M.A. 2010. The impact of agricultural residue yield range on the delivered cost to a biorefinery in the Peace River region of Alberta, Canada. Biosystems Engineering, 105(3), 298-305.

Sträuber, H., Lucas, R., Kleinsteuber, S. 2016. Metabolic and microbial community dynamics during the anaerobic digestion of maize silage in a two-phase process. Applied Microbiology and Biotechnology, 100(1), 479-491.

Sun, S., Jones, R.B., Fodor, A.A. 2020. Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome, 8(1), 46.

Sun, X., Wang, Q., Zhao, W., Ma, H., Sakata, K. 2006. Extraction and purification of lactic acid from fermentation broth by esterification and hydrolysis method. Separation and Purification Technology, 49(1), 43-48.

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., Khanal, S. 2015. Anaerobic Digestion-Based Biorefinery for Bioenergy and Biobased Products. Industrial Biotechnology, 11(2), 103-112.

Tait, S., Harris, P.W., McCabe, B.K. 2021. Biogas recovery by anaerobic digestion of Australian agro-industry waste: A review. Journal of Cleaner Production, 299, 126876.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y. 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management, 52, 278-285.

Tashiro, Y., Inokuchi, S., Poudel, P., Okugawa, Y., Miyamoto, H., Miayamoto, H., Sakai, K. 2016. Novel pH control strategy for efficient production of optically active l-lactic acid from kitchen refuse using a mixed culture system. Bioresource Technology, 216, 52-59.

Teglia, C., Tremier, A., Martel, J.-L. 2011. Characterization of Solid Digestates: Part 1, Review of Existing Indicators to Assess Solid Digestates Agricultural Use. Waste and Biomass Valorization, 2(1), 43-58.

Thang, V.H., Novalin, S. 2008. Green Biorefinery: Separation of lactic acid from grass silage juice by chromatography using neutral polymeric resin. Bioresource Technology, 99(10), 4368-4379.

Tung, A.L., Judson King, C. 1994. Sorption and extraction of lactic and succinic acids at pH > pKa1. I. Factors governing equilibria. Industrial & Engineering Chemistry Research, 33, 3217-3223.

Turnley, D., Hopwood, L., Burns, C., Maio, D.D. 2016. Assessment of digestate drying as an eligible heat use in the Renewable Heat Incentive.

Ujor, V.C., Okonkwo, C.C., Rush, B.B., McCrea, G.E., Ezeji, T.C. 2020. Harnessing the Residual Nutrients in Anaerobic Digestate for Ethanol Fermentation and Digestate Remediation Using Saccharomyces cerevisiae. Fermentation, 6(2), 52.

UN. 2019. Resolution Adopted by the General Assembly on 25 September 2015, (Eds.) J. Walker, A. Pekmezovic, G. Walker, John Wiley & Sons, Ltd. Chichester, UK, pp. 333-374.

UN. 2022. Sustainable Development Goals, Vol. 2022.

Van Hecke, W., Kaur, G., De Wever, H. 2014. Advances in in-situ product recovery (ISPR) in whole cell biotechnology during the last decade. Biotechnology Advances, 32(7), 1245-1255.

Van Wouwe, P., Dusselier, M., Vanleeuw, E., Sels, B. 2016. Lactide Synthesis and Chirality Control for Polylactic acid Production. ChemSusChem, 9(9), 907-921.

Vaneeckhaute, C., Lebuf, V., Michels, E., Belia, E., Vanrolleghem, P.A., Tack, F.M.G., Meers, E. 2017. Nutrient Recovery from Digestate: Systematic Technology Review and Product Classification. Waste and Biomass Valorization, 8(1), 21-40.

Vasco-Correa, J., Khanal, S., Manandhar, A., Shah, A. 2018. Anaerobic digestion for bioenergy production: Global status, environmental and techno-economic implications, and government policies. Bioresource Technology, 247, 1015-1026.

Vijayakumar, J., Thangavelu, V., Rajendran, A. 2008. Recent Trends in the Production, Purification and Application of Lactic Acid.

Wang, Q., Yang, L., Feng, K., Li, H., Deng, Z., Liu, J. 2021. Promote lactic acid production from food waste fermentation using biogas slurry recirculation. Bioresour Technol, 337, 125393.

Wang, Y., Tashiro, Y., Sonomoto, K. 2015. Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits. Journal of Bioscience and Bioengineering, 119(1), 10-18.

Wasewar, K. 2005. Separation of lactic acid: Recent advances.

WBA. 2018. Global Food Waste Management: An Implementation Guide For Cities.

Wee, Y.-J., Kim, J.-N., Ryu, H.-W. 2006. Biotechnological Production of Lactic Acid and Its Recent Applications. Food Technol. Biotechnol, 44(2), 163-172.

Wischral, D., Arias, J.M., Modesto, L.F., de França Passos, D., Pereira Jr, N. 2019. Lactic acid production from sugarcane bagasse hydrolysates by Lactobacillus pentosus: Integrating xylose and glucose fermentation. Biotechnology Progress, 35(1), e2718.

WRAP. 2017. BSI PAS 110: Producing Quality Anaerobic Digestate, Vol. 2022, The Waste and Resources Action Programme. The Waste and Resources Action Programme.

Wu, Y., Wang, C., Liu, X., Ma, H., Wu, J., Zuo, J., Wang, K. 2016. A new method of two-phase anaerobic digestion for fruit and vegetable waste treatment. Bioresource Technology, 211, 16-23.

Xu, H., Li, B., Mu, X. 2016. Review of Alkali-Based Pretreatment To Enhance Enzymatic Saccharification for Lignocellulosic Biomass Conversion. Industrial & Engineering Chemistry Research, 55(32), 8691-8705.

Xu, K., Xu, P. 2014. Efficient calcium lactate production by fermentation coupled with crystallization-based in situ product removal. Bioresource Technology, 163, 33-39.

Yang, S.-T., El-Ensashy, H., Thongchul, N. 2013. Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers : In Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers. American Institute of Chemical Engineers, Somerset, UNITED STATES.

Yankov, D., Jj, M., Gg, K., Albet, J., Gg, M. 2005. Improvement of the Lactic Acid Extraction. Extraction From Aqueous Solutions and Simulated Fermentation Broth by Means of Mixed Extractant and TOA, Partially Loaded with HCl.

Ye, T., Li, X., Zhang, T., Su, Y. 2018. Copper (II) addition to accelerate lactic acid production from co-fermentation of food waste and waste activated sludge: Understanding of the corresponding metabolisms, microbial community and predictive functional profiling. Waste management (Elmsford), 76, 414-422.

Yousuf, A., Bastidas-Oyanedel, J.-R., Schmidt, J.E. 2018. Effect of total solid content and pretreatment on the production of lactic acid from mixed culture dark fermentation of food waste. Waste Management, 77, 516-521.

Yuan, S.-F., Hsu, T.-C., Wang, C.-A., Jang, M.-F., Kuo, Y.-C., Alper, H.S., Guo, G.-L., Hwang, W.-S. 2018. Production of optically pure l(+)-lactic acid from waste plywood chips using an isolated thermotolerant Enterococcus faecalis SI at a pilot scale. Journal of industrial microbiology & biotechnology, 45(11), 961-970.

Zaman, A., Reynolds, C. 2015. The economic and bio-energy production potential of South Australian food waste using Anaerobic digestion. Unmaking Waste, May 22-24, Adelaide, South Australia. Unmaking Waste.

Zeng, J., Zheng, Y., Yu, X., Yu, L., Gao, D., Chen, S. 2013. Lignocellulosic biomass as a carbohydrate source for lipid production by Mortierella isabellina. Bioresource Technology, 128, 385-391.

Zhang, C., Yang, H.-Q., Wu, D.-J. 2019. Study on the reuse of anaerobic digestion effluent in lactic acid production. Journal of Cleaner Production, 239, 118028.

Zhang, W., Li, X., He, Y., Xu, X., Chen, H., Zhang, A., Liu, Y., Xue, G., Makinia, J. 2020a. Ammonia amendment promotes high rate lactate production and recovery from semi-continuous food waste fermentation. Bioresource Technology, 302, 122881.

Zhang, W., Li, X., Zhang, T., Li, J., Lai, S., Chen, H., Gao, P., Xue, G. 2017. High-rate lactic acid production from food waste and waste activated sludge via interactive control of pH adjustment and fermentation temperature. Chemical Engineering Journal, 328, 197-206.

Zhang, W., Xu, X., Yu, P., Zuo, P., He, Y., Chen, H., Liu, Y., Xue, G., Li, X., Alvarez, P.J.J. 2020b. Ammonium Enhances Food Waste Fermentation to High-Value Optically Active l-Lactic acid. ACS Sustainable Chemistry & Engineering, 8(1), 669-677.

Zhang, Y., Qian, Z., Liu, P., Liu, L., Zheng, Z., Ouyang, J. 2018. Efficient in situ separation and production of l-lactic acid by Bacillus coagulans using weak

basic anion-exchange resin. Bioprocess and Biosystems Engineering, 41(2), 205-212.

Zhao, Q., Leonhardt, E., MacConnell, C., Frear, C., Chen, S. 2010. Purification technologies for biogas generated by anaerobic digestion.

Zheng, J., Rehmann, L. 2014. Extrusion Pretreatment of Lignocellulosic Biomass: A Review. International Journal of Molecular Sciences, 15(10), 18967-18984.

Zilio, M., Pigoli, A., Rizzi, B., Geromel, G., Meers, E., Schoumans, O., Giordano, A., Adani, F. 2021. Measuring ammonia and odours emissions during full field digestate use in agriculture. The Science of the total environment, 782, 146882-146882.

Zou, L., Ouyang, S., Hu, Y., Zheng, Z., Ouyang, J. 2021. Efficient lactic acid production from dilute acid-pretreated lignocellulosic biomass by a synthetic consortium of engineered Pseudomonas putida and Bacillus coagulans. Biotechnology for Biofuels, 14(1), 227.

CHAPTER 3 MONITORING AN INDUSTRIAL FACILITY

Foreword

Chapter 2 explored and reviewed literature on LA production and AD focusing on LA fermentation, its extraction, and how such systems may be integrated into AD by exploring potential advantages and challenges. It was identified there were no studies exploring LA production within a commercial AD facility and the following knowledge gaps were identified:

1. Lack of understanding how operation of an AD facility would impact LA fermentation
2. Influence of varying feed composition and rate of feeding would impact LA fermentation
3. How the absence of pH of temperature control would influence commercial scale LA fermentation
4. How would unsterilised food wastes impact the community composition at industrial scale.

It is crucial to understand the impact of commercial AD operating procedures on LA fermentation before integrating the two technologies. Accordingly, Chapter 3 explores the LA fermentation performance of an industrial scale pre-fermenter within a commercial scale AD facility, aiming to identify the impact of process conditions and feed composition on LA production.

The following chapter has been drafted in accordance with the Journal of Cleaner Production.

The current manuscript is published.

Christopher H. Bühlmann, Bede S. Mickan, Stephan Tait, Michael Renton, Parisa A. Bahri, Lactic acid from mixed food wastes at a commercial biogas facility: Effect of feedstock and process conditions, Journal of Cleaner Production, Volume 284, 2021, 125243, ISSN 0959-6526, https://doi.org/10.1016/j.jclepro.2020.125243.

The following authors contributed to this manuscript as outlined below.

Authorship order	Contribution (%)	Concept Development	Data Collection	Data Analyses	Drafting of manuscript
Christopher H. Buhlmann	75	X	X	X	X
Prof. Parisa A. Bahri	10	X		X	
Dr Bede S. Mickan	5	X		X	
Dr Stephan Tait	5	X		X	
Michael Renton	5	X		X	

Contribution indicates the total involvement each author has had in this project. Placing an 'X' in the remaining boxes indicates which aspect(s) of the project each author engaged in.

By signing this document, the Candidate and Principal Supervisor acknowledge that the above information is accurate and has been agreed to by all other authors.

Candidate **Principal Supervisor**

Journal Metrics (as of June 2022)

Journal Name: Journal of Cleaner Production

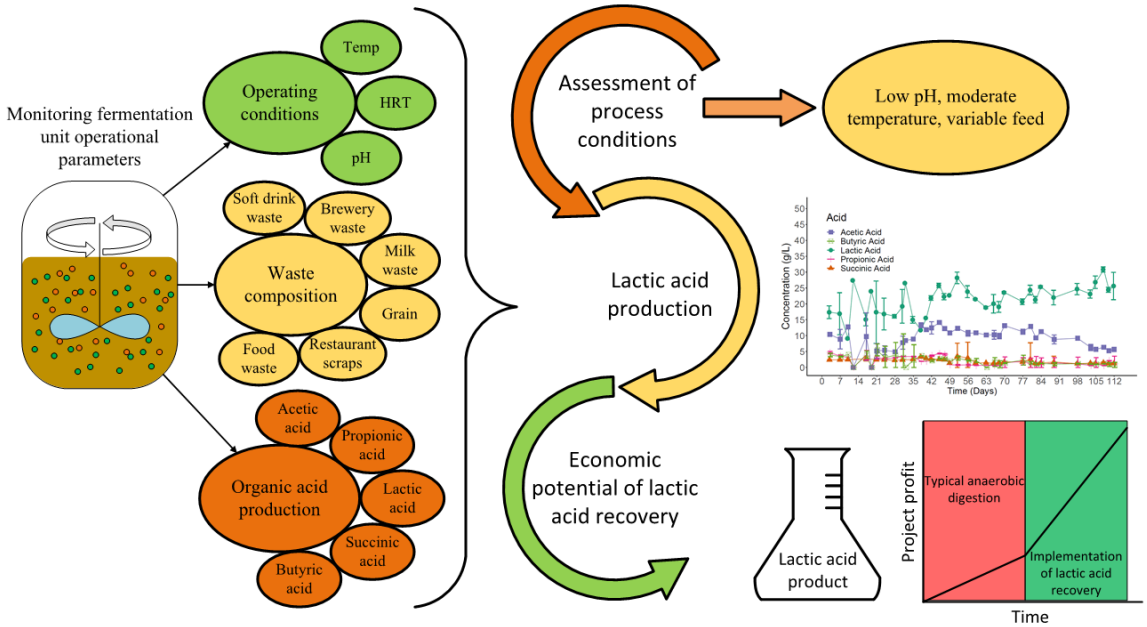
Peer Reviewed: Yes

Impact Factor: 11.072

Cite Score: 15.8

ABSTRACT

Anaerobic digestion facilities can become biorefineries to produce higher-value products together with biogas energy and nutrient-rich digestate. To inform future biorefinery concepts with lactic acid recovery, the current study monitored organic acids in a pre-fermentation stage at a commercial anaerobic digestion facility. The study assessed lactic acid production performance and the impact of mixed food waste feedstocks and process conditions. Feed rate and feedstock composition varied weekly with waste availability. Normal operating conditions of the pre-fermentation stage included warm ambient conditions (24-35 °C), low pH (3.45 ± 0.03), a short hydraulic retention time (1-3.5 days) and stable organic loading rate (12 ± 2 $kg_{VS} \cdot m^{-3} \cdot day^{-1}$). These conditions favoured lactic acid, being dominant at an encouraging average concentration of 21.70 $g \cdot L^{-1}$, notably without any process optimisation or control. *Lactobacillus* constituted the majority of the microbial community in the pre-fermentation stage (98.1-99.1% relative abundance) with an unknown *Lactobacillus* species and *L. reuteri* being the major species present. Grain processing waste and milk paste were positive influencers of LA concentration. The monitoring results, together with a simple economic evaluation, indicated that lactic acid recovery from a commercial food waste anaerobic digestion facility had baseline feasibility. In addition, there would be significant opportunities to increase economic performance by targeted process control and optimisation.



3.1 INTRODUCTION

In 2016-17, 76% of all food waste (FW) in Australia was landfilled, where it may anaerobically decay to emit fugitive methane (Pickin et al., 2018). Moreover, according to a study by the World Biogas Association, 8% of global annual anthropogenic greenhouse gas emissions (4.4 giga-tonnes of CO_2 eq.) originate from FW alone (WBA, 2018). Fortunately, FWs may instead be diverted away from landfill and, due to its nutrient-rich composition, has attracted increasing interest as a potential feedstock for biorefineries to produce chemicals, value-add materials, and fuels (Kwan et al., 2016).

Anaerobic digestion (AD) is a traditional technology that has been used to produce renewable biogas energy from FWs, as well as to produce a nutrient-rich digestate. However, high construction, operation, and management costs of AD can lead to questionable economic benefit (Kim et al., 2016). For this reason, commercial AD projects have been somewhat reliant on subsidies or policy incentives to remain profitable (Cucchiella & D'Adamo, 2016). AD can instead be utilised as a centrepiece technology within a larger integrated biorefinery, simultaneously producing various bioproducts (Surendra et al., 2015).

Lactic acid (LA) is one such bioproduct and has been identified as one of the twelve most promising chemical building blocks produced from sugars (Kwan et al., 2016). It is a highly versatile chemical with uses in the food, pharmaceutical, and cosmetic industries, and emerging applications including the production of biodegradable plastics (Lin & Wang, 2007). The potential application of LA is dependent on the isomeric form (L or D) as specific isomers may be preferred for certain applications, such as L-lactate for food and pharmaceutical industries (Alves de Oliveira et al., 2018).

Production of LA from various alternate sources, such as wood chips, rice straw, soybean vinasse, recycled paper sludge, and coffee pulp has also been explored as summarised by Alves de Oliveira et al. (2018). FW may be a particularly promising substrate for LA production due to its rich nutrient and carbohydrate content (Kim et al., 2003a; Kim et al., 2016), including lactose from dairy food waste. In the case of two-stage AD, LA could be produced via pre-fermentation of FW with the solid fermentation residues produced from extraction then undergoing subsequent AD processing (Demichelis et al., 2017; Kim et al., 2016).

The production of LA from FWs has been explored by various researchers and, while the reported concentrations vary (e.g. 58 g L^{-1} (Pleissner et al., 2017), 94 g L^{-1} (Kwan et al., 2016), 66.5 g L^{-1} (Demichelis et al., 2017), and 40 g L^{-1} (Kim et al., 2016)), high reported LA concentrations are encouraging and indicate baseline feasibility. LA recovery from FW fermentation broth has also been suggested to be technically feasible via various methods (Hu et al., 2017; Pleissner et al., 2017; Yousuf et al., 2016) and has been identified as potentially highly profitable (Kwan et al., 2015).

Recent literature has indicated that the production and recovery of LA, combined with subsequent AD of the fermented residue, can improve the overall revenue per tonne of FW processed by 122% - 180%, as compared to only processing by AD (Demichelis et al., 2017; Kim et al., 2016). LA production in combination with AD has also been shown to significantly outperform both sole AD (Bastidas-Oyanedel & Schmidt, 2018) and sole LA production from FWs (Demichelis et al., 2018). Whilst these reports are promising, they dealt with development of greenfield LA-AD biorefineries rather than integration of LA production into an existing FW AD facility. The use of existing infrastructure at existing AD facilities could significantly boost the profitability of biorefineries at such facilities.

Whilst previous studies identified LA production from FW as reasonable and potentially profitable, the impact of complex and highly variable FW feedstocks introduces significant uncertainty. For example, the feedstock mix of a commercial AD facility could vary with location, surrounding industries, climate, season, population density and socio-demographics, and government policy such as landfill waste diversion (Ghatak, 2011). While some FW ingredients would logically be promising feedstocks for LA production (e.g. milk and dairy by-products), other wastes may instead be detrimental to LA production. Tailored LA fermentation processes are typically operated at pH 5.0-6.0 and temperature 35-40 ^{o}C (Alves de Oliveira et al., 2018), and with short hydraulic retention times (HRTs) in continuous systems to promote LA accumulation (Komemoto et al., 2009; Tang et al., 2016). However, the pre-fermentation stage within a two-stage AD facility may function with limited control, with pH, temperature, HRT, and organic loading rate (OLR) being naturally determined by the feedstock and surrounding environmental conditions, themselves subject to variability. Understanding how complex and dynamic waste mixtures and operating conditions may influence LA production is of high importance to successfully integrate LA production into a mixed FW AD facility.

To inform future biorefinery concepts, the present study monitored organic acids production (including LA) in the pre-fermentation stage of a commercial AD plant. Analyses explored the effects of mixed feedstock composition and fermentation operating conditions on resulting LA concentrations. The results from the study were used together with a high-level techno-economic evaluation to assess baseline feasibility of integrating LA recovery into a commercial AD facility.

3.2 METHOD

3.2.1 The anaerobic digestion facility under study

The commercial AD facility under study was located in Western Australia (WA) and designed to process up to 50,000 tonnes year^{-1} of FW (source radius of 50 km). With an estimated 2.4 tonnes of FW produced per person in Perth (year 2014-15; (Spagnolo, 2019)), the plant capacity equates to roughly 20,800 inhabitants.

Waste is received both packaged and unpackaged, or in tankers. Unpackaged solid waste materials received are placed in large storage bins until use. These materials are fed, along with packaged FW, into a de-packager to remove any plastics and large non-organic contaminants (Fig. A1). All material fed into the de-packager is mixed with rainwater collected onsite at an approximate 1:1 volume ratio to produce a pumpable mixture (estimated dry matter at 15.7%). Packaged material that cannot be directly run through the de-packager is de-packaged by hand and then combined with other solid feedstock material. Packaged soft drink waste is pumped into a small storage vessel for subsequent use. Bulk liquid wastes, received in tankers are piped directly into a blending tank, with the connection port to tankers being located outside the main waste storage shed (Fig. A1). This waste blending tank is a 350 m^3 open-top steel tank, with a design liquid operating capacity of 290 m^3, to which the various wastes described above are fed in proportion to their availability. Mixing of the blending tank is automatically controlled by two propeller mixers operated approximately every hour. Temperature or pH in the blending tank are not controlled.

Following homogenisation of the waste in the blending tank, the feedstock mixture is fed into a fermentation reactor (pre-fermenter) for organic acid production by natural biological fermentation. No external reagents are added, and pH and temperature is not controlled. The reactor is a cylindrical 350 m^3 steel closed roof reactor, with a design liquid operating capacity of 290 m^3, and is periodically mixed with a single mounted

propeller mixer. The rate of feeding from the blending tank to the pre-fermenter is controlled based on blending tank liquid height; feeding at a rate of approximately 10 m^3 of waste every hour until the level in the blending tank reaches a pre-set minimum value, or the pre-fermenter is full. Standard operational procedures aims to fill the pre-fermenter over an operational week to provide a feed buffer for the downstream anaerobic digesters over the weekend when the facility is not supervised.

Subsequent methane fermentation occurs in two mesophilic anaerobic digesters (37 ^oC) operating in parallel (Fig. A1), each with a design HRT of 30 days and a design OLR of 2.3 $kg_{VS}.m^{-3}.day^{-1}$. The digesters are intermittently fed every hour and continuously mixed via Landia Gas Mix systems (Landia, Denmark). The temperature of the digester contents is continuously maintained utilising excess heat produced from onsite combined heat and power (CHP) engine generators running on biogas produced by the anaerobic digesters. The pH in the digesters is not controlled, but is monitored continuously using Knick Stratos MS A405 meters equipped with Knick SE571X/1-NMSN pH probes. The digesters typically operate at a pH close to 7.5 and, due to the nature of the feed material, operate at elevated ammonium concentrations (3383 - 4541 ppm total ammoniacal nitrogen over the 16 week monitoring period), typical of commercial FW digestion (Banks et al., 2011; Buhlmann et al., 2018; Serna-Maza et al., 2015).

3.2.1.1 Food waste feedstocks

During the monitoring period, the feed received by the AD facility typically consisted of shredded solid FW, such as food scraps, expired food from grocery stores, and organic waste from food processing plants. The process was also fed with a variety of liquid wastes, such as brewery and milk processing waste, which provided a significant amount of the process water required for preparation of the mixed feedstock pulp. Due to the large variety of waste materials received, feedstocks were sub-categorised into 11 broader categories as shown in Table 3.1.

3.2.1.2 Full-scale sampling, analysis and data collection

Routine sampling was conducted 2-3 times a week between 2nd November and 22nd February (i.e. summer in Australia). To obtain a representative sample, mixers for the blending tank or the pre-fermenter were first manually activated and allowed to run for a few minutes before sampling. Samples were taken from ports on the vessel walls approximately 0.5 m off the floor of the blending tank and pre-fermenter (Sample port 1

and 2; Fig. A1), and in each case, only after 3-4 L of liquid was discharged from the port prior to sampling. Unless otherwise stated, three samples were taken at each sampling event, at the start, middle, and end of the working day (8am - 4pm). The pH and temperature of the samples were measured immediately after sampling using a pre-calibrated pH meter (Model pH100A, YSI EcoSense®, USA). Samples were tested for total solids (TS) and volatile solids (VS) (See Section 3.2.2). Subsamples were centrifuged at 5,800 rpm for 15 minutes, filtered with a 0.45µm PES Millipore® filter and frozen for later analysis of volatile organic acids, including LA (See Section 3.2.2).

3.2.1.3 Online monitoring data collection

All online monitoring data was collected from a SCADA system, including; operating flow rates into the blending tank and out of the pre-fermenter, and tank liquid volumes (based on liquid heights), tank temperature and pH, biogas composition continuously measured online by an AwiFLEX COOL+ system (AWITE, Germany) and cross-checked for calibration weekly by an external laboratory. pH was intermittently monitored offline using the pre-calibrated pH probe mentioned in Section 3.2.1.2.

3.2.2 Analytical methods

TS and VS were measured according to Standard Methods (APHA, 1995). LA, succinic acid, acetic acid, propionic acid, and butyric acid were quantified via HPLC using standards prepared from high purity L-LA (purity >98%), acetic acid (purity >99%), propionic acid (purity >99.5%), butyric acid (purity >99%), and succinic acid (purity >98%), all purchased from Sigma (Sigma-Aldrich Corporation, USA). The HPLC was equipped with a UV-VIS detector set at 210 nm, Aminex® HPX-87H column (300 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA) and guard column with a Micro-Guard Cation H Cartridge installed (Bio-Rad Laboratories, Hercules, CA). Measurements were carried out using an aqueous mobile phase with 5 mM sulphuric acid at a constant flow rate of 0.6 ml/min and a column temperature of 50^{o}C.

To analyse for microbial community composition, samples on days 3, 52, and 112 were collected and immediately stored at -20 ^{o}C before DNA extraction. Methods for DNA extraction, amplification, and screening have been previously described elsewhere (Buhlmann et al., 2018). DNA sequencing was done on the Illumina® Mi-seq platform. A detailed description of the bioinformatics and PICRUSt analysis utilised to analyse

functional genes is presented elsewhere (Buhlmann et al., 2018). All genes were identified using the KEGG database (KEGG, 2022).

3.2.3 Statistical analyses

All statistical analyses were conducted in R (R Development Core Team, 2022). Where three replicate measurements were performed, the mean and 95% confidence intervals determined by a student t-test have been reported. Multiple linear regression models were used to explore the effect of various waste materials on the LA concentration from day 36-112 (when the blending tank was in operation (Section 3.3.2)). In each model, measured LA concentration within the vessel (i.e. blending tank or pre-fermenter) was modelled as the independent variable, and tonnages of the 11 distinct categories of feedstocks (Table 3.1) were 11 dependent/explanatory variables. In order to account and test for time lags between when a certain tonnage of material had been received and documented by the facility and when the same material might have influenced LA concentration, it was considered in the statistical model, the feedstock tonnage recorded a set nominal number of days prior to a LA concentration measurement. For example, a nominal zero time-lag implies that a feedstock was fed into a vessel without substantial delay after receival and had a near-immediate effect on measured LA concentration, whereas a nominal 1, 2, or 3-day time lag implies a delayed effect from a feedstock on measured LA concentration within a specific vessel (i.e. blending tank or pre-fermenter), whether by a delay before processing, by hydraulic lag (e.g. time taken for material to flow from blending tank into pre-fermenter) or by other mechanisms (e.g. delayed fermentation). The lags considered were 0, 1, and 2 days for the blending tank, and 1, 2, and 3 days for the pre-fermenter, generating three separate statistical models for each of the two vessels. A 0-day time lag was not considered for the pre-fermenter because the blending tank has a non-zero mean HRT. In order to avoid overfitting, the step() function in R was applied to sequentially remove parameters based on the model Akaike Information Criterion (AIC).

Table 3.1: Outline of waste material categories, minor compositional data, and anticipated effects on LA production

Feedstock name	Description/ composition	Estimated TS% (VS/TS ratio)	Anticipated effects on LA fermentation
WFS1	Out of date/rejected FW from supermarkets and manufacturers-Bread crumbs, crumbed chicken, "out of date" bread, general FW, fruit, vegetables, meat, dough, pies, salad. Highly variable composition, large variety of sources.	16-20% (0.935-0.964)	Beneficial-High carbohydrate and nutrient content (Kim et al., 2016; Komemoto et al., 2009; Tang et al., 2016)
Milk	Waste / "out of date" milk products received in bottles and pouches	11.6-15.0% (0.844-0.923) *	Beneficial - Native growth medium for LA bacteria. Carbohydrates and proteins required for LA production (Hati et al., 2018; Liptáková et al., 2017)
Milk Paste	Thick paste produced from milk product processing. Concentrated milk curdles	-	Beneficial - As directly above
Brewery liquid waste	Consisting of out of date or reject beer with liquid wastes produced from the flush or fermenting process.	4.0-5.8% (0.332-0.857) *	Unknown
Sugary liquid mix	High Sugar content liquid. Waste soft drink, "out of date" or reject packaged soft drink. Large variation in TS and VS. Occasionally mixed with other solid waste like popcorn.	5.4-45.8% (0.788-0.976) *	Beneficial-Contains pure sugars. However, may be nutrient deficient (Varsamis et al., 2017)
Grain processing waste	Composed of grain dust, spilt grain, straw and stalks.	-	Beneficial-Contains β-glucans, which improve growth and viability of microbes, and essential B vitamins (Han et al., 2019; Russo et al., 2012; Skrede et al., 2003)
Spent grain	Grain utilised in a micro-brewery producing craft beer	21.1-24.7% (0.951-0.953) (Bochmann et al.,	Potentially Beneficial-Sufficient nitrogen and carbohydrates must be available (Pejin et al., 2017)

Feedstock name	Description/ composition	Estimated TS% (VS/TS ratio)	Anticipated effects on LA fermentation
		2015; Bougrier et al., 2018)	
Restaurant scraps	Waste food from restaurants and cafes, including some compostable coffee cups and compostable plastics. Diverse variety. Material was sampled after being shredded by the de-packager onsite and after being mixed with site water	13.5-15.4% (0.893-0.909) *	Potentially Beneficial-Anticipated influence of the compostable fraction is unknown.
Liquid waste	FW having a very high water content. Waste material from food processing industries	3.2% (0.588) *	Beneficial-However, nutrients and carbohydrates are significantly more diluted compared to raw FW.
Bleaching Earth	Spent acid activated bentonite clay containing various fats and oils from cooking oil bleaching	-	Minimal- Predominantly spent clay not expected to be utilised during fermentation, but may cause sorption effects.
Unknown	Any material not categorised as above because its form or origin was unidentifiable. Macro-composition unknown	-	Unknown

* Indicates authors' own measurement

3.3 RESULTS AND DISCUSSION

3.3.1 Waste feedstocks received and processed

The quantity and types of wastes received during the monitoring period were variable (Fig. 3.1). As the biogas facility was designed to receive mixed FWs, it was not surprising that Waste Feedstock No. 1 (WFS1), restaurant scraps, and liquid wastes constituted the majority of the wastes regularly received by the facility (Fig. 3.1; Table A1). Sugary liquid mix contributed the second-largest tonnage of weekly waste received over the monitoring period, with deliveries remaining relatively regular. An exception was weeks 13 - 15, with no deliveries of sugary liquid mix. Other organic wastes (i.e. spent grain, grain processing waste, and brewery liquid wastes) were more irregular in supply, with varying tonnages over the 16 weeks period (Fig. 3.1). However, deliveries for the majority of the other wastes remained reasonably consistent (Fig. 3.1). An abnormally large delivery of milk was noted in week 11 (i.e. 58.7 tonnes).

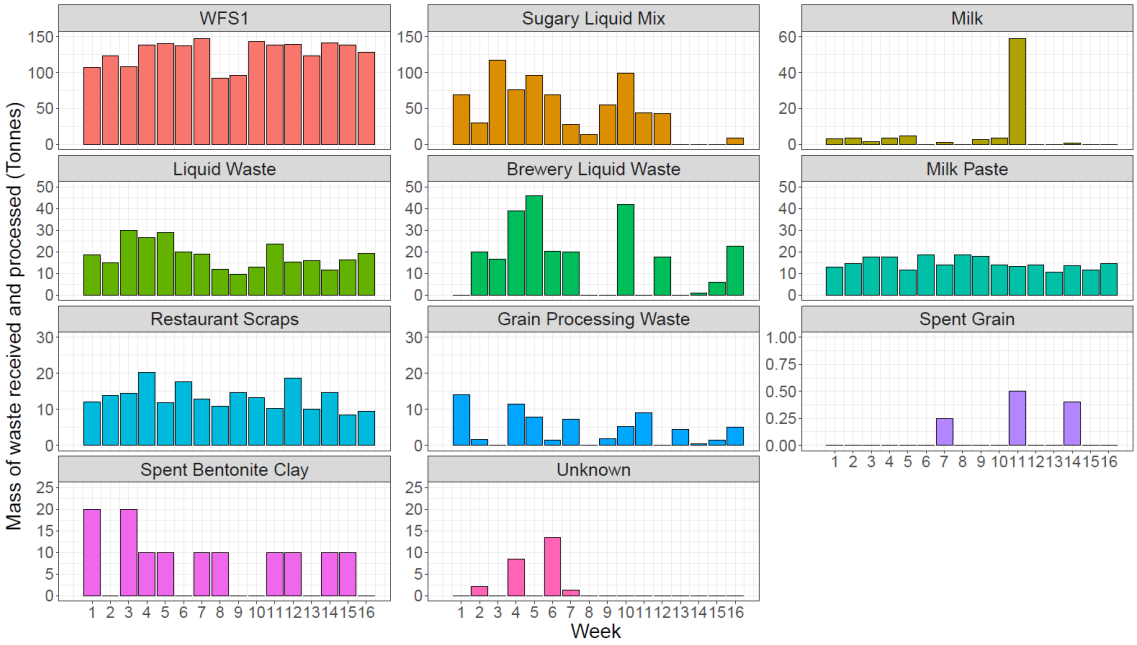


Figure 3.1: Weekly waste material received by the AD plant over the monitoring period. Waste Feedstock No. 1 (WFS1) consists of mixed food wastes. Note the y-axis scale changes with waste type.

Of the various wastes processed, the majority was anticipated to be beneficial for LA production (Table 3.1). WFS1, milk waste, milk paste, restaurant scraps, and the sugary liquid mix were of particular interest. For example, FWs have been shown to contain many of the essential nutrients and fermentable carbon sources required for LA production and for the proliferation of LA bacteria (Kim et al., 2003a), resulting in

reasonable LA concentrations 58.0 - 94.0 g L^{-1} (Demichelis et al., 2017; Kwan et al., 2016; Pleissner et al., 2017). LA bacteria can naturally proliferate in food products such as milk, meats, cereals, and vegetables (Kim et al., 2016). Moreover, milk contains many of the essential nutrients and carbohydrates required for LA production and would readily support the growth of LA bacteria (Hati et al., 2018; Liptáková et al., 2017). Concentrations of up to 143.70 g L^{-1} LA have been reported previously for fermentation of dairy wastes (Bernardo et al., 2016).

To the authors' knowledge, the fermentation of soft drinks for the production of LA has not been previously investigated. However, the sugars within Australian soft drinks, namely glucose (0.96 $g_{glucose}$/100 ml) and fructose (0.97 $g_{fructose}$/100 ml) (Varsamis et al., 2017), would be expected to be highly fermentable. Mixed fermentation with other nutrient-rich waste feedstocks could provide the essential nutrients for LA bacteria, such as nitrogen, vitamins and minerals (Alves de Oliveira et al., 2018), to overcome the nutrient-deficiency of sugary drink wastes mostly comprised of simple sugars in water.

3.3.2 Fermentation operating conditions

Both the blending tank and pre-fermenter displayed dynamic HRT variations (Fig. 3.2A), mainly due to fluctuations in the volume of waste received whilst maintaining a constant feed to the digesters. As both the blending tank and pre-fermenter operated at similar conditions, LA fermentation was likely occurring in both vessels, with the sum of the mean HRTs of both vessels being 3.6 days on average. An optimum HRT for LA production may vary depending on numerous factors such as waste composition, reactor design, and other operational parameters such as pH and temperature (Gu et al., 2018; Kim et al., 2003b; Tang et al., 2016). However, it is generally accepted that short HRTs tend to promote LA accumulation, whilst longer HRTs favour the conversion of LA to other organic acids (Choi et al., 2016; Tang et al., 2016). From when sampling commenced until day 36 of the monitoring period, the blending tank was taken offline to replace its internal lining. When the blending tank was brought back online, it remained fully operational for the remainder of the monitoring period (until day 112). Comparison of data collected with and without the blending tank in operation allowed some evaluation of the need for pre-homogenisation and its impact on LA production (See Section 3.3.3).

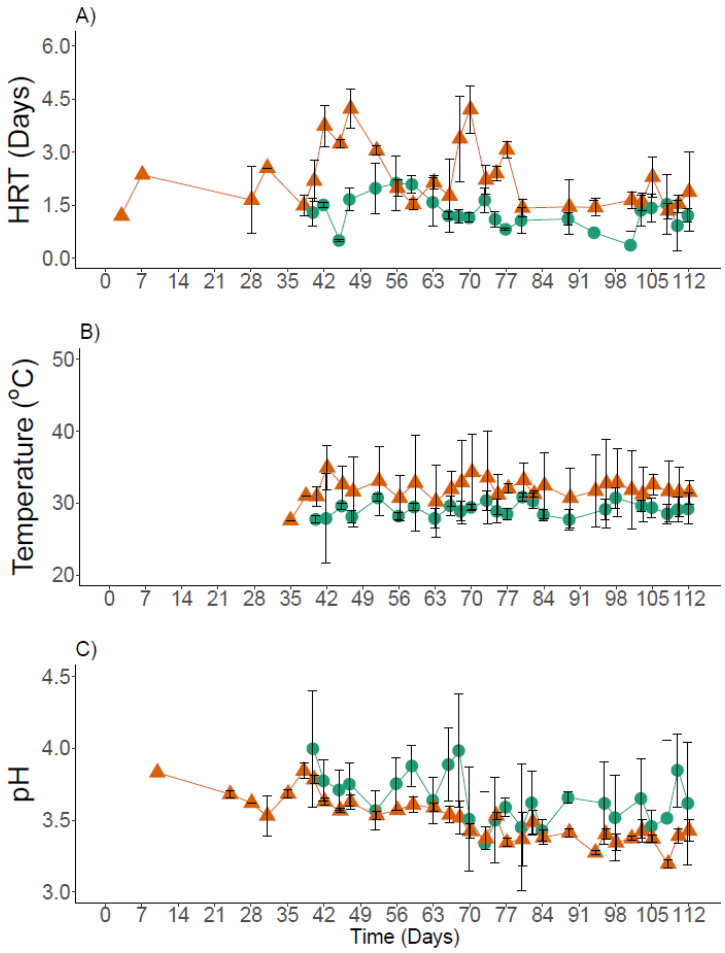


Figure 3.2: Process operating parameters of the blending tank (•) and pre-fermenter (▲). Values are expressed as the mean of triplicates ± the 95% confidence interval. Values without error bars are expressed as the mean of duplicates or as single values.

Both vessels followed similar trends for average temperature and pH (Fig. 3.2B/C); however, as the pre-fermenter was located outside a temperature-controlled waste storage shed (Fig. A1), daily temperature variations in the pre-fermenter were more notable than in the blending tank, reflecting the warm ambient climate of WA during summer. The average daily temperatures of the blending tank (29.0 ^{o}C) and pre-fermenter (32.0 ^{o}C) were at the lower end of the wide temperature range typically utilised for LA production (i.e. 30 - 50 ^{o}C; (Abdel-Rahman et al., 2013)). While the optimal temperature is dependent on microbial community (Kim et al., 2016; Tang et al., 2016), it is known that fermentation temperature directly impacts the maximum achievable LA concentration, influencing both the rate of LA production and the rate of LA conversion to other organic acids (Komemoto et al., 2009). Temperature control may assist in the stabilisation of a fermentation process, while a higher temperature (up to an optimum) would likely lead to increased LA productivity (Gulfam et al., 2017; Kim et al., 2016; Tang et al., 2016). The pH of the blending tank experienced more significant fluctuations than the pre-

fermenter. This was likely due to varying pH and composition of incoming waste stream mixtures, and dilution effects, which were not hydraulically buffered for the blending tank. In contrast, the feed to the pre-fermenter was hydraulically buffered by the blending tank. Overall, the pH of the two vessels remained low for the duration of the monitoring period, with the highest being pH 4 observed in the blending tank on days 40 and 68. pH would be a crucial operational parameter for optimisation and control to maximise LA yield and productivity as low operational pH can have inhibitory effects on cellular metabolism (Abdel-Rahman & Sonomoto, 2016). Accordingly, LA fermentation processes are typically conducted at a pH of 5.0 - 6.0 (Abdel-Rahman et al., 2013). However, higher pH may also increase competition between LA production and the production of other organic acids (Bo & Pin-jing, 2014; Gu et al., 2018; Wu et al., 2015). Maintaining slightly acidic pH conditions at higher operating temperatures has been shown to favour some LA producers (Kim et al., 2012; Kim et al., 2016). pH control strategies may improve LA productivities and yields from that observed in the current study (See Section 3.3.3), but should be further investigated in future fermentation studies with FW.

High concentrations of ammonia can be produced during the degradation of FW feedstocks (Serna-Maza et al., 2015). During the monitoring period, routine on-plant measurements by operators suggested that TAN concentrations in the pre-fermenter varied between 615 - 821 mg L^{-1}. Using the method outlined by Wang et al. (2017) to convert TAN to NH_3-N, the NH_3-N concentration was 0.0018 - 0.0024 mg L^{-1} at 32 ^oC and pH 3.5. These NH_3-N concentrations were well below the reported inhibitory threshold (Zhang et al., 2019), so the effects of TAN and NH_3-N on LA were not further considered in the current work.

TS and VS in the blending tank and pre-fermenter varied minimally over the monitoring period, averaging 15.7±0.7% TS and 13.9±0.6% VS for the blending tank, and 14.4 ± 0.5% TS and 12.2±0.3% VS for the pre-fermenter. The pre-fermenter OLR also remained relatively stable due to the hydraulic buffering provided by the blending tank. The average OLR in the pre-fermenter was 12.0 ± 2.4 $kg_{VS}\,m^{-3}\,day^{-1}$ between days 35 and 112 of the monitoring period. Utilised OLRs for LA production vary (Kim et al., 2012; Luongo et al., 2019; Tang et al., 2016), however, high OLRs generally favour the accumulation of LA over other organic acids (Bo & Pin-jing, 2014; Luongo et al., 2019). Maximising the OLR is preferred for commercial reasons by increasing FW throughput,

but may lead to reduced performance or process instability if a maximum tolerable OLR is exceeded in the fermentation process (Tang et al., 2016).

3.3.3 Organic acid production

Fig. 3.3 presents measured concentrations of various organic acids (including LA) within the blending tank and pre-fermenter. LA and acetic acid were dominant, with only minor observed quantities of propionic acid, butyric acid and succinic acid (Fig. 3.3).

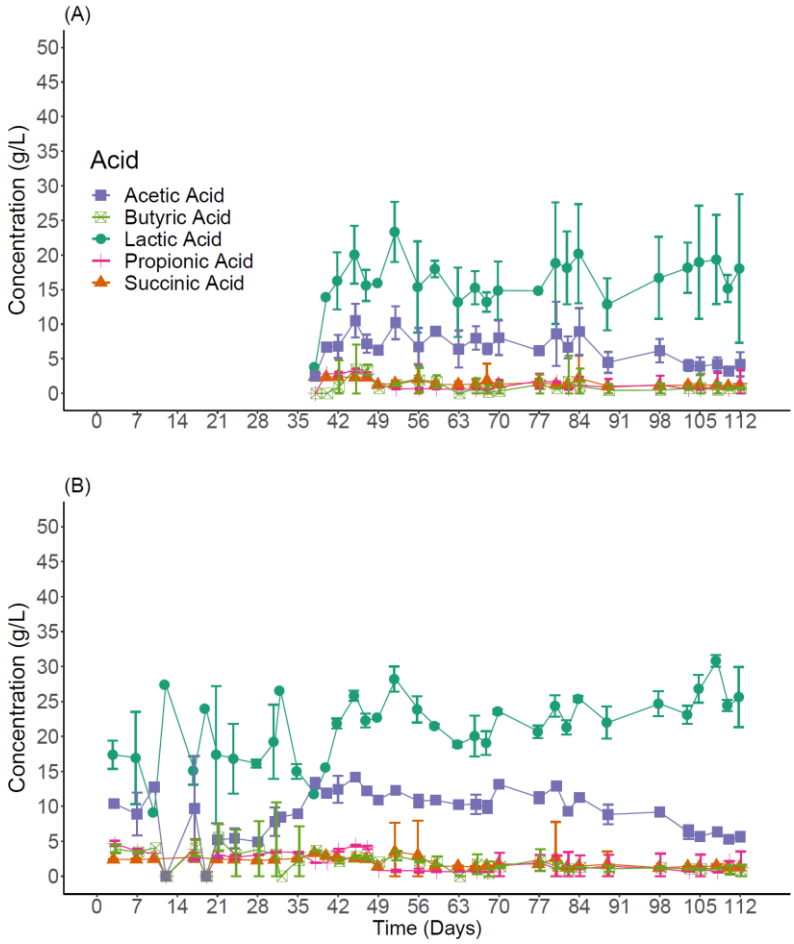


Figure 3.3: Measured concentration of organic acids within the A) Blending tank and B) Pre-Fermenter. Values are expressed as the mean ± 95% confidence interval. Values without error bars are expressed as the mean of duplicates or as single values.

Aside from the rapid changes in LA concentration experienced in the pre-fermenter between days 12 and 19, and again between days 31 and 35 (Fig. 3.3B), concentrations of the various organic acids were reasonably stable for both vessels during the monitoring period. This was despite the dynamic operating conditions of these vessels (Section 3.3.3.2). An observed increase in LA concentration between days 10 - 12 from 9.12 to 27.39 g L^{-1} may have been due to reduced daily feeding from 64.3 to 25.3 tonnes day^{-1}. Similarly, from day 17 to 19, LA concentration rose from 15.12 to 23.97 g L^{-1},

corresponding to a small reduction in feed by 13.0 tonnes day^{-1}. The same effects were observed between days 31 and 35. However, once the blending tank was brought back online (from day 36), LA in the pre-fermenter appeared to stabilise (Fig. 3.3B), resulting in an average yield of 0.047 ± 0.005 g_{LA}/g_{VS} in the pre-fermenter between 42 - 110 days. An improvement in the LA selectivity was also experienced following the reintroduction of the blending tank, increasing from 43.2% between days 36 - 42, to 74.2% by the end of the monitoring period. Reduced daily feeding variations combined with reduced dilution effects introduced by the blending tank likely aided process stability in the pre-fermenter. This indicates the overall benefits of upfront flow and composition buffering by the blending tank at the monitored facility.

3.3.4 Microbial composition analysis

Although microbial community dynamics couldn't be fully explored with only a limited number of microbial analyses for days 3, 52, and 112, the analysis did provide a measure of community diversity and the PICRUSt analysis an indication of potential functional gene pathways for LA production. The results indicated that *Lactobacillus* was the dominant genus within the pre-fermenter, accounting for 98.1% - 99.1% relative abundance for the total microbial population over the three samples (data not shown). The proliferation of *Lactobacillus* could have been facilitated by the low operating pH in the pre-fermenter (Bonk et al., 2017; Tang et al., 2017; Wu et al., 2015). At species level, an unknown *Lactobacillus* species and *Lactobacillus reuteri* represented 67% - 88% of all *Lactobacillus* species (Fig. A2). *L. reuteri* was not intentionally seeded into the blending tank or pre-fermenter during the start-up of the commercial facility and likely originated from one of the feedstocks (considering moderate background LA concentrations in some feedstocks (Table A2)) and considering the rapid establishment of the blending tank once brought back online (Fig. 3.3A). It would not be possible to reliably identify a feedstock inoculant source due to the feedstock variability of the commercial facility. Some *L. reuteri* strains are known to produce reuterin, a broad-spectrum anti-microbial compound (Morita et al., 2008) which, if produced by this species, may have worked in cooperation with the low pH conditions to maintain the low microbial community diversity observed in this study. In addition, Pancheniak et al. (2012) identified a *L. reuteri* strain which was aciduric, growing better under uncontrolled and low pH conditions, which may have been an additional factor contributing to the dominance of *L. reuteri* in the studied fermenter.

Utilising the KEGG database (KEGG, 2022), and the results from the PICRUSt analysis, relevant functional genes were identified. *L. reuteri* is an obligate heterofermentative bacteria, producing ethanol, acetic acid, carbon dioxide and LA during glucose fermentation (Morita et al., 2008), which was anticipated due to the coproduction of both LA and acetic acid in the current study (Fig. 3.3B). The PICRUSt analysis indicated the presence of hetero-fermentative bacteria due to the presence of phosphoketolase, a key gene required for the phosphoketolase pathway (PKP) (Alves de Oliveira et al., 2018). Fructose 1,6-diphosphate aldolase was also identified, a key gene required in the Embden-Meyerhof-Parnas pathway (EMP) utilised for homofermentative LA production (Alves de Oliveira et al., 2018). Årsköld et al. (2008) found that both the EMP and PKP were utilised by a strain of *L. reuteri*, however, the main flux was through the PKP. While the optical purity was not assessed in the current study, the presence of both L-lactate dehydrogenase and D-lactate dehydrogenase suggests that both lactate stereoisomers could have been produced in this uncontrolled commercial scale system. Future studies should aim to clarify optical purity, including the ability to control optical purity by operator intervention.

3.3.5 Statistical analysis

For the blending tank, there was no correlation between LA concentration and any of the waste materials introduced on earlier days (Table 3.2). However, four variables were retained in the simplified model testing for the effect of recorded waste material tonnages on LA concentration measured on the same day. Note that materials not retained in the simplified model may have had native LA concentrations (Table A2) close to the average measured LA concentration in the blending tank, while for the fermenter, material not included was thought to drive the LA concentration to the average concentration of the vessel (intercept; Table 3.2). Otherwise, a received mass of a particular waste may not have been large enough to have a significant net impact on LA.

Bleaching earth was positively correlated with LA concentration in the blending tank (Table 3.2), which could be due to the release of some native adsorbed LA from its surface (Zakaria et al., 2009). The simplified model also suggested that milk paste had a net positive effect on LA concentration, however, restaurant scraps were negatively correlated with LA. The fact that these were retained in the simplified model, based on AIC, but were not significant according to a marginal t-test, suggests that these

components may be interacting with each other or that the results should be interpreted with caution. The milk paste could have contained native LA produced by fermentation during transport and storage, as milk can natively contain LA bacteria (Widyastuti et al., 2014). Similarly, FWs within restaurant scraps were expected to also be highly fermentable during storage and transport, including to produce LA (Gu et al., 2018; Tang et al., 2016). However, the LA concentration within the restaurant scraps (Table A2) may have been below the average LA concentration in the blending tank (Table 3.2; intercept), causing a dilution effect on LA and a net negative influence in the statistical model (Table 3.2). The Unknown waste material appeared to have a significant, but negative influence on LA concentration within the blending tank, for reasons that could not be identified.

Table 3.2: Statistical model output for the blending tank and pre-fermenter, indicating significance and size of the influence on base lactic acid concentration (intercept).

Tank	Time lag	Model R^2	Parameter	Coefficient estimate [a]
Blending tank	0 days	0.5868	Intercept	16.4787 ***
			Bleaching Earth	0.4088 ·
			Milk Paste	0.4173 ·
			Restaurant Scraps	-0.6091 ·
			Unknown	-0.4005 **
	1 day	-	-	No significant predictors found
	2 days	-	-	No significant predictors found
Pre-Fermenter	1 day	-	-	No significant predictors found
	2 days	0.1433	Intercept	23.0674 ***
			Unknown	-0.7071 ·
	3 days	0.4878	Intercept	20.9411 ***
			Brewery Liquid Waste	0.2808 ·
			Grain processing waste	1.4606 **
			Milk	-2.8107 ·
			Milk Paste	0.7234 *
			Restaurant Scraps	-0.5222 ·

[a]: Superscripts represent significance according to a marginal t-test, (.) $P < 0.1$, (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$

For the pre-fermenter, the model with a 2-day time lag indicated that the Unknown waste material had a negative impact on the LA concentration; however, no correlations with any of the other waste materials were apparent. The 3-day time lag model suggested that grain processing waste was a significant positive influencer of LA concentration. LA fermentation of grain has been shown to increase the solubility of β-glucans, which can improve the growth and viability of probiotic microbes after a few hours of fermentation (Russo et al., 2012; Skrede et al., 2003). The inhibitory conditions caused by low pH, and

low biodegradability of the material, may have reduced the rate at which β-glucans were released or utilised, potentially leading to a delayed positive response after grain waste addition. Additionally, wheat straw has been shown to contain various B vitamins which are known to be required during LA fermentation (Han et al., 2019), even though it is expected to be difficult to break down itself. With a modelled 3-day time lag, milk paste had a positive net effect on LA concentration. As outlined in Table 3.1, milk paste mainly consisted of milk curdles, which were agglomerated proteins, and waste milk. As many LA bacteria have limited capability to synthesise amino acids (Alves de Oliveira et al., 2018), the addition and delayed hydrolysis of these concentrated proteins may have provided additional amino acids required by LA bacteria, allowing for more efficient LA production and bacterial growth. Milk, brewery liquid waste, and restaurant scraps were also retained in the simplified model, even though they were not significant according to the marginal t-test. As mentioned previously (Section 3.3.1), restaurant scraps were expected to be beneficial for LA fermentation, being potentially capable of producing high LA titres. However, as in the case of the blending tank, the statistical model suggested a net negative influence of restaurant scraps on LA concentration in the pre-fermenter (Table 3.2). The cause for this predicted negative response is unknown, but may have been partly due to large quantities of spent coffee grains, which may require pre-treatment prior to LA fermentation (Hudeckova et al., 2018). Separation of this waste stream during collection and direct utilisation in AD for biogas production may aid LA fermentation. However, this would require future testing to confirm the extent of the benefit from targeted side-stream fermentation.

Whilst milk is logically an ideal growth medium for LA bacteria (Liptáková et al., 2017), with high quantities of lactose and proteins (Hati et al., 2018), milk was identified as a net negative influencer of LA concentration in the pre-fermenter. Whilst somewhat unexpected, the effect of milk may have been cross-correlated with the effect of other feedstocks and the volume of milk received and processed was noted to be relatively small as compared to that of other waste materials that were processed (Fig. 3.1). No significant effects were found when a time lag of 1-day was implemented for the pre-fermenter model (Table 3.2), likely due to the HRT of the blending tank, on average, being longer than one day (1.45 days). Overall, the statistical model results should be considered as indicative and preliminary, but may indicate interesting net feedstock influences that could be further explored in future studies.

3.3.6 Feasibility of integrating LA production into an existing FW AD facility

Overall, the stable observed LA production for much of the monitoring period was somewhat surprising and encouraging, considering the variability in feed composition (Section 3.3.1), low fermentation pH (Section 3.3.2), and uncontrolled temperature and HRT (Section 3.3.2) (Fig. 3.2). The recovery of LA from fermentation broths of FW has been shown to be technically feasible by various methods (Hu et al., 2017; Pleissner et al., 2017; Yousuf et al., 2016). In the current work, a high-level simple economic evaluation was conducted on a facility to better understand potential economic benefits of LA recovery. While it is anticipated that LA recovery would likely reduce the biomethane potential of the liquid fraction, revenue losses due to reduced biogas yields were not considered in the simple economic evaluation. A previous study has suggested that LA fermentation increased the methane produced from FW compared to direct utilisation in AD (Demichelis et al., 2017). Future studies should consider the overall net effect LA recovery would have on methane production following LA fermentation.

Using an average LA concentration of 23.4 g L^{-1} measured during a stable period of monitoring (42 - 112 days) (Section 3.3.3), an average combined HRT of 3.59 days (blending tank + pre-fermenter), and average combined volume of 269.5 m^3, a LA production rate of 1.8 tonne day^{-1} was estimated. Assuming an overall LA recovery efficiency of 51.1%, as has been observed for a combination of ultrafiltration, electrodialysis and multi-effect vacuum evaporation (Demichelis et al., 2018) and an estimated LA production cost of 0.83 USD kg^{-1}_{LA} (1.11 AUD kg^{-1}_{LA}) (Jantasee et al., 2017), a production rate of 1.02 tonne $_{88wt\%\ LA}$ day^{-1} would result in a corresponding production cost of 0.36 million AUD $year^{-1}$. Assuming a LA value of 2.18 AUD kg^{-1} $_{88\ wt\%\ LA}$ (Demichelis et al., 2018), an estimated conservative value of 0.45 million AUD $year^{-1}$ (0.32 million USD $year^{-1}$) could be generated from LA recovery alone at the studied AD plant. The simultaneous recovery and utilisation of biogas energy produced by the facility could further reduce production costs and enhance profitability.

The monitoring data did indicate that the system under study may have been underperforming, with other fine-tuned systems able to achieve higher LA titres of 33.8 - 40 g L^{-1} through process optimisation and control (Kim et al., 2016; Tang et al., 2016; Wang et al., 2005). LA production could also benefit from utilising fungal hydrolysis in a separate hydrolysis and fermentation system, potentially yielding 94.0 g L^{-1} (Kwan et

al., 2016). Assuming that the current process can achieve a higher titre of 40.0 g L^{-1} through process control, an estimated 0.77 million AUD $year^{-1}$ (0.55 million USD $year^{-1}$) may instead be generated, utilising the same feedstock. These estimates are in line with similar high level economic assessments conducted by others on similar biorefinery processes (Demichelis et al., 2017; Kim et al., 2016). Lastly, it is important to note that the blending tank and pre-fermenter in the current study were considered to be underloaded hydraulically, operating at approximately half of their combined volumetric capacity (580 m^3). Accordingly, LA production at the facility could have been doubled, if feedstock was available to maintain the same HRT and organic loading rate at twice the liquid hold-up volume in these vessels, provided that stable performance of the downstream anaerobic digesters could be maintained.

3.4 CONCLUSION

The fermentation system at the commercial facility under study performed well in terms of LA production during the monitoring period, averaging a LA concentration of 21.70 g L^{-1}. Fermentation pH remained low (3.45 ± 0.025), while temperature and HRT fluctuated. *Lactobacillus* constituted the majority of the microbial community (98.1% - 99.1%). The statistical model indicated grain processing waste and milk paste were the leading positive influencers of LA concentration. A high-level economic evaluation suggested there is economic potential to integrate LA recovery into the current AD infrastructure, albeit a more detailed cost-benefit analysis is needed. Fermentation tests on mixed complex food wastes with optimised process conditions and feed composition are recommended. The results and simple economic evaluation indicated that integration of LA recovery into an existing commercial FW AD facilities would be feasible, and that economic performance would benefit from process optimisation and control.

3.5 REFERENCES

Abdel-Rahman, M.A., Sonomoto, K. 2016. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. Journal of biotechnology, 236, 176-192.

Abdel-Rahman, M.A., Tashiro, Y., Sonomoto, K. 2013. Recent advances in lactic acid production by microbial fermentation processes. Biotechnology Advances, 31(6), 877-902.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From

economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

APHA. 1995. Standard methods for the examination of water and wastewater. American Public Health Association American Water Works Association/Environmental Federation, Washington, DC.

Årsköld, E., Lohmeier-Vogel, E., Cao, R., Roos, S., Rådström, P., Ed, W.J.v.N. 2008. Phosphoketolase Pathway Dominates in Lactobacillus reuteri ATCC 55730 Containing Dual Pathways for Glycolysis. Journal of Bacteriology, 190(1), 206-212.

Banks, C.J., Chesshire, M., Heaven, S., Arnold, R. 2011. Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance. Bioresource Technology, 102(2), 612-620.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

Bernardo, M.P., Coelho, L.F., Sass, D.C., Contiero, J. 2016. l-(+)-Lactic acid production by Lactobacillus rhamnosus B103 from dairy industry waste. Brazilian Journal of Microbiology, 47(3), 640-646.

Bo, Z., Pin-jing, H. 2014. Performance assessment of two-stage anaerobic digestion of kitchen wastes. Environmental Technology, 35(10), 1277-1285.

Bochmann, G., Drosg, B., Fuchs, W. 2015. Anaerobic digestion of thermal pretreated brewers' spent grains. Environmental Progress & Sustainable Energy, 34(4), 1092-1096.

Bonk, F., Bastidas-Oyanedel, J.-R., Yousef, A.F., Schmidt, J.E. 2017. Exploring the selective lactic acid production from food waste in uncontrolled pH mixed culture fermentations using different reactor configurations. Bioresource Technology, 238, 416-424.

Bougrier, C., Dognin, D., Laroche, C., Cacho Rivero, J.A. 2018. Use of trace elements addition for anaerobic digestion of brewer's spent grains. Journal of Environmental Management, 223, 101-107.

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum: Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

Choi, G., Kim, J., Lee, C. 2016. Effect of low pH start-up on continuous mixed-culture lactic acid fermentation of dairy effluent. Applied Microbiology and Biotechnology, 100(23), 10179-10191.

Cucchiella, F., D'Adamo, I. 2016. Technical and economic analysis of biomethane: A focus on the role of subsidies. Energy Conversion and Management, 119, 338-351.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization

through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Ghatak, H.R. 2011. Biorefineries from the perspective of sustainability: Feedstocks, products, and processes. Renewable and Sustainable Energy Reviews, 15(8), 4042-4052.

Gu, X.Y., Liu, J.Z., Wong, J.W.C. 2018. Control of lactic acid production during hydrolysis and acidogenesis of food waste. Bioresource Technology, 247, 711-715.

Gulfam, A., Guo, G., Tajebe, S., Chen, L., Liu, Q., Yuan, X., Bai, Y., Saho, T. 2017. Characteristics of lactic acid bacteria isolates and their effect on the fermentation quality of Napier grass silage at three high temperatures. Journal of the Science of Food and Agriculture, 97(6), 1931-1938.

Han, X., Li, L., Wei, C., Zhang, J., Bao, J. 2019. Facilitation of l-Lactic Acid Fermentation by Lignocellulose Biomass Rich in Vitamin B Compounds. Journal of Agricultural and Food Chemistry, 67(25), 7082-7086.

Hati, S., Patel, N., Mandal, S. 2018. Comparative Growth Behaviour and Biofunctionality of Lactic Acid Bacteria During Fermentation of Soy Milk and Bovine Milk. Probiotics and Antimicrobial Proteins, 10(2), 277-283.

Hu, Y., Kwan, T.H., Daoud, W.A., Lin, C.S.K. 2017. Continuous ultrasonic-mediated solvent extraction of lactic acid from fermentation broths. Journal of Cleaner Production, 145, 142-150.

Hudeckova, H., Neureiter, M., Obruca, S., Frühauf, S., Marova, I. 2018. Biotechnological conversion of spent coffee grounds into lactic acid. Letters in Applied Microbiology, 66(4), 306-312.

Jantasee, S., Kienberger, M., Mungma, N., Siebenhofer, M. 2017. Potential and assessment of lactic acid production and isolation – a review. Journal of Chemical Technology & Biotechnology, 92(12), 2885-2893.

KEGG. 2022. Kyoto Encyclopedia of Genes and Genomes.

Kim, D.-H., Lim, W.-T., Lee, M.-K., Kim, M.-S. 2012. Effect of temperature on continuous fermentative lactic acid (LA) production and bacterial community, and development of LA-producing UASB reactor. Bioresource Technology, 119, 355-361.

Kim, K.I., Kim, W.K., Seo, D.K., Yoo, I.S., Kim, E.K., Yoon, H.H. 2003a. Production of Lactic Acid from Food Wastes. in: Biotechnology for Fuels and Chemicals: The Twenty-Fourth Symposium, (Eds.) B.H. Davison, J.W. Lee, M. Finkelstein, J.D. McMillan, Humana Press. Totowa, NJ, pp. 637-647.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Kim, M., Gomec, C.Y., Ahn, Y., Speece, R.E. 2003b. Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. Environmental Technology, 24(9), 1183-1190.

Komemoto, K., Lim, Y.G., Nagao, N., Onoue, Y., Niwa, C., Toda, T. 2009. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Management, 29(12), 2950-2955.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2016. Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with Lactobacillus casei Shirota. Bioresource Technology, 217, 129-136.

Kwan, T.H., Pleissner, D., Lau, K.Y., Venus, J., Pommeret, A., Lin, C.S.K. 2015. Techno-economic analysis of a food waste valorization process via microalgae cultivation and co-production of plasticizer, lactic acid and animal feed from algal biomass and food waste. Bioresource technology, 198, 292-299.

Lin, H.-T., Wang, F.-S. 2007. Optimal design of an integrated fermentation process for lactic acid production. AIChE Journal, 53(2), 449-459.

Liptáková, D., Matejčeková, Z., Valík, Ľ. 2017. Lactic Acid Bacteria and Fermentation of Cereals and Pseudocereals, IntechOpen.

Luongo, V., Policastro, G., Ghimire, A., Pirozzi, F., Fabbricino, M. 2019. Repeated-Batch Fermentation of Cheese Whey for Semi-Continuous Lactic Acid Production Using Mixed Cultures at Uncontrolled pH. Sustainability, 11(12).

Morita, H., Toh, H., Fukuda, S., Horikawa, H., Oshima, K., Suzuki, T., Murakami, M., Hisamatsu, S., Kato, Y., Takizawa, T., Fukuoka, H., Yoshimura, T., Itoh, K., O'Sullivan, D.J., McKay, L.L., Ohno, H., Kikuchi, J., Masaoka, T., Hattori, M. 2008. Comparative Genome Analysis of Lactobacillus reuteri and Lactobacillus fermentum Reveal a Genomic Island for Reuterin and Cobalamin Production. DNA Research, 15(3), 151-161.

Pancheniak, E.d.F.R., Maziero, M.T., Rodriguez-León, J.A., Parada, J.L., Spier, M.R., Soccol, C.R. 2012. Molecular characterisation and biomass and metabolite production of Lactobacillus reuteri LPB P01-001: A potential probiotic. Brazilian journal of microbiology, 43(1), 135-147.

Pejin, J., Radosavljević, M., Kocić-Tanackov, S., Mladenović, D., Djukić-Vuković, A., Mojović, L. 2017. Fed-batch l-(+)-lactic acid fermentation of brewer's spent grain hydrolysate. Journal of the Institute of Brewing, 123(4), 537-543.

Pickin, J., Randell, P., Trinh, J., Grant, B. 2018. National Waste Report 2018.

Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, 143, 615-623.

R Development Core Team. 2022. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.

Russo, P., López, P., Capozzi, V., de Palencia, P.F., Dueñas, M.T., Spano, G., Fiocco, D. 2012. Beta-glucans improve growth, viability and colonization of probiotic microorganisms. International journal of molecular sciences, 13(5), 6026-6039.

Serna-Maza, A., Heaven, S., Banks, C.J. 2015. Biogas stripping of ammonia from fresh digestate from a food waste digester. Bioresource Technology, 190, 66-75.

Skrede, G., Herstad, O., Sahlstrøm, S., Holck, A., Slinde, E., Skrede, A. 2003. Effects of lactic acid fermentation on wheat and barley carbohydrate composition and production performance in the chicken. Animal Feed Science and Technology, 105(1), 135-148.

Spagnolo, J. 2019. War on waste: Three-bin strategy for Perth to boost recycling by 2025. in: PerthNow, Seven West Media (WA). PerthNow.

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., Khanal, S.K. 2015. Anaerobic digestion-based biorefinery for bioenergy and biobased products. Industrial Biotechnology, 11(2), 103-112.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y. 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management, 52, 278-285.

Tang, J., Wang, X.C., Hu, Y., Ngo, H.H., Li, Y. 2017. Dynamic membrane-assisted fermentation of food wastes for enhancing lactic acid production. Bioresource Technology, 234, 40-47.

Varsamis, P., Larsen, R.N., Dunstan, D.W., Jennings, G.L.R., Owen, N., Kingwell, B.A. 2017. The sugar content of soft drinks in Australia, Europe and the United States. Medical Journal of Australia, 206(10), 454-455.

Wang, Q., Wang, X., Wang, X., Ma, H., Ren, N. 2005. Bioconversion of Kitchen Garbage to Lactic Acid by Two Wild Strains of Lactobacillus Species. Journal of Environmental Science and Health, Part A, 40(10), 1951-1962.

Wang, W., Ren, X., Yang, K., Hu, Z., Yuan, S. 2017. Inhibition of ammonia on anaerobic digestion of synthetic coal gasification wastewater and recovery using struvite precipitation. Journal of Hazardous Materials, 340, 152-159.

WBA. 2018. Global Food Waste Management: An Implementation Guide For Cities.

Widyastuti, Y., Rohmatussolihat, Febrisiantosa, A. 2014. The Role of Lactic Acid Bacteria in Milk Fermentation. Food and Nutrition Sciences, 5(4), 435-442.

Wu, Y., Ma, H., Zheng, M., Wang, K. 2015. Lactic acid production from acidogenic fermentation of fruit and vegetable wastes. Bioresource Technology, 191, 53-58.

Yousuf, A., Bonk, F., Bastidas-Oyanedel, J.-R., Schmidt, J.E. 2016. Recovery of carboxylic acids produced during dark fermentation of food waste by adsorption on Amberlite IRA-67 and activated carbon. Bioresource Technology, 217, 137-140.

Zakaria, R.M., Hassan, I., El-Abd, M.Z., El-Tawil, Y.A. 2009. Lactic acid removal from wastewater by using different types of activated clay. Thirteenth International Water Technology Conference, Hurghada, Egypt.

Zhang, C., Yang, H.-Q., Wu, D.-J. 2019. Study on the reuse of anaerobic digestion effluent in lactic acid production. Journal of Cleaner Production, 239, 118028.

CHAPTER 4 FERMENTATION OPTIMISATION

Foreword

Chapter 3 explored LA production performance at a commercial two-stage FW AD facility. While it was identified LA production was relatively stable, and encouragingly high LA concentrations were produced from FW, fermentation would likely benefit from targeted optimisation and control. The current chapter explores the influence of pH and temperature control on LA production from a synthetic FW feedstock. Furthermore, the study utilised an adapted industrial inoculum, obtained from the pre-fermenter monitored in Chapter 3, to explore the influence of these conditions on the microbial community, product spectrum, and metabolic pathways for LA production.

The following chapter has been drafted in accordance with the Journal of Cleaner Production.

The current manuscript is published

Bühlmann, C.H., Mickan, B.S., Tait, S., Batstone, D.J., Mercer, G.D., Bahri, P.A., 2022. Lactic acid from mixed food waste fermentation using an adapted inoculum: Influence of pH and temperature regulation on yield and product spectrum. Journal of Cleaner Production, 133716.

The following authors contributed to this manuscript as outlined below.

Authorship order	Contribution (%)	Concept Development	Data Collection	Data Analyses	Drafting of manuscript
Christopher H. Buhlmann	75	X	X	X	X
Prof. Parisa A. Bahri	10	X		X	
Dr Bede S. Mickan	5	X		X	
Dr Stephan Tait	5	X		X	
Dr Damien Batstone	3.5	X		X	
George D. Merger	1.5		X		

Contribution indicates the total involvement each author has had in this project. Placing an 'X' in the remaining boxes indicates which aspect(s) of the project each author engaged in.

By signing this document, the Candidate and Principal Supervisor acknowledge that the above information is accurate and has been agreed to by all other authors.

Candidate **Principal Supervisor**

Journal Metrics (as of June 2022)

Journal Name: Journal of Cleaner Production

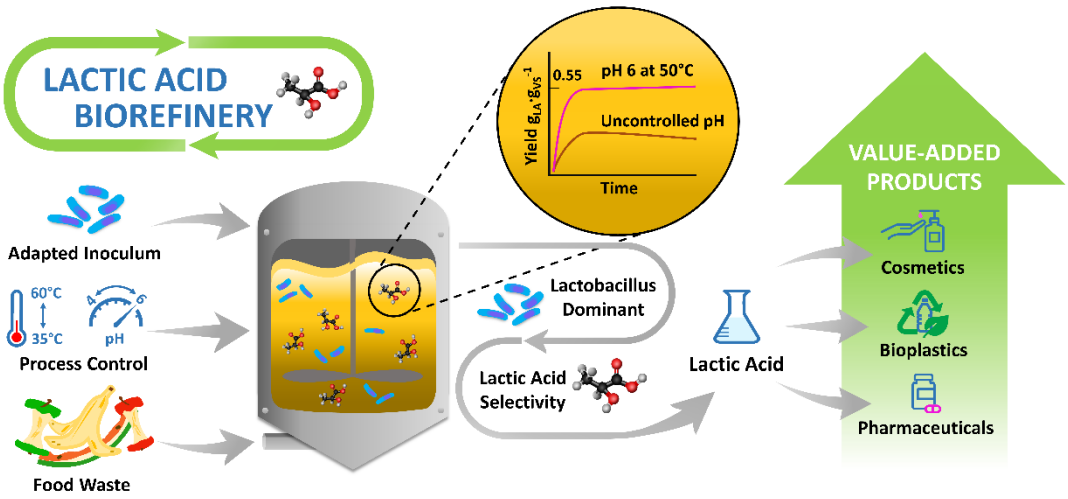
Peer Reviewed: Yes

Impact Factor: 11.072

Cite Score: 15.8

ABSTRACT:

Environmental conditions (pH and temperature) are expected to influence microbial community composition and product spectrum in mixed-culture food waste (FW) fermentation. However, some conditions may favour growth of multiple organisms that compete for common substrates or consume target metabolites. The inoculum plays an integral role in mixed-culture fermentation, but it is currently unknown how an adapted inoculum, known to selectively produce the target metabolite, would influence fermentation, and how environmental conditions could control fermentation outcomes. Therefore, this study assessed the effects of pH (uncontrolled vs. controlled pH 4-6) and temperature (35-60°C) on lactic acid (LA) from synthetic mixed FW batch fermentation (80 $g_{VS} \cdot L^{-1}$) utilising an adapted fermentation inoculum known to produce significant LA (10% inoculum volume). Concentrations of LA and competing organic acids were measured. Uncontrolled pH encouraged *Lactobacillus* growth but resulted in a low LA yield due to inhibitory conditions. Controlled pH 6 improved LA production but introduced LA consumption and competitive butyrate production. Observed butyrate production was dependent on pH and temperature and correlated with the growth of *Clostridium Sensu Stricto 12*. At pH 6 and 50°C, observable LA consumption was eliminated, and the LA yield was maximised at 0.55 $g_{LA} \cdot g_{VS}^{-1}$ (39 $g_{LA} \cdot L^{-1}$) while *Lactobacillus* remained dominant. The adapted inoculum effectively promoted LA production, while pH and temperature regulation were effective control levers to target LA.



4.1 INTRODUCTION

Food waste (FW), which is defined as the loss of any healthy edible substances such as fruit, meat, vegetables, and dairy products, occurring along the food production and supply chain (Braguglia et al., 2018). Estimates have placed global yearly FW production at 1.3-1.6 billion tonnes and have associated FW with an economic loss of USD990 billion, and production of 3.3 billion tonnes of CO2 eq. emissions (Demichelis et al., 2018). In an attempt to reduce global FW production, the United Nations have set a goal to halve the per-capita FW at the consumer and retail levels by 2030 (UN, 2019). However, unavoidable losses occur during the processing stage for some food products, such as milk, where losses may occur during pasteurisation, and production of cheese and yogurt, or losses from industrial processing and packaging of fish (Ishangulyyev et al., 2019). It is therefore desirable to reclaim value from unavoidable FW via processing and biorefining concepts.

Anaerobic mixed-culture fermentation is a prospective technology with widespread commercial applications and has the capability to recover value from FWs. Lactic acid (LA) is one possible product from FW fermentation (Kim et al., 2016; Tang et al., 2016), and is a high-value reagent used in numerous industrial applications including in the food and beverage sector, pharmaceutical and chemical industries, and in the production of biodegradable plastics (Demichelis et al., 2017). The utility of LA is dependent on the specific isomeric form (L or D) as certain industries may require a specific isomer. For example, food and pharmaceutical industries generally requires L-LA, as D-LA in high dosages can be harmful to humans (Alves de Oliveira et al., 2018). As the food industry demands the majority of LA currently produced (~85%) (Ahmad et al., 2020), L-LA is generally the target isomer for LA fermentation.

While LA production from FW has been previously explored (Table 4.1), mixed FW fermentation is still challenged by a low L-LA optical selectivity and low LA yield (Zhang et al., 2020b). This is, at least partly, due to the competitive uptake of substrate and LA by mixed-cultures for alternate metabolic products. LA is primarily viewed as an intermediate in fermentation which is consumed following substrate depletion (Hoelzle et al., 2021; Regueira et al., 2021; Rombouts et al., 2020) or via the growth of microbes that take up LA as substrate (Feng et al., 2018; Rombouts et al., 2020). Manipulation of fermentation pH and temperature has been shown to somewhat target preferential LA

production, however, inconsistencies in the response of LA to variations in these process conditions have been reported. For example, Tang et al. (2016) reported optimal LA production from mixed FW fermentation at pH 6.0, having observed low competitive production of alternate volatile organic acids; in contrast, Feng et al. (2018) outlined that pH 3.2-4.5 was preferred, because LA was said to be quickly consumed at higher pH values by competitive biological processes. For temperature, Tang et al. (2016) reported that LA production at 37 °C was optimal (i.e. highest LA yield and productivity), thought to be due to LA bacteria not acclimating to thermophilic conditions. However, Kim et al. (2012) and Akao et al. (2007) reported optimal LA production at thermophilic conditions linked to obvious microbial community shifts towards thermophilic LA producers. These reported inconsistencies in the response of fermentation to process operating conditions (e.g. pH and temperature) could be partly due to differences in microbial communities, also influenced by seed cultures (Arras et al., 2019), and differences in the FW composition.

Studies exploring LA production from FW predominantly utilised seed cultures comprised of diverse sludges from AD treatment plants (Arras et al., 2019; Feng et al., 2018) or microbes present within FW substrates themselves (Kim et al., 2016; Tang et al., 2016; Yang et al., 2022). However, the seed culture is expected to be a key determining factor impacting the evolution of fermentation pathways and LA yields, productivities, and competitive uptake of LA by other biological processes (Arras et al., 2019; Tang et al., 2017; Wang et al., 2014). While pure cultures aim to improve yields of target products, mixed cultures may be preferred owing to their resilience to fluctuations in environmental conditions, relatively lower cost (Feng et al., 2018), and improved capacity to receive a diverse feedstock owing to their improved hydrolytic capability and potentially diverse flanking community. For engineered biorefinery concepts, it is logical and potentially favourable to use an inoculum that is adapted to diverse mixed FW fermentation conditions and is known to produce significant quantities of LA. Such an inoculum may promote LA production and reduce competitive LA consumption while having a general tolerance to a diverse FW fermentation matrix. However, it is currently unknown how such an adapted inoculum could influence LA production, and if operational pH and temperature could be used as process levers to influence the developing microbial community, LA production, and fermentation product spectrum.

Table 4.1: Recent literature surrounding fermentation of FW and some other waste sources for lactic acid production

Inoculum source	Waste	Operation mode	Temperature (ºC)	pH	Lactic acid			Reference
					Y_{max}	C_{max}	$OP_{L\text{-}LA}$	
Indigenous microbiome	Urban bio-waste [a]	Batch	37	6.2	0.56 $g \cdot g_{total\ sugars}^{-1}$	16.59 $g \cdot L^{-1}$	-	(Tsapekos et al., 2020)
Inoculated with *Lactobacillus delbrueckii*				6.2	0.65 $g \cdot g_{total\ sugars}^{-1}$	18.41 $g \cdot L^{-1}$	-	
	Hydrolysed urban bio-waste			6.2	0.72 $g \cdot g_{total\ sugars}^{-1}$	19.73 $g \cdot L^{-1}$	-	
Anaerobic Sludge	Simulated FW	Continuous	35	3.2-4.5	-	5.7-13.5 $g \cdot L^{-1}$	-	(Feng et al., 2018)
Indigenous microbiome	FW from a canteen	Batch	37	6.0	0.46 $g \cdot g_{TS}^{-1}$	30.4 $g \cdot L^{-1}$	-	(Tang et al., 2016)
Indigenous microbiome	FW from a cafe	Batch	37	5.0	0.46 $g \cdot g_{TS}^{-1}$	28.4 $g \cdot L^{-1}$	-	(Tang et al., 2017)
Methanogenic sludge [b]		Batch	37	5.0	0.36 $g \cdot g_{TS}^{-1}$	20.7 $g \cdot L^{-1}$	-	
Anaerobic sludge [c]		Batch	37	5.0	0.41 $g \cdot g_{TS}^{-1}$	22.6 $g \cdot L^{-1}$	-	
Indigenous microbiome	Synthetic FW	Continuous	52	5.5	0.57 $g \cdot g_{VS}^{-1}$	54.3 $g \cdot L^{-1}$	-14.4%	(Yang et al., 2022)
Methanogenic sludge [b]	Synthetic FW + industrial biogas slurry	Semi-continuous	36	Uncontrolled	0.45 $g \cdot g_{VS}^{-1}$	21.7 $g \cdot L^{-1}$	-	(Wang et al., 2021)
Waste activated sludge [c]	Canteen FW	Repeat-batch	35	9.0	0.54 $g \cdot g_{TCOD}^{-1}$	24.5 $g_{COD} \cdot L^{-1}$	76.8 %	(Zhang et al., 2020b)
Marine-animal-resources compost	Saccharified model kitchen refuse	Batch	50	7.0	1.38 $g \cdot g_{total\ sugar\ utilised}^{-1}$	39.2 $g \cdot L^{-1}$	100%	(Tashiro et al., 2016)
Indigenous microbiome	Cafeteria	Continuous	50	5.0	1.6 $mol_{LA} \cdot mol_{hexose}^{-1}$	40 $g \cdot L^{-1}$	-	(Kim et al., 2016)
Streptococcus sp. strain A620	Hydrolysed Canteen FW	Batch	35	6.0	0.33 $g \cdot g_{TS}^{-1}$	66.5 $g \cdot L^{-1}$	-	(Demichelis et al., 2017)

a. Consisting of the organic fraction of municipal solid waste, which was source sorted, supermarket waste, and FW from restaurants, kitchens, and cafes. b. from an Anaerobic Digester. c. From a wastewater treatment plant.

To address the above knowledge gaps, the current study assessed the influence of pH and temperature regulation on LA production rates and yields from mixed synthetic FW, utilising an inoculum obtained from a pre-fermenter of a commercial two-stage FW AD facility known to produce LA (Bühlmann et al., 2021). While previous studies have explored the effects of pH and temperature on FW fermentation for LA (Akao et al., 2007; Tang et al., 2016; Tashiro et al., 2016; Yang et al., 2022), there have been no reported studies, to the authors knowledge, using adapted inoculum and investigating the effect of pH and temperature on LA production performance. This would be relevant for engineered mixed FW fermentation processes. Furthermore, the current study uniquely explored fermentation pathways for LA, its consumption, and related these findings to shifts in the microbial community, including those related to L-LA optical purity. The current study aims to facilitate the development of future FW biorefinery concepts.

4.2 METHOD

4.2.1 Substrate and inoculum

To ensure a consistent FW feed composition across all treatments, a synthetic FW mixture was prepared as outlined by Capson-Tojo et al. (2017) (Table B1). Each component was blended separately in a kitchen blender, mixed together with tap water at a ratio of 1 w/w, and then screened at 1.18 mm to prevent blockage of the needles used for sampling (Section 4.2.2). This synthetic FW was refrigerated (1-4 °C) for up to 1 week until use. The microbial inoculum was obtained from the pre-fermenter of a commercial two-stage mesophilic FW AD facility located in Perth Western Australia (WA) (Bühlmann et al., 2021), and was refrigerated at 1-4 °C for up to 1 week until use. The inoculum used in this study was considered adapted for two primary reasons, 1) the source facility primarily processed mixed FW, and 2) the source fermenter was already producing moderately high LA concentrations (Bühlmann et al., 2021).

4.2.2 Batch fermentation tests

The effects of pH were evaluated in triplicate in eighteen identical glass bottle batch reactors (250 ml total volume). Each vessel was inoculated with 20 ml (10% v/v) inoculum, added to 180 ml of synthetic FW (Section 4.2.1) to a total working volume of 200 ml. The initial pH of each vessel was then adjusted to either 4.0, 4.5, 5.0, 5.5, or 6.0 with 10 M NaOH or HCl and promptly sealed with a butyl rubber septa and screw cap lids. An uncontrolled pH test was run in parallel for comparison.

For sampling, pH control, and purging of the vessels, the rubber septum was pierced with a thick gauge needle (gauge 21; BD Microlance) fitted with a three-way luer-lock valve. To ensure anaerobic conditions, the rubber septum was pierced with a second needle of same gauge and the headspace purged with high purity nitrogen (99.992 %; BOC). The second needle was promptly removed following purging and the luer lock valve closed to provide a gas-tight seal for incubation at 35^{o}C for 6 days.

For pH correction in the controlled-pH tests, excess gas produced by a test vessel was initially released to the atmosphere via the luer lock valve. The valve was subsequently closed to atmosphere, the vessel inverted (rotated so the bottle cap faced the bench), and a 5 ml liquid sample collected via the luer-lock valve using a syringe. The valve was then closed off to prevent any gas from subsequently entering the vessel when it was turned up-right. pH of the liquid sample was then measured with a calibrated benchtop pH meter and probe (Rowe Scientific, Australia; IP1400 and IP1163) and acid or alkali demand determined by dropwise addition of concentrated HCl or NaOH. Following the measurement, the liquid sample was reinjected back into the vessel along with a proportional quantity of concentrated HCl or NaOH for pH correction of the whole vessel contents. Following pH correction, the vessel was thoroughly mixed by swirling, and a second sample drawn to confirm the corrected pH. If required, the process was repeated (max. 4 times) until the measured pH had reached the required value. This procedure was repeated approximately every 6 hours.

To explore the effects of fermentation temperature, inoculum and FW was added to each vessel, pH initially adjusted to pH 6.0 using 10 M NaOH or HCl as needed, the vessel promptly sealed, and then purged with nitrogen as above. The vessels were then incubated at 35, 40, 45, 50, 55, or 60 °C for 7 days. The results of the pH-trial indicated that pH depression due to the formation of organic acids was most significant within the first 1.5 days of fermentation; therefore, in the temperature tests, pH was adjusted back to 6.0 every 6 hours in the first 1.5 days of fermentation, and then every 12 hours thereafter.

In all tests, liquid samples were periodically collected (approximately every 12 hours) for analysis of LA and other volatile organic acids analysis (Section 4.2.3). For this, the test vessels were inverted (rotated so the bottle cap faced the bench), and 5 ml liquid samples collected. In the pH-controlled tests, this was typically done every 12 hours, prior to pH correction. The samples were stored in 15 ml sterile centrifuge vials at 1-4 °C for a

maximum of 2 days prior to further processing and analysis of volatile fatty acids (VFAs) and LA (Section 4.2.3), or longer-term storage at -20 °C for microbial analysis (Section 4.2.3).

4.2.3 Analytical methods

Total solids (TS) and volatile solids (VS) were measured according to Standard Methods (APHA, 1995). Prior to organic acids analyses, parts of the liquid samples were centrifuged at 10,000 g for 10 minutes and the supernatant collected for analysis, and the pellet discarded. The supernatant was diluted with deionised water as required to be within measurement range, and then filtered through a 0.45 µm PES Millipore® filter before measuring LA, succinic acid, acetic acid, propionic acid, and butyric acid concentrations by High Performance Liquid Chromatography (HPLC) as previously described elsewhere (Bühlmann et al., 2021). The D-LA concentration was also measured for samples pertaining to the maximum measured LA concentration from each test vessel. This analysis used a D-LA assay kit following the manufacturer's instructions (K-DATE: Megazyme, Ireland). L-LA concentration was determined by difference between total LA measured by HPLC and D-LA measured by the assay. The L-LA optical purity ($OP_{L\text{-}LA}$) was calculated using Eq. 4.1 (Tashiro et al., 2013):

$$OP_{L-LA}(\%) = \frac{C_{L-LA} - C_{D-LA}}{C_{L-LA} + C_{D-LA}} \times 100 \qquad \text{(Eq. 4.1)}$$

where $C_{L\text{-}LA}$ and $C_{D\text{-}LA}$ are the respective L-LA and D-LA concentrations ($g \cdot L^{-1}$). The $OP_{L\text{-}LA}$ is a measure of the enantiomeric purity, indicating the relative concentration of each form of LA. Values less than zero indicate D-LA is the dominant stereoisomer, while values larger than zero suggest L-LA is the dominant form. A value of zero indicates that both isomers are present in equal quantities (i.e. a racemic mixture). The LA selectivity was calculated using Eq. 4.2;

$$LA(\%) = \frac{C_{LA}}{C_{LA}+C_{SA}+C_{AA}+C_{PA}+C_{BA}} \qquad \text{(Eq. 4.2)}$$

where C_{LA}, C_{SA}, C_{AA}, C_{PA}, and C_{BA} are the concentration of LA, succinic acid, acetic acid, propionic acid, and butyric acid ($g \cdot L^{-1}$), respectively.

The remainder of each liquid sample (the part not centrifuged as above) was stored at -20 °C prior to analysis of microbial community composition. For this analysis, the frozen samples collected on day 5 were thawed and vortexed for 15 seconds. DNA was extracted

from 250 µl of liquid sample using DNeasy® Powersoil® Pro Kit (QIAGEN, Germany) as per manufacturer's instructions. Bacterial and archaeal 16S rRNA genes were amplified using modified universal core primers (Mori et al., 2014) 515f (5' GTGYCAGCMGCCGCGGTAA 3') and 806R (5' GACTACHVGGGTWTCTAATCC 3') under the following thermocycling parameters: 98 °C for 2 min (preheat) then 25 cycles of 98 °C for 10 s, 55 °C for 30 s, 72 °C for 30 s, then 72 °C for 2 min. Each reaction contained 12.5 µl Q5® High-Fidelity 2X Master Mix (New England BioLabs, Australia), 1.25 µl of specific forward and reverse primer at a concentration of 10 µM (synthesised by Integrated DNA Technologies, Australia), 2.5 µl template DNA and 7.5 µl sterile water. PCR products were screened for size and specificity using 2% agarose E-Gel™ SizeSelect™ II (Thermo Fisher Scientific, Australia) and sent for preparation and sequencing on an Illumina® Mi-seq platform by the Australian Centre for Ecogenomics (ACE) at The University of Queensland, Brisbane, Australia.

4.2.4 Bioinformatics

4.2.4.1 Taxonomy analysis

Sequence data was processed using Mothur version 1.46.1 with a slightly modified standard operating procedure (Schloss et al., 2009). Sequences were aligned with the Silva database (Release 132) and assigned operational taxonomic units (OTU- based taxonomic analysis) based on 97% similarity. Refer to Appendix B for a detailed outline of methods undertaken also mostly described previously elsewhere (Buhlmann et al., 2018).

4.2.4.2 Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis (PICRUSt)

Sequence data for PICRUSt analysis was processed using Mothur version 1.46.1 with a slightly modified standard operating procedure as above. Sequences were aligned with the Greengenes database (gg_13_5) and assigned to OTUs based on 97% similarity. NSTI values for this study ranged from 0.05 to 0.14 with an average of 0.10 ± 0.024 s.d. Lower NSTI values are associated with higher similarity between the reference genome database and the sample genome. The average weighted NSTI for this study is similar to those for environmental communities and lower than the 0.15 threshold used to indicate similarity with the reference genome database (Langille et al., 2013; Louvado et al., 2020). All genes were identified via the KEGG database (KEGG, 2022).

4.2.5 Data analysis and statistical methods

Because background LA and other organic acids were present in the inoculum and potentially in the synthetic FW, all acid concentrations and yields presented below are net values calculated as the difference between the initial starting value (at $t = 0$) and a value at a particular time point (t, hours) during the experiment. Where replicate measurements were performed, mean values and 95% confidence intervals determined by a two-tailed student t-test are reported.

The maximum LA yield was visually identified and where it held stable for 2 or more points (rather than immediately decreasing), the maximum yield was calculated based on the average of these multiple points (with relevant statistical analysis for uncertainty). Maximum substrate uptake rates were determined based on the linear section of the substrate production curve as previously outlined (Buhlmann et al., 2018). To investigate the impact of pH and temperature on the product spectrum at the maximum LA yield, a continuous ANOVA was conducted in Excel with individual acid yields as the response variable and pH and temperature as predictors.

To explore the impact of pH and temperature on the microbial community composition, a Principal Component Analysis (PCA) was performed. Principal components (PC) assignment, calculations, and visualisation of the outputs were conducted in R version 4.1.1 (R Development Core Team, 2022). To ensure the magnitude of the relative abundance of varying microbes did not impact on the resulting PC values, variables were normalised using "scale = TRUE" within the "prcomp" function in R. An ANOVA followed with the post-hoc Tukey's HSD pairwise comparison method was utilised to assess the impact of pH and temperature on the maximum LA yield, $OP_{L\text{-}LA}$, and relative abundance of select genes inferred from the PICRUSt analysis.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of regulated pH

In all tests at 35 °C (variable pH), LA accumulation occurred rapidly (Fig. 4.1A), without substantial delay. As expected, this resulted in a rapid pH depression in the uncontrolled pH tests within the first 12 hours down to a value of 3.5 (Fig. B1). LA fermentation continued for a further 48 hours, reaching a maximum LA concentration of 20.3 $g \cdot L^{-1}$ (yield of 0.28 $g_{LA} \cdot g_{VS}^{-1}$) (Fig. 4.1A). The industrial fermenter from which the inoculum

was sourced routinely operates at a similar depressed pH (Bühlmann et al., 2021), so it was somewhat unsurprising that fermentation continued at these inhibitory conditions. Furthermore, the concentration achieved was similar to that observed for the inoculum-source industrial fermenter, suggesting that the inoculum mimicked the large-scale operation. However, continued fermentation led to a continual decline in the LA yield after about 60 hours (Fig. 4.1A) accompanied by a continual increase in acetic acid (Fig. 4.2A).

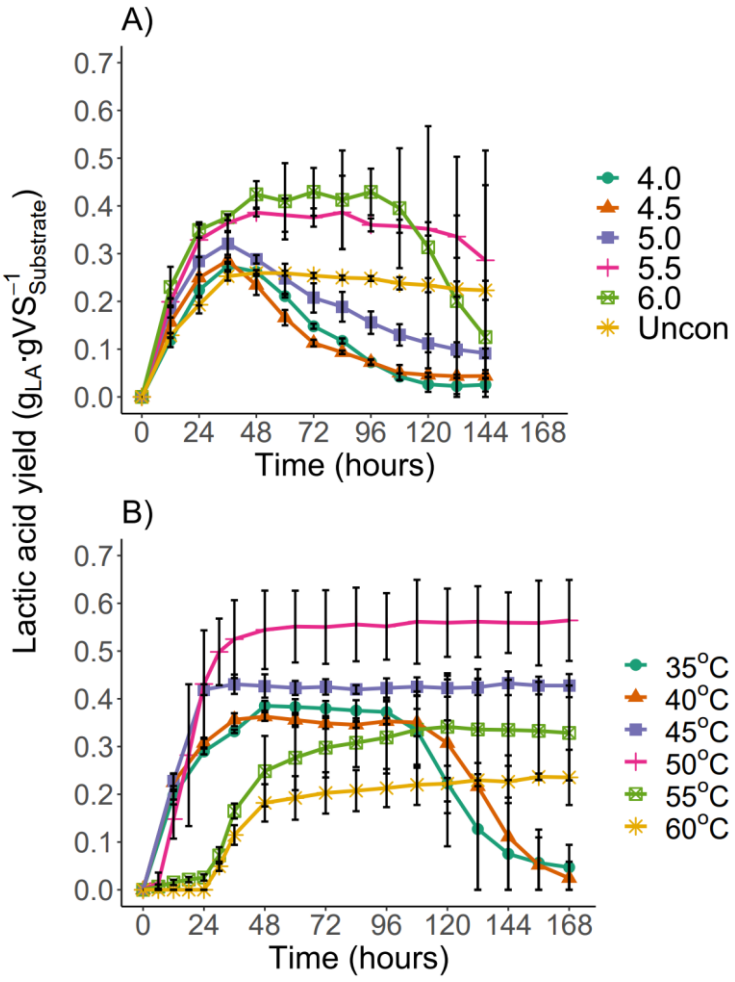


Figure 4.1: Effect of A) pH and B) Temperature on net yield of LA from fermentation of synthetic FW. pH tests were conducted at a constant temperature 35 °C, while temperature tests were conducted at controlled pH 6.0. Error bars show 95% confidence intervals. For pH 4.0, the data point at 24 hours is displayed as the mean of duplicates (without error bars).

Introduction of pH control had a significant effect on the peak LA yield ($P < 0.001$). At pH 4.5-5.0, minor improvements in the LA yield (Table 4.2) were experienced compared to uncontrolled pH ($P < 0.01$) but, compared to the uncontrolled pH tests, resulted in a more rapid subsequent consumption of LA, after about 36 hours, with simultaneous acetate production (Fig. 4.1A; Section 4.3.3). In contrast, at pH 5.5 and 6.0, the LA yield

peaked and then remained constant for approximately 70 hours thereafter, but eventually declined, accompanied by butyric acid production (Fig. 4.1A and Fig. 4.2D; Section 4.3.3). At pH 6.0, the highest LA yield achieved was 0.42 $g \cdot g_{VS}^{-1}$, approximately 1.6 times higher than that at uncontrolled pH. This maximum yield was comparable to that reported by other similar studies, e.g. 0.33-0.46 $g \cdot g_{TS}^{-1}$ (Wang et al., 2005), 0.46 $g \cdot g_{TS}^{-1}$ (Tang et al., 2016), 0.35-0.39 $g \cdot g_{TS}^{-1}$ (Pleissner et al., 2017). Maximum uptake rates across all pH levels were comparable at 0.6 $g_{COD} \cdot d^{-1}$ at all pH levels. Only LA yield appeared to be affected, due to subsequent competitive microbial processes causing a net consumption of LA, and possibly an increase in product inhibition at low (compared to high) pH (Section 4.3.3). Analysis of L-LA optical purity ($OP_{L\text{-}LA}$) (Section 4.2.3) revealed that the ratio of L-LA and D-LA did not significantly change with controlled pH (Table 4.2) albeit that L-LA optical purity was just significantly lower (i.e. lower proportion of L-LA) under controlled pH 5.0-5.5 when compared with that at uncontrolled pH ($P = 0.01\text{-}0.03$; Table 4.2). $OP_{L\text{-}LA}$ appeared to be less variable under uncontrolled pH, which may be a result of low pH constraining the variable metabolic pathways that influence optical purity. Overall, correlation between pH and $OP_{L\text{-}LA}$ in the current study was minimal. Alkaline pH has been reported to promote L-LA production (Li et al., 2015; Zhang et al., 2017b), however, application of alkaline pH could increase operational costs for chemical pH correction and may adversely affect downstream processing. For example, the recovery efficiency of adsorption processes are heavily influenced by operational pH, and can require acidic pH values (pH 2-4) for optimal LA recovery (Bühlmann et al., 2022).

Table 4.2: Maximum LA yield for the temperature and pH tests. Values are displayed as their mean ± 95 % confidence interval.

pH	Temperature (°C)	LA selectivity (%) [a]	$OP_{L\text{-}LA}$ (%)	Max concentration ($g_{LA} \cdot L^{-1}$) [b]	Time at Max. Yield (hour) [c]	Max Yield ($g_{LA} \cdot g_{VS}^{-1}$)	Lag phase (hour)
Uncontrolled	35	76 ± 4	13.8 ± 1.5	18.4 ± 0.18	48	0.26 ± 0.003	-
4.0		79 ± 0	7.6 ± 7.8	19.6 ± 0.6 [d]	36	0.27 ± 0.009 [d]	-
4.5		77 ± 5	7.3 ± 12.8	20.4 ± 0.5 [d]	36	0.29 ± 0.006 [d]	-
5.0		81 ± 4	5.4 ± 3.3	23.0 ± 1.7 [d]	36	0.32 ± 0.024 [d]	-
5.5		75 ± 1	7.4 ± 5.2	27.2 ± 0.5	48	0.38 ± 0.007	-
6.0		80 ± 4	16.9 ± 5.0	30.1 ± 1.2	48	0.42 ± 0.02	-
6.0	40	67 ± 2	20.4 ± 7.9	28.3 ± 0.7	48	0.36 ± 0.006	-
	45	75 ± 1	-14.6 ± 27.8	33.6 ± 0.4	48	0.42 ± 0.005	-
	50	87 ± 3	4.9 ± 3.2	39.3 ± 0.3	60	0.55 ± 0.021	6
	55	66 ± 12	5.6 ± 15.1	18.5 ± 1.6	120	0.34 ± 0.029	24
	60	67 ± 8	5.2 ± 17.0	12.4 ± 0.5	120	0.23 ± 0.008	24

a) Calculated based on measured VFAs (Eq. 4.2), b) Measured concentrations at the max LA yield, c) initial time selected as max yield, d) selected as a single point as substantial LA consumption occurs after max yield.

4.3.2 Effect of temperature

LA accumulated rapidly at all temperatures assessed, except for 55-60 °C where a substantial initial time lag was observed (Fig. 4.1B). Minimal differences in LA production were observed between 35 °C and 40 °C (Fig. 4.1B), although a delay in the onset of butyrate production was noted (Section 4.3.3). Overall, the LA yield was impacted by fermentation temperature ($P = 0.012$), with fermentation at 45 °C and 50 °C improving the yield of LA, and fermentation at 50 °C displaying the highest measured LA yield of 0.55 $g \cdot g_{VS}^{-1}$ (following a short lag phase) (Table 4.2). At temperatures above 45 °C, LA was maintained with minimal subsequent conversion to butyrate (Fig. 4.1B and Section 4.3.3) and could be linked to a shift in microbial community and/or metabolic pathways (Section 4.3.4 and 4.3.5). At 55-60 °C, LA yield was notably reduced, and some butyrate production was observed initially at 55 °C and 60 °C, and throughout the experiment at 60 °C.

Like in the pH tests, $OP_{L\text{-}LA}$ in the temperature tests was highly variable. $OP_{L\text{-}LA}$ (Section 4.2.3) showed slightly more L-LA than D-LA at all test temperatures, except at 45 °C for which $OP_{L\text{-}LA}$, while negative, was not significantly different from zero. The highest proportion of L-LA was observed at 40 °C ($P = 0.003$, comparing 35 to 40 °C; Table 4.2). Previous research has indicated a positive correlation between temperature and $OP_{L\text{-}LA}$, with studies showing that higher temperatures favoured L-LA production (Gu et al., 2014; Tashiro et al., 2013), suggested to be due to a higher thermostability of L-lactate dehydrogenase (Gu et al., 2014). However, such a correlation was not observed in the current study, which may be due to microbial community composition or metabolic pathways utilised for LA production (Section 4.3.5).

4.3.3 Organic acid product spectrum

The production of VFAs were dynamically influenced by operational pH, temperature, and fermentation time (Fig. 4.2 and Fig. 4.3). In the pH tests, acetate primarily accumulated (Fig. 4.2A) with LA, which resulted in a reduced LA selectivity (Table 4.2). Following 60 hours, LA was slowly consumed while acetic acid continued to accumulate. However, at the peak LA yield, the operational pH had a weak to minimal effect on the acetate yield ($P = 0.039$). Introduction of pH control at 4.0-5.0 saw LA consumed following 36 hours, whilst predominantly acetic acid accumulated (Fig. 4.2A). Acetate has been suggested to improve mixed culture LA production when present at 10 $g \cdot L^{-1}$

(Khor et al., 2016), but was generally correlated with reduced LA yields in the current study (Section 4.3.1). Elferink et al. (2001) reported that *Lactobacillus buchneri* and other LA bacteria may be capable of degrading LA to acetate under anoxic conditions when alternative electron acceptors are present, which may have occurred in the current study. Introduction of pH control increased the production of succinic acid and generally remained unaffected by changing pH, with the exception of pH 4.0 for which production was reduced compared to other values, while propionic acid generally saw a gradual increase in production with pH (Fig. 4.2C).

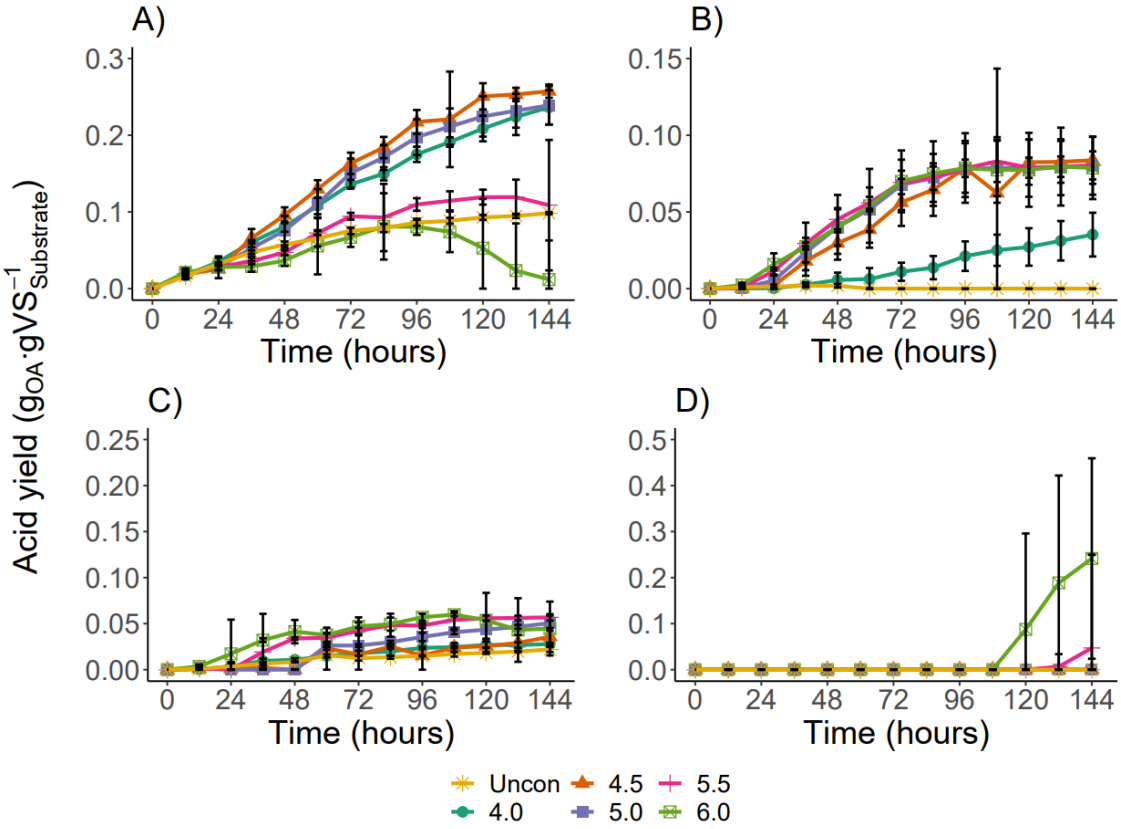


Figure 4.2: Effect of pH on the net yield of various organic acids (OA), including: A) Acetic acid, B) Succinic acid, C) Propionic acid, and D) Butyric acid. Error bars show the 95% confidence interval. For pH 4.0, point at 24 hours is displayed as the mean of duplicates (i.e. without error bars). Note the different vertical axes scales.

At 35 °C (pH 5.5 and 6.0), and at 40 °C (pH 6.0), production of acetic, succinic, and propionic acids was similar (Fig. 4.2 and 4.3), although higher temperatures slightly increased succinic acid production (Fig. 4.3). At the peak LA yield, temperature also had a significant influence in the acetic ($P = 0.006$), succinic ($P < 0.001$), and propionic acid ($P < 0.001$) yields, with fermentation at 50 °C displaying the lowest production of alternate organic acids (Fig. 4.3). Following 108-132 hours the consumption of LA, acetic acid, and propionic acid, and production of butyric acid were observed for 35 and 40 °C

(Figs. 2 and 3). Furthermore, a notable increase in gas production occurred when fermentation switched to butyrate. Note, butyric acid production occurred at slightly different times within replicates, resulting in large error bars for these treatments. Studies have reported the conversion of LA and acetate to butyrate at similar conditions (Shetty et al., 2020; Tashiro et al., 2013), and this has been suggested to be induced by a lack of viable substrate during mixed culture (Hoelzle et al., 2021) and pure culture fermentation (Shetty et al., 2020). Recent literature has shown that if sufficient substrate is available for LA formation, the conversion of LA into butyrate is suppressed (Hoelzle et al., 2021). At 45-50 °C acetic and succinic acid production was significantly reduced while propionic acid saw an increase in production at 50 °C (Fig. 4.3). No butyric acid was observed at 45-50 °C (Fig. 4.3D), which could be related to a shift in the community structure (Section 4.3.4). At 55-60 °C, acetic acid, propionic acid, and butyric acid were produced with LA, and acetic acid subsequently consumed to variable extents (Fig. 4.3A). Only minor quantities of succinic acid were detected at 55-60 °C (Figs. 2B and 3B).

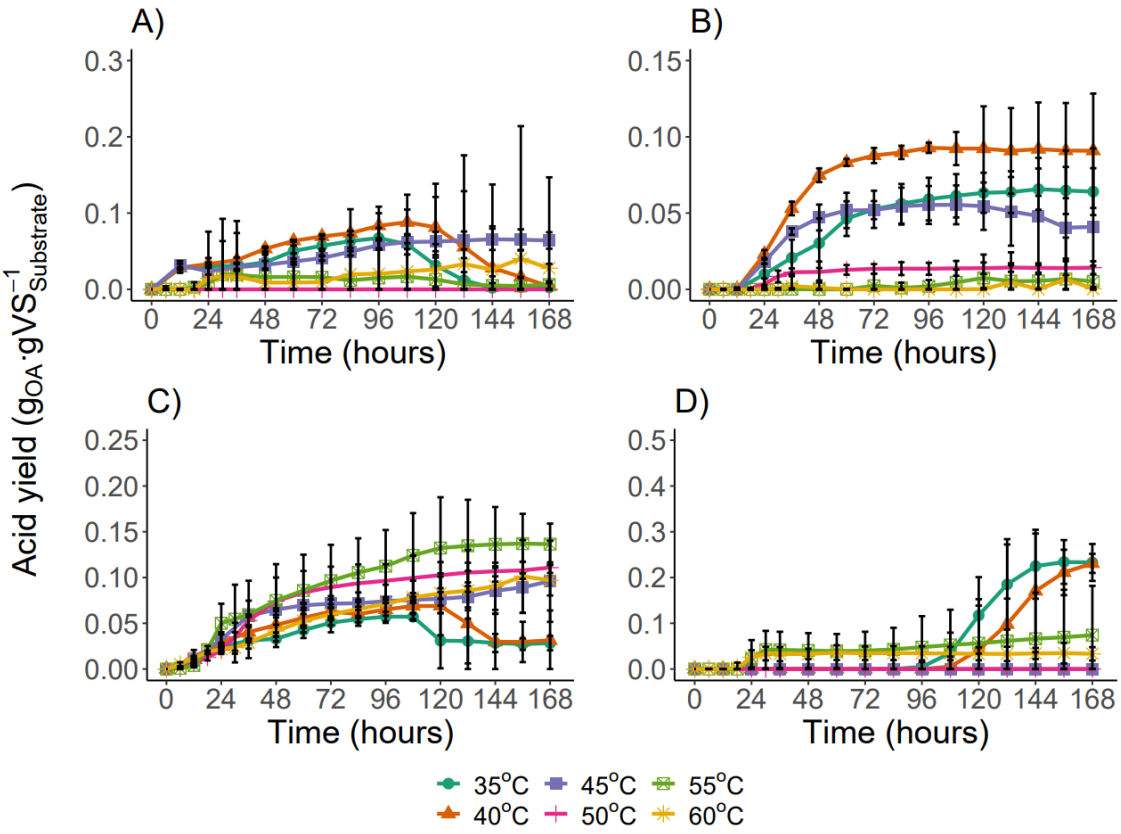


Figure 4.3: Effect of temperature on the net yield of various organic acids (OA), including: A) Acetic acid, B) Succinic acid, C) Propionic acid, and D) Butyric acid. Error bars show the 95% confidence interval. Note the different vertical axes scales.

4.3.4 Microbial community relative abundance

To investigate the effect of pH and temperature on the microbial community, samples representing the inoculum and samples at day 5 of fermentation for each treatment were collected and sequenced. While the inoculum was relatively diverse, likely due to the high feedstock diversity (Bühlmann et al., 2021), the majority of identified bacteria fell under the "Other" label as their individual relative abundance were below the 1% threshold (Fig. 4.4). This was likely due to the inhibitory conditions of the industrial fermenter at the inoculum source facility, the diversity in feedstocks received by the same facility (Bühlmann et al., 2021), and some periodic recycling of a portion of digestate back to the fermenter at the same facility. *Lactobacillus* represented a large fraction of the microbial community within the inoculum (50%) and was the largest single genus present. Other minor genera included *Prevotella_7* (6.7%), an unclassified Prevotellaceae (4.8%), *Muribaculaceae_ge* (3.7%), an unclassified bacterium (1.5%), *Prevotella_1* (1.5%), *Methanoculleus* (1.4%), and an unclassified Lactobacillus (1.2%).

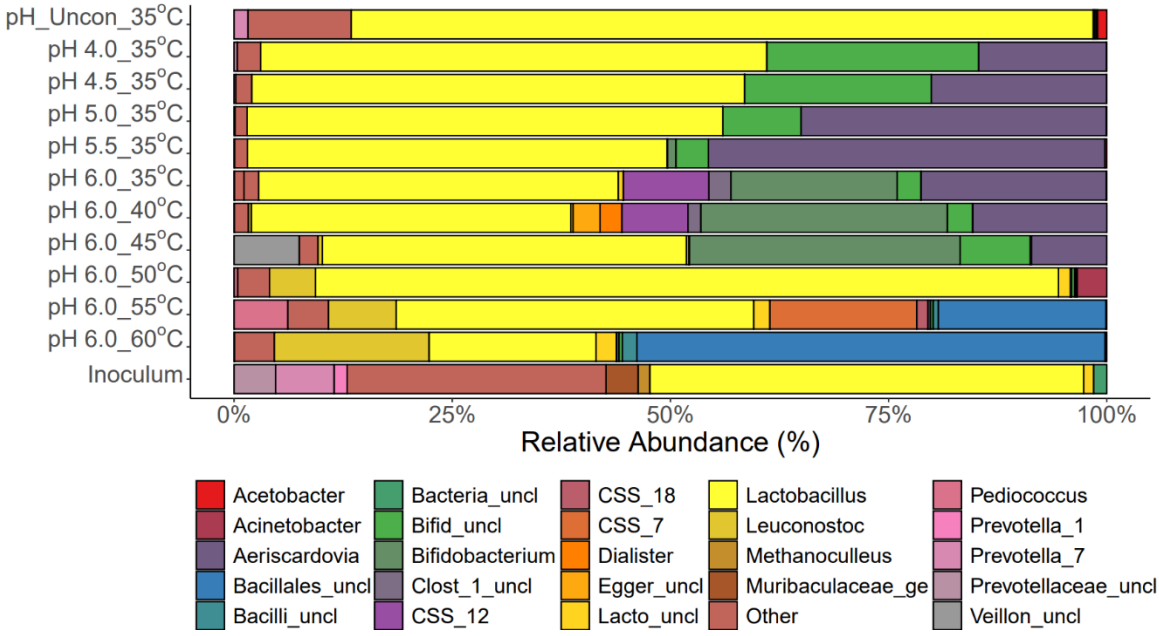


Figure 4.4: Relative abundance of microbial genera (>1%) within the inoculum and following 5-days of FW fermentation at different pH and temperatures. The names of various genera have been shorted to fit them within the legend. Full names are as follows: Bifid (Bifidobacteriaceae), Clost_1 (Clostridiaceae_1), CSS (Clostridium sensu stricto), Egger (Eggerthellaceae), and Lacto (Lactobacillales). All genera containing "uncl" were unclassified.

During uncontrolled pH fermentation, the community converged to the inoculum reflecting the uncontrolled pH operation of the source fermenter, and to Firmicutes dominating the community, primarily comprising of *Lactobacillus* (Fig. 4.4). With

controlled pH, convergence towards the inoculum microbial composition was not directly observed, but *Lactobacillus* still dominated as with the inoculum. Distinct shifts in the microbial community composition were observed at different pH values (Fig. 4.4 and Fig. 4.5A), with controlled pH 4-5.5 distinctly correlating with *Aeriscardovia* and an unclassified *Bifidobacteriaceae* (Fig. 4.5A). At pH 6 the community shifted to *Bifidobacterium*, *CSS_12*, and an unclassified Clostridium. While LA production performance was highest at pH 6.0, the relative abundance of *Lactobacillus* dropped sharply at pH 6.0 (Fig. 4.4). *Lactobacillus* is a bacterium closely associated with LA formation (Bühlmann et al., 2021; Kim et al., 2016; Tang et al., 2016). However, improvements in LA production in the current work appeared to be associated with increased relative abundance of *Bifidobacterium* (Fig. 4.5A), previously reported to produce LA through the bifidus pathway (Feng et al., 2018). The relative abundance of *Aeriscardovia*, whilst decreasing from pH 5.5 to 6.0, may have also aided LA production in the controlled pH tests, as it has been reported to be involved in LA production from FW (De Groof et al., 2021).

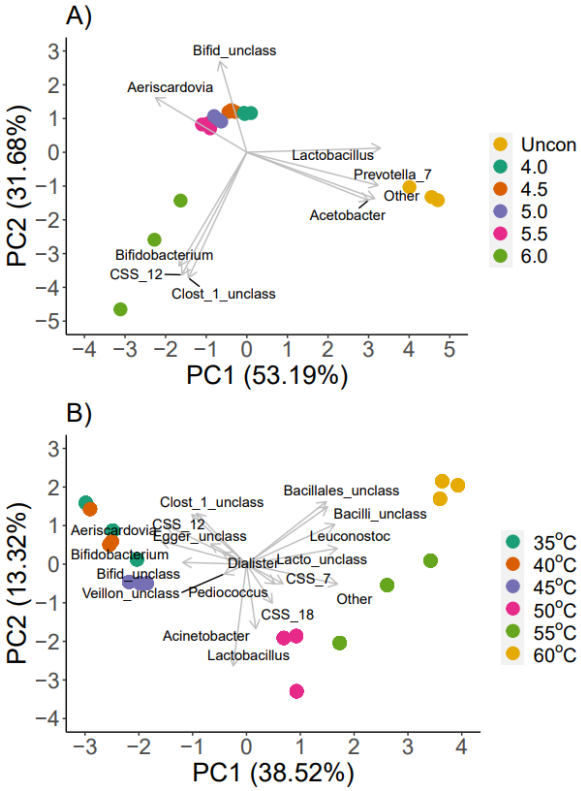


Figure 4.5: Principal component analysis (PCA) to show the impact of A) pH, and B) Temperature on the relative abundance of microbial genera (>1%).

In the controlled temperature tests, at 35-40 °C, the observed butyrate formation (Section 4.3.3) correlated with the relative abundance of CSS_12. These correlations extended to

the replicates which had initiated butyrate production earlier, showing a higher relative abundance of CSS_12 (P = 0.001). A previous study by Detman et al. (2021) reported the conversion of LA and acetate into butyrate and hydrogen at pH 5-6, with a community composition similar to the current study (Fig. 4.4), comprising primarily of *Lactobacillus*, *Bifidobacterium*, and *CSS_12*. Furthermore, *Bifidobacterium* has been suggested to form a syntrophic relationship with *Clostridium* for butyrate formation (Xiong et al., 2019) which may have occurred in the current study. At 50 °C, the community diversity again reduced significantly (as compared to the inoculum and lower temperature tests) with Firmicutes dominating the community with a 95% relative abundance, comprised mostly of *Lactobacillus* with a relative abundance of over 85% at the genus level (Fig. 4.4 and Fig. 4.5B). However, at 55 and 60 °C the growth of *Lactobacillus* was significantly hindered, dropping below 20% at 60 °C (Fig. 4.4), while the abundance of an unclassified Bacillales genus and *Leuconsotoc* increased (Fig. 4.4 and Fig. 4.5B). This was expected as *Lactobacillus* species are primarily mesophiles, with some thermophilic strains capable of surviving at 53 °C (Śliżewska & Chlebicz-Wójcik, 2020). Studies have reported *Bacillus* strains, such as *Bacillus coagulans* (a bacterium known to utilise a wide range of carbon sources to produce high purity L-LA (Zhang et al., 2017a)), are important LA producers at thermophilic conditions (Tashiro et al., 2013; Yang et al., 2022). While the in-silico analysis did not predict the presence of the *Bacillus* genera, the unclassified Bacillales genera may be composed of an unclassified *Bacillus* which could have aided LA production at these conditions. Higher temperatures of 45-50 °C seemingly eliminated butyrate formation which was accompanied by a disappearance of *CSS_12*. However, this did not result in a continual increase in the LA yield with LA production ceasing after 48 hours, possibly due to substrate limited conditions. A COD balance on measured VFAs supports this, showing no significant change in the total COD (VFAs + LA) once the maximum LA concentration was achieved. However, other products (e.g. alcohols and gaseous products) could have been formed but could not be measured in this study. Consequently, substrate limiting conditions could not be confirmed. In the case of substrate limited conditions, pre-treatment or supplementation of simple carbohydrates to the FW may further increase LA production under these conditions.

4.3.5 Functional gene analysis

To maximise LA fermentation, metabolic pathways need to both maximise the production of LA and minimise its consumption and conversion into other organic acids.

Homo-lactate fermentation maximises the LA yield with LA nearly exclusively produced via this pathway in pure culture fermenters, while hetero-fermentation produces acetate, ethanol, and carbon dioxide along with LA, lowering the overall LA yield (Alves de Oliveira et al., 2018).

Genes related to homo- and hetero- LA fermentation, namely 1,6-diphosphate aldolase (*fbaA*; homo-fermentation) and phosphoketolase (*xfp*; hetero-fermentation) (Alves de Oliveira et al., 2018), were predicted at a relative abundance of 0.04 – 0.05% and 0.06×10^{-4} – 1.17×10^{-4} % for *fbaA* and *xfp*, respectively. Introduction of pH control or higher fermentation temperatures displayed no significant change in the relative abundance of either *fbaA* or *xfp* ($P > 0.05$), with the exception of 55-60 °C, which saw a 19 times increase in the relative abundance of *xfp* as compared to at 35 °C ($P < 0.004$).

Major shifts in the relative abundance of acid producing genes occurred at pH 5.5 and 6.0 (at 35 °C) and at 50-60 °C (at pH 6.0) (Fig. B5). With increasing pH, the proportion of genes related to metabolism of carbon in FW, specifically glycolysis, glyoxylate, pyruvate, fructose and mannose, galactose, and starch and sucrose metabolism, were observed to decrease in the current study, albeit that the change was marginal (Fig. B6). The exception was those of the butonate pathway, for which, at pH 6.0, the abundance of genes increased by 14%, relative to that at uncontrolled pH conditions (Section 4.3.3). Increasing fermentation temperatures saw an increase in genes associated with glycolysis, and galactose, starch and sucrose metabolism, while those of glyoxylate, pyruvate, butonate, and galactose metabolism saw a reduction in relative abundance (Fig. B6). An increase in the relative abundance of genes associated with butyric acid production (*buk*, *atoD*, and *atoA*) was identified at pH 5.5 and 6 and were similarly correlated with fermentation at 40 °C, which aligned with the butyrate production observed at these conditions (Section 4.3.3). Additionally, fermentation at pH 5.5 and 6 also aligned with an increase in the relative abundance of lactate dehydrogenase (*ldh*) (Fig. B5A), a gene commonly explored when assessing the performance of LA fermentation (Alves de Oliveira et al., 2018; Zhang et al., 2020a; Zhang et al., 2020b). At 50 °C, LA was primarily correlated with *porA-porD* (Fig. B5B), which are enzyme encoding genes responsible for catalysing the reversable conversion of acetyl-CoA to pyruvate in a number of pathways ((KEGG, 2022); Fig. 4.6). Furthermore, LA was also correlated with *acdA* which may have catalysed the reversible conversion of acetate to acetyl-CoA (Wang et al., 2022), allowing for the conversion of acetate to pyruvate (Fig. 4.6). Pyruvate

is an important intermediate in LA production (Zhang et al., 2020a), and an observed increase in the relative abundance of genes related to pyruvate formation may have correlated with conditions promoting LA formation.

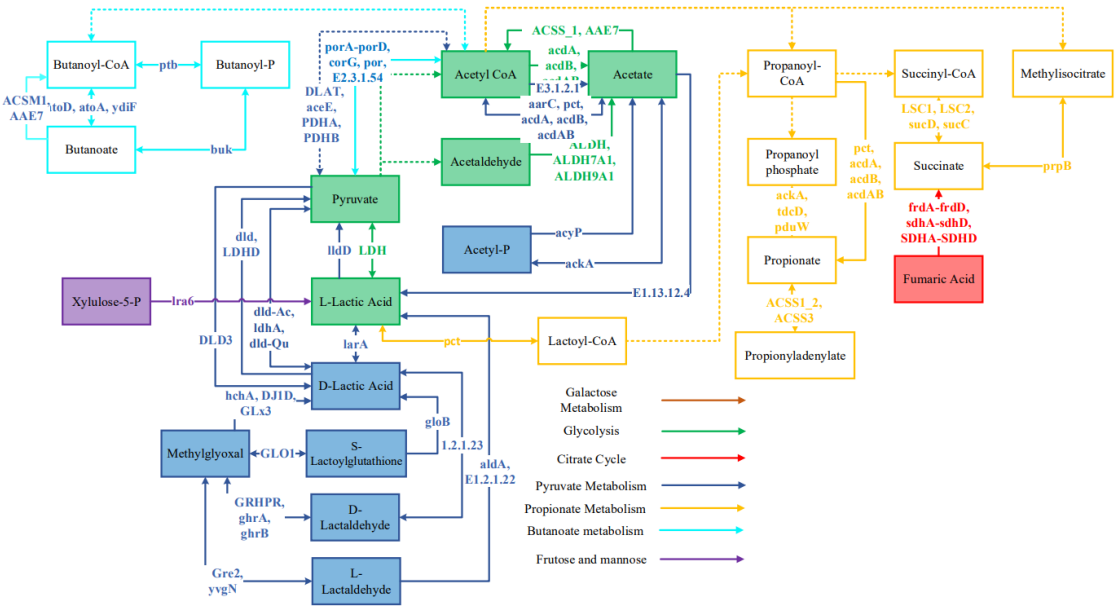


Figure 4.6: Metabolic pathway diagram outlining the selected genes involved in the production of lactic acid, succinic acid, acetic acid, propionic acid, and butyric acid. Specific genes were identified from the pyruvate, galactose, fructose and mannose, propionate, and butanoate, metabolisms as well as the citrate cycle, and glycolysis pathways. Dotted lines represent simplification in the metabolic pathway.

D-LA producing genes were predicted to be in higher relative abundance than L-LA genes at all pH and temperature conditions (Fig. 4.7). This may indicate a role of the rate of various metabolic pathways on the overall racemic outcomes of fermentation. It is however important to note that sequencing occurred on samples taken on day 5, and the $OP_{L\text{-}LA}$ was determined on samples from prior days, so that the relative abundance of L- or D-LA producing genes may not have strictly aligned with observed $OP_{L\text{-}LA}$ values. Introduction of pH control at pH 5.5-6.0 saw a 92-130% increase in the relative abundance of D-LA genes, compared to uncontrolled pH (Fig. 4.7A), whereas no significant change in L-LA genes was observed ($P = 0.199$). *gloB* accounted for 94-99% of identified genes related to LA production (i.e. *gloB*, *ldhA*, and *dld* for D-LA and *pct*, *ldh*, and *aldA* for L-LA) over the pH range tested. *gloB* catalyses the conversion of S-Lactoylglutathione to D-LA following the detoxification of methylglyoxal through the glyoxalase pathway (Jain et al., 2018). Methylglyoxal is a toxic by-product from glycolysis which is produced from dihydroxyacetone-P when glucose consumption overtakes phosphate uptake (Hoelzle et al., 2021). Furthermore, methylglyoxal

detoxification leads to the production of a racemic LA mixture (Mazumdar et al., 2013), which was primarily observed in the current study (Table 4.2) and suggests LA production primarily occurred via this pathway. At 55-60 °C, a 46-52 times increase in the relative abundance of L-LA genes was observed as compared to at 35 °C ($P < 0.01$). Previous research has indicated a positive correlation between temperature and $OP_{L\text{-}LA}$, with studies showing higher temperatures tending towards L-LA production (Gu et al., 2014; Tashiro et al., 2013). This has been suggested to be due to the higher thermostability of L-lactate dehydrogenase (Gu et al., 2014). However, whilst higher temperatures saw an increase in the relative abundance of L-LA genes in the current study, no significant change in D-LA genes was observed, with the exception of the 50 °C test whereat a 19% reduction occurred in comparison to the 35 °C test ($P = 0.02$). Regardless, D-LA genes remained dominant in all cases and the LA mixture remained racemic (Section 4.3.1 and 4.3.2).

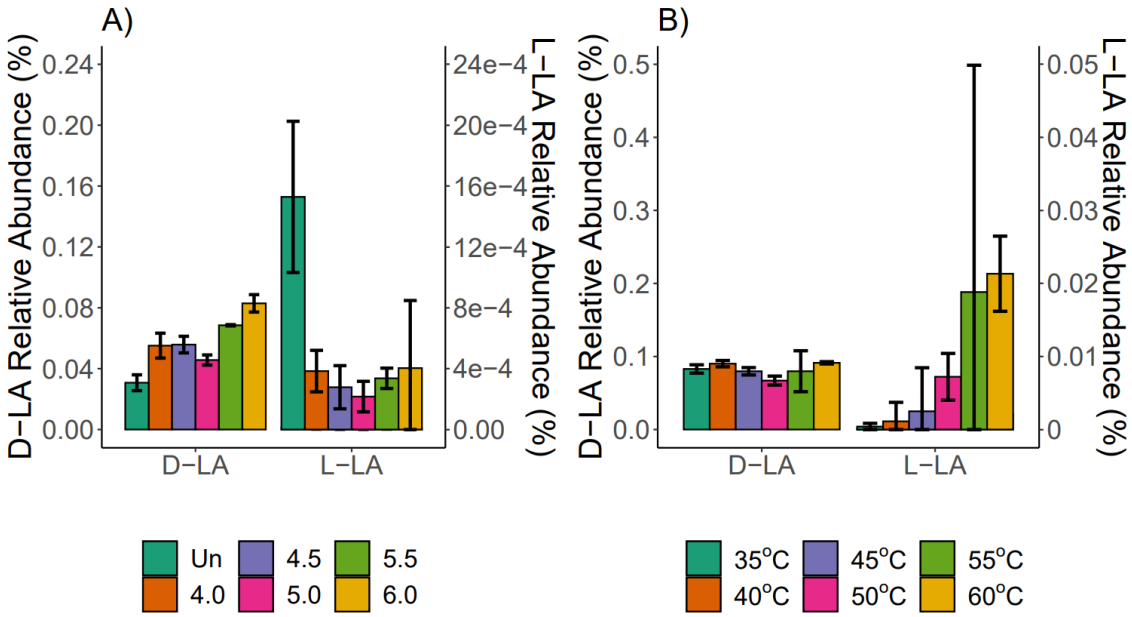


Figure 4.7: Sum of the relative abundance of identified L-LA and D-LA producing genes at A) different controlled pH values (at 35 °C), and B) various temperatures (at pH 6.0). Second axis in plot A displays the L-LA relative abundance ×100, while the second axis in plot B displays the L-LA relative abundance ×10. Error bars represent the 95% confidence interval.

4.3.6 Implications for biorefinery production of lactate

Overall, LA was effectively produced from a complex FW mixture with the operational pH and temperature dynamically altering the fermentation product spectrum and LA yield. While LA as an end-product was partially selected by the adapted inoculum, control of the operational pH and temperature effectively regulated the growth of

alternate LA consuming microbes and development of alternate metabolic pathways, allowing sustained production of LA. Furthermore, at the optimum conditions for LA production, the production of many measured metabolic products was significantly reduced (Fig. 4.3), which would reduce costs associated with downstream processing. Moreover, the results of this study show dynamic shifts in the product spectrum can be controlled through operator intervention, allowing downstream changes in separation efficiency to be managed by upstream shifts in operational conditions.

The results of this study also indicate future biorefinery applications can effectively promote LA formation using an adapted inoculum and can selectively target specific metabolic products by simple adjustment of the fermentation pH and temperature. This can potentially be utilised by operators to maintain production of LA during the anticipated variation in feedstock composition with seasonal and market changes. However, while previous studies have related shifts in the $OP_{L\text{-}LA}$ to changes in operational pH (Section 4.3.1) and temperature (Section 4.3.2), this study shows manipulation of such conditions may not result in such anticipated changes, depending on the dominant pathways which are present. In such a case, it may be appropriate to inoculate the FW with a second inoculum containing LA bacteria known to produce optically pure L- or D-LA, or for which the $OP_{L\text{-}LA}$ is influenced by the process levers of pH and temperature.

4.4 CONCLUSION

In conclusion, LA was effectively promoted using an adapted fermentation inoculum while pH and temperature regulation were effective and practical process levers to improve LA production from mixed food waste fermentation. Fermentation at pH 6 and 50 °C maximised the LA yield at 0.55 $g_{LA} \cdot g^{-1}_{VS}$, being approximately double that observed at uncontrolled pH and 35 °C, and promoted the growth of *Lactobacillus*. Mesophilic conditions somewhat promoted LA formation, but introduced a LA consumption phase, which was dependent on pH. In contrast, thermophilic fermentation eliminated net LA consumption for butyrate formation and reduced the production of alternate volatile organic acids. With an adapted inoculum, temperature and pH control can effectively control the evolution of microbial communities and target fermentation products of future biorefineries.

4.5 REFERENCE

Ahmad, A., Banat, F., Taher, H. 2020. A review on the lactic acid fermentation from low-cost renewable materials: Recent developments and challenges. Environmental Technology & Innovation, 20, 101138.

Akao, S., Tsuno, H., Horie, T., Mori, S. 2007. Effects of pH and temperature on products and bacterial community in l-lactate batch fermentation of garbage under unsterile condition. Water Research, 41(12), 2636-2642.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

APHA. 1995. Standard methods for the examination of water and wastewater. American Public Health Association American Water Works Association/Environmental Federation, Washington, DC.

Arras, W., Hussain, A., Hausler, R., Guiot, S.R. 2019. Mesophilic, thermophilic and hyperthermophilic acidogenic fermentation of food waste in batch: Effect of inoculum source. Waste Management, 87, 279-287.

Braguglia, C.M., Gallipoli, A., Gianico, A., Pagliaccia, P. 2018. Anaerobic bioconversion of food waste into energy: A critical review. Bioresource Technology, 248, 37-56.

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum: Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

Bühlmann, C.H., Mickan, B.S., Tait, S., Bahri, P.A. 2022. Developing a food waste biorefinery: Lactic acid extraction using anionic resin and impacts on downstream biogas production. Chemical Engineering Journal, 431, 133243.

Bühlmann, C.H., Mickan, B.S., Tait, S., Renton, M., Bahri, P.A. 2021. Lactic acid from mixed food wastes at a commercial biogas facility: Effect of feedstock and process conditions. Journal of Cleaner Production, 284, 125243.

Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Steyer, J.-P., Delgenès, J.-P., Escudié, R. 2017. Dry anaerobic digestion of food waste and cardboard at different substrate loads, solid contents and co-digestion proportions. Bioresource Technology, 233, 166-175.

De Groof, V., Coma, M., Arnot, T., Leak, D.J., Lanham, A.B. 2021. Selecting fermentation products for food waste valorisation with HRT and OLR as the key operational parameters. Waste Management, 127, 80-89.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization

through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Detman, A., Laubitz, D., Chojnacka, A., Kiela, P.R., Salamon, A., Barberán, A., Chen, Y., Yang, F., Błaszczyk, M.K., Sikora, A. 2021. Dynamics of dark fermentation microbial communities in the light of lactate and butyrate production. Microbiome, 9(1), 1-158.

Elferink, S., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., ehuis, F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. Applied and environmental microbiology, 67, 125-132.

Feng, K., Li, H., Zheng, C. 2018. Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresource Technology, 270, 180-188.

Gu, S.-A., Jun, C., Joo, J.C., Kim, S., Lee, S.H., Kim, Y.H. 2014. Higher thermostability of l-lactate dehydrogenases is a key factor in decreasing the optical purity of d-lactic acid produced from Lactobacillus coryniformis. Enzyme and microbial technology, 58-59, 29-35.

Hoelzle, R.D., Puyol, D., Virdis, B., Batstone, D. 2021. Substrate availability drives mixed culture fermentation of glucose to lactate at steady state. Biotechnology and Bioengineering, 118(4), 1617-1629.

Ishangulyyev, R., Kim, S., Lee, S.H. 2019. Understanding Food Loss and Waste-Why Are We Losing and Wasting Food? Foods, 8(8), 297.

Jain, M., Nagar, P., Sharma, A., Batth, R., Aggarwal, S., Kumari, S., Mustafiz, A. 2018. GLYI and D-LDH play key role in methylglyoxal detoxification and abiotic stress tolerance. Scientific Reports, 8(1), 5451.

KEGG. 2022. Kyoto Encyclopedia of Genes and Genomes.

Khor, W.C., Roume, H., Coma, M., Vervaeren, H., Rabaey, K. 2016. Acetate accumulation enhances mixed culture fermentation of biomass to lactic acid. Applied microbiology and biotechnology, 100(19), 8337-8348.

Kim, D.-H., Lim, W.-T., Lee, M.-K., Kim, M.-S. 2012. Effect of temperature on continuous fermentative lactic acid (LA) production and bacterial community, and development of LA-producing UASB reactor. Bioresource Technology, 119, 355-361.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology, 31(9), 814-821.

Li, X., Chen, Y., Zhao, S., Chen, H., Zheng, X., Luo, J., Liu, Y. 2015. Efficient production of optically pure l-lactic acid from food waste at ambient temperature by regulating key enzyme activity. Water Research, 70, 148-157.

Louvado, A., Coelho, F.J.R.C., Palma, M., Tavares, L.C., Ozorio, R.O.A., Magnoni, L., Viegas, I., Gomes, N.C.M. 2020. Effect of glycerol feed-supplementation on seabass metabolism and gut microbiota. Applied microbiology and biotechnology, 104(19), 8439-8453.

Mazumdar, S., Blankschien, M.D., Clomburg, J.M., Gonzalez, R. 2013. Efficient synthesis of L-lactic acid from glycerol by metabolically engineered Escherichia coli. Microbial cell factories, 12(1), 7-7.

Mori, H., Maruyama, F., Kato, H., Toyoda, A., Dozono, A., Ohtsubo, Y., Nagata, Y., Fujiyama, A., Tsuda, M., Kurokawa, K. 2014. Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes. DNA Res, 21(2), 217-27.

Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, 143, 615-623.

R Development Core Team. 2022. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.

Regueira, A., Rombouts, J.L., Wahl, S.A., Mauricio-Iglesias, M., Lema, J.M., Kleerebezem, R. 2021. Resource allocation explains lactic acid production in mixed-culture anaerobic fermentations. Biotechnology and bioengineering, 118(2), 745-758.

Rombouts, J.L., Kranendonk, E.M.M., Regueira, A., Weissbrodt, D.G., Kleerebezem, R., van Loosecht, M.C.M. 2020. Selecting for lactic acid producing and utilising bacteria in anaerobic enrichment cultures. Biotechnology and bioengineering, 117(5), 1281-1293.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F. 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology, 75(23), 7537-7541.

Shetty, S.A., Boeren, S., Bui, T.P.N., Smidt, H., Vos, W.M. 2020. Unravelling lactate-acetate and sugar conversion into butyrate by intestinal Anaerobutyricum and Anaerostipes species by comparative proteogenomics. Environmental microbiology, 22(11), 4863-4875.

Śliżewska, K., Chlebicz-Wójcik, A. 2020. Growth Kinetics of Probiotic Lactobacillus Strains in the Alternative, Cost-Efficient Semi-Solid Fermentation Medium. Biology (Basel, Switzerland), 9(12), 423.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y. 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management, 52, 278-285.

Tang, J., Wang, X.C., Hu, Y., Zhang, Y., Li, Y. 2017. Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. Bioresource Technology, 224, 544-552.

Tashiro, Y., Inokuchi, S., Poudel, P., Okugawa, Y., Miyamoto, H., Miayamoto, H., Sakai, K. 2016. Novel pH control strategy for efficient production of optically active l-lactic acid from kitchen refuse using a mixed culture system. Bioresource Technology, 216, 52-59.

Tashiro, Y., Matsumoto, H., Miyamoto, H., Okugawa, Y., Pramod, P., Miyamoto, H., Sakai, K. 2013. A novel production process for optically pure l-lactic acid from kitchen refuse using a bacterial consortium at high temperatures. Bioresource Technology, 146, 672-681.

Tsapekos, P., Alvarado-Morales, M., Baladi, S., Bosma, E.F., Angelidaki, I. 2020. Fermentative Production of Lactic Acid as a Sustainable Approach to Valorize Household Bio-Waste. Frontiers in Sustainability, 1.

UN. 2019. Resolution Adopted by the General Assembly on 25 September 2015, (Eds.) J. Walker, A. Pekmezovic, G. Walker, John Wiley & Sons, Ltd. Chichester, UK, pp. 333-374.

Wang, F., Luo, J., Fang, S., Huang, W., Zhang, Y., Zhang, L., Cheng, X., Du, W., Fang, F., Cao, J., Wu, Y. 2022. Mechanisms of allicin exposure for the sludge fermentation enhancement: Focusing on the fermentation processes and microbial metabolic traits. Journal of Environmental Sciences, 115, 253-264.

Wang, K., Yin, J., Shen, D., Li, N. 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: Effect of pH. Bioresource technology, 161, 395-401.

Wang, Q., Wang, X., Wang, X., Ma, H., Ren, N. 2005. Bioconversion of Kitchen Garbage to Lactic Acid by Two Wild Strains of Lactobacillus Species. Journal of Environmental Science and Health, Part A, 40(10), 1951-1962.

Wang, Q., Yang, L., Feng, K., Li, H., Deng, Z., Liu, J. 2021. Promote lactic acid production from food waste fermentation using biogas slurry recirculation. Bioresour Technol, 337, 125393.

Xiong, Z., Hussain, A., Lee, J., Lee, H.-S. 2019. Food waste fermentation in a leach bed reactor: Reactor performance, and microbial ecology and dynamics. Bioresource Technology, 274, 153-161.

Yang, L., Chen, L., Li, H., Deng, Z., Liu, J. 2022. Lactic acid production from mesophilic and thermophilic fermentation of food waste at different pH. Journal of Environmental Management, 304, 114312.

Zhang, C., Zhou, C., Assavasirijinda, N., Yu, B., Wang, L., Ma, Y. 2017a. Non-sterilized fermentation of high optically pure d-lactic acid by a genetically modified thermophilic Bacillus coagulans strain. Microbial Cell Factories, 16(1), 213.

Zhang, W., Li, X., He, Y., Xu, X., Chen, H., Zhang, A., Liu, Y., Xue, G., Makinia, J. 2020a. Ammonia amendment promotes high rate lactate production and recovery from semi-continuous food waste fermentation. Bioresource Technology, 302, 122881.

Zhang, W., Li, X., Zhang, T., Li, J., Lai, S., Chen, H., Gao, P., Xue, G. 2017b. High-rate lactic acid production from food waste and waste activated sludge via interactive control of pH adjustment and fermentation temperature. Chemical Engineering Journal, 328, 197-206.

Zhang, W., Xu, X., Yu, P., Zuo, P., He, Y., Chen, H., Liu, Y., Xue, G., Li, X., Alvarez, P.J.J. 2020b. Ammonium Enhances Food Waste Fermentation to High-Value Optically Active l-Lactic acid. ACS Sustainable Chemistry & Engineering, 8(1), 669-677.

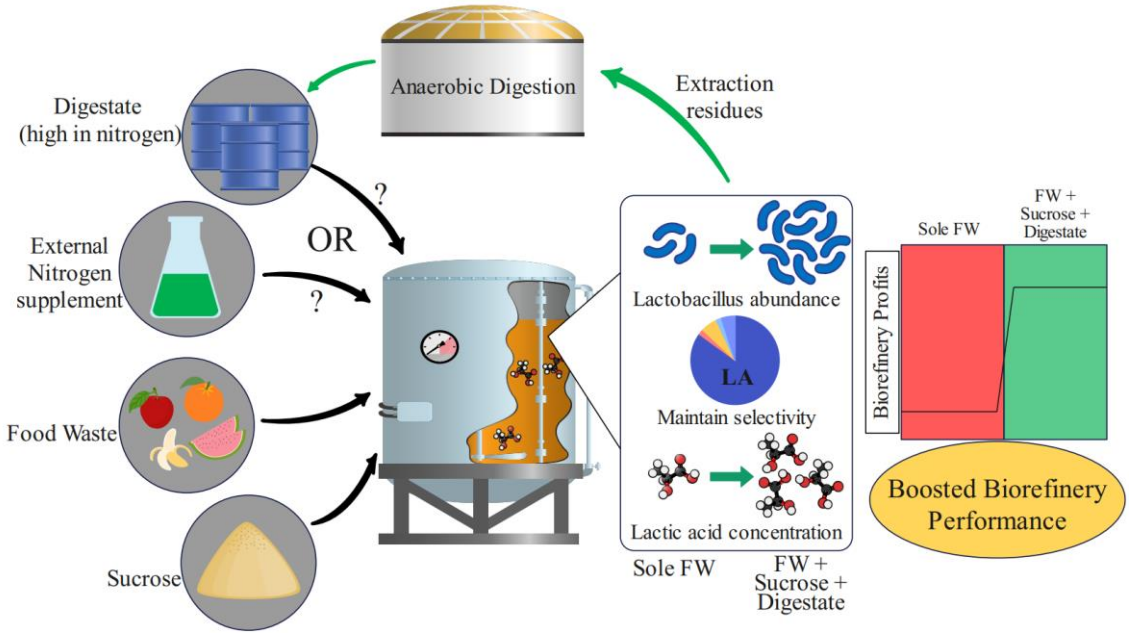
CHAPTER 5 ENHANCING LACTIC ACID FERMENTATION

Foreword

Chapter 4 explored LA fermentation from FWs using an adapted inoculum, known to produce LA as its primary product (Chapter 3), and optimised operational conditions to maximise LA production. The current chapter utilises the data obtained from Chapter 4 to explore methods to further boost LA fermentation performance from an LA-AD biorefinery, specifically through supplementing FW with a simple carbohydrate and implementing partial digestate recirculation as a nitrogen supplement. In addition to LA, the impact of carbohydrate and digestate addition on the product spectrum, microbial community, and degradation pathways for LA are also explored in this chapter.

ABSTRACT

Food waste anaerobic digestion (AD) facilities can become high-value biorefineries when integrated with lactic acid (LA) production. Supplementing with nitrogen as a nutrient source and sucrose as a low-value bioavailable carbon source during fermentation, may improve LA production and enhance the value of LA-AD biorefineries. Therefore, this work explored the effects of sucrose addition (0-150 $g \cdot L^{-1}$) and nitrogen supplementation (0-400 $mgN \cdot L^{-1}$) as NH_4Cl or digestate on LA fermentation, utilising a bi-factorial experimental design. Response surface methodology analysis showed nitrogen supplementation incrementally improved the rate and final LA concentration by 0.03±0.02 $hour^{-1}$ and 5.2±4.6 $g \cdot L^{-1}$, respectively, as compared to the modelled base case (i.e. no sucrose or nitrogen). The same analysis showed that digestate addition led to a similar incremental rate improvement of 0.04±0.02 $hour^{-1}$. Sucrose addition at 107 $g.L^{-1}$ enhanced the final LA concentration from 25-30 $g \cdot L^{-1}$ to 59-68 $g \cdot L^{-1}$, depending on nitrogen dosage and source, but led to substrate inhibition at higher dosages. Digestate as nitrogen source increased microbial diversity and increased the production of competitor organic acids, while sucrose enhanced the growth of *Lactobacillus*, reduced microbial diversity, and improved LA selectively. Overall, digestate recirculation can be effective at promoting LA production, but its influence on acid product mix may need to be constrained by limiting dosages; further, sucrose dosed up to a certain level, can improve LA concentration, LA selectivity, and promote growth of favourable LA bacteria for FW fermentation.



5.1 INTRODUCTION

Food waste (FW), is any healthy edible food lost along the production, supply and consumption chain, produced in large quantities, and continues to increase with a growing global population (Wolka & Melaku, 2015). Recent estimates have suggested an annual global economic loss associated with FW nearing USD 1.0 trillion (FAO, 2021) and that 8% of global anthropogenic greenhouse gas emissions is associated with FW (WBA, 2018). For these reasons, there is a pressing need to develop closed-loop technologies that beneficially utilise and reduce FW to mitigate associated adverse impacts.

Anaerobic digestion (AD) is a technology now widely used to process FWs into renewable biogas energy, and digestate as a fertilizer nutrient source. However, the economic feasibility of FW AD heavily relies on gate-fees, which are subject to government policy or government subsidies (Bastidas-Oyanedel & Schmidt, 2018; Edwards et al., 2015). To address this, recent research has proposed and demonstrated pairing AD with lactic acid (LA) production and recovery, aimed at generating additional revenue via a LA-AD biorefinery concept to make FW AD facilities more economically feasible (Bühlmann et al., 2022; Demichelis et al., 2018; Kim et al., 2016).

LA is a valuable commodity chemical with uses in various industries including the food, pharmaceutical, and textile industries or as a raw material for the production of biodegradable bioplastic (Kim et al., 2016). Literature has explored LA production from FW and identified that production is technically feasible at both lab (Kim et al., 2016; Pleissner et al., 2017) and commercial scale (Bühlmann et al., 2021). However, barriers which limit LA production from FWs still exist including 1) the slow hydrolysis rate of FW (Zhang et al., 2020b), 2) formation of competitor metabolite by-products, and 3) the low LA yield (Yousuf et al., 2018). Arguably, the last listed is currently one of the most significant constraints on the development of commercial LA biorefineries, as it directly limits the final LA concentration achieved, and therefore elevates costs of recovery (Alves de Oliveira et al., 2018).

LA production by fermentation of FW is thought to be limited by readily bioavailable carbohydrates and hence the inability of LA bacteria to fully utilise the FW as substrate. Higher substrate concentrations promote LA formation (Pleissner et al., 2017; Pu et al., 2019; Yousuf et al., 2018) and the growth of LA bacteria (Pu et al., 2019), but can be

subject to lower overall yields. When FW has been pre-treated prior to fermentation, LA production has been enhanced (Demichelis et al., 2017; Kwan et al., 2016; Pleissner et al., 2016), likely by increasing the bioavailability of the FW. However, pre-treatment is costly to operate (Surendra et al., 2015). Alternatively FWs may be supplemented with simple carbohydrates to increase the concentration of readily available substrate for LA bacteria, such as with sucrose, which is commonly used for LA production (Olszewska-Widdrat et al., 2020). Supplementing a complex substrate with a simple carbohydrate is realistic for a FW LA-AD biorefinery. Moreover, promoting LA formation and ensuring that high target LA concentrations are reached would be important to minimise downstream processing costs. This is especially important within the FW context, where a variable feedstock composition is anticipated and would likely influence LA fermentation (Chapter 3). However, past research has not yet explored the effects of sucrose addition on LA fermentation from FW, looking at associated microbial community dynamics, and metabolic pathways that influence LA production.

Limited nitrogen (N) availability can also adversely impact on LA production, being required for the synthesis of proteins and nucleic acids for bacterial growth (Zhang et al., 2019). Recent literature has shown NH_4Cl can be an effective N supplement to enhance FW fermentation to LA, yielding a 2.0-2.4 fold increase in LA concentration following supplementation with 300-400 $mgN \cdot L^{-1}$ (Zhang et al., 2020a; Zhang et al., 2020b). However, FW AD digestate naturally contains elevated ammonium concentrations (3,280-5,000 ppm N, (Buhlmann et al., 2018; Serna-Maza et al., 2015)), and so may be a promising low-cost N source for LA fermentation. In fact, digestate is often considered a cost liability for many AD facilities, despite its imbedded carbon and nutrient value. This is partly due to its bulky nature, with a high moisture content, diluting its nutrient value, and increasing its associated costs of storage, transport, and land application (Turnley et al., 2016). Digestate utilisation within LA fermentation instead provides an opportunity for this low value by-product of AD to enhance the feasibility of a FW LA-AD biorefinery concept.

Limited available research has shown benefits of digestate on FW fermentation, improving pH stability, increasing microbial diversity, maintaining a low oxidation reduction potential (Wang et al., 2021), or simply being a process water source for LA fermentation following pre-treatment (Zhang et al., 2019). While these reports are promising, these available studies seeded LA fermentation with waste activated sludge

(Wang et al., 2021) or a specific strain of LA bacteria (Zhang et al., 2019). However, these inoculum sources either provide resilience in terms of a diverse microbial community (i.e. waste activated sludge), or the targeted performance of a pure culture, but not both. Instead, an adapted inoculum would more likely be used in future FW biorefinery concepts, for which the commercial mixed FW context can be highly variable (Bühlmann et al., 2021), that can reliably produce high LA concentrations, as well as having adequate microbial diversity to accommodate imminent process changes. For this reason, it would be vital to understand the impact of digestate for an adapted mixed culture inoculum.

To address the above knowledge gaps, this study tested the effect of sucrose and N addition (as digestate or NH_4Cl) on FW LA fermentation, for an acclimatised inoculum, sourced from the pre-fermentation stage of a commercial FW AD facility. The study aimed to resolve individual and combined effects of substrate availability (via sucrose addition) and N supplementation, and the distinct effects of N source as digestate or NH_4Cl. The impact on microbial community and fermentation pathways were also explored. The aim was to improve LA fermentation from FWs to enable future LA-AD biorefinery concepts.

5.2 METHOD

5.2.1 Substrate and inoculum

A synthetic mixed FW feedstock was used in this study and prepared following the recipe of Capson-Tojo et al. (2017) (Table C1). Preparation of the FW included maceration, blending, and screening as previously described elsewhere (Chapter 4). The prepared FW was stored overnight at 1-4 °C before use. The inoculum was obtained from the pre-fermentation tank of a commercial two-stage FW AD facility, previously described elsewhere (Bühlmann et al., 2021). Anaerobic digestate was sourced from an anaerobic digester at the same facility. The inoculum and digestate were stored at 1-4 °C before use. Compositional analysis of the prepared synthetic FW, inoculum, and digestate was conducted at the Analytical Reference Laboratory (Perth, Australia) using standard methods (Table C2). Reagent grade sucrose (Chem-Supply, Australia; SA030) and analytical reagent grade ammonium chloride (Chem-Supply, Australia; AA049) were used as carbohydrate and model N source in the experiments, respectively.

5.2.2 Batch fermentation tests

Batch fermentation tests were performed in 250 ml glass serum vials. Vials were filled with 20 ml inoculum and 180 ml synthetic FW, and digestate (see below) or tap water up to a total working volume of 234 ml. Sucrose crystals were added at dosages of 0, 43, 107, or 150 $g_{sucrose} \cdot L_{mixture}^{-1}$ to align with similar conditions of relevant past studies (Reddy et al., 2015). In line with Zhang et al. (2020b) (Section 5.1), fermentation vessels were supplemented with N at 0, 300, or 400 $mgN \cdot L_{mixture}^{-1}$ (excluding background), added as NH_4Cl powder (Chem-Supply; AA049) or digestate. Levels in each test vial was set by a bi-factorial experimental design, assessing the effects of sucrose and N addition. The tests were conducted in four blocks (Table C3), which did introduce an additional time factor, due to progressive aging of the inoculum from block 1 to 4, and this was added as a separate variable in the subsequent analysis.

After reagent addition (as relevant), the test vessels were sealed with a butyl rubber septum and screw top cap, the headspace was purged with high purity nitrogen, and the fermentation mixtures were adjusted to pH 6.0 and maintained at this pH by the method previously reported elsewhere (Chapter 4). The vessels were then incubated at 50 °C for 5 days. A previous study by the authors had been identified this test pH and temperature as being preferred for LA fermentation by the same adapted inoculum (Chapter 4).

pH was measured using a calibrated benchtop pH meter and probe (Rowe Scientific, Australia; IP1400 and IP1163). Liquid samples were periodically collected for measurements of volatile fatty acids (VFAs) and LA (Section 5.2.3). For this, the vessel was inverted, and a 5 ml sample was extracted and stored in 15 ml centrifuge vials at 1-4 °C for a maximum of 2 days prior to analysis (Section 5.2.3). At the end of fermentation (5 days), an additional 10 ml sample was taken and immediately stored at -20°C for DNA sequencing (Section 5.2.4).

5.2.3 Analytical methods

Total solids (TS) and volatile solids (VS) were measured according to Standard Methods (APHA, 1995). Prior to organic acid analysis, part of each liquid sample was centrifuged at 10,000 g for 10 minutes and the remainder part was immediately put into longer term storage at -20 °C. The supernatant from the centrifuged sample was collected for analysis and the pellet discarded. The supernatant was diluted with deionised water to within measurement range and filtered through a 0.45 mm PES Millipore® filter before

measurement of LA, acetic acid, succinic acid, butyric acid, and propionic acid by High Performance Liquid Chromatography using the methods previously described elsewhere (Bühlmann et al., 2021). LA selectivity was calculated using Eq. 5.1 after first converting acid concentrations from $g \cdot L^{-1}$ to $gCOD \cdot L^{-1}$ using theoretical COD to mass ratios.

$$LA(\%) = \frac{C_{LA}}{C_{LA}+C_{SA}+C_{AA}+C_{PA}+C_{BA}} \quad \text{(Eq. 5.1)}$$

where C_{LA}, C_{SA}, C_{AA}, C_{PA}, and C_{BA} are the concentrations of LA, succinic acid, acetic acid, propionic acid, and butyric acid ($gCOD \cdot L^{-1}$), respectively.

5.2.4 DNA extraction and amplification

The frozen whole liquid samples collected on day 5 were thawed and vortexed for 15 seconds, and DNA subsequently extracted for analysis. Methods for DNA extraction, amplification, and screening have been previously described elsewhere (Chapter 4). The extracted DNA was sequenced at the Australian Centre for Ecogenomics (ACE), The University of Queensland (Brisbane, Australia), on the Illumina® Mi-seq platform.

5.2.5 Bioinformatics

5.2.5.1 Taxonomy analysis

Taxonomic assignment used Mothur 1.46.1 with a slightly modified standard operating procedure (Schloss et al., 2009). Sequences were aligned with the Silva database (Release v132) and assigned operational taxonomic units based on 97% similarity. Detailed methods used for the taxonomy analysis were as previously described elsewhere (Chapter 4).

5.2.5.2 Phylogenic investigation of Communities by Reconstruction of Unobserved States (PICRUSt)

Sequenced data for PICRUSt was processed using Mothur 1.46.1 with a slightly modified standard operating procedure as above and as previously described elsewhere (Chapter 4). NSTI values for this study ranged from 0.06±0.003 to 0.12±0.019 with an average of 0.099±0.019 s.d, similar to those for environmental communities and lower than the 0.15 threshold used to indicate similarity with the reference genome database (Langille et al., 2013; Louvado et al., 2020). All genes were identified via the KEGG database (KEGG, 2022).

5.2.6 Data analysis and statistics

As LA and other organic acids were present within the inoculum, all acid yields and concentrations presented below are net values after subtracting initial concentrations measured at time t=0. All measurements are presented as the mean of triplicates ±95% confidence intervals determined by a two-tailed student t-test. Acid yields were normalized with respect to the initial VS of FW and sucrose added (not including VS from added inoculum or digestate).

The rate of LA formation and maximum LA yield were estimated using a first-order plus lag model (Eq. 5.2).

$$P(t) = P_{max}(1 - \exp(-k(t - \theta)) \quad \text{(Eq. 5.2)}$$

where $P(t)$ is LA yield ($g_{LA} \cdot g_{VS}^{-1}$) at time t (hours), P_{max} is the maximum LA yield ($g_{LA} \cdot g_{VS}^{-1}$), k is the first-order rate constant ($hour^{-1}$), and $\boldsymbol{\theta}$ is an initial time lag (hours). This analysis was conducted in AQUASIM 2D (Reichert, 1994) and included all data up to the visually identified maximum measured LA yield. Parameter uncertainty was estimated at the 95% confidence limit based on a two-tailed t-test on parameter standard error around the optimum, as determined by AQUASIM 2D. The coefficient of determination (R^2) of the model fits were calculated in Microsoft Excel.

Response surface methodology (RSM) was used to identify single and interactive effects of N supplementation and sucrose addition on the maximum LA concentration and rate of LA formation (k values from Eq. 5.2). Independent variables were sucrose (X_S), N-_dosage (X_N), and the N_source (X_{NS}). The raw triplicate data of measured LA concentration (individual observations) and the model estimates of k, were the response variables in separate analyses. For the statistical analysis, the numerical independent variables were normalised linearly (Table C3), to ensure each predictor had an equal weighting. N source was included as a categorical variable (X_{NS}; N_Source) in the model (0=NH_4Cl, 1=Digestate). As the tests were conducted in runs in time sequence (4 blocks in total), a block factor (R_B) was included within the regression analysis as a continuous factor (1-4) to test for aging of the inoculum (Table 5.1). The standard scores were fitted to a second order regression model (Eq. 5.3) via least squares regression analysis, as follows:

$$Y = \beta_0 + R_B X_B + (\beta_S X_S) + (\beta_N X_N) + (\beta_{NS} X_{NS}) + (\beta_{S\_N} X_S X_N) + (\beta_{S\_NS} X_S X_{NS}) + (\beta_{N\_NS} X_N X_{NS}) + (\beta_{S^2} X_S^2) + (\beta_{N^2} X_N^2)$$ (Eq. 5.3)

where β_0 is an intercept, β_S, β_N, and β_{NS} are linear terms, β_{S_N}, β_{S_NS}, and β_{N_NS} are two-way interaction terms, and $\beta_{S\wedge 2}$ and $\beta_{N\wedge 2}$ are squared effects. Model parameters were determined using the RSM function in R (R Development Core Team, 2022). To avoid overfitting and ensure the most significant parameters remained within the model, the step() function was applied to sequentially remove parameters from the model based on the model Akaike Information Criterion (AIC) as previously described elsewhere (Bühlmann et al., 2021). The 95% confidence intervals for each parameter estimate were determined using confint() in R, and 95% confidence intervals for the model predictions were determined using the predict() function in R.

To assess the effects of N supplementation and sucrose addition on other measured organic acids, microbial community composition, and putative metabolic pathways, the RSM described above was further applied to individual VFA concentrations achieved at the visually selected maximum LA concentration, the relative abundance of genera (>1%), and select genes related to LA formation, as respective response variables in separate analyses. The relative abundance of all genes included in the analysis was arbitrarily multiplied by a factor of 1,000 to improve the sensitivity of the model fit. Predictor variables remained unchanged from that described above.

To estimate the fraction of sucrose utilised, the RSM was applied to interpolate and estimate the maximum LA concentration achieved for selected conditions, from which a theoretical yield on sucrose was subsequently calculated. For this, the LA yield on FW was assumed to be that achieved when no sucrose had been added (accounting for the separate effects of N, N_source and block). For sucrose, the theoretical LA yield was assumed to be the same as that for glucose, specifically 1 $g_{LA} \cdot g_{glucose}^{-1}$ (Yang et al., 2013), because a theoretical yield on sucrose could not be found in the literature.

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of sucrose and nitrogen addition on lactic acid production

All test conditions showed similar LA production profiles, with the LA concentration initially rising rapidly to an asymptotic final value, with minimal to no subsequent LA depletion observed over the 120 hours test period (Fig. 5.1 – 5.4) (depletion of LA would

result from the conversion of LA into other organic acids, Chapter 4). Consequently, all tests were appropriately described by 1st order kinetics with an initial time lag (Table 5.1). With no sucrose or N addition, LA accumulated rapidly within the first 24 hours, and then slowed significantly, reaching a maximum yield of 0.63 $g_{LA} \cdot g_{VS}^{-1}$ by 60 hours (Table 5.1). This yield was similar to those reported by studies conducted at similar conditions e.g. 0.57 $g_{LA} \cdot g_{VS}^{-1}$ (Yang et al., 2022), 0.58 $g_{LA} \cdot g_{VS}^{-1}$ (Akao et al., 2007), and 0.55 $g_{LA} \cdot g_{VS}^{-1}$ (Chapter 4).

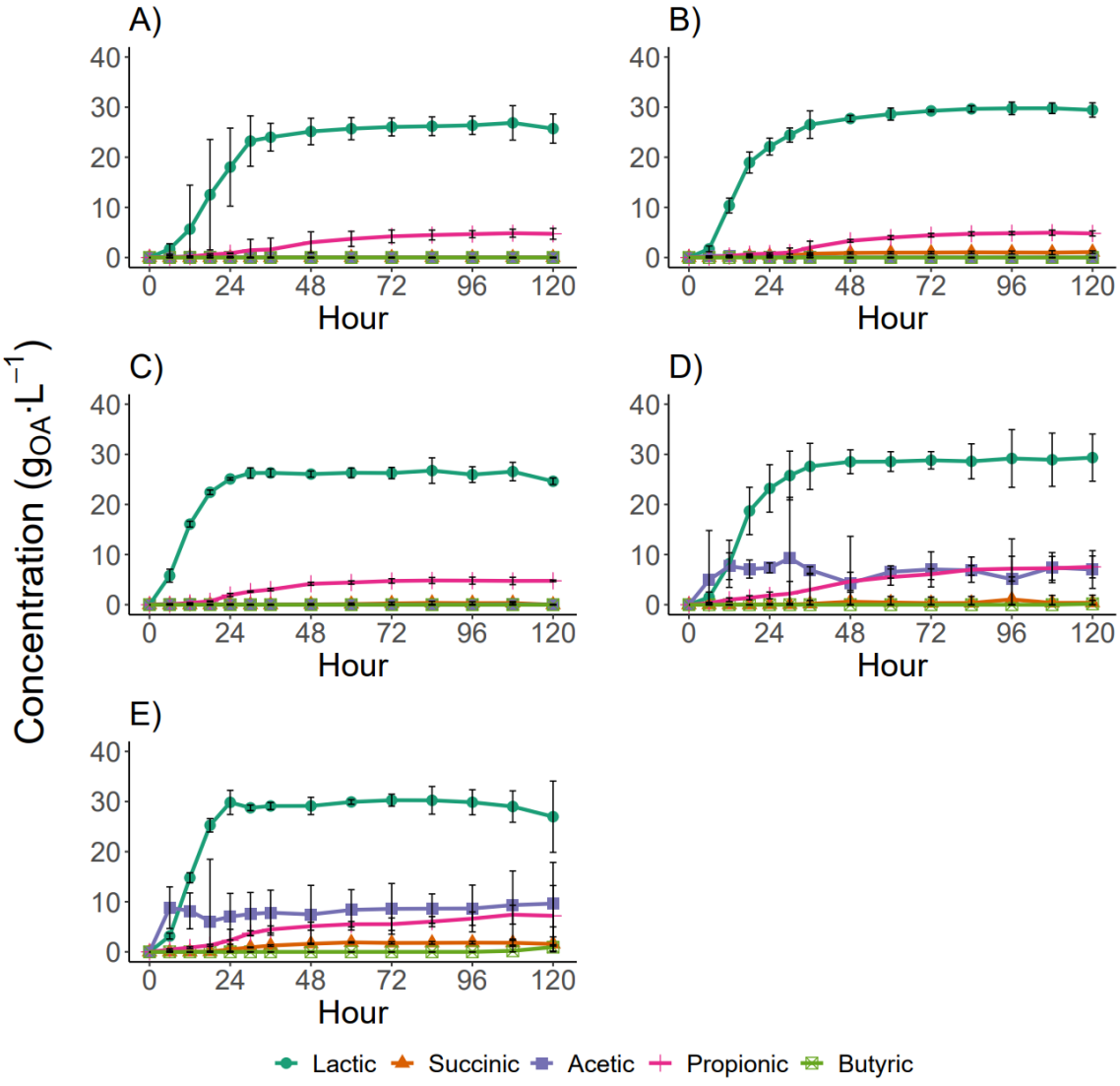


Figure 5.1: Plot of organic acid production with 0 g·L-1 sucrose addition with A) 0 $mgN \cdot L^{-1}$ supplement, B) 300 $mgN \cdot L^{-1}$ supplement with NH4Cl, C) 400 $mgN \cdot L^{-1}$ supplement with NH4Cl, D) 300 $mgN \cdot L^{-1}$ supplement with digestate, and E) 400 $mgN \cdot L^{-1}$ supplement with digestate. Error bars represent the mean ±95% confidence interval.

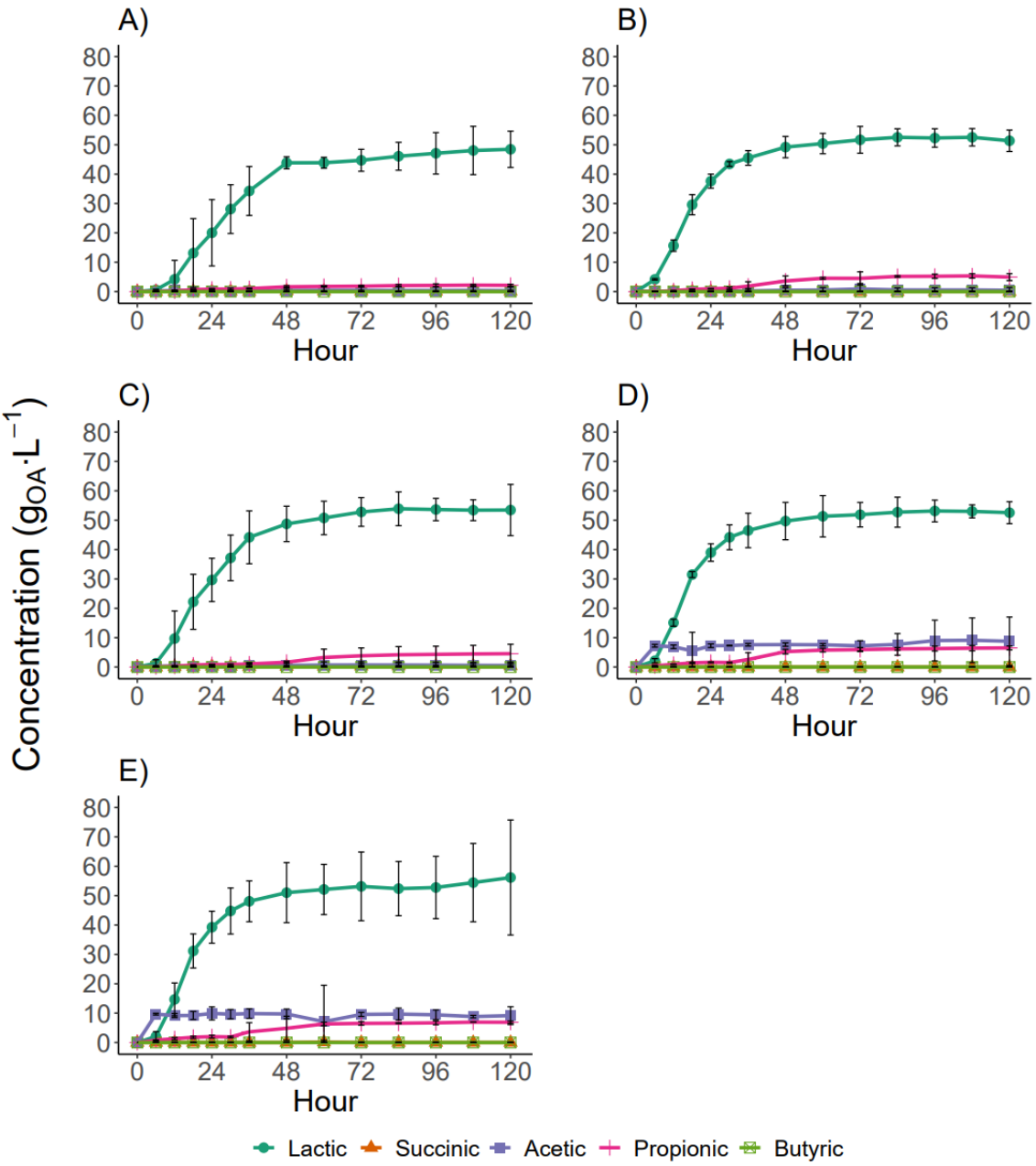


Figure 5.2: Plot of organic acid production with 43 g·L-1 sucrose addition with A) 0 $mgN \cdot L^{-1}$ supplement, B) 300 $mgN \cdot L^{-1}$ supplement with NH4Cl, C) 400 $mgN \cdot L^{-1}$ supplement with NH4Cl, D) 300 $mgN \cdot L^{-1}$ supplement with digestate, and E) 400 $mgN \cdot L^{-1}$ supplement with digestate. Error bars represent the mean ±95% confidence interval.

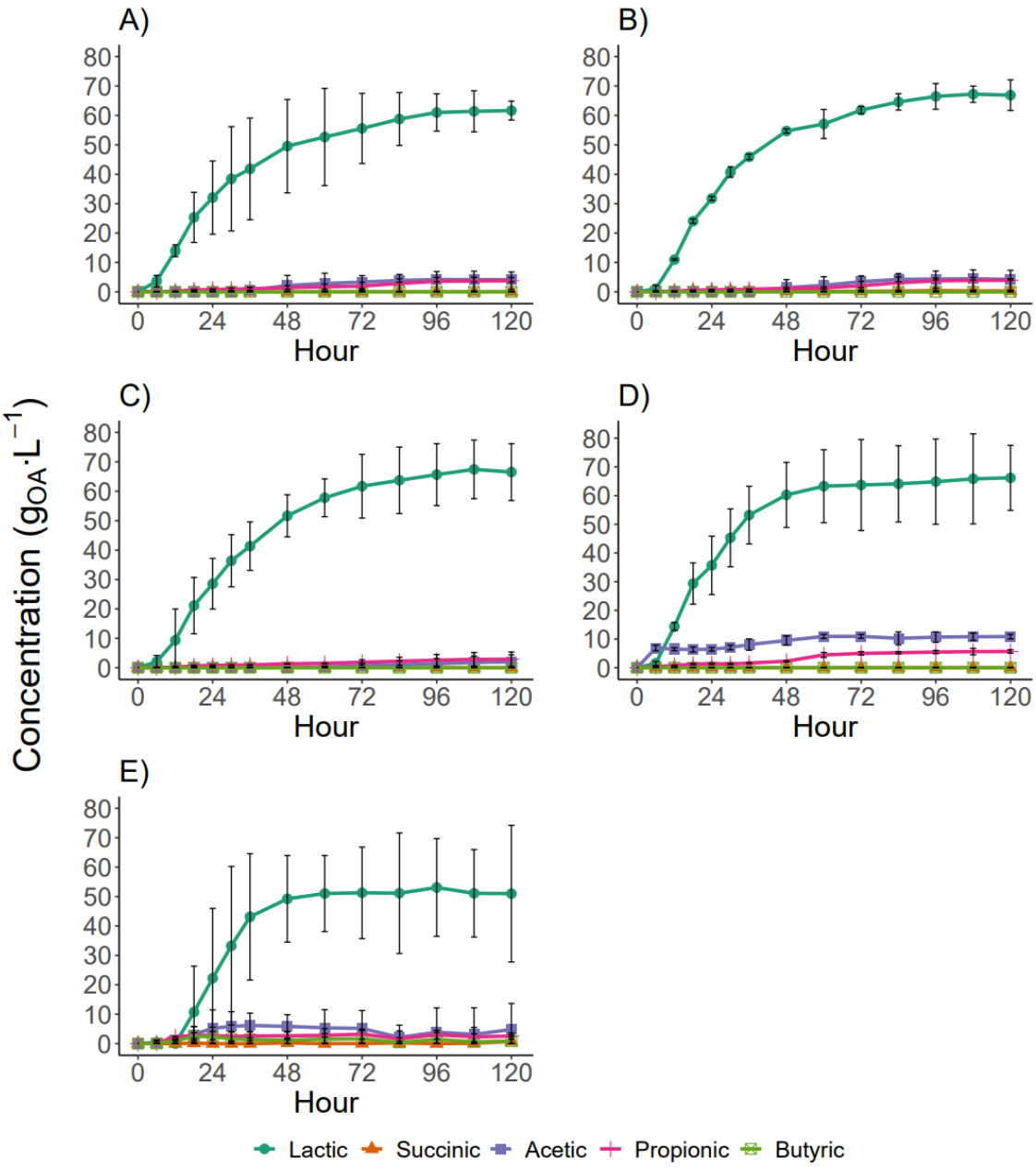


Figure 5.3: Plot of organic acid production with 107 g·L-1 sucrose addition with A) 0 mgN·L^{-1} supplement, B) 300 mgN·L^{-1} supplement with NH4Cl, C) 400 mgN·L^{-1} supplement with NH4Cl, D) 300 mgN·L^{-1} supplement with digestate, and E) 400 mgN·L^{-1} supplement with digestate. Error bars represent the mean ±95% confidence interval.

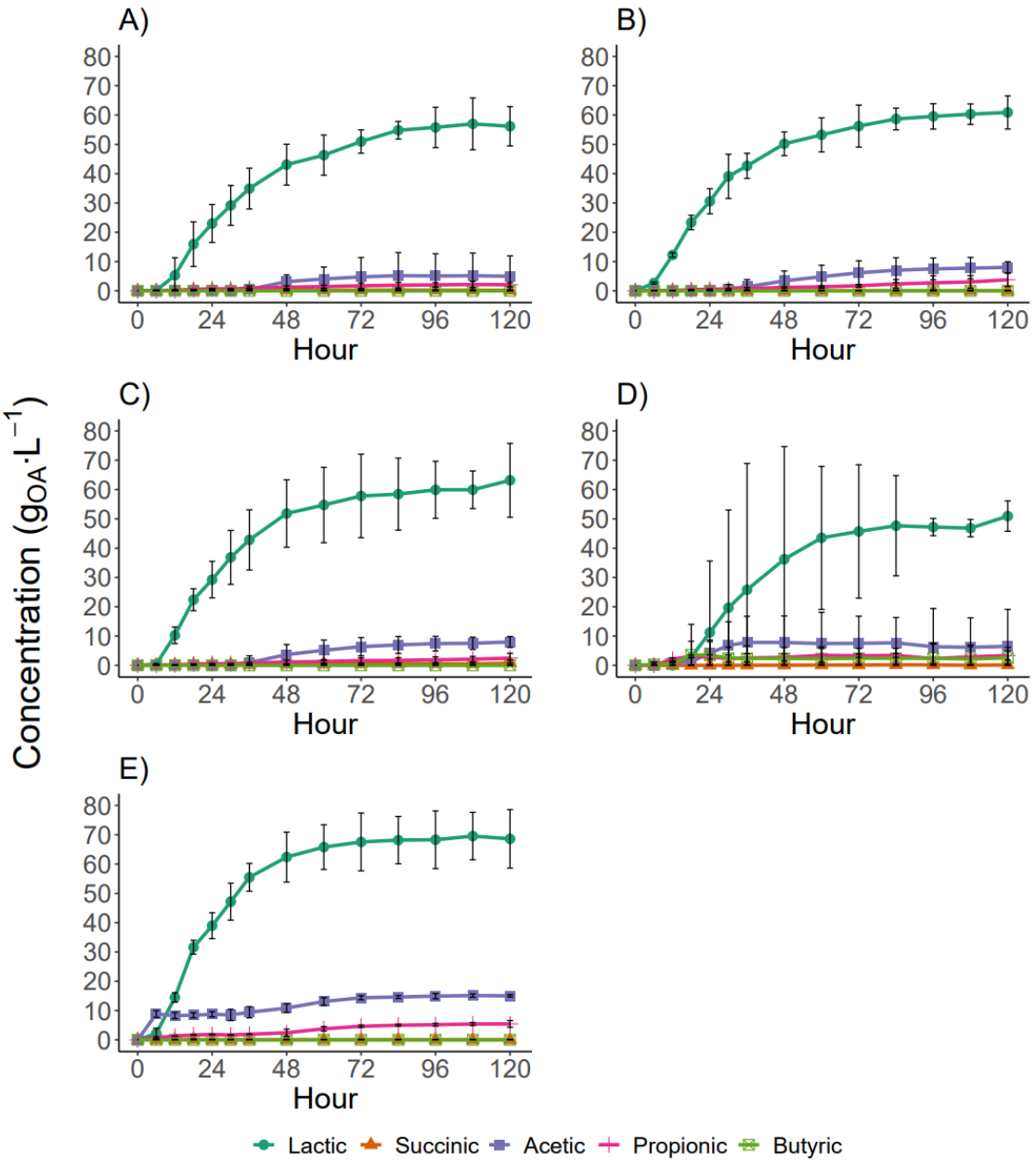


Figure 5.4: Plot of organic acid production with 150 g·L-1 sucrose addition with A) 0 mgN·L^{-1} supplement, B) 300 mgN·L^{-1} supplement with NH4Cl, C) 400 mgN·L^{-1} supplement with NH4Cl, D) 300 mgN·L^{-1} supplement with digestate, and E) 400 mgN·L^{-1} supplement with digestate. Error bars represent the mean ±95% confidence interval.

The final simplified RSM models described the observed data well, having an adjusted R^2 of 0.89 and 0.88 (Table 5.2). No two-way interactions were retained by the step() function, except for a single interaction term within the rate model, albeit that its coefficient estimate was not found to be significant (i.e. not significantly different from zero) (Table 5.2).

Sucrose displayed a strong positive linear effect and a strong negative second order effect on LA concentration (Table 5.2), indicating that LA concentration was increased by sucrose addition up to a certain dosage (Table 5.1), but that higher dosages led to a reduction in LA concentration, possibly partly due to substrate inhibition. In contrast, sucrose displayed a strong negative linear effect on LA formation rate (k), indicating rate inhibition at all levels. Sucrose also had a minor positive second-order effect on k (Table 5.2).

Table 5.1: Kinetic parameters for the first-order model. Errors(±) represent 95% confidence intervals.

Block	Sucrose ($g \cdot L^{-1}$)	N ($mgN \cdot L^{-1}$)[a]	Max. time (hours)[b]	Max. LA ($g_{LA} \cdot L^{-1}$)	Max. yield ($g_{LA} \cdot g_{VS}^{-1}$)	k ($hour^{-1}$)	Lag phase (hours)
1	0	0	60	25.7(±2.2)	0.63(±0.06)	0.08(±0.03)	9.4(±2.0)
1	107	0	120	61.7(±3.2)	0.45(±0.01)	0.04(±0.01)	4.3(±0.8)
1	43	300	72	51.7(±4.6)	0.62(±0.03)	0.08(±0.02)	7.4(±1.4)
1	150	300	120	60.9(±5.7)	0.35(±0.01)	0.04(±0.01)	6.4(±1.4)
1	0	400	48	26.1(±0.6)	0.64(±0.02)	0.13(±0.02)	4.1(±0.7)
1	107	400	120	66.5(±9.7)	0.50(±0.01)	0.03(±0.01)	7.6(±1.0)
2	43	0	72	44.7(±3.7)	0.60(±0.08)	0.04(±0.02)	10.6(±2.2)
2	150	0	120	56.2(±6.7)	0.33(±0.01)	0.03(±0.01)	8.9(±0.9)
2	0	300	84	29.7(±0.6)	0.69(±0.03)	0.08(±0.02)	6.3(±2.1)
2	107	300	120	66.9(±5.2)	0.49(±0.01)	0.04(±0.01)	7.2(±0.9)
2	43	400	72	52.8(±4.9)	0.66(±0.03)	0.05(±0.01)	8.5(±1.3)
2	150	400	96	59.9(±9.7)	0.35(±0.01)	0.04(±0.01)	7.7(±1.0)
3	0	300_D	48	28.5(±2.4)	0.69(±0.05)	0.11(±0.04)	9.0(±1.2)
3	43	300_D	60	51.3(±7.0)	0.62(±0.03)	0.09(±0.02)	8.1(±1.2)
3	107	300_D	72	63.7(±15.9)	0.49(±0.03)	0.05(±0.01)	7.3(±2.1)
3	0	400_D	60	29.9(±0.5)	0.72(±0.05)	0.13(±0.05)	5.3(±1.2)
3	43	400_D	60	52.1(±8.6)	0.64(±0.02)	0.09(±0.02)	8.9(±1.0)
3	150	400_D	84	68.2(±8.1)	0.40(±0.02)	0.05(±0.01)	7.4(±1.4)
4	150	300_D	96	47.2(±2.9)	0.29(±0.01)	0.04(±0.01)	17.1(±1.1)
4	107	400_D	72	51.3(±15.6)	0.38(±0.02)	0.07(±0.02)	15.2(±1.5)

a) N source is indicated as either NH_4Cl (no subscript) or digestate (subscript D); b) Corresponds to the time at which the LA concentration was at its maximum value.

N_Amount retained a positive linear effect on both LA yield and k, with the model estimating an incremental concentration increase of 5.2±4.6 $g \cdot L^{-1}$ LA at the highest level (400 $mgN \cdot L^{-1}$) compared to a modelled base case with no sucrose or N addition (i.e. 27.3±3.4 $gLA \cdot L^{-1}$). Similar studies by Zhang et al. (2020b) and Zhang et al. (2020a) outlined a 2-2.4 fold increase in LA concentration resulting from N supplementation using NH_4Cl, much higher than that observed in the current study (1.2-fold). This difference may be, at least partially, due to different inoculum sources and FW utilised.

The seed material has been suggested to be a key determining factor for the evolution of relevant metabolic pathways, LA yields, productivities, and competing biological processes (Arras et al., 2019; Tang et al., 2017; Wang et al., 2014). Both Zhang et al. (2020b) and Zhang et al. (2020a) utilised waste activated sludge for inoculation and reported NH_4Cl significantly increasing the relative abundance of LA producers. However, the adapted mixed inoculum utilised in the current study had LA bacteria naturally dominant, even in the zero N test (Section 5.3.3). The use of an adapted inoculum could have promoted LA production, leading to a lower overall response from N addition as NH_4Cl. A lower background N concentration (more limited N conditions) could also have caused the larger response to N observed by Zhang et al. (2020b) and Zhang et al. (2020a) but they did not report compositional data for their FW, so background N levels in their study could not be estimated.

Table 5.2: Simplified RSM model parameters with associated 95% confidence intervals.

Variable	Symbol	LA Concentration Model	k (Rate) Model
Intercept	β_0	32.32(±4.88)***	0.09(±0.02)***
Block	R_B	-4.97(±2.69)***	-0.01(±0.01)
Sucrose	β_S	95.52(±12.47)***	-0.12(±0.06)***
N_Amount	β_N	5.21(±3.62)**	0.03(±0.02)*
N_Source	β_{NS}	5.99(±5.43)*	0.03(±0.02)*
Sucrose^2	$\beta_{S\wedge2}$	-63.91(±12.02)***	0.08(±0.05)**
N_Amount^2	$\beta_{N\wedge2}$	-[a]	-[a]
TWI(Sucrose:Ammonia)	β_{S_N}	-[a]	-0.03(±0.04)
TWI(Sucrose:N_Source)	β_{S_NS}	-[a]	-[a]
TWI(Ammonia:N_Source)	β_{N_NS}	-[a]	-[a]
Adj.R^2	-	0.89	0.88

a: Removed by step() function (Section 5.2.6); ***=(P<0.001), **=(P<0.01), *=(P<0.05).

The RSM model showed that digestate led to a 1.3±4.5 $g \cdot L^{-1}$ incremental improvement in LA concentration, compared to the modelled base case, and, at the highest digestate level resulted in an increased k of 0.13±0.01 $hour^{-1}$, compared to the base case of 0.08±0.02 $hour^{-1}$. Similar to the current study, Wang et al. (2021) outlined that industrial digestate improved LA fermentation when utilised at a ratio of 0.2 $L_{digestate} \cdot L_{feedstock}^{-1}$ (current study used a ratio of 0.19 $L_{digestate} \cdot L_{feedstock}^{-1}$, at 400 $mgN \cdot L^{-1}$). While digestate contains high concentrations of NH_4^+-N, its complex matrix also contains various other nutrients (Table C2) and additional fermentative bacteria which may further aide LA fermentation.

The second order effect for N was not significant in either of the two RSM models (Table 5.2), suggesting that, unlike for sucrose, inhibitory concentrations for NH_4Cl and digestate were not reached in the current study. Previous research by Zhang et al. (2020b) outlined a reduction in LA production with NH_4^+-N supplementation above 500 $mgN \cdot L^{-1}$, which is higher than the maximum added dose in the current study (400 $mgN \cdot L^{-1}$).

Limited research is available exploring LA fermentation with added digestate, however, it has been suggested that excessive ammonia-N, zinc, iron, sulphur, and manganese within digestate could inhibit *Lactobacillus casei* during batch LA fermentation from starch, when the digestate is being used as a process water source (Zhang et al., 2019). Comparably, Wang et al. (2021) suggested that excessively high dosages of digestate would alter fermentation pathways, lowering LA selectively; however, these same authors did not report any inhibition of fermentation, possibly because of relatively lower digestate dosages and a mixed culture utilised for fermentation in their study.

Overall, yield on sucrose was highest at the lowest sucrose level and N supplementation at all N dosage rates, as indicated by the RSM model (Table 5.2). With NH_4Cl, at the lowest sucrose level and 300 $mgN \cdot L^{-1}$, LA yield on sucrose was at a maximum, at 63%. An increase in N dosage as NH_4Cl saw a reduction back to a baseline yield on sucrose of 40% also observed when no N was added. With digestate, the RSM in contrast suggested an improved yield on sucrose to 52% with an increase in N to 400 $mgN \cdot L^{-1}$. At the higher sucrose dose of 107 $g \cdot L^{-1}$, LA yield on sucrose saw no change with N dosage (i.e. remained at ~33%), except for 400 $mgN \cdot L^{-1}$ whereat digestate caused a reduction in LA yield on sucrose. At the highest sucrose level, overall yields were similar across treatments, at around 18%, except for 300 $mgN \cdot L^{-1}$ as NH_4Cl and 300 $mgN \cdot L^{-1}$ as digestate, for which LA yields on sucrose were 24% and 21%, respectively.

5.3.2 Product spectrum

FW fermentation can also lead to the production of competitor organic acids, via the competitive uptake of available substrate, or by the consumption of LA as the substrate, resulting in a lower LA yield/selectivity (Arras et al., 2019). To minimise downstream processing costs and increase LA output, it is important to tune LA fermentation to minimise the production of competitor acids wherever possible.

The observed production of various competitor VFAs varied dynamically with sucrose, NH_4Cl, and digestate addition. Acetic acid and propionic acid production profiles differed

the most between treatments, displaying variations in both apparent production rate and maximum concentration achieved (Fig. 5.1-5.4; Fig. C1). In contrast, succinic and butyric acid production generally followed similar production profiles with different treatments (Fig. C1).

As LA would likely be recovered at a LA-AD facility when it is at its peak concentration (Table 5.1), it was appropriate to carry out an analysis of variable effects on VFA concentrations measured for samples of each experiment for which LA concentration was at its maximum. The resulting RSM model (Table 5.3) interestingly showed that while N_Source was retained within all models by the step() function, its effect was not significant in the acetic and propionic acid models. However, acetic acid was generally higher in the digestate treatments (Fig. C1A).

Table 5.3: Simplified RSM model parameters for competitor VFAs given with ±95% confidence intervals

Variable	Succinic	Acetic	Propionic	Butyric
Intercept	-0.19(±0.37)	3.18(±2.47)*	4.49(±1.1)***	-0.74(±0.61)*
Block	0.37(±0.19)***	-2.10(±1.40)**	-1.13(±0.52)***	0.52(±0.36)**
Sucrose	-1.87(±0.88)***	-[a]	1.39(±2.66)	-0.08(±0.54)
N_Amount	0.20(±0.26)	0.40(±1.95)	7.05(±3.54)***	-[a]
N_Source	-1.03(±1.03)*	3.90(±7.7)	-2.69(±3.82)	-0.95(±0.76)*
Sucrose^2	1.69(±0.83)***	6.21(±1.67)***	-1.60(±2.30)	-[a]
N_Amount^2	-[a]	-[a]	-6.02(±3.57)**	-[a]
TWI(Sucrose:Ammonia)	-[a]	-[a]	-1.37(±1.66)	-[a]
TWI(Sucrose:N_Source)	-1.04(±0.46)***	-[a]	-[a]	1.08(±0.86)*
TWI(Ammonia:N_Source)	1.12(±1.1)*	5.68(±8.33)	6.47(±4.2)**	-[a]
Adj.R^2	0.54	0.67	0.6	0.34

a: Removed by step() function (Section 5.2.6). ***=(P<0.001), **=(P<0.01), *=(P<0.05).

The highest competitor VFA levels were at 22.1 gCOD·L^{-1} for 150 g·L^{-1} sucrose and 400 mgN·L^{-1} as digestate. In contrast only 3.9 gCOD·L^{-1} of VFAs were produced with 43 g·L^{-1} sucrose and no N added, and only increased to 5.5 gCOD·L^{-1} with 400 mgN·L^{-1} as NH_4Cl, resulting in selectivities (91-92%) that were higher than the modelled base case (no sucrose, no added N, 83%). All digestate treatments produced more competitor VFAs than those without added N or with NH_4Cl (Table C4). However, like NH_4Cl, higher sucrose dosages increased the production of LA and, while competitor VFA production may have also increased, sucrose generally increased LA production more than it increased competitor acids, increasing the overall LA selectivity. Previous work by Zhang et al. (2020b) suggested that proteins present within waste activated sludge can be

a substrate for VFA production, elevating undesired metabolite levels, and this may have occurred in the current study. Furthermore, as the digestate was not sterilised in the current study, alternative fermentative bacteria introduced with the digestate could have competed with LA bacteria for substrate and contributed to the observed increase in VFA production (Section 5.3.3).

Butyric acid production generally remained low treatments, except for the digestate treatment without sucrose and inconsistently for some digestate containing vessels with sucrose at 107-150 $g \cdot L^{-1}$ (Fig. C1 and Table C4). At 400 $mgN \cdot L^{-1}$ without sucrose, butyric acid production appeared to be accompanied by a slight reduction in the LA yield (Fig. C1). Previous research has suggested that the formation of butyric acid from LA may be related to substrate availability (Detman et al., 2019; Hoelzle et al., 2021), with the addition of substrate shown to prevent the conversion of LA to butyric acid (Hoelzle et al., 2021). While it cannot be confirmed that butyrate production was prevented through sucrose addition in the current study, butyric acid was not detected with sucrose and NH_4Cl addition and was only observed in some treatments with both sucrose and digestate.

5.3.3 Microbial community analysis

The most abundant phyla across all treatments were Firmicutes (66-99%), with other minor phyla including Actinobacteria (0.2-29%), Bacteroidetes (0.0-2.4%), Euryarchaeota (0-2.0%), Chloroflexi (0.0-1.4%), and Thermotogae (0-0.8%) (Fig. C2). While all phyla were detected in nearly all treatments, digestate likely also acted as a secondary inoculum. For example, Bacteroidetes, Thermotogae, Actinobacteria, Euryarchaeota, and Chloroflexi were primarily enriched in the digestate treatments (Fig. C2). Chloroflexi, and Bacteroidetes are commonly found within FW AD systems (Buhlmann et al., 2018; St-Pierre & Wright, 2014), and were likely inoculated when digestate was added to test vessels. Thermotogae have been reported to form a syntrophic relationship with hydrogenotrophic methanogens for the oxidation of acetate during methanogenesis at high total ammoniacal N concentrations (Li et al., 2016). The digesters from which digestate was sourced in the current study, have been reported to operate at elevated total ammoniacal N concentrations (Buhlmann et al., 2018), which could have caused an increased relative abundance of Thermotogae in the digestate treatments.

Lactobacillus was the dominant genus with all treatments but showed a reduced relative abundance in the RSM model when digestate was added without sucrose (Table C5), down to 50% and 30% with digestate dosages of 300 and 400 mgN·L^{-1}, respectively (Fig. 5.5). *Clostridium Sensu Stricto 15* (*CSS_15*) proliferated with the addition of 400 mgN·L^{-1} as NH_4Cl, while *Bifidobacterium*, *CSS_18*, and *Proteiniphilum*, primarily grew in digestate containing environments without sucrose (See Sucrose and N_Source effects; Table C5). Research detailing the metabolic process of *CSS_15* are limited, however, *Clostridium* include a variety of bacteria which are specialised in utilising multiple sugars to generate methanogenic precursors, such as acetate, butyrate, carbon dioxide, and hydrogen (Song et al., 2021). *Bifidobacterium* form short chain fatty acids (e.g., LA and acetate) from carbohydrates and may form a syntrophy with *Clostridium* for butyrate formation (Xiong et al., 2019), which may have occurred in the current study (Section 5.3.2). *Proteiniphilum* plays an important role in protein degradation and has been isolated from biogas plants, particularly those treating FW, brewery waste, and wheat straw (Orellana et al., 2022), such as the plant from where the digestate was sourced (Bühlmann et al., 2021).

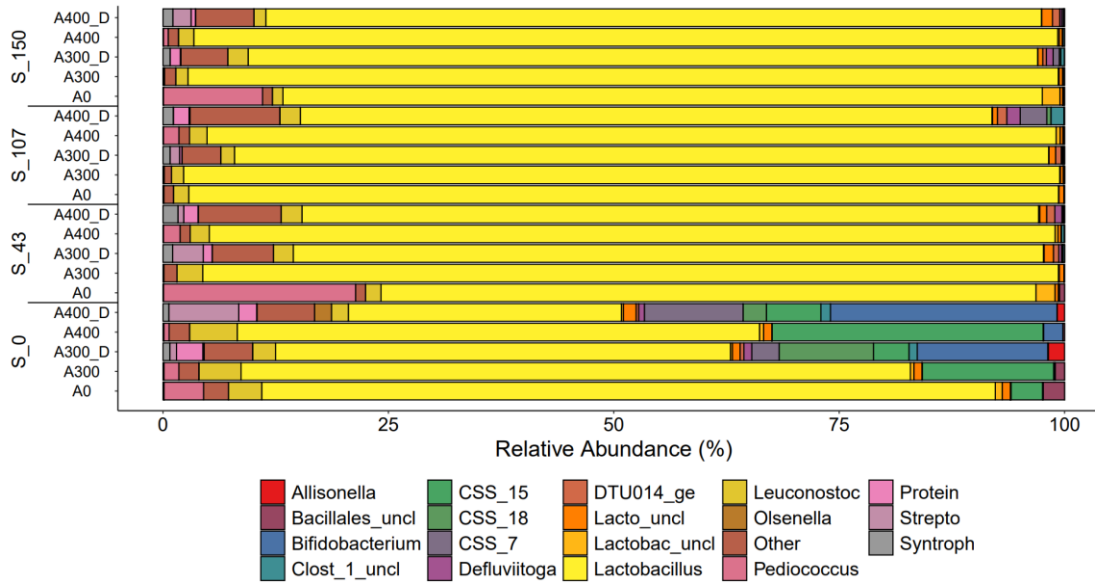


Figure 5.5: Relative abundance of microbial genera (>1%) following 5-days of FW fermentation supplemented with sucrose and N (NH_4Cl or digestate). Sucrose level is denoted by an "S" followed by the initial supplement concentration, while supplemented N is denoted by an "A" and N added as digestate is denoted by "D". The names of various genera have been shorted to fit them within the legend. Full names are as follows: Bifid (Bifidobacteriaceae), Clost_1 (Clostridiaceae_1), CSS (Clostridium sensu stricto), Lacto (Lactobacillales), Lactobac (Lactobacillaceae), Protein (Proteiniphilum), Strepto (Streptococcus), and Syntroph (Syntrophaceticus). All genera containing "uncl" were unclassified.

For both NH_4Cl and digestate treatments, sugar addition at any level significantly suppressed the growth of the flanking community and promoted the growth of *Lactobacillus* (Table C5; and Fig. 5.5). While research exploring supplementation of sugar during FW fermentation could not be found, previous studies have reported that higher substrate concentrations promote LA production and the growth of LA bacteria during FW fermentation (Pu et al., 2019; Simonetti et al., 2021).

5.3.4 Functional gene analysis

To better understand the impact of sucrose and N supplementation on metabolic pathways for LA production, a conceptual pathway diagram (Fig. 5.6) was constructed based on various relevant metabolic pathways (Table C6). The resulting predicted genes from the PICRUSt analysis were then utilised to explore the effect of sucrose and N addition on relevant microbial degradation pathways.

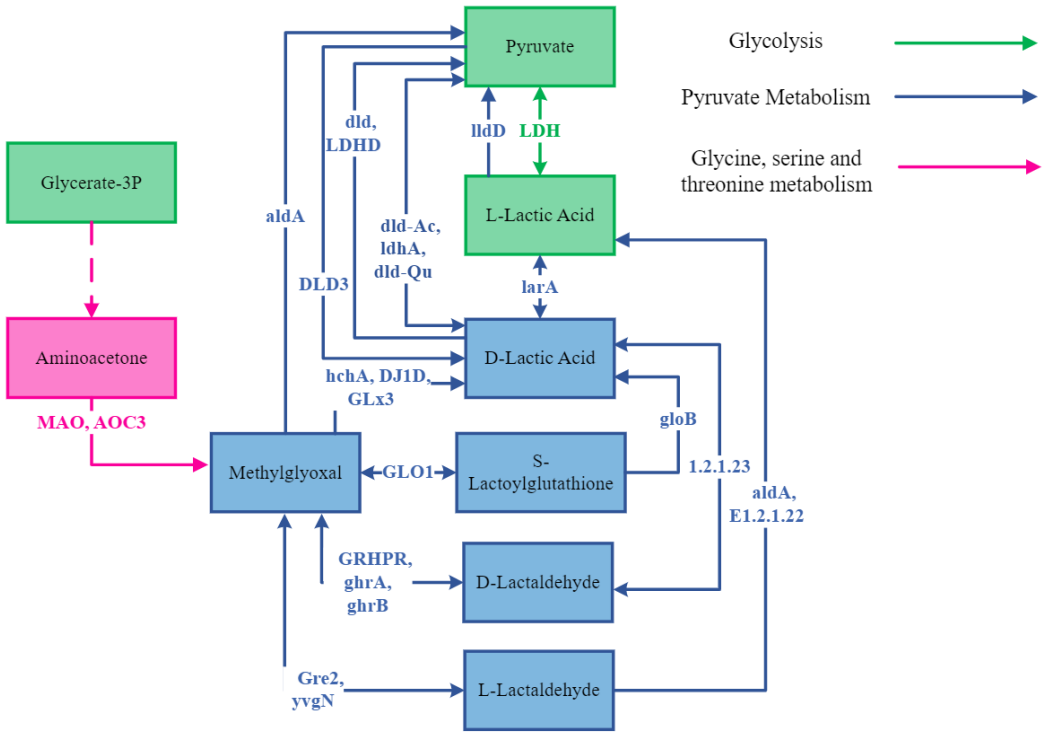


Figure 5.6: Identified genes within selected metabolic pathways associated with LA production and consumption as well as other organic acids. All genes were identified via the KEGG database. To simplify the diagram, only pathways directly related to LA formation have been included (Modified from (KEGG, 2022)).

The *in-silco* prediction suggested LA production likely occurred through multiple pathways working in tandem. The abundance of Lactate dehydrogenase (*LDH*) tended to increase with the addition of digestate ($P<0.001$; Table C6) which aligns with the

increased LA concentration associated with those treatments (Section 5.3.1). Genes utilised within the glycine, serine and threonine metabolism associated with the production of methylglyoxal, namely *AOC3* and *MAO*, fluctuated with the addition of sucrose and N (Table C6). *AOC3* was primarily enriched with NH_4Cl addition, though the combined addition of digestate and sucrose increased the relative abundance of this gene ($P<0.001$). In contrast, the combination of sucrose and digestate tended to reduce the relative abundance of *MAO* ($P=0.04$; Table C6). Methylglyoxal is a toxic by-product produced from glycolysis from dihydroxyacetone-P when glucose consumption overtakes phosphate uptake (Hoelzle et al., 2021). *Lactobacillus* strains generally carry out methylglyoxal detoxification through the production of either acetol (Hydroxyacetone) or 1,2-propanediol (Gandhi et al., 2018), of which a gene related to acetol formation (*yqhD*) was predicted to be present, but tended to reduce in relative abundance with sucrose ($P<0.001$) or N addition ($P=0.03$), and this effect was exacerbated with digestate ($P<0.001$). Detoxification can also occur through the glyoxalase pathway which consists of two enzymes, *GLO1* and *gloB*, which convert the toxic methylglyoxal to D-LA (Jain et al., 2018). The high relative abundance of *gloB* in this study, as compared to alternate LA producing genes (accounting for 81-95% of all identified LA producing genes, i.e. *gloB*, *ldhA*, *dld*, *pct*, *ldh*, and *aldA*), suggests methylglyoxal detoxification could have been a major pathway for LA production at the test conditions. However, the relative abundance of *GLO1* primarily reduced with higher sucrose dosages ($P<0.001$; Table C6), which may have reduced the capacity of the fermentation system to reduce methylglyoxal, possibly allowing it to accumulate to toxic levels at higher sucrose dosages. Such accumulation may have contributed to the reduced LA yields observed at higher sucrose dosages (Section 5.3.1)

5.3.5 The integrated LA-AD biorefinery

Overall, LA was effectively produced from the complex FW feedstock with N, digestate, and sucrose dynamically impacting on LA production, product spectrum, and microbial community composition. While digestate improved the LA concentration, digestate simultaneously increased the production of alternate organic acids and increased microbial community diversity. However, supplementation with sucrose effectively promoted LA formation and steered the product spectrum towards LA, and selectively promoted the growth of the desired LA bacteria. Digestate is considered a cost liability to many AD facilities, generally requiring pre-treatment (primarily solid-liquid

separation) before agricultural land application and tends to be expensive to transport and apply to land because of its moisture content (Lu & Xu, 2021; Turnley et al., 2016). However, the results from the current study showed that, along with sucrose supplementation, digestate can effectively aid LA production without needing prior sterilisation or pre-treatment. Furthermore, recirculation of digestate at a LA-AD biorefinery can save on freshwater consumption and costs for LA fermentation, by using the digestate as a process water source.

While sucrose increased the LA concentration and steered fermentation towards LA in the presence of digestate, it is important to explore additional costs associated with its use. Utilising the RSM developed in Section 5.3.1, the cost to implement sucrose supplementation was estimated at 0.54, 0.85, and 1.33 AUD·kg_{LA}^{-1} (based on additional LA produced) for scaled sucrose levels of 0.29, 0.71, and 1, respectively, and assuming a sucrose price of 0.28 AUD·kg^{-1} (0.21 USD·kg^{-1} (Efe et al., 2013)). With the price of LA previously estimated at 2.18 AUD·$kg_{60wt\%\ LA}^{-1}$ (1.36 Euro·$kg_{60wt\%\ LA}^{-1}$ (Demichelis et al., 2018)) and assuming a recovery efficiency of 51% (Demichelis et al., 2018), the additional cost of sucrose would be easily justified by the value of additional LA product, at all sucrose dosages applied in the current study. Adaptation of the fermentation inoculum to higher sucrose dosages may also improve LA yield on sucrose, thereby increasing the associated economic benefits. However, it is important to note that while fermentation efficiency may have been improved, a fraction of the added sucrose will remain in the fermentation broth. Downstream AD of solid and liquid extraction residues would likely utilise this residual sucrose for methane generation, which can offset energy requirements of LA separation and recovery. This can be important because recovery of LA is known to be energy intensive (Din et al., 2021).

5.4 CONCLUSION

Overall, LA was effectively produced from the complex FW mixture aided by addition of digestate as a relatively low-value N source and supplementing with sucrose as a readily bioavailable substrate. Digestate addition improved both the rate and yield of LA production. However, digestate also increased the microbial diversity which promoted the production of competitor organic acids. Sucrose effectively improved the LA concentration, steered the product spectrum towards LA, and selectively promoted the growth of the desired *Lactobacillus*, while suppressing the flanking community when

either NH_4Cl or digestate were added. A simple evaluation indicated that the value of additional LA produced with sucrose addition outweighed the costs of the sucrose. Overall, the results indicated that an integrated LA-AD biorefinery can effectively implement digestate recirculation without prior pre-treatment or sterilisation, and benefit from sucrose supplementation as a relatively low-value carbon source. This could increase the viability of future LA-AD biorefinery concepts.

5.5 REFERENCE

Akao, S., Tsuno, H., Horie, T., Mori, S. 2007. Effects of pH and temperature on products and bacterial community in l-lactate batch fermentation of garbage under unsterile condition. Water Research, 41(12), 2636-2642.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

APHA. 1995. Standard methods for the examination of water and wastewater. American Public Health Association American Water Works Association/Environmental Federation, Washington, DC.

Arras, W., Hussain, A., Hausler, R., Guiot, S.R. 2019. Mesophilic, thermophilic and hyperthermophilic acidogenic fermentation of food waste in batch: Effect of inoculum source. Waste Management, 87, 279-287.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum: Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

Bühlmann, C.H., Mickan, B.S., Tait, S., Bahri, P.A. 2022. Developing a food waste biorefinery: Lactic acid extraction using anionic resin and impacts on downstream biogas production. Chemical Engineering Journal, 431, 133243.

Bühlmann, C.H., Mickan, B.S., Tait, S., Renton, M., Bahri, P.A. 2021. Lactic acid from mixed food wastes at a commercial biogas facility: Effect of feedstock and process conditions. Journal of Cleaner Production, 284, 125243.

Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Steyer, J.-P., Delgenès, J.-P., Escudié, R. 2017. Dry anaerobic digestion of food waste and cardboard at different substrate loads, solid contents and co-digestion proportions. Bioresource Technology, 233, 166-175.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Detman, A., Mielecki, D., Chojnacka, A., Salamon, A., Błaszczyk, M.K., Sikora, A. 2019. Cell factories converting lactate and acetate to butyrate: Clostridium butyricum and microbial communities from dark fermentation bioreactors. Microbial Cell Factories, 18(1), 36.

Din, N.A.S., Lim, S.J., Maskat, M.Y., Mutalib, S.A., Zaini, N.A.M. 2021. Lactic acid separation and recovery from fermentation broth by ion-exchange resin: A review. Bioresources and Bioprocessing, 8(1), 1-23.

Edwards, J., Othman, M., Burn, S. 2015. A review of policy drivers and barriers for the use of anaerobic digestion in Europe, the United States and Australia. Renewable and Sustainable Energy Reviews, 52, 815-828.

Efe, Ç., van der Wielen, L.A.M., Straathof, A.J.J. 2013. Techno-economic analysis of succinic acid production using adsorption from fermentation medium. Biomass and Bioenergy, 56, 479-492.

FAO. 2021. FAO calls for action against food loss, waste in Asia-Pacific. in: The Nation - Thailand, Knowledge Bylanes.

Gandhi, N.N., Cobra, P.F., Steele, J.L., Markley, J.L., Rankin, S.A. 2018. Lactobacillus demonstrate thiol-independent metabolism of methylglyoxal: Implications toward browning prevention in Parmesan cheese. Journal of dairy science, 101(2), 968-978.

Hoelzle, R.D., Puyol, D., Virdis, B., Batstone, D. 2021. Substrate availability drives mixed culture fermentation of glucose to lactate at steady state. Biotechnology and Bioengineering, 118(4), 1617-1629.

Jain, M., Nagar, P., Sharma, A., Batth, R., Aggarwal, S., Kumari, S., Mustafiz, A. 2018. GLYI and D-LDH play key role in methylglyoxal detoxification and abiotic stress tolerance. Scientific Reports, 8(1), 5451.

KEGG. 2022. Kyoto Encyclopedia of Genes and Genomes.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2016. Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with Lactobacillus casei Shirota. Bioresource Technology, 217, 129-136.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology, 31(9), 814-821.

Li, L., He, Q., Ma, Y., Wang, X., Peng, X. 2016. A mesophilic anaerobic digester for treating food waste: process stability and microbial community analysis using pyrosequencing. Microbial Cell Factories, 15, 65.

Louvado, A., Coelho, F.J.R.C., Palma, M., Tavares, L.C., Ozorio, R.O.A., Magnoni, L., Viegas, I., Gomes, N.C.M. 2020. Effect of glycerol feed-supplementation on seabass metabolism and gut microbiota. Applied microbiology and biotechnology, 104(19), 8439-8453.

Lu, J., Xu, S. 2021. Post-treatment of food waste digestate towards land application: A review. Journal of cleaner production, 303, 127033.

Olszewska-Widdrat, A., Alexandri, M., López-Gómez, J.P., Schneider, R., Venus, J. 2020. Batch and Continuous Lactic Acid Fermentation Based on A Multi-Substrate Approach. Microorganisms (Basel), 8(7), 1084.

Orellana, E., Guerrero, L.D., Davies-Sala, C., Altina, M., Pontiggia, R.M., Erijman, L. 2022. Extracellular hydrolytic potential drives microbiome shifts during anaerobic co-digestion of sewage sludge and food waste. Bioresource Technology, 343, 126102.

Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, 143, 615-623.

Pleissner, D., Neu, A.-K., Mehlmann, K., Schneider, R., Puerta-Quintero, G.I., Venus, J. 2016. Fermentative lactic acid production from coffee pulp hydrolysate using Bacillus coagulans at laboratory and pilot scales. Bioresource Technology, 218, 167-173.

Pu, Y., Tang, J., Wang, X.C., Hu, Y., Huang, J., Zeng, Y., Ngo, H.H., Li, Y. 2019. Hydrogen production from acidogenic food waste fermentation using untreated inoculum: Effect of substrate concentrations. International Journal of Hydrogen Energy, 44(50), 27272-27284.

R Development Core Team. 2022. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.

Reddy, L.V., Park, J.-H., Wee, Y.-J. 2015. Homofermentative production of optically pure l-lactic acid from sucrose and mixed sugars by batch fermentation of Enterococcus faecalis RKY1. Biotechnology and bioprocess engineering, 20(6), 1099-1105.

Reichert, P. 1994. AQUASIM – A TOOL FOR SIMULATION AND DATA ANALYSIS OF AQUATIC SYSTEMS. Water science and technology, 30(2), 21-30.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F. 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology, 75(23), 7537-7541.

Serna-Maza, A., Heaven, S., Banks, C.J. 2015. Biogas stripping of ammonia from fresh digestate from a food waste digester. Bioresource Technology, 190, 66-75.

Simonetti, S., Martín, C.F., Dionisi, D. 2021. Anaerobic fermentation for the production of short chain organic acids: Product concentration, yield and productivity in batch experiments at high feed concentration. Journal of Environmental Chemical Engineering, 9(5), 106311.

Song, C., Li, W., Cai, F., Liu, G., Chen, C. 2021. Anaerobic and Microaerobic Pretreatment for Improving Methane Production From Paper Waste in Anaerobic Digestion. Frontiers in Microbiology, 12(1520).

St-Pierre, B., Wright, A.-D.G. 2014. Comparative metagenomic analysis of bacterial populations in three full-scale mesophilic anaerobic manure digesters. Applied Microbiology and Biotechnology, 98(6), 2709-2717.

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., Khanal, S. 2015. Anaerobic Digestion-Based Biorefinery for Bioenergy and Biobased Products. Industrial Biotechnology, 11(2), 103-112.

Tang, J., Wang, X.C., Hu, Y., Zhang, Y., Li, Y. 2017. Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. Bioresource Technology, 224, 544-552.

Turnley, D., Hopwood, L., Burns, C., Maio, D.D. 2016. Assessment of digestate drying as an eligible heat use in the Renewable Heat Incentive.

Wang, K., Yin, J., Shen, D., Li, N. 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: Effect of pH. Bioresource technology, 161, 395-401.

Wang, Q., Yang, L., Feng, K., Li, H., Deng, Z., Liu, J. 2021. Promote lactic acid production from food waste fermentation using biogas slurry recirculation. Bioresour Technol, 337, 125393.

WBA. 2018. Global Food Waste Management: An Implementation Guide For Cities.

Wolka, K., Melaku, B. 2015. Exploring selected plant nutrient in compost prepared from food waste and cattle manure and its effect on soil properties and maize yield at Wondo Genet, Ethiopia. Environmental Systems Research, 4(1), 9.

Xiong, Z., Hussain, A., Lee, J., Lee, H.-S. 2019. Food waste fermentation in a leach bed reactor: Reactor performance, and microbial ecology and dynamics. Bioresource Technology, 274, 153-161.

Yang, L., Chen, L., Li, H., Deng, Z., Liu, J. 2022. Lactic acid production from mesophilic and thermophilic fermentation of food waste at different pH. Journal of Environmental Management, 304, 114312.

Yang, X., Lai, Z., Lai, C., Zhu, M., Li, S., Wang, J., Wang, X. 2013. Efficient production of l-lactic acid by an engineered Thermoanaerobacterium aotearoense with broad substrate specificity. Biotechnology for biofuels, 6(1), 124-124.

Yousuf, A., Bastidas-Oyanedel, J.-R., Schmidt, J.E. 2018. Effect of total solid content and pretreatment on the production of lactic acid from mixed culture dark fermentation of food waste. Waste Management, 77, 516-521.

Zhang, C., Yang, H.-Q., Wu, D.-J. 2019. Study on the reuse of anaerobic digestion effluent in lactic acid production. Journal of Cleaner Production, 239, 118028.

Zhang, W., Li, X., He, Y., Xu, X., Chen, H., Zhang, A., Liu, Y., Xue, G., Makinia, J. 2020a. Ammonia amendment promotes high rate lactate production and recovery from semi-continuous food waste fermentation. Bioresource Technology, 302, 122881.

Zhang, W., Xu, X., Yu, P., Zuo, P., He, Y., Chen, H., Liu, Y., Xue, G., Li, X., Alvarez, P.J.J. 2020b. Ammonium Enhances Food Waste Fermentation to High-Value Optically Active l-Lactic acid. ACS Sustainable Chemistry & Engineering, 8(1), 669-677.

CHAPTER 6 LACTIC ACID RECOVEY AND DOWNSTREAM EFFECTS

Foreword

The preceding chapters (Chapters 3-5) explored LA production at a commercial two-stage FW AD facility, optimised fermentation, and improved LA production performance. While it was shown LA production could be effectively produced from FWs and production enhanced, it is unclear if LA can be recovered from complex fermentation media, such as would be produced at a commercial AD facility. Furthermore, the impact of LA recovery on downstream methane production is unclear.

Therefore, to address the knowledge gaps, the following chapter explores the feasibility of recovering LA from complex real fermentation from the AD facility explored in Chapter 3 using ion exchange. The chapter aims to optimise LA recovery through optimisation of extraction conditions and explores the impact of LA recovery on downstream methane production from the extraction residues.

The following chapter has been drafted in accordance with the Journal of Cleaner Production.

The current manuscript is published.

Christopher H. Bühlmann, Bede S. Mickan, Stephan Tait, Parisa A. Bahri, Developing a food waste biorefinery: Lactic acid extraction using anionic resin and impacts on downstream biogas production, Chemical Engineering Journal, Volume 431, Part 3, 2022, 133243, ISSN 1385-8947, https://doi.org/10.1016/j.cej.2021.133243

The following authors contributed to this manuscript as outlined below.

Authorship order	Contribution (%)	Concept Development	Data Collection	Data Analyses	Drafting of manuscript
Christopher H. Buhlmann	75	X	X	X	X
Prof. Parisa A. Bahri	10	X		X	
Dr Bede S. Mickan	7.5	X		X	
Dr Stephan Tait	7.5	X		X	

Contribution indicates the total involvement each author has had in this project. Placing an 'X' in the remaining boxes indicates which aspect(s) of the project each author engaged in.

By signing this document, the Candidate and Principal Supervisor acknowledge that the above information is accurate and has been agreed to by all other authors.

Candidate **Principal Supervisor**

Journal Metrics (as of July 2022)

Journal Name: Chemical Engineering Journal

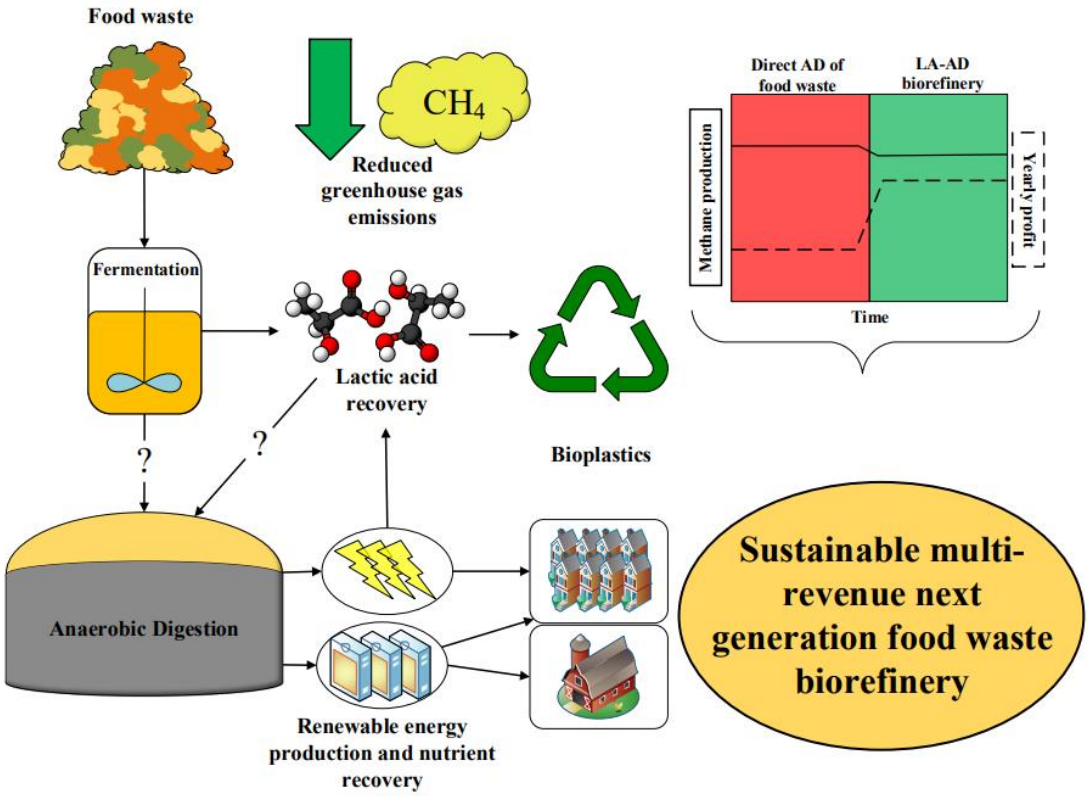
Peer Reviewed: Yes

Impact Factor: 16.744

Cite Score: 19.4

ABSTRACT

Coupling lactic acid (LA) production with food waste (FW) anaerobic digestion (AD) can facilitate the next generation biorefinery to increase revenue and economic viability of FW AD. For this, LA should be effectively extracted from complex fermentation broths with minimal adverse effects on subsequent AD to maximise economic benefit. This study evaluated LA recovery by adsorption using a polymeric resin (BA765), not previously tested for LA, to explore adsorption capacity and kinetics. Furthermore, biochemical methane potential (BMP) tests were utilised to assess effect of LA extraction on subsequent AD by measuring biogas production from the solid and liquid extraction residues. Optimal adsorption conditions yielded a maximum capacity of 0.21 $g_{LA} \cdot g^{-1}$ from pure solutions at pH 2-4, which was insensitive to temperature. However, real mixed fermentation broth impurities reduced LA uptake by 37%. BMP tests showed that the solid and liquid extraction residues had significant methane potential, with only a 21% reduction in overall methane yield compared to the raw fermentation broth prior to LA extraction. LA production outweighed loss in methane energy in terms of relative value and indicated a FW biorefinery concept could be commercially attractive and technically feasible.



6.1 INTRODUCTION

Food waste (FW) is a relentless and unstructured problem facing the modern world (Närvänen et al., 2020). The Food and Agricultural Organisation estimated that FW causes an economic loss of as much as $400 billion annually and identified that 14% of food produced globally is lost before reaching markets (FAO, 2020). With 8% of global yearly anthropogenic greenhouse gas emissions originating from FW alone (Demichelis et al., 2017; WBA, 2018), the correct disposal and processing of FW for resource recovery is becoming a global challenge and responsibility.

Anaerobic digestion (AD) is a mature technology using biological processes to convert FW into bioenergy (e.g. CH_4) and a nutrient rich by-product (digestate) which can be used as a fertiliser (Edwards et al., 2015). However, high construction and operational costs combined with a relatively low value of biogas and digestate, has generally resulted in FW AD being uneconomical without government subsidy and policy support (Edwards et al., 2015; Kim et al., 2016). Integrating biorefinery technologies into AD for the production of valuable bioproducts can provide significant economic benefit to boost profitability, for example by incorporating lactic acid (LA) production and recovery (Bastidas-Oyanedel & Schmidt, 2018; Demichelis et al., 2018; Demichelis et al., 2017; Kim et al., 2016).

LA is a highly versatile chemical and has received increased attention as a feedstock for the production of poly-lactic acid (PLA), a biodegradable bioplastic (Kim et al., 2016). PLA is a particularly interesting bioplastic as it can be easily processed using conventional equipment to produce moulded parts, films, and fibres, and it ultimately degrades into inert carbon dioxide and water (Li et al., 2020; Nduko & Taguchi, 2021). However, LA used for PLA is currently mainly produced from refined sugars and starch, which results in unwanted competition with food resources (Abdel-Rahman & Sonomoto, 2016).

At commercial FW AD facilities, an acidic first-stage fermentation can be applied to produce a pre-fermented FW feedstock to promote subsequent methanogenesis (De Gioannis et al., 2017). LA is a natural intermediate produced in such a first-stage FW fermentation step (Bühlmann et al., 2021), and this can result in the accumulation of moderate LA quantities of 21.7 - 40 $g \cdot L^{-1}$ (Bühlmann et al., 2021; Kim et al., 2016; Tang et al., 2016). Higher concentrations of 58 - 94 $g \cdot L^{-1}$ have also been reported when

hydrolysis and fermentation steps were separate (Demichelis et al., 2017; Kwan et al., 2016; Pleissner et al., 2017). Furthermore, literature has indicated the economic benefits from combining LA production with subsequent AD of extraction residues can be substantial (Demichelis et al., 2018; Demichelis et al., 2017; Kim et al., 2016). The integration of LA production has even been able to eliminate the need for subsidy support to make AD feasible (Bastidas-Oyanedel & Schmidt, 2018).

While production of LA from FW is promising, the variability of mixed FW complicates the fermentation product mix and the downstream recovery of LA (Din et al., 2021). Adsorption is a promising method for the recovery of LA despite an anticipated complex fermentation media. Adsorption processes are widely used within industrial biotechnology as they are robust, and are relatively easy to operate (da Silva & Miranda, 2013). A wide variety of resins have been assessed for LA recovery (Gao et al., 2010), with Amberlite IRA-67 consistently reported to be one of the best performers (Luongo et al., 2018; Moldes et al., 2003; Patel et al., 2008), showing a high capacity for LA (Table 6.1).

BA765 is an adsorbent similar to IRA-67 in structure and functional group (i.e. weak base gel-type polyacrylic resin with a tertiary amine functional group (Gao et al., 2012; Gluszcz et al., 2004)), but, to the authors' knowledge, has not been previously assessed for LA recovery. Moreover, LA recovery by adsorption has been previously explored for synthetic broths, broths of relatively pure feedstocks (Luongo et al., 2018; Song et al., 2017; Zhang et al., 2018), and experimental simple FW broths (Yousuf et al., 2016), but not for mixed fermented FW of a commercial AD facility. The mixed fermented FWs at such facilities would resemble the complex reality of a future commercial FW biorefinery, making it important to test the capability of new adsorbents to recover LA from such complex mixtures.

LA recovery with AD in a future biorefinery concept would be innately sustainable, being able to supply biogas energy from FW AD to meet the thermal and electrical energy requirements of LA production and recovery. However, LA extraction will remove organic matter and thereby logically reduce the methane yield from AD. Previous work has identified the methane potential of the solid extraction residues could be improved by the LA extraction (Demichelis et al., 2017), which may reduce overall methane losses due to extraction. However, no reported studies have explored the methane yield from

the liquid extraction residues from LA extraction, even though liquid residues could be important for re-dilution and preparation of the residue feedstock mixture for subsequent AD. It is important to understand how LA recovery impacts on overall methane yield to quantify the net benefits of a future biorefinery concept.

To address the key knowledge gaps above, the objectives of this work were: to evaluate and optimise LA recovery with BA765 resin; to evaluate BA765 resin with mixed fermented FW from a commercial AD facility; and to explore the impact of LA recovery on subsequent methane yield from AD of liquid and solid extraction residues.

Table 6.1: Lactic acid loading capacity of various ion exchanges resins from literature.

Resin	Basicity	Matrix	LA Solution type	Adsorption pH	Temperature (ºC)	Resin capacity	Reference
Amberlite IRA-67	weak	Gel	Pure	2	25	60.10 $mg_{LA} \cdot g^{-1}$	(Yousuf et al., 2016)
			Synthetic FW fermentation broth	2	25	84.03 $mg_{LA} \cdot g^{-1}$	
			Pure	Uncontrolled	24	326.30 $mg_{LA} \cdot g^{-1}$ dry resin	(Patel et al., 2008)
			Pure	4.85	25	276.00 $mg_{LA} \cdot g^{-1}$ dry resin	(Moldes et al., 2003)
			Cassava bagasse starch hydrolysate fermentation broth	5	25	126.00 $mg_{LA} \cdot g^{-1}$ wet resin	(John et al., 2008)
			Pure	Uncontrolled	50	203.80 $mg_{LA} \cdot g^{-1}$ wet resin	(Garrett et al., 2015)
			Fermented corn stover hydrolysate	Uncontrolled	50	170.20 $mg_{LA} \cdot g^{-1}$ wet resin	
			Pure	Uncontrolled	50	192.30 $mg_{LA} \cdot g^{-1}$ wet resin	(Gao et al., 2010)
Activated carbon	-	-	Pure	2	25	20.75 $mg_{LA} \cdot g^{-1}$	(Yousuf et al., 2016)
			Synthetic FW fermentation broth	2	25	18.63 $mg_{LA} \cdot g^{-1}$	
			Model fermentation broth	2	30	126.60 $mg_{LA} \cdot g^{-1}$	(Pradhan et al., 2017)
Amberlite IRA-400	Strong	Gel	Model fermentation broth	5	30	63.50 $mg_{LA} \cdot g^{-1}$	
			Pure	4.85	25	161.00 $mg_{LA} \cdot g^{-1}$ dry resin	(Moldes et al., 2003)
Reilex® 425	Weak	-	Model fermentation broth	2	30	108.70 $mg_{LA} \cdot g^{-1}$	(Pradhan et al., 2017)
Amberlite IRA-96	Weak	Macroretic ular	Pure	4.85	25	270.00 $mg_{LA} \cdot g^{-1}$ dry resin	(Moldes et al., 2003)
Amberlite IRA 900	Strong	Macroretic ular	Pure	4.85	25	172.00 $mg_{LA} \cdot g^{-1}$ dry resin	
Amberlite IRA-402	Strong	-	Cassava bagasse starch hydrolysate fermentation broth	5	25	119.00 $mg_{LA} \cdot g^{-1}$ wet resin	(John et al., 2008)

6.2 METHOD

BA765 was purchased from Haihang Industry Co. Ltd (Shandong Province, China). BA765 is a weak base gel-type resin with an acrylic acid matrix and a tertiary amine functional group (Table D1). The specificaiton sheet for the resin can be found on the Bestion® website (http://www.bojieresin.com/). Before utilisation, the resin was prepared following the procedure outlined by Moldes et al. (2003). Briefly, the resin was washed with 1 M NaOH solution, then with distilled water, then with 1 M HCl solution, then with distilled water, then with 1 M NaOH solution, and finally with distilled water until the pH of the solution was 7. Conventional lab-grade LA solution (~80 wt%) was sourced as a pure reagent for comparative testing from Merck (Germany). Microcrystalline cellulose from Sigma-Aldrich Corporation (USA) was used as a positive control substrate for biochemical methane potential testing. Other reagents used in the experiments included sulphuric acid (H_2SO_4) (Ajax Finechem, Australia; AJA534), hydrochloric acid (HCl) (Rowe Scientific, Western Australia; CH1680), sodium chloride (NaCl) (Chem-Supply, Australia; SA046), and sodium hydroxide (NaOH) (Chem-Supply, Australia; SL000), which were all at or above laboratory reagent grade (98% purity).

The raw fermentation broth and anaerobic inoculum for testing were obtained from a commercial scale two-stage mesophilic AD facility treating mixed FW near Perth, WA (Buhlmann et al., 2018; Bühlmann et al., 2021). The sampled broth and anaerobic inoculum were stored at 4 ^{o}C for up to 2 days before use.

To prepare substrates for the methane potential testing (Section 6.2.2), the raw fermentation broth was centrifuged at 10,000 g for 10 minutes, and a liquid supernatant was separated by first removing a floating oily fraction that formed prior to decanting of the liquid supernatant. The settled solid and oily fractions were then combined and diluted with tap water to the same total solids (TS) content as the original broth and stored for up to 2 days at 4 ^{o}C until use. This mixture of solid and oily fractions is termed solid extraction residue from hereon. The liquid supernatant was further vacuum filtered with a glass fibre filter (Merk, Germany; 1822-047) and a 0.45 µm cellulose filter (Merk, Germany; 1001-150), and then a 0.22 µm nitrocellulose membrane filter (Millipore, Germany; GSWP04700). The resulting filtered broth was stored for 1 day at 4 ^{o}C until use for LA extraction testing (Section 6.2.1.5). The raw fermentation broth, solid

extraction residue, and filtered broth were analysed for volatile solids (VS), TS, volatile fatty acids (VFAs), and LA (Section 6.2.4).

6.2.1 Adsorption test method

6.2.1.1 Adsorption capacity testing

Pure LA test solutions were prepared by diluting the pure LA reagent (Section 6.2) with deionised water to a measured starting concentration of 1.05, 5.38, 14.16, 20.47, 35.47, 54.75, 64.22, 88.87 $g \cdot L^{-1}$ at either pH 2.5 or pH 3.5. The pH of each LA solution was adjusted using 10 M NaOH or 10 M HCl. One gram of resin was added to 10 ml of each LA test solution which were then lightly agitated for 12 hours while maintained at 36 ^{o}C to ensure equilibrium had been reached. Equilibration time was identified in the kinetic study (Section 6.2.1.2). A sample of solution was then collected to measure LA by high performance liquid chromatography (HPLC) (Section 6.2.4). Test data from the experiment were fitted with adsorption equilibrium isotherms (Section 6.2.1.6).

6.2.1.2 Adsorption kinetics study

LA adsorption kinetics were studied over a 24-hr period. To ensure the ratio of solution to resin remained constant, a sacrificial experimental approach was adopted for the kinetic study. For this, one gram of wet BA765 resin was mixed with 10 ml of 61.15 $g \cdot L^{-1}$ pure LA solution at pH 2.5 (adjusted using 10 M HCl and 10 M NaOH) and 36 ^{o}C with light agitation. At 0.02, 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 16, and 24 hours, select adsorption vessels were removed and sampled for LA analysis by HPLC (Section 6.2.4) shortly after allowing the resin to settle. The resin readily settled following agitation, allowing near immediate sampling of the vessel following target contact time. Test data from the experiment were fitted with kinetic rate expressions (Section 6.2.1.6).

6.2.1.3 Effect of pH and temperature

To evaluate the effect of pH, one gram of BA765 resin was added to 10 ml of pure LA solution with a measured starting concentration of 86.82 $g \cdot L^{-1}$. LA fermentation has typically operated at uncontrolled pH conditions (pH < 4.0) (Bonk et al., 2017; Bühlmann et al., 2021; Gao et al., 2011; Luongo et al., 2019), or at controlled conditions to promote LA production (pH ~4.5 - 6.0) (Feng et al., 2018; Kim et al., 2016; Pleissner et al., 2017; Tang et al., 2016). Consequently, a pH of 1, 2, 3, 4, 5, and 6 were elected to cover the relevant range of test pH values to assess the requirement for pH correction prior to

adsorption separation that follows on from such controlled and uncontrolled LA fermentation systems. The pH of the test solution was adjusted to the target value using 10 M HCl and 10 M NaOH prior to the addition of the resin. The samples were lightly agitated and maintained at 36 ^{o}C for 12 hours.

To evaluate the effect of temperature, one gram of resin was added to 10 ml of pure LA solution with a measured starting concentration of 68.85 g·L^{-1}. The pH of the test solution was adjusted to 2.5 using 10 M HCl and 10 M NaOH prior to resin addition. The majority of LA fermentation processes are conducted at mesophilic conditions (Alves de Oliveira et al., 2018), therefore, to assess the requirement for heating and cooling prior to adsorption, 26, 36, or 46 ^{o}C were elected as the relevant test temperatures. Samples were lightly agitated and maintained at the selected temperature for 12 hours. Each pH and temperature test was conducted in triplicate. For both the pH and temperature tests, a sample of solution was collected at the end of the test to measure LA by HPLC (Section 6.2.4). The experimental data were analysed as described in Section 6.2.1.6.

6.2.1.4 Desorption

Desorption experiments were conducted to determine the optimal reagent for LA removal from BA765 resin, which was determined as the reagent that achieved the highest extent of LA removal at the lowest reagent concentration. For the desorption trials, 50 g of resin was loaded with 500 ml of pure LA solution at a concentration of 62.49 g·L^{-1} and pH 2.5 and was lightly agitated for 12 hours at 36 ^{o}C as before (Section 6.2.1.1). The resin was then separated from the liquid fraction via filtration and briefly washed with distilled water to remove any residual LA on its outer surface. 1 g of the loaded resin was then desorbed with 10 ml of either pure H_2SO_4, NaOH, or NaCl at 0.1, 0.5, 1.0, or 2.0 M. The solutions were allowed to equilibrate with the desorbent for 24 hours while gently agitated. A sample of solution was then collected to measure LA by HPLC (Section 6.4). Data were analysed as described in Section 6.2.6.

6.2.1.5 Adsorption of LA from a real mixed FW fermentation broth

LA adsorption from industrially produced mixed FW fermentation broth was conducted to examine the impact of real-world contaminants on LA adsorption. 1 gram of the prepared BA765 resin was mixed with 10 ml of the prepared filtered broth (Section 6.2) and allowed to equilibrate for 12 hours at 36 ^{o}C under light agitation. Samples were then taken for VFA and LA measurements by HPLC (Section 6.2.4).

For preparation of liquid extraction residues for the proceeding methane potential experiments (Section 6.2.2), 700 ml of the filtered broth (Section 6.2.1) was treated with 70 grams of resin and allowed to equilibrate for 24 hours at 36 °C under light agitation. Samples were taken following adsorption for analysis of VFAs and LA by HPLC (Section 6.2.4).

6.2.1.6 Theoretical analysis

The amount of LA adsorbed at equilibrium q_e ($g_{LA}.g^{-1}_{resin}$) in each case was calculated by equation 6.1:

$$q_e = \frac{(C_0 - C_e) \cdot V_i}{W} \quad \text{(Eq. 6.1)}$$

where C_0 is the initial LA concentration ($g \cdot L^{-1}$), C_e is the LA concentration at equilibrium ($g \cdot L^{-1}$), V_i is the initial solution volume (L), and W is the weight of the wet resin initially added (g).

To identify an appropriate equilibrium model description, the capacity test data (Section 6.2.1.1) were fitted with Langmuir (Eq. 6.2), Freundlich (Eq. 6.3), or Redlich-Peterson (Eq. 6.4) isotherms (Foo & Hameed, 2010):

$$q_e = \frac{Q_0 \cdot b \cdot C_e}{1 + b \cdot C_e} \quad \text{(Eq. 6.2)}$$

$$q_e = K_F \cdot C_e^{\frac{1}{n}} \quad \text{(Eq. 6.3)}$$

$$q_e = \frac{K_R \cdot C_e}{1 + (a_R \cdot C_e^{g})} \quad \text{(Eq. 6.4)}$$

where q_e is the amount of LA adsorbed at equilibrium ($mg \cdot g^{-1}$), C_e is the measured equilibrium LA concentration ($mg \cdot L^{-1}$), b is a Langmuir isotherm parameter ($L \cdot mg^{-1}$), Q_0 is a capacity parameter ($mg \cdot g^{-1}$), K_F is the Freundlich isotherm parameter ($mg \cdot g^{-1}$), n is a fitted parameter representing adsorption intensity, K_R is the Redlich-Peterson isotherm parameter ($L \cdot g^{-1}$), a_R is a Redlich-Peterson parameter ($L \cdot mg^{-1}$), and g is a fitted exponent between 0 and 1. The model fits were performed in AQUASIM (Reichert, 1994) (Section 6.2.5).

To identify an appropriate kinetic model description, the kinetic test data (Section 6.2.1.2) were fitted with pseudo-first order (Eq. 6.5), pseudo-second order (Eq. 6.6), or intra-particle diffusion (Eq. 6.7) kinetic models (Riahi et al., 2017; Wang & Guo, 2020).

$$\frac{dq_t}{dt} = k_1 \cdot (q_e - q_t) \quad \text{(Eq. 6.5)}$$

$$\frac{dq_t}{dt} = k_2 \cdot (q_e - q_t)^2 \quad \text{(Eq. 6.6)}$$

$$q_t = k_{id} \cdot t^{\frac{1}{2}} + C \quad \text{(Eq. 6.7)}$$

where q_t is LA adsorbed on the resin ($mg_{LA} \cdot g^{-1}_{Resin}$) at time t (min), k_1 is a pseudo-first order rate parameter (min^{-1}), k_2 is the pseudo-second order rate parameter ($g_{Resin} \cdot mg^{-1}_{LA} \cdot min^{-1}$), k_{id} is the intra-particle diffusion rate parameter ($mg \cdot g^{-1} \cdot min^{-1/2}$), and C is a fit parameter ($mg \cdot g^{-1}$) said to be related to the thickness of the diffusion boundary layer. The intra-particle diffusion model was of interest as it could provide an expanded mechanistic analysis of adsorption behaviour (Fierro et al., 2008; Riahi et al., 2017). The model fits were performed in AQUASIM (Reichert, 1994) (Section 6.2.5).

The amount of LA desorbed q_d ($g_{LA} \cdot g^{-1}_{resin}$) was calculated using the desorption test data (Section 6.2.1.4) and equations 6.8 and 6.9:

$$q_d = \frac{C_d \cdot V_d}{W} \quad \text{(Eq. 6.8)}$$

$$D = \frac{q_d}{q_e} \times 100 \quad \text{(Eq. 6.9)}$$

where C_d is the concentration of LA in the desorption solution at equilibrium ($g \cdot L^{-1}$), V_d is the desorption solution volume (L), D is the percentage of LA desorbed from the resin (%).

6.2.2 Biochemical methane potential assays

6.2.2.1 Experimental method

Biochemical methane potential (BMP) assays were performed with an Automatic Methane Potential Test System (AMPTS II) (Bioprocess Control AB, Lund, Sweden). All vessels had working liquid volumes of 400 ml. Test mixtures initially added comprised of anaerobic inoculum (Section 6.2) and substrate, consisting of either cellulose, raw fermentation broth prior to LA extraction, solid extraction residue or liquid extraction residue. Cellulose was included as the positive control to assess microbial activity and validate the results of the BMP tests (Weinrich et al., 2018). Predetermined quantities of substrate and inoculum were fed into the vessels to ensure an inoculum-to-substrate ratio of 2:1 (on a VS basis) to prevent overload or inhibition of the microbial

population (Weinrich et al., 2018). The AMPTS II digestion vessels were then promptly sealed, purged with a gaseous mixture of nitrogen and carbon dioxide (60% N_2:40% CO_2 by volume) to ensure anaerobic conditions, and then placed in a water bath at 38 ^oC, which falls within the typical temperature range for mesophilic AD (Raposo et al., 2011), for the duration of the digestion experiment (30 days). A blank triplicate containing only inoculum was run in parallel to determine the background methane production of the inoculum (Weinrich et al., 2018). The volume of methane from the blank was subtracted from that produced by each of the treatments in proportion to relative amounts of inoculum added to each vessel. The resulting background-corrected methane production was normalised with respect to VS added as substrate to each vessel, as outlined by equation 6.10.

$$B(t) = \frac{M(t) - (m_I \times I)}{VS_S} \qquad \text{(Eq. 6.10)}$$

Where *B(t)* is the cumulative methane produced by the substrate (mL $CH_4 \cdot gVS_{substrate}^{-1}$, 0°C and 1 atm), *M(t)* is the cumulative methane produced from the substrate and inoculum mixture (mL CH_4) at time *t* (days), m_I is the mass of inoculum added to the digestion vessel (g), *I* is the cumulative methane produced by the inoculum per mass of inoculum (mL $CH_4 \cdot g^{-1}_{inoculum}$) as measured by the inoculum blank, and VS_s is the VS mass of substrate added to the vessel (g).

6.2.3 Data analysis

Methane production was fitted with a first-order plus time lag kinetic model (Eq. 6.11) in AQUASIM (Reichert, 1994) (Section 6.2.5):

$$B(t) = B_0 \cdot (1 - \exp(-k \cdot (t - \lambda))) \qquad \text{(Eq. 6.11)}$$

Where *B(t)* is the blank-corrected and normalized cumulative methane produced (mL $CH_4 \cdot gVS^{-1}_{substrate}$, 0°C and 1 atm) at time t (days), B_0 is specific biochemical methane potential (mL $CH_4 \cdot gVS^{-1}_{substrate}$, 0°C and 1 atm), k is a first order rate parameter (day^{-1}) and λ is an initial time lag (days). B_0, k and λ were the fitted parameters.

To assess methane losses by LA extraction, a TS balance was used to estimate theoretical amounts of solid and liquid residues that would be produced by LA extraction from a nominal mass of raw fermentation broth. Accordingly, estimated methane potential for the raw fermentation broth (= $B_0 \times VS_{mass}$) and the extraction residues (calculated

similarly) were calculated and compared to estimate a theoretical methane yield loss resulting from LA extraction.

6.2.4 Analytical methods

TS and VS were measured according to Standard Methods (APHA, 1995). VFAs and LA were measured using HPLC equipped with a BioRad Aminex HPX-87H (300 mm × 7.8 mm) column maintained at 50 ^{o}C, with a UV detector set at 210 nm. The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL·min^{-1}. Standards of L-LA (purity >98%), acetic acid (purity >99%), propionic acid (purity >99.5%), butyric acid (purity >99%), and succinic acid (purity >98%) sourced from Sigma-Aldrich Corporation (USA) were used to calibrate the HPLC. Prior to analysis, samples were diluted with deionized water to within the calibration range and then filtered at 0.45 um using a PES Millipore® syringe filter.

6.2.5 Statistics

All tests (adsorption, desorption, and methane potential) were conducted in triplicate, unless otherwise stated, and results are presented as calculated mean values for triplicates ± the 95% confidence intervals determined using a student's t-distribution (n=3, Degrees of Freedom = 2). The correlation coefficient (R^2) was calculated in Microsoft Excel using the standard calculation procedure (Simonin, 2016).

To examine significant differences between final equilibrium adsorption and desorption concentrations (Sections 6.2.1.3 and 6.2.1.4), an analysis of variance (ANOVA) was conducted in R (R Development Core Team, 2022) at the 5% significance threshold. The findings were utilised within a more detailed analysis examining the significance of variance between treatments using the post-hoc Tukey's HSD pairwise comparison method, as previously described by Mickan et al. (2018).

All model fits used non-linear regression analysis with a secant search method in AQUASIM (Reichert, 1994), and errors in fitted parameter values were estimated as 95% confidence intervals using a student's t-distribution and appropriate Degrees of Freedom.

6.3 RESULTS AND DISCUSSION

6.3.1 Lactic acid adsorption kinetics

Sorption kinetics, which are related to the rate of LA uptake onto the resin, are an important property defining sorption efficiency (Zhao et al., 2011). Adsorption onto BA765 in pure LA solutions was observed to occur rapidly during the initial stages, and then slowed down after a contact time greater than about 0.5 hours to attain equilibrium after about 1 hour of adsorption (Fig. 6.1A). Accordingly, the 12-hr equilibration time used in the other adsorption experiments (Section 6.2) was deemed conservative and valid.

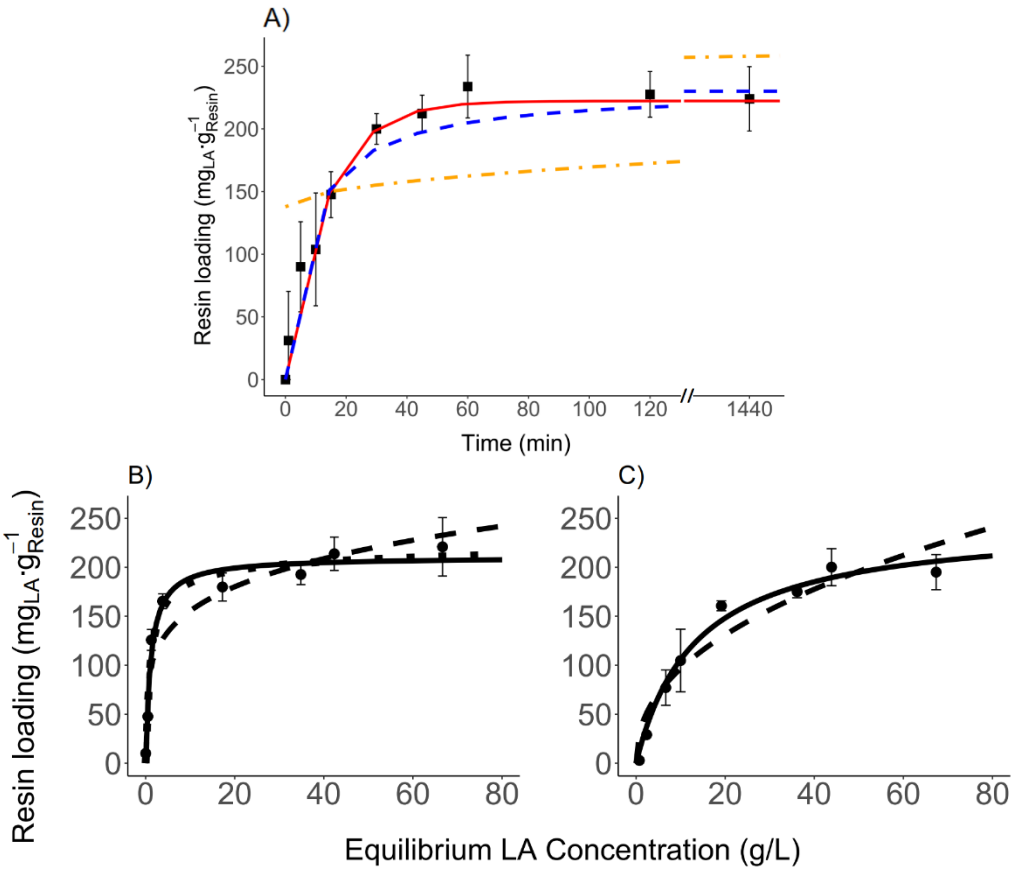


Figure 6.1: Plot of A) adsorption kinetics and adsorption isotherms at pH B) 2.5, and C) 3.5. The black symbols represent the experimental results (mean of triplicates). For the kinetics plot (Plot A), the red solid line, blue dashed line, and orange dot-dashed line represent the fitted Pseudo First Order, Pseudo Second Order, and Intra-Particle Diffusion models, respectively. For the isotherm plots (Plot B and C), the solid lines represent the fitted Langmuir isotherm, dotted lines the Redlich-Peterson isotherm, and the dashed lines the Freundlich isotherm. Please note the different x-axis units between plots. Error bars show the 95% confidence intervals on the experimental data.

The first order and second order models provided appropriate descriptions of the observed test data in contrast to the intraparticle diffusion model (see R^2 values, Table D2). The first order model assumes physisorption is the rate controlling step whilst the second order model represents a process limited by the rate of chemisorption (Mousavi et al., 2021). Weak base resins (like BA765) adsorb volatile fatty acids (like LA) via weak ionic bonding (Gao et al., 2010; Pradhan et al., 2017), so it is logical that a pseudo-second order model indicating chemisorption-controlled mechanisms would apply. Overall, the kinetic study indicated adsorption of LA on BA765 occurred rapidly and was competitive with that observed for other similar resins (Gao et al., 2010; Pradhan et al., 2017; Zhang et al., 2018).

6.3.2 Lactic acid adsorption equilibrium

As expected, the equilibrium studies indicated that the quantity of LA adsorbed increases with an increase in the LA concentration of the solution (Fig. 6.1B and C). The capacity of an adsorption medium is similarly important to its sorption kinetic behaviour because it dictates adsorption system size and capital costs. A sorption capacity measure was provided by a fit of the Langmuir sorption isotherm, which showed a reasonable description of the observed adsorption behaviour (high R^2 value, Table D3). The sorption capacity Q_0 parameter value was 0.211 $g \cdot g^{-1}$ (Table D3), which indicated that the capacity of BA765 for LA recovery was competitive to that of Amberlite IRA-67 and other similar resins (Table 6.1).

The appropriateness of equilibrium isotherm models as descriptions of adsorption data can give an indication of equilibrium adsorption mechanisms. The observed equilibrium adsorption behaviour (Fig. 6.1B and C) showed type 1 characteristics suggesting monolayer adsorption on a homogeneous microporous adsorbent with no interaction between adsorbed species (Bhandari & Ranade, 2014; Ruthven, 1984). The Langmuir and Redlich-Peterson isotherms provided a better fit of the adsorption data at pH 2.5 than the Freundlich isotherm (lower R^2 value, Table D3); similarly, for the adsorption data at pH 3.5, with the exception of the Redlich-Peterson isotherm for which parameters could not be estimated at pH 3.5, suspected to be due to issues with model parameter correlation. Behaviour consistent with a Langmuir isotherm suggests that adsorption is chemisorption controlled (Lee et al., 2018), whilst behaviour consistent with a Redlich-Peterson can indicate multilayered adsorption if the value of *g* is less than 1 (Ayawei et

al., 2017). Because the observed values of *g* were not significantly different from 1 (Table D3), the simpler Langmuir isotherm likely provides the most parsimonious description of the observed equilibrium adsorption by BA765.

6.3.3 Effect of pH and temperature on LA adsorption

The preferred pH and temperature conditions for LA recovery by adsorption are important, as these will influence any heating or cooling and acid or alkali chemical addition required to integrate LA extraction with the upstream fermentation step. The adsorption experiments with pure LA solutions (Section 6.2.1.3) indicated an optimal pH range of 2 - 4, with deviations away from this pH range notably decreasing the resin loading (Fig. 6.2A). This behaviour is similar to that reported for other weak base resins (Luongo et al., 2018; Pradhan et al., 2017). Fermentation for LA is generally conducted at pH 5 - 6 for pH controlled fermentation systems that target higher LA titres (Alves de Oliveira et al., 2018). However, the adsorption results favoured upstream fermentation at uncontrolled pH, where LA would be allowed to naturally depress pH to near its pKa value, falling within the observed optimal pH range for adsorption with BA765 resin.

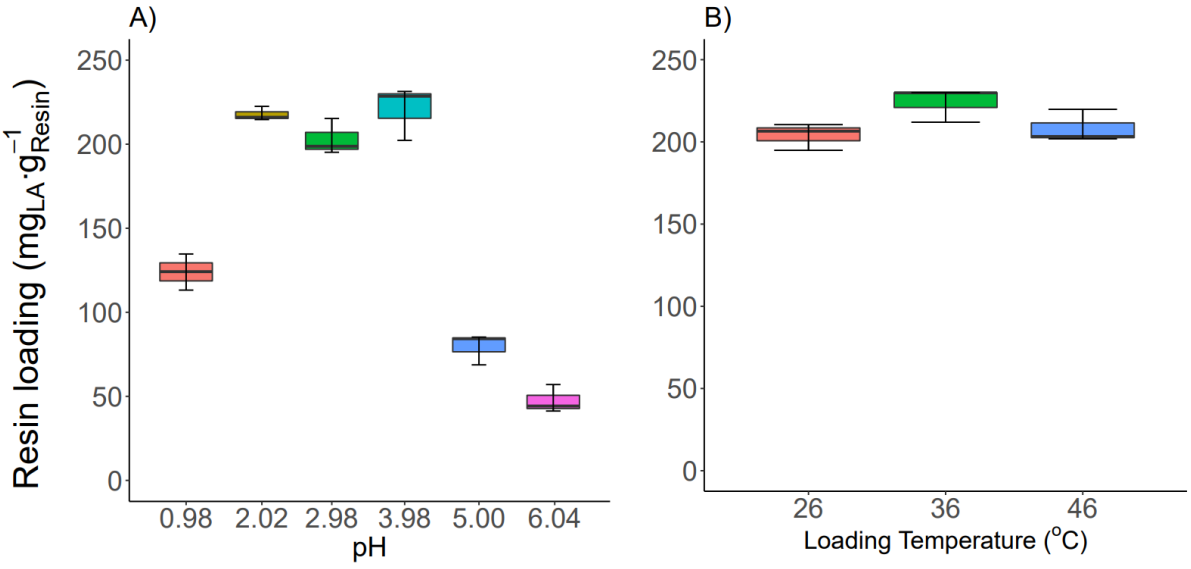


Figure 6.2: Effect of A) pH, and B) Temperature, on LA sorption capacity of the BA765 Resin from pure LA solutions. The pH of the LA solution for the temperature tests was adjusted to 2.5 by alkali addition.

Temperature showed no significant effect on equilibrium LA adsorption over the tested range (Fig. 6.2B). These results are in accordance with other research which has typically reported minimal to no change in performance with temperature for other weak base resins (Arcanjo et al., 2015; Gao et al., 2010; Gluszcz et al., 2004; Patel et al., 2008).

While the effect of temperature on LA adsorption kinetics was not assessed in this study, it was anticipated that the 12 hours adsorption time would have been adequate for the system to reach equilibrium at all temperatures tested, as a previous study has shown minimal impact on adsorption kinetics with temperature for a similar resin (Gao et al., 2010). BA765 is expected to adsorb and hold onto LA via weak ionic bonding. This type of bonding could reduce in strength with increasing temperature (Patel et al., 2008), so at temperatures exceeding 46 ^{o}C the capacity of BA765 may begin to decrease. However, fermentation for LA is generally conducted at mesophilic or low thermophilic temperatures (Alves de Oliveira et al., 2018), so that the raw broth is unlikely to enter a LA extraction step at higher temperatures than 46 ^{o}C. Hence, LA fermentation may be conducted at its optimal temperature without requiring heating or cooling prior to LA extraction with BA765.

6.3.4 Lactic acid desorption

Desorption experiments were conducted (Section 6.2.1.5) to determine the optimal desorbant to recover LA from BA765 resin. The results indicated that H_2SO_4 was the optimal desorbant, achieving the highest recovery of LA at the lowest desorbant concentration (Fig. 6.3). As sulphate is a divalent anion, it may have had a stronger binding affinity to the resin than hydroxide and chloride anions (Cao et al., 2002), allowing for higher LA recoveries at lower desorbant concentrations. Unfortunately, H_2SO_4 may pose potential problems for downstream AD. Depending on the integration strategy for LA fermentation and AD, the stripped broth may be utilised as process water for preparation of the AD feedstock to save on fresh water and utility costs. Moreover, with the use of H_2SO_4, the stripped broth may contain elevated levels of sulphate which can exacerbate hydrogen sulphide and decrease methane yield in subsequent AD (Lackner et al., 2020).

High recoveries were also achieved using NaOH at concentrations at or above 1.0 M (Fig. 6.3). Higher required concentrations could be attributed to the depression of pH resulting from LA desorption. While the solution pH following LA desorption was not measured, at 0.1 M NaOH (10 ml of solution) the available hydroxide would have been inadequate to completely neutralise all desorbed LA, resulting in pH depression and low pH which could have limited further desorption of LA. However, at >0.5 M NaOH, the higher hydroxide concentration was adequate to fully neutralise all the desorbed LA, which may

explain the observed higher desorption efficiency. While NaOH did not provide the highest elution efficiency, utilising this reagent for elusion would simultaneously strip and regenerate the resin, saving on operational costs and reducing the process time. This could be why NaOH is commonly used as an eluent with LA and other resins (Gao et al., 2010; Gao et al., 2012; Luongo et al., 2018; Zhang et al., 2018). As this was a baseline study, the purpose was to explore the general functionality of BA765, and the regeneration efficiency was not explored and should be assessed in future studies. The efficiency of the regeneration processes is a major contributor to the specific economics of ion exchange systems, often being the major indictor of overall ion exchange process economics (Arup K. SenGupta, 2017). Weak base exchangers, such as BA765, are advantageous in this regard, as they may be regenerated effectively by dilute basic solutions, due to their high affinity for OH-, while having high regeneration efficiencies compared to their strong base counterparts (Arup K. SenGupta, 2017; Tung & Judson King, 1994). However, these aspects should be clarified by future studies for business case definition for particular sites/applications.

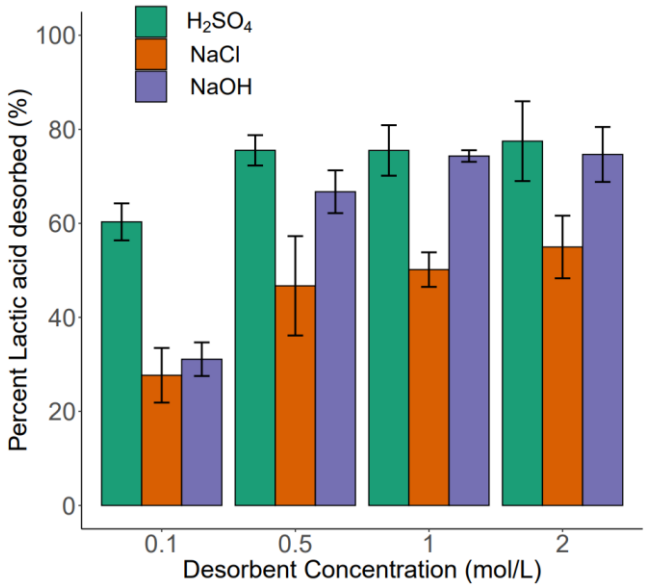


Figure 6.3: Percent LA desorbed from the resin following contact with H_2SO_4, NaOH, or NaCl at the concentrations indicated. Error bars indicate the 95% confidence interval of calculated mean values (n=3).

6.3.5 Adsorption of LA from a real mixed FW fermentation broth

To explore the impact of complex real-world contaminants on LA adsorption with BA765 resin, LA was extracted from a commercial FW fermentation broth (Section 6.2.1.5). The results showed competitive adsorption of other VFAs along with LA (**Error! Reference source not found.**), with 61% less LA adsorbed from the filtered broth at pH 2.5 than from pure LA solutions at the same pH and similar starting concentration (32.45 $g \cdot L^{-1}$). Interestingly, LA adsorption significantly improved at unadjusted pH 3.5, resulting in a loading capacity of 110.1 $mg_{LA} \cdot g^{-1}_{resin}$ (Table 6.2). The reason for this observed improvement in performance at pH 3.5 was uncertain but could have been partly due to competition between LA and HCl (Din et al., 2021) used for pH depression, or competition with other contaminants adsorbed from the filtered broth.

Table 6.2: Organic acids loaded onto BA765 from filtered broth at pH 2.5 and pH 3.5, and 36 ^{o}C, and comparison results for pure LA solution at pH 2.5 and 3.5. Values are expressed as calculated mean values ± the 95% confidence intervals.

	Lactic acid	**Succinic acid**	**Acetic acid**	**Propionic acid**	**Butyric acid**
Initial Concentration ($g \cdot L^{-1}$)	32.16	1.97	11.10	5.90	1.94
Amount @ pH 2.5 ($mg \cdot g^{-1}$)	69.59 ± 13.40	7.94 ± 4.11	12.48 ± 21.67	7.34 ± 11.89	5.53 ± 13.31
Removal extent (%)	22.27 ± 4.29	49.30 ± 25.53	11.34 ± 19.69	13.85 ± 22.41	27.36 ± 65.85
Amount @ pH 3.5 ($mg \cdot g^{-1}$)	110.11 ± 6.20	8.28 ± 6.80	25.12 ± 7.42	10.89 ± 9.38	1.76 ± 0.58
Removal extent (%)	34.58 ± 1.21	42.42 ± 34.00	22.84 ± 6.26	18.62 ± 15.60	9.14 ± 3.16
Amount @ pH 2.5 ($mg \cdot g^{-1}$) [a.1]	179.89 ± 14.32	-	-	-	-
Removal extent (%) [a.1]	51.39 ± 4.34	-	-	-	-
Amount @ pH 3.5 ($mg \cdot g^{-1}$) [a.2]	175.67 ± 10.99	-	-	-	-
Removal extent (%) [a.2]	54.21 ± 4.42	-	-	-	-

a: Pure LA solution used for comparative adsorption with a starting concentration of 1) 35.47 $g \cdot L^{-1}$, or 2) 32.45 $g \cdot L^{-1}$. Extraction efficiencies were determined by dividing the difference between the initial and final organic acid concentration by the initial organic acid concentration.

6.3.6 Impact of Lactic acid recovery on Anaerobic Digestion

Biochemical methane potential tests were conducted to explore the impacts of LA recovery on methane yield from downstream AD. The data for cellulose (Fig. 6.4, Table 6.3) indicated that the test conditions (e.g. inoculum, and other) had been valid. B_0 values were determined both by kinetic modelling (Eq. 6.9) (method 1) as well as by calculating the mean of the triplicate cumulative methane yield at the end of the experiment (@ 30 days, Fig. 6.4) (method 2). In all cases, the B_0 value determined by these two methods were not significantly different, so the B_0 values determined by method 2 are presented in Table 6.3 and that determined by method 1 were used for further methane yield analysis (Section 6.2.2). B_0 (on a VS basis) (Fig. 6.4) of the liquid extraction residue was not

significantly different to that of the untreated broth (P = 0.07), indicating similar biodegradability of organic matter in both; albeit the amount of VS in the liquid extraction residue was minimal compared to that in the untreated broth (Table 6.3). The rate of digestion (k values, Table 6.3) was similar and just significantly different between the solid and liquid extraction residues and cellulose as the model substrate but was slightly lower for the untreated broth.

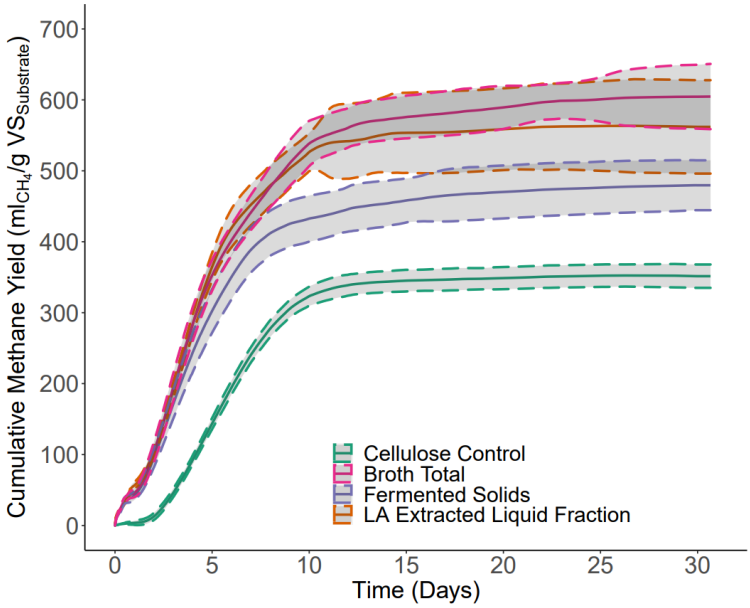


Figure 6.4: Normalized cumulative methane produced over time in the biochemical methane potential tests. The solid centre lines represent the measured cumulative methane yield as the mean of triplicates (n=3), while the surrounding dashed lines represent the 95% confidence intervals. The positive control (cellulose) had a calculated B_0 (method 2) of 351 ± 17 ml $CH_4 \cdot g_{VS}^{-1}$, indicating that the digestion results were reliable (Weinrich et al., 2018).

The methane potential balance (Section 6.2.2) indicated that 31% of the LA was extracted by adsorption at pH 3.5 (Table 6.2) with a total of 15.5 $gCOD \cdot L^{-1}$ extracted as VFAs (including LA), and corresponded to 5% of the expected methane yield of the untreated broth. This analysis assumed that all VFAs (including LA) were 100% biodegradable (350 ml $CH_4 \cdot g_{COD}^{-1}$ at 1 atm and 0 K). The methane potential balance further estimated that LA extraction resulted in an overall 21% reduction in methane yield from the untreated broth to the extraction residues, including the 5% decrease above due to VFAs extracted. The remainder loss in methane yield could have been due to uncertainties in various measurements used in the methane balance (i.e. TS, B_0) or due to other biodegradable compounds being extracted but not able to be measured by HPLC due to

standards not being available to identify and quantity these other compounds present (Fig. D1).

Table 6.3: Summary of results for the biochemical methane potential tests, including initial and final digestion pH, B_0, and fitted parameters of a first order plus time lag kinetic model. All BMP values are presented as the average of triplicates ± 95% confidence intervals.

Substrate	pH		TS [a]	VS [a]	Maximum Specific Methane Yield (B_0) (ml $CH_4 \cdot gVS_{substrate}^{-1}$) [b]	Kinetic model fit parameters	
	Initial	Final				First order rate constant (day^{-1})	Lag phase (day)
Cellulose	7.54±0.01	7.86±0.03	95.1%	94.9%	351 ± 17	0.286 ± 0.005	2.86 ± 0.04
Fermentation broth	6.65±0.04	7.86±0.07	12.6%	11.7%	605± 46 (431 ± 33)	0.228 ± 0.003	1.14 ± 0.03
Solid extraction residue	7.05±0.01	7.83±0.01	28.5%	27.5%	480 ± 35 (420 ± 31)	0.262 ± 0.003	1.10 ± 0.03
Liquid extraction residue	6.34±0.05	7.89±0.03	5.1%	4.2%	562 ± 67 (292 ± 34)	0.275 ± 0.004	1.26 ± 0.03

a: percent wet mass basis, reported as single measured values, typical variability in repeat measurements are 0.25% for TS and 0.16% for VS; b: Values within brackets represent corrected B_0 values where VFAs were added to measured VS of substrate before the measured cumulative methane produced was normalised. This correction accounts for volatilisation losses of VFAs that typically occurs during the drying step of the standard VS measurement.

6.3.7 Implications, economic impact of LA recovery at a FW biorefinery

To estimate the relative economic benefit from LA extraction (Section 6.3.5) vs. the loss in methane yield due to extraction (Section 6.3.6), mass balance modelling was performed for a real full-scale scenario described by Bühlmann et al. (2021). The fermenter size, retention time, and the resulting LA concentration utilised within the scenario are outlined in Table 6.4. This gives a total LA production rate of 2.11 tonne·day^{-1}. With an extraction efficiency of 34.6% (Table 6.2), and a LA price and production cost of 1.8 USD·kg^{-1} and 0.5 USD·kg^{-1}, respectively (Table 6.6), the estimated profit from LA recovery would be an estimated 949 USD·day^{-1} (346,413 USD·$year^{-1}$). For AD, the methane produced was estimated by multiplying the measured methane potential of the broth (Table 6.3) with the anticipated equivalent digester feed rate in the scenario (Bühlmann et al., 2021) [i.e. $603.7 \frac{m^3_{CH_4}}{tonne_{VS}} \times \left(\frac{269.5\ m3}{3.59\ days}\right) \times 0.117 \frac{g_{VS}}{g^{-1}_{Feed}}$], which equates to 5,302 $m^3{}_{CH4}$ per day. The AD system described in Bühlmann et al. (2021) has a 30 day retention time. This retention time was utilised together with the corresponding first order degradation rate of the fermentation broth (Table 6.3) to estimate the fraction of methane

yield recoverable in a well-mixed heated digester, which was [$\frac{30\ \text{day} \times 0.228\ \text{day}^{-1}}{1+(30\ \text{day} \times 0.228\ \text{day}^{-1})}$ = 0.87]. Finally, by multiplying the estimated methane produced, the recoverable fraction of the methane yield (i.e. 0.87), and the percentage estimated methane yield loss by LA extraction (21%, Section 6.3.6), the amount of methane lost by LA extraction was estimated at 960 $m^3_{CH4}.day^{-1}$. The corresponding value of the biogas and its cost of production have been previously estimated at 0.013 $USD \cdot MJ^{-1}$ and 0.075 $USD \cdot m^3_{biogas}{}^{-1}$, respectively (Table 6.4). For a typical CH_4 content in biogas from FW of 60% and with a corresponding heating value for CH_4 of 35.85 $MJ \cdot m^3_{CH4}{}^{-1}$ at these conditions (Table 6.4), the equivalent value of the methane energy lost was estimated at 327 $USD \cdot day^{-1}$. Accordingly, the 949 $USD \cdot day^{-1}$ in value from LA extraction outweighs the 327 $USD \cdot day^{-1}$ loss in methane energy. This is in agreement with findings reported by other authors (Bastidas-Oyanedel & Schmidt, 2018; Demichelis et al., 2018; Kim et al., 2016).

Table 6.4: Economic parameters utilised for the evaluation of the LA integration into AD.

	Value	**Reference**
LA production cost ($USD \cdot kg^{-1}$)	0.5	(Kim et al., 2016)
Value of LA ($USD \cdot kg^{-1}$)	1.8	(Kim et al., 2016)
Value of biogas energy ($USD \cdot MJ^{-1}$)	0.013	(Kim et al., 2016)
Lower heating value of CH_4	35.85	(Tranter et al., 2011)
Biogas production cost ($USD \cdot m^{-3}_{biogas}$)	0.075	(Kim et al., 2016)
Fermenter conditions		
HRT (Days)	3.59	(Bühlmann et al., 2021) or Chapter 3
Size (m^3)	269.5	(Bühlmann et al., 2021) or Chapter 3
LA liquid phase concentration ($g \cdot L^{-1}$)	32.16	(Bühlmann et al., 2021) or Chapter 3

It is important to note that there would be significant opportunity to further improve the economic performance of the biorefinery concept through; 1) optimisation and control of the LA fermentation stage which was not under any process control in the reference study of Bühlmann et al. (2021), 2) increasing the number of adsorption stages to increase LA recovery, and 3) further optimising the conditions of the adsorption process to increase LA selectivity and reduce removal of competing components partly responsible for the observed methane yield loss.

6.4 CONCLUSION

Overall, the results from this study showed LA recovery via adsorption from complex mixed FW fermentation broth is technically feasible. The BA765 resin optimally adsorbed LA from pure solutions at conditions favourable for uncontrolled upstream LA

fermentation at acidic pH 2-4, eliminating costs associated with pH and temperature correction prior to recovery. However, impurities in real FW fermentation broth reduced LA recovery onto the resin by 37%, suggesting targeted LA production during fermentation and control of adsorption conditions to promote LA adsorption could improve overall process efficiency. The methane potential tests showed that both the solid and liquid extraction residues contained significant methane potential and LA recovery only resulted in a 21% reduction in overall methane yield. Moreover, LA recovery outweighed the loss in methane yield, in terms of relative value, indicating that a LA-AD biorefinery would be technically feasible and commercially attractive.

6.5 REFERENCES

Abdel-Rahman, M.A., Sonomoto, K. 2016. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. Journal of biotechnology, 236, 176-192.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

APHA. 1995. Standard methods for the examination of water and wastewater. American Public Health Association American Water Works Association/Environmental Federation, Washington, DC.

Arcanjo, M.R.A., Fernandes, F.A.N., Silva, I.J. 2015. Separation of Lactic Acid Produced by Hydrothermal Conversion of Glycerol Using Ion-Exchange Chromatography. Adsorption Science & Technology, 33(2), 139-151.

Arup K. SenGupta, S. 2017. Ion exchange: environmental processes. Wiley-Blackwell.

Ayawei, N., Ebelegi, A.N., Wankasi, D. 2017. Modelling and Interpretation of Adsorption Isotherms. Journal of Chemistry, 2017, 1-11.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

Bhandari, V.M., Ranade, V.V. 2014. Chapter 2 - Advanced Physico-chemical Methods of Treatment for Industrial Wastewaters. in: Industrial Wastewater Treatment, Recycling and Reuse, (Eds.) V.V. Ranade, V.M. Bhandari, Butterworth-Heinemann. Oxford, pp. 81-140.

Bonk, F., Bastidas-Oyanedel, J.-R., Yousef, A.F., Schmidt, J.E. 2017. Exploring the selective lactic acid production from food waste in uncontrolled pH mixed culture fermentations using different reactor configurations. Bioresource Technology, 238, 416-424.

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum:

Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

Bühlmann, C.H., Mickan, B.S., Tait, S., Renton, M., Bahri, P.A. 2021. Lactic acid from mixed food wastes at a commercial biogas facility: Effect of feedstock and process conditions. Journal of Cleaner Production, 284, 125243.

Cao, X., Yun, H.S., Koo, Y.-M. 2002. Recovery of l-(+)-lactic acid by anion exchange resin Amberlite IRA-400. Biochemical Engineering Journal, 11(2), 189-196.

da Silva, A.H., Miranda, E.A. 2013. Adsorption/Desorption of Organic Acids onto Different Adsorbents for Their Recovery from Fermentation Broths. Journal of Chemical & Engineering Data, 58(6), 1454-1463.

De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R., Spiga, D. 2017. Energy recovery from one- and two-stage anaerobic digestion of food waste. Waste Management, 68, 595-602.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Din, N.A.S., Lim, S.J., Maskat, M.Y., Mutalib, S.A., Zaini, N.A.M. 2021. Lactic acid separation and recovery from fermentation broth by ion-exchange resin: A review. Bioresources and Bioprocessing, 8(1), 1-23.

Edwards, J., Othman, M., Burn, S. 2015. A review of policy drivers and barriers for the use of anaerobic digestion in Europe, the United States and Australia. Renewable and Sustainable Energy Reviews, 52, 815-828.

FAO. 2020. Food loss and waste must be reduced for greater food security and environmental sustainability.

Feng, K., Li, H., Zheng, C. 2018. Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresource Technology, 270, 180-188.

Fierro, V., Torné-Fernández, V., Montané, D., Celzard, A. 2008. Adsorption of phenol onto activated carbons having different textural and surface properties. Microporous and Mesoporous Materials, 111(1), 276-284.

Foo, K.Y., Hameed, B.H. 2010. Insights into the modeling of adsorption isotherm systems. Chemical Engineering Journal, 156(1), 2-10.

Gao, M.-T., Shimamura, T., Ishida, N., Takahashi, H. 2011. pH-uncontrolled lactic acid fermentation with activated carbon as an adsorbent. Enzyme and Microbial Technology, 48(6), 526-530.

Gao, Q., Liu, F., Zhang, T., Zhang, J., Jia, S., Yu, C., Jiang, K., Gao, N. 2010. The Role of Lactic Acid Adsorption by Ion Exchange Chromatography. PLoS ONE, 5(11), e13948.

Gao, Q., Pan, C., Liu, F., Lu, F., Wang, D., Zhang, J., Zhu, Y. 2012. Adsorption characteristics of malic acid from aqueous solutions by weakly basic ion-exchange chromatography. Journal of Chromatography A, 1251, 148-153.

Garrett, B.G., Srinivas, K., Ahring, B.K. 2015. Performance and stability of Amberlite™ IRA-67 ion exchange resin for product extraction and pH control during homolactic fermentation of corn stover sugars. Biochemical Engineering Journal, 94, 1-8.

Gluszcz, P., Jamroz, T., Sencio, B., Ledakowicz, S. 2004. Equilibrium and dynamic investigations of organic acids adsorption onto ion-exchange resins. Bioprocess and biosystems engineering, 26(3), 185-190.

John, R.P., Nampoothiri, K.M., Pandey, A. 2008. L(+)-Lactic acid recovery from cassava bagasse based fermented medium using anion exchange resins. Brazilian Archives of Biology and Technology, 51, 1241-1248.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2016. Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with Lactobacillus casei Shirota. Bioresource Technology, 217, 129-136.

Lackner, N., Wagner, A.O., Illmer, P. 2020. Effect of sulfate addition on carbon flow and microbial community composition during thermophilic digestion of cellulose. Applied Microbiology and Biotechnology, 104(10), 4605-4615.

Lee, Y., Lee, H., Jung, D., Chen, Z., Lim, S. 2018. Chloride Ion Adsorption Capacity of Anion Exchange Resin in Cement Mortar. Materials (Basel, Switzerland), 11(4), 560.

Li, G., Zhao, M., Xu, F., Yang, B., Li, X., Meng, X., Teng, L., Sun, F., Li, Y. 2020. Synthesis and Biological Application of Polylactic Acid. Molecules, 25(21), 5023.

Luongo, V., Palma, A., Rene, E.R., Fontana, A., Pirozzi, F., Esposito, G., Lens, P.N.L. 2018. Lactic acid recovery from a model of Thermotoga neapolitana fermentation broth using ion exchange resins in batch and fixed-bed reactors. Separation Science and Technology, 54(6), 1008-1025.

Luongo, V., Policastro, G., Ghimire, A., Pirozzi, F., Fabbricino, M. 2019. Repeated-Batch Fermentation of Cheese Whey for Semi-Continuous Lactic Acid Production Using Mixed Cultures at Uncontrolled pH. Sustainability, 11(12).

Mickan, B.S., Abbott, L.K., Fan, J., Hart, M.M., Siddique, K.H.M., Solaiman, Z.M., Jenkins, S.N. 2018. Application of compost and clay under water-stressed conditions influences functional diversity of rhizosphere bacteria. Biology and Fertility of Soils, 54(1), 55-70.

Moldes, A.B., Alonso, J.L., Parajó, J.C. 2003. Recovery of lactic acid from simultaneous saccharification and fermentation media using anion exchange resins. Bioprocess and Biosystems Engineering, 25(6), 357-363.

Mousavi, S.A., Kamarehie, B., Almasi, A., Darvishmotevalli, M. 2021. Removal of Rhodamine B from aqueous solution by stalk corn activated carbon: adsorption and kinetic study. Biomass conversion and biorefinery.

Närvänen, E.e., Mesiranta, N.e., Mattila, M.e., Heikkinen, A.e., SpringerLink. 2020. Food Waste Management: Solving the Wicked Problem. 1st 2020. ed. Springer International Publishing, Cham.

Nduko, J.M., Taguchi, S. 2021. Microbial Production of Biodegradable Lactate-Based Polymers and Oligomeric Building Blocks From Renewable and Waste Resources. Frontiers in Bioengineering and Biotechnology, 8(1548).

Patel, M., Bassi, A.S., Zhu, J.J.-X., Gomaa, H. 2008. Investigation of a dual-particle liquid–solid circulating fluidized bed bioreactor for extractive fermentation of lactic acid. Biotechnology Progress, 24(4), 821-831.

Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, 143, 615-623.

Pradhan, N., Rene, E., Lens, P., Dipasquale, L., D'Ippolito, G., Fontana, A., Panico, A., Esposito, G. 2017. Adsorption Behaviour of Lactic Acid on Granular Activated Carbon and Anionic Resins: Thermodynamics, Isotherms and Kinetic Studies. Energies, 10(5), 665.

R Development Core Team. 2022. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I., de Wilde, V. 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. Journal of Chemical Technology & Biotechnology, 86(8), 1088-1098.

Reichert, P. 1994. AQUASIM – A TOOL FOR SIMULATION AND DATA ANALYSIS OF AQUATIC SYSTEMS. Water science and technology, 30(2), 21-30.

Riahi, K., Chaabane, S., Thayer, B.B. 2017. A kinetic modeling study of phosphate adsorption onto Phoenix dactylifera L. date palm fibers in batch mode. Journal of Saudi Chemical Society, 21, S143-S152.

Ruthven, D.M. 1984. Principles of Adsorption and Adsorption Processes. John Wiley & Sons.

Simonin, J.-P. 2016. On the comparison of pseudo-first order and pseudo-second order rate laws in the modeling of adsorption kinetics. Chemical Engineering Journal, 300.

Song, M., Jiao, P., Qin, T., Jiang, K., Zhou, J., Zhuang, W., Chen, Y., Liu, D., Zhu, C., Chen, X., Ying, H., Wu, J. 2017. Recovery of lactic acid from the pretreated fermentation broth based on a novel hyper-cross-linked meso-micropore resin: Modeling. Bioresource Technology, 241, 593-602.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y. 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management, 52, 278-285.

Tranter, R.B., Swinbank, A., Jones, P.J., Banks, C.J., Salter, A.M. 2011. Assessing the potential for the uptake of on-farm anaerobic digestion for energy production in England. Energy policy, 39(5), 2424-2430.

Tung, A.L., Judson King, C. 1994. Sorption and extraction of lactic and succinic acids at pH > pKa1. I. Factors governing equilibria. Industrial & Engineering Chemistry Research, 33, 3217-3223.

Wang, J., Guo, X. 2020. Adsorption kinetic models: Physical meanings, applications, and solving methods. Journal of Hazardous Materials, 390, 122156.

WBA. 2018. Global Food Waste Management: An Implementation Guide For Cities.

Weinrich, S., Schäfer, F., Liebetrau, J. 2018. Value of batch tests for biogas potential analysis; method comparison and challenges of substrate and efficiency evaluation of biogas plants. IEA Bioenergy Task 37.

Yousuf, A., Bonk, F., Bastidas-Oyanedel, J.-R., Schmidt, J.E. 2016. Recovery of carboxylic acids produced during dark fermentation of food waste by adsorption on Amberlite IRA-67 and activated carbon. Bioresource Technology, 217, 137-140.

Zhang, Y., Qian, Z., Liu, P., Liu, L., Zheng, Z., Ouyang, J. 2018. Efficient in situ separation and production of l-lactic acid by Bacillus coagulans using weak basic anion-exchange resin. Bioprocess and Biosystems Engineering, 41(2), 205-212.

Zhao, X., Song, L., Fu, J., Tang, P., Liu, F. 2011. Adsorption characteristics of Ni(II) onto MA–DTPA/PVDF chelating membrane. Journal of Hazardous Materials, 189(3), 732-740.

CHAPTER 7 TECHNOECONOMIC STUDY

Foreword

Chapters 3 – 6 explored LA production from FWs at a commercial AD facility, optimisation of the fermentation process to maximise LA production, and explored the impact of its extraction on downstream AD performance. The current chapter utilises the data acquired from these previous chapters and the literature to explore the economic feasibility of integrating LA production into existing two-stage FW AD facilities. The economic impact of various control and optimisation measures discussed in the preceding chapters are also explored in the analysis of this chapter.

ABSTRACT

Integration of lactic acid (LA) production and recovery technologies into Anaerobic digestion (AD) can yield a higher value biorefinery to boost AD economic performance, increase valorisation of food wastes, and reduce reliance on subsidies for AD economic viability. To evaluate the economic feasibility of the LA-AD biorefinery, various integration scenarios were assessed, including, Brownfield and Greenfield scenarios, mainline or side-stream LA fermentation, carbohydrate supplementation with partial digestate recirculation, and integration of PLA synthesis. Brownfield mainline LA fermentation combined with carbohydrate supplementation and partial digestate recirculation provided the most economically attractive scenario, displaying a cost-benefit ratio (20.3), a Return on Investment (ROI) (17%), an Internal Rate of Return (IRR) (1.71 years), and a simple payback period, of 1.6 years. In general, Greenfield scenarios yielded a higher Net Present Value (NPV), primarily due to the inclusion of gate-fees not included in the Brownfield scenarios (not additional), but displayed lower overall economic feasibility resulting from higher capital and operational costs. A sensitivity analysis showed the economic feasibility of the LA-AD biorefinery was most sensitive to the price and yield of LA. PLA synthesis was largely infeasible and required higher PLA sale price or lower capital costs for feasibility. Overall, the LA-AD biorefinery is financially feasible and attractive method to increase AD profitability.

7.1 INTRODUCTION

Anaerobic digestion (AD) is a simple and effective biorefinery technology for organic waste recycling, and has been proven for FW treatment (Edwards et al., 2015). An AD system can enable the simultaneous recovery of bioenergy and biofertilizer from FW (Kim et al., 2016). However, due to high costs for construction, operation, and management, the economic feasibility of AD is often uncertain and typically relies on government subsidies or gate fees (Bastidas-Oyanedel & Schmidt, 2018; Cucchiella & D'Adamo, 2016; Edwards et al., 2015; Kim et al., 2016). Integration of biorefinery technologies, such as lactic acid (LA) production and recovery together with AD, can provide an opportunity to recover additional higher-value products and improve the economic performance of FW processing (Chapter 3-6). Depending on its application, the price of LA will vary, but has recently been reported to be of significant value (up to 4.0-5.33 $AUD.kg^{-1}{}_{88wt\%LA}$) with global demand projected to grow into the future (Abedi & Hashemi, 2020; Alves de Oliveira et al., 2018).

Recent research (Demichelis et al., 2017; Kim et al., 2016) has indicated combined LA production and AD of FWs may yield a 120-180% increase in the value from a FW biorefinery as compared to only using FW AD. Furthermore, recent technoeconomic analyses have outlined a potential revenue increase of 110-395%, depending on plant capacity and other economic factors (Bastidas-Oyanedel & Schmidt, 2018; Demichelis et al., 2018). These reports suggest LA recovery is potentially attractive from a techno-economic perspective. However, generally the technoeconomic analyses have implemented pre-treatment methods within their analysis to increase the LA yield from FW fermentation (Bastidas-Oyanedel & Schmidt, 2018; Demichelis et al., 2018; Kwan et al., 2018). In contrast, existing commercial FW AD facilities already have a significant upfront sorting and pre-processing of wastes, potentially making additional pre-treatment unnecessary and benefiting from existing capital. This is important not only because pre-treatment methods can be costly, but they also often lead to the generation of large quantities of solid and liquid wastes which require further treatment prior to ultimate disposal (Surendra et al., 2015). Therefore, alternative cost-effective methods should be implemented to improve LA production from FW fermentation, which generate minimal wastes for ultimate disposal.

Nitrogen supplementation with ammonium has been shown to promote hydrolysis and LA production from FW fermentation (Zhang et al., 2020a; Zhang et al., 2020b). FW AD digestate naturally contains elevated concentrations of ammonium (Banks et al., 2011; Buhlmann et al., 2018; Serna-Maza et al., 2015) and has been shown to be an effective nutrient supplement to improve LA fermentation of FWs, provided its use does not exceed critical levels ((Wang et al., 2021; Zhang et al., 2019) and Chapter 6). Furthermore, recirculation of digestate as a nutrient and moisture supply, and adding sucrose as a highly fermentable carbon source, has been shown to increase LA production significantly compared to a controlled FW fermentation system (Chapter 6). Despite these anticipated benefits, the economic implications of digestate recirculation and sugar addition have not been explored by previous studies. Furthermore, previous studies have only examined LA recovery from mainline fermentation, whereas a dedicated side-stream fermentation system, only processing selected feedstocks known to promote or enhance LA production, may offer opportunities to better optimise LA production and recovery and thus enhance techno-economic feasibility. The integration of LA fermentation and recovery into existing two-stage AD facilities (i.e. pre-fermentation followed by AD) has not been explored previously from a techno-economic perspective, nor has side-stream vs. mainline fermentation within this context. Such integration and side-stream optimisation could provide significant cost benefits from utilising existing process infrastructure and achieving higher LA production.

To this end, this Chapter aims to explore the technoeconomic feasibility of integrating LA production technologies into two-stage FW AD (Greenfield vs. Brownfield) utilising mainline or side-stream fermentation approaches. The techno-economic impact of supplementing FW with sucrose and recirculating digestate was also explored and evaluated. Finally, the economic feasibility of further processing of the produced LA into polylactic acid (PLA) was evaluated. The analysis used various common economic indicators, such as the net present value, cost benefit ratio, internal rate of return, and simple payback period, to assess technoeconomic performance.

7.2 METHOD

7.2.1 Fermentation scenario descriptions

Scenarios were developed and analysed based on the existing FW AD facility described in Chapter 3. The current study compared two main operation scenarios during the techno-economic analysis, namely:

1) Mainline Fermentation (MLS): all wastes received are utilised within a single fermenter operated to target LA production and accumulation.
2) Side-stream Fermentation (SDS): wastes received are separated with those deemed to be preferable/favourable for LA production (Chapter 3) being utilised within a separate smaller fermenter operated to target LA production and accumulation, while the remainder wastes are utilised for mainline AD for biogas production. For this scenario, a second fermenter was sized and costed for side-stream fermentation.

An average feedstock mixture composition was provided by the existing facility and was assumed to represent conditions for all scenarios (Table E.1). This average feedstock composition was also used to select a subset of preferred feedstocks for SDS. These selected feedstocks were: FW (46.45 $t.day^{-1}$), milk waste (6.27 $t.day^{-1}$), grain processing waste (1.81 $t.day^{-1}$), and soft drink waste (15.91 $t.day^{-1}$). Further details of these selected feedstocks are reported in Chapter 3.

In addition, two separate LA fermentation scenarios were also explored, namely, conventional fermentation (CNF) consisting of a standard fermenter under pH and temperature control (Chapter 4), and conventional fermentation enhanced with sucrose and digestate addition (SDAF, Chapter 5). The yield of LA and other competing organic acids along with operational conditions for these scenarios are outlined in Table 7.1. Both fermentation scenarios were assumed to be pH- and temperature-controlled at pH 6.0 and 50 ^{o}C, respectively, to provide optimal fermentation conditions (Chapters 4 and 5).

The thermal energy required to maintain a fermenter temperature at 50 ^{o}C (compared to base case 35 ^{o}C) was determined in the current work, accounting for convective heat loss only through the cylindrical fermenter outer wall. This used a conventional first-order heat transfer approach to determine if the heat generated from AD would be sufficient to heat the fermenter. The convective heat loss in this case (h(W)) for vessel was calculated using Equation 7.1.

$$h(W) = \frac{Y \times k \times \Delta T}{X} \times A \quad (7.1)$$

where A is the external surface area of the tank (ft^2), π is pi, r_0 is the external radius of the vessel (m) assumed to be 5.05 m, and h_1 is the working height of the vessel (m) assumed to be 6.00 m. To determine if heat produced by the CHP was capable of heating the fermenter, the calculated electrical energy produced by a Combined Heat and Power engine (Section 7.2.2) was then multiplied by a factor of 1.9 to determine the amount of waste heat available for fermenter heating (i.e. common energy output of a CHP engine is 65% heat and 35% electrical energy; 65% / 35% = 1.9 (Bastidas-Oyanedel & Schmidt, 2018)).

Table 7.1: Process parameters used for the technoeconomic assessment

Process parameter	CNF	SDAF
Fermenter		
Controlled pH [a]	6.0	6.0
Controlled Temperature (°C) [a]	50	50
Total plant feed rate ($tonne_{FW} \cdot year^{-1}$) [b]	35,000	35,000
Lactic acid yield ($kg \cdot kg_{VS}^{-1}$)	0.56	0.60
Succinic acid yield ($kg \cdot kg_{VS}^{-1}$)	0.01	-
Acetic acid yield ($kg \cdot kg_{VS}^{-1}$)	-	0.09
Propionic acid yield ($kg \cdot kg_{VS}^{-1}$)	0.09	0.06
Butyric acid yield ($kg \cdot kg_{VS}^{-1}$)	-	-
Sucrose dose rate ($kg \cdot kg_{VS\text{-}FW}^{-1}$)	-	0.7
Digestate dose rate ($kg \cdot kg_{VS\text{-}FW}^{-1}$)	-	1.79
Separation		
Lactic acid recovery efficiency (%)	51.1	51.1
PLA synthesis		
Final product recovery efficiency (%)	42.5	42.5

[a] Fermentation conditions selected based on Chapters 4 and 5, [b] FW VS content of 16.6%

7.2.2 Downstream processing

Downstream processing encompassed all separation, purification, and synthesis processes necessary for the production of the final LA or PLA product. The extraction and purification system used in this study was based on the technoeconomic assessment conducted by Demichelis et al. (2018). Briefly, the extraction method consisted of centrifugation, microfiltration, ultrafiltration, mono and bipolar electrodialysis, followed by multi-effect vacuum evaporation (Demichelis et al., 2018), each suitably sized for the required scenario capacities. Solid and liquid extraction residues produced by the LA recovery and separation were assumed to be utilised fully within AD for methane recovery (Chapter 6). The separation and purification system was assumed to have an

overall LA recovery efficiency of 51.1% and produced a LA product with a concentration of 702 $g \cdot L^{-1}$ (60 wt%) as per (Demichelis et al., 2018).

PLA synthesis followed the ring-open polymerisation of lactide which was modelled after the technoeconomic assessment conducted by Kwan et al. (2018). Briefly, the process consisted of lactide synthesis from LA as raw material, purification of lactide via dissolution in ethyl acetate and recrystallisation, and PLA synthesis using ring-open polymerisation with lactide as raw material (Kwan et al., 2018). The PLA in that study (Kwan et al., 2018) was purified by dissolving in chloroform and precipitation in methanol. This process required a LA feed concentration of 80 wt% and as such, additional thermal energy was invested to further up-concentrate the 60 wt% LA by simple evaporation. This analysis calculated the additional energy required via a mass and energy balance and the enthalpy of evaporation for water at 0.2 bar (2359.74 $kJ \cdot kg^{-1}$ (Felder & Rousseau, 2000)), which was the pressure utilised in the final evaporation stage by Kwan et al. (2018). The overall conversion/recovery ratio for PLA synthesis (80% LA to PLA) was assumed to be 0.425 weight PLA/weight LA.

Capital cost (CAPEX) estimations included fixed capital and working capital based on the work of Demichelis et al. (2018) who conducted detailed costings for LA-AD biorefineries with capacities ranging from 286-143,000 $t_{FW}.annum^{-1}$. Demichelis et al. (2018) provided a percentage breakdown of contributions of individual system components to overall plant costs specifically for; downstream processing, AD infrastructure, LA fermenter, and CHP costs vs. plant capacity. Percentage break-down of OPEX were also similarly provided. In the current study, linear interpolation lines were fitted to the CAPEX data of Demichelis et al. (2018) for the LA fermenter, AD infrastructure and CHP engines, vs. plant size, to determine individual CAPEX amounts for each of these components. A similar approach was used for OPEX of the LA fermenter, downstream processing, digester, CHP, and waste management. However, a linear interpolation was found to be not appropriate for CAPEX of downstream recovery costs. As such, a power-law scaling rule (Eq. 7.1) (Tribe & Alpine, 1986) was tested instead:

$$\frac{C_1}{C_2} = \left(\frac{V_1}{V_2}\right)^a \qquad \text{(Eq. 7.1)}$$

where c_1 is the initial cost of equipment, V_1 is the initial capacity of the equipment, c_2 is the estimated capital cost of equipment, V_2 is the target equipment capacity, and a is an empirical scale factor. A fitted value for a was determined using the lm() function in R and was found to be 0.02 ± 0.01, which, while significant, results in only a 10% increase in CAPEX with a 500 times increase in capacity (Demichelis et al., 2018). Consequently, the CAPEX was instead set to a fixed value equal to the average cost reported by Demichelis et al. (2018). OPEX included the costs for labour, which were assumed to be 58,552 AUD·year^{-1}·operator^{-1} (ABS, 2021) with 2 additional operators required for MLS and SDS, and a total of 5 for the Greenfield scenarios (Towler & Sinnott, 2013). For scenarios including sucrose supplementation, the cost of sucrose was assumed to be 0.28 AUD·kg^{-1} (Efe et al., 2013).

All CAPEX and OPEX costs were included for Greenfield scenarios. For the Brownfield scenarios, CAPEX and OPEX of the fermenter, Digester and CHP were excluded for MLS, and CAPEX and OPEX for the Digester and CHP were excluded for SDS, assuming these individual components were pre-existing (will not incur additional CAPEX) and already fully operational (no additional OPEX). As per Demichelis et al. (2018), the techno-economic evaluation considered 300 days of continuous operation with equipment sized to operate at 90% of their maximum capacity.

For PLA synthesis, the plant costs were scaled based on size (i.e. the annual volume of LA solution processed (at 80 wt%)) using a single available data-point found in the literature (Kwan et al., 2018) and Equation 7.2 with a set to a value of 0.6 (Tribe & Alpine, 1986).

Revenue/benefits included the sale of LA and gate fees from the feedstock waste received and processed by the facility. However, in scenarios for MLS and SDS, gate fees were only included for additional waste received to compensate for the organic matter/methane yield lost due to the extraction of LA extracted. For this, the methane production rate of the digesters was estimated assuming that the FW being processed had a VS content of 13% and a biochemical methane potential (BMP) of 519 m^3_{CH4}·$tonne_{VS}^{-1}$ (Holliger et al., 2017), and was being processed at a total feed rate of 117 $tonnes_{FW}$·day^{-1} (35,000 $tonnes_{FW}$·year^{-1}). It was assumed the digesters were well mixed with a mean residence time of 30 days and first order rate constant of 0.24 day^{-1} (Gao et al., 2021), providing a fractional yield of 0.88 [$= \frac{30*0.24}{1+(30*0.24)}$] to provide a total methane output of 7,233 m^3_{CH4}

$_{(STP)}$·day^{-1}. To estimate methane yield lost and quantity of additional FW required, a simple methane balance was performed, assuming that the solid and liquid extraction residues had BMP yields of 479.64 and 292.00 m^3_{CH4}·$tonne_{VS}^{-1}$ (Chapter 6) and that LA was fully biodegradable with a conventional carbohydrate BMP of 0.415 m^3_{CH4}·$tonne_{VS}^{-1}$. The methane balance aimed to sustain the total methane output of the digesters (i.e. 7233.21 $m^3_{CH4\ (STP)}$·day^{-1}) by adding additional FW to offset the LA extracted. The gate fee charged for incoming waste was assumed to be 78.82 AUD.t^{-1}, based on the 2019-2020 average landfill levy rates across metropolitan and regional Australia (Carlu et al., 2019).

The sale value of LA was assumed to be 2.18 AUD.kg^{-1} $_{60\ wt\%\ LA}$ (Demichelis et al., 2018), and the value of PLA of 2.97 AUD.kg^{-1} (Madhavan Nampoothiri et al., 2010) as a conservative estimate.

In the case of Brownfield MLS and SDS, biogas electricity was excluded as a benefit because in this case the AD plant was fully operational, and the analysis excluded the associated CAPEX. For GFS, plant electricity output was estimated (Eq. 7.2):

$$P = B \cdot LHV \cdot I \cdot 0.28 \qquad \text{(Eq. 7.2)}$$

Where, P is the power output from the CHP engine (kWh·day^{-1}), B is the biogas production rate (m^3·day^{-1}), LHV is the lower heating value of biogas (taken as 22.35 MJ·m^{-3} biogas with 60% methane (Ghosh & Bhattacherjee, 2013)), I is the electrical efficiency of a CHP engine (taken as 36% (Tait et al., 2021)), and 0.28 is a units conversion factor. To account for the parasitic power consumption, it was assumed 60% of the produced power was sold as an electricity surplus (Bastidas-Oyanedel & Schmidt, 2018). A power sale price of 80 AUD.MWh^{-1} was assumed (Edwards et al., 2015). Installation and commissioning were assumed to occur over 1-year (start in 2020), during which all revenue was set to zero.

7.2.3 Techno-economic calculations

Calculations used the interpolated relationships described above for CAPEX and OPEX at different plant sizes. Interpolation of the economic data obtained from Demichelis et al. (2018) and Kwan et al. (2018) gave the relationships in Table 7.2, which were used for the cost estimation with various plant capacities (x).

Table 7.2: Interpolation equations used for estimating system capital and operational costs.

System component	Equation	R^2	Factor used for scaling
	CAPEX		
Lactic acid fermenter (×1000 AUD)	(27.19*x + 61497) *1.66	1.00	Raw FW yearly feed rate to fermenter (tonnes/year)
Downstream Processing (×1000 AUD)	2,314,509 [a]	-	**Utilises a set value**
Anaerobic digester (×1000 AUD)	136.97*x + 55561.83	1.00	Total FW feed rate to the plan (tonnes/year)
CHP (x1000 AUD)	15.13*x + 7505.65	1.00	Total FW feed rate to the plan (tonnes/year)
PLA plant (AUD)	$5{,}377{,}333 * \left(\frac{24{,}983}{x}\right)^{0.6}$	-	Yearly 80 wt% LA to be processed into PLA (tonnes/year)
	OPEX		
Lactic acid fermenter	(0.62*x + -21.43) *1.66	1.00	Raw FW yearly feed rate to fermenter (tonnes/year)
Downstream Processing	134.99*x + 890.76	1.00	Mass of unclean LA produced from fermentation (tonnes/year)
Anaerobic digester	16.63*x + -10.39	1.00	Total FW feed rate to the plan (tonnes/year)
CHP	20.05*x + -56.78	1.00	Total FW feed rate to the plan (tonnes/year)
Waste management	28.03*x + 8.7	1.00	Total FW feed rate to the plan (tonnes/year)
Lactide synthesis	31.84*x	-	Yearly mass of LA processed for PLA (kg 80 wt% LA/year)
PLA synthesis	55.22*x	-	mass of LA processed for PLA production (kg 80 wt% LA /year)
PLA plant Reagents	**Unit cost (USD/kg)**	**Usage ($kg \cdot kg_{LA}^{-1}$)**	
Ethyl acetate	1058	33.30	$kg/LA_{80\ wt\%}$ processed
Zinc oxide nanoparticle	20000	2.52	$kg/LA_{80\ wt\%}$ processed
Polyglyceryl-10	264	0.33	$kg/LA_{80\ wt\%}$ processed
Stannous octoate	12000	0.68	$kg/LA_{80\ wt\%}$ processed
Chloroform	581	33.30	$kg/LA_{80\ wt\%}$ processed
Methanol	300	53.28	$kg/LA_{80\ wt\%}$ processed

a: average value for downstream processing cost

Taxation and decommissioning benefits/costs were excluded, and sale value of plant after a nominal 20-year project life (expected life of process infrastructure) was assumed to be nil. For the Brownfield scenarios, revenue from AD were not included, because the AD CAPEX and OPEX had been excluded (as above). For the Greenfield scenarios all costs and revenue associated with AD were included in the NPV calculations, because associated CAPEX and OPEX had been included (as above).

Cost feasibility was quantified using common calculated metrics: (1) net present value (NPV) for a nominal 20-year project life, using a 5% discount rate on future cash flows;

$$NPV\ [AUD] = \sum_{n-1}^{T} \frac{C_n}{(1+d)^n} - C_0 \qquad \text{(Eq. 7.3)}$$

where; C_n is the net cash flow during the time period n, d is the discount rate, and C_0 is initial capital investment (including fixed and working capital). (2) Return on Investment (ROI) and annualised ROI, which were calculated using Eq. 7.4 and Eq. 7.5, respectively:

$$ROI[\%] = \frac{Annual\ Net\ Cash\ flow}{C_o} \times 100 \qquad \text{(Eq. 7.4)}$$

$$Annualised\ ROI\ (\%) = \left((1 + ROI[\%])^{\frac{1}{n}} - 1\right) * 100 \qquad \text{(Eq. 7.5)}$$

where; $ROI[\%]$ is the return on investment over a nominal plant life n = 20 years and does not include debt finance. 3) cost-benefit analysis (CBA), 4) internal rate of return (IRR), 5) minimum sale price of LA or PLA for a zero NPV, and 6) payback period which was the time taken to recoup the initial capital investment using annual undiscounted cash flows from the project (Garrison & Noreen, 2000). All monetary values were quantified in AUD, using average exchange rates in 2021 for conversion between literature values and AUD of 0.64 AUD.Euro^{-1} and 0.75 AUD.USD^{-1} (RBA, 2021). A sensitivity analysis determined the effect of a ±25% variation in LA price, capital costs, annual operating costs, nominal LA yield, power price, plant capacity, and gate fees on calculated NPV.

7.3 RESULTS AND DISCUSSION

7.3.1 Plant energy and material inputs

The maximum thermal energy requirement for heating the fermenter to 50 °C (i.e. for MLS) was estimated at 11.4 kW, while for up-concentration of 60% LA to 80% for PLA production was 56.2 kW. These requirements were anticipated to be covered by the estimated 2,151 kW in waste heat generated (i.e. 65% of total energy produced (Section 7.2.1)) by the combustion of biogas and were not included as additional operational costs.

Control of the fermentation pH is known to be vital for LA fermentation (Chapter 4), and was included within operational costs presented by Demichelis et al. (2018). With 0.44 kg $_{NaOH}$ required per 1 kg $_{LA}$ produced from fermentation (for complete neutralisation) at a price of 0.44 AUD·kg^{-1} (Demichelis et al., 2018), pH control can form a significant portion of the operational costs. However, the use of bipolar electrodialysis provides an

opportunity to reduce costs associated with pH correction during fermentation as alkali is produced with the recovery of LA (Alves De Oliveira et al., 2019).

Partial recirculation of digestate and supplementation of sucrose (Table 7.1) increased LA production by 84% for both side stream and mainline fermentation but was associated with an operational cost increase of 3,622 AUD·day^{-1}. However, as only 1.0 tonne·day^{-1} of LA (or 1.7 tonnes·day^{-1} of 60 wt% LA) is required to offset the additional operational costs, sucrose supplementation was vastly offset by the additional LA produced. Within an integrated LA-AD biorefinery, digestate is a free input, produced during the AD of the solid and liquid extraction residues following LA separation and recovery. Furthermore, as the results of Chapter 5 indicated digestate did not require pre-treatment prior to use within LA fermentation, the costs associated with the implementation of digestate recirculation are expected to be negligible (i.e. purchasing and installation of piping).

7.3.2 Brownfield vs. Greenfield

The Brownfield scenarios benefited from significant capital savings of 59-79% and 24-43% for mainline vs. side-stream recovery, respectively, as compared to the Greenfield scenarios (Fig. 7.1). While the overall revenue was lower in the Brownfield LA scenarios, the higher revenue achieved in Greenfield scenarios was primarily due to the inclusion of gate-fees, which accounted for 55-59% of the total revenue received. However, following the integration of LA production into AD, the financial benefits from gate-fees were no longer required to provide a positive NPV. In contrast, for sole AD a minimum gate fee of 61.60 AUD·tonne$_{FW}^{-1}$ was required to break even, so economic feasibility is expected to be sensitive to feedstock supply. This also suggests that the integration of LA production into AD can reduce dependency on the need for subsidy support to remain profitable. Similar results were reported by Bastidas-Oyanedel and Schmidt (2018) who explored the profitability of various biorefinery options, including the LA-AD biorefinery.

For all Brownfield scenarios, while SDAF elevated operational costs compared to CNF (Fig. 7.1A), economic feasibility was improved, resulting in a higher NPV, CBA ratio, IRR, and ROI (Fig. 7.1). While all Greenfield scenarios had a higher NPV compared to their respective Brownfield scenario (i.e. Brownfield MLS compared with Greenfield MLS) it was primarily due to the inclusion of gate-fees with power sale providing a minor increase to revenue. However, due to the lower capital costs, Brownfield scenarios were

more economically feasible, displaying a higher CBA ratio, ROI, IRR, and lower payback period. However, compared to Greenfield AD, a Greenfield LA-AD biorefinery is much more economically feasible, displaying a higher CBA ratio and much higher NPV than sole FW AD (Fig. 7.1B).

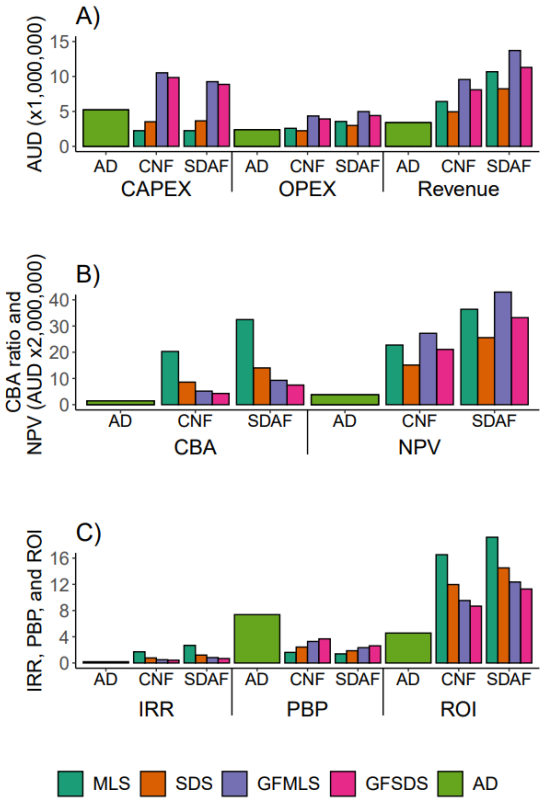


Figure 7.1: Economic evaluation of Brownfield and Greenfield LA production scenarios for Greenfield AD, conventional fermentation (CNF) and sucrose and digestate added fermentation (SDAF). ROI is the yearly ROI. Not the different y-axis extents.

7.3.3 Mainline vs. Side-stream

MLS was the most economically feasible scenario for LA production, displaying the highest CBA ratio, IRR, and ROI (Fig. 7.1). Side-stream recovery performed worse in all economic indicators assessed (Fig. 7.1), due to a lower LA production rate, and higher CAPEX due the requirement of a second smaller fermentation unit. However, if the feed rate to the side-stream fermenter could be increased from 90 to 132 tonne.day^{-1}, SDS would have the same NPV as MLS, provided that the LA yield remained relatively unchanged.

Utilisation of a secondary fermentation unit also allows for more strict control of the feed composition with selected wastes known to be beneficial to LA fermentation used to improve overall LA yield. Modelling identified if the LA yield within SDS reached 0.74

or 0.94 $g_{LA} \cdot g^{-1}_{substrate\ Dry}$, for CNF and SDAF, respectively, this scenario would have the same NPV as the MLS. While such high yields would pose a challenge for optimisation of LA fermentation, as many reports give yields in the range 0.33-0.46 $g \cdot g^{-1}_{FW\ Dry}$ (Pleissner et al., 2017; Tang et al., 2016; Wang et al., 2005), a yield of 0.74 $g_{LA} \cdot g^{-1}_{FW\ Dry}$ could be achievable based on the results of Chapter 6, where implementation of digestate recirculation rose the yield to 0.72 $g_{LA} \cdot g^{-1}_{FW\ Dry}$. Moreover, a highly optimised and controlled LA fermenter may allow for a higher organic loading rate or reduced retention time (Kim et al., 2016; Tang et al., 2016) owing to improved productivity and microbial growth. This can have additional process intensification benefits.

7.3.4 LA vs. PLA production

While PLA is a higher value product compared to LA (Section 7.2.2), the higher sale price did not offset the additional capital and operational costs associated with PLA synthesis, resulting in a negative NPV for all Brownfield scenarios (Fig. 7.2B). Furthermore, the improved production of LA from integrating sucrose supplementation and digestate recirculation was not able to improve the economic feasibility, but rather further reduced the NPV (Fig. 7.2B). Overall, economic feasibility of integrating PLA production into AD was weak, with all economic indicators showing poor performance.

In contrast, the additional revenue generated from power production and gate-fees from Greenfield scenarios significantly improved the economics of the PLA-AD biorefinery (Fig. 7.2), leading to positive NPVs for CNF Greenfield scenarios. As discussed in Section 7.3.3, a yield of 0.74 $g_{LA} \cdot g^{-1}_{FW\ Dry}$ may be achievable via conventional fermentation. Such a yield could increase the project NPV to 2,370,592 and 9,664,581 for mainline and side-stream CNF scenarios, respectively. However, Greenfield scenarios including sucrose supplementation, while experiencing improved economic feasibility compared to Brownfield scenarios, were still infeasible, displaying a negative NPV, CBA ratio, and a payback period >20 years.

The minimum required PLA price to break even for Brownfield scenarios without sucrose supplementation were 3.34 and 4.02 $AUD \cdot kg^{-1}$, for MLS and SDS, respectively. While these values fall below previously reported prices for PLA (6.93 $AUD.kg^{-1}$; 5.2 $USD.kg^{-1}$ (Kwan et al., 2018)), they were not considered to be sustainable, even over the nominal biorefinery lifetime of 20 years. For PLA to compete with fossil-fuel-based plastics, it has been reported the price should decrease by approximately half of its present price of

2.93 AUD·kg^{-1} (2.2 USD·kg^{-1}) (Abdel-Rahman et al., 2013; Madhavan Nampoothiri et al., 2010). Consequently, alternative, more cost-effective methods for PLA production or LA fermentation from FWs are required to improve economic feasibility. It should be noted that, while the NPV for PLA was lower than for the LA-only scenarios, the integration with AD also plays an integral role in PLA production, providing a method of disposal for residues and producing renewable energy to meet the demands of processing equipment.

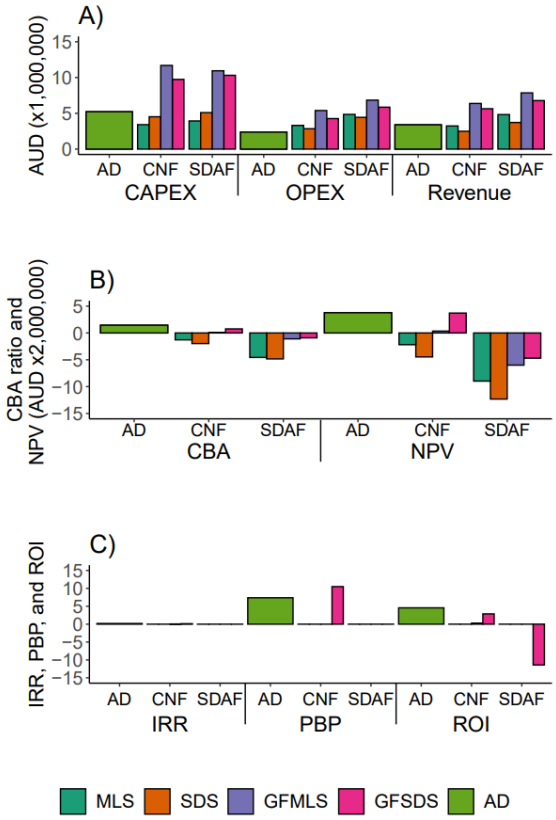


Figure 7.2: Economic evaluation of Brownfield and Greenfield LA production scenarios for Greenfield AD, conventional fermentation (CNF) and sucrose and digestate added fermentation (SDAF). ROI is the yearly ROI. Not the different y-axis extents.

7.3.5 Sensitivity and Opportunities to Increase Feasibility

Economic feasibility in all LA production scenarios was primarily driven by the LA price, yield, and plant capacity (Fig. 7.3). While, it is not anticipated the price and yield of LA will pose a great risk to projected feasibility, as the LA market is expected to have an annual growth rate of 8% between 2021-2028 (Grand View Research, 2021), higher yields could significantly boost the economic feasibility of LA-AD biorefineries. Literature has shown LA yields could be effectively increased through implementing FW pre-treatment, such as enzymatic, fungal, acidic, alkali pre-treatment (Ahmad et al., 2020;

Demichelis et al., 2017; Kim et al., 2003; Kwan et al., 2016; Pleissner et al., 2016). However, these processes are often costly and require additional processes for waste disposal (Surendra et al., 2015). In contrast, inoculation with select LA bacteria may be an effective method to promote LA production (Pleissner et al., 2017). Utilisation of a select LA bacteria or engineered inoculum aimed at promoting FW conversion to LA could be achievable at an engineered biorefinery.

While previous studies have shown economies of scale favours larger LA biorefinery capacities (Demichelis et al., 2018; Kwan et al., 2018), its influence was primarily driven by the fixed capital cost for downstream separation and recovery of LA in this chapter, due to the inability to model the CAPEX vs capacity (Section 7.2.2). It is unlikely CAPEX will remain constant as plant capacity increases, even so, results show the CAPEX for LA recovery and separation can increased 10-fold (keeping capacity constant), while still producing a respectable NPV of 49 and 28 million AUD for MLS and SDS, respectively. However, it should be noted that FW biorefineries may experience greater economic risk from potential seasonal changes in feedstock composition and availability (Chapter 3) and from future competing biorefinery operations, potentially impacting the overall plant capacity. Strict seasonal feedstock management and development of waste contracts to secure available wastes will likely be a necessity for future FW biorefinery concepts.

Variations in the price of sucrose in SDAF scenarios had a minor overall influence on project NPV and was near negligible for some scenarios (Fig. 7.3). Carbohydrates are the primary substrate for LA production but the resulting LA concentration from FW fermentation is limited due to the exhaustion of available substrate (Chapter 4). The low overall sensitivity to the sucrose price suggests sucrose supplementation could be an effective, economically feasible method to improve the economic performance of FW LA-AD biorefineries. Variations in the power price had no effect on Brownfield scenarios as they were excluded from feasibility calculations but were included within Greenfield scenarios. While power sales had a minor effect on projected feasibility (Fig. 7.3), the benefits from on-site power production are anticipated to primarily manifest in lower operational costs, due to the reduced reliance on the grid-based power.

Gate-fees were another major driver of economic feasibility for Greenfield scenarios, having a similar effect to the price and yield of LA. While gate-fees provide an opportunity for biorefineries to significantly boost their revenue, it is not anticipated to

be a sustainable revenue stream. As biorefineries develop and compete to secure waste contracts, gate-fees are likely to reduce and are become a cost as waste manufactures may assign value to their waste stream as competition develops. For example, AD is prolific in Europe due to the financial incentives provided by governments (Chapter 2). However, as a consequence utilisation of many biowastes are associated with an upfront cost to purchase the waste from the producer (BIRMAN et al., 2021), which could be further elevated by additional costs for transport and logistics (BIRMAN et al., 2021; Demichelis et al., 2018). Consequently, biorefineries should target economical operation without the support of gate-fees.

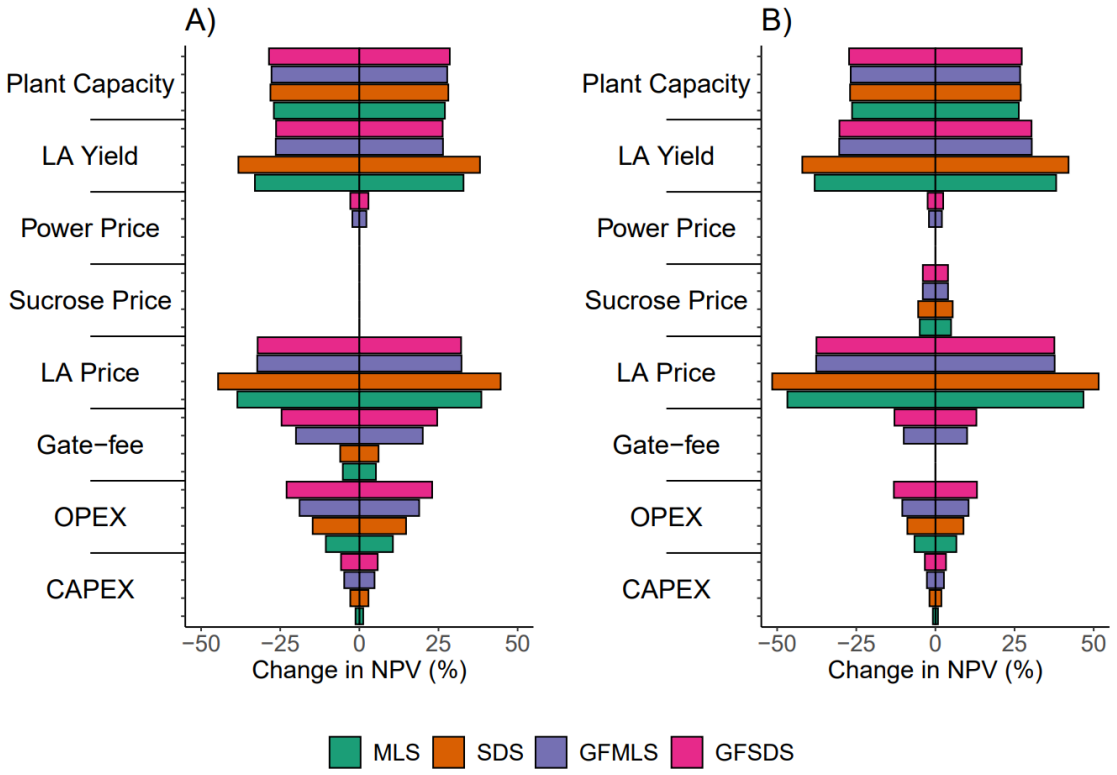


Figure 7.3: Sensitivity analysis (±25%) of LA production scenarios for A) conventional fermentation (CNV), and B) sucrose and digestate fermentation (SDAF). MLS and SDS are Brownfield mainline and side-stream recovery, respectively, and GFMLS and GFSDS are Greenfield mainline and side-stream recovery, respectively. All scenarios were evaluated over a timeframe of 20 years.

For all PLA integration scenarios, the PLA price and OPEX were the major influencers of project feasibility (Fig. 7.4) and led to much larger variation in economic feasibility compared to LA production scenarios (Fig. 7.3). This was, at least partly, due to the relatively higher price of PLA, as compared to LA, and the increased costs associated reagent purchases and equipment operational costs for PLA synthesis. It is anticipated

that the price of PLA can pose a significant risk to profitability of the biorefinery as mentioned previously above (Section 7.3.4). Integration of methods to improve the economics of LA fermentation or PLA production may aid the economic feasibility of the PLA-AD biorefinery.

In the case of Greenfield scenarios, gate-fees had a strong impact on all PLA production scenarios and were crucial for economic feasibility, requiring a minimum value of 77.50 or 64.73 AUD·tonne^{-1} for MLS and SDS, respectively, to break even. However, such values may not be sustainable throughout the biorefinery lifetime as mentioned previously. When utilising sugar and digestate fermentation, the price of PLA and operational costs were again the major divers in project feasibility, while gate-fees had a moderate effect (Fig. 7.4B). Interestingly the economic sensitivity to the PLA price increased with SDAF (Fig. 7.4B), as compared to CNF (Fig. 7.4A), which may be due to a combination of the relatively lower NPV for SDAF, as compared to CNF (Fig. 7.2), increased operational costs associated with sucrose addition, and higher PLA production rate. Similar to LA scenarios, power had a minor effect on economic feasibility.

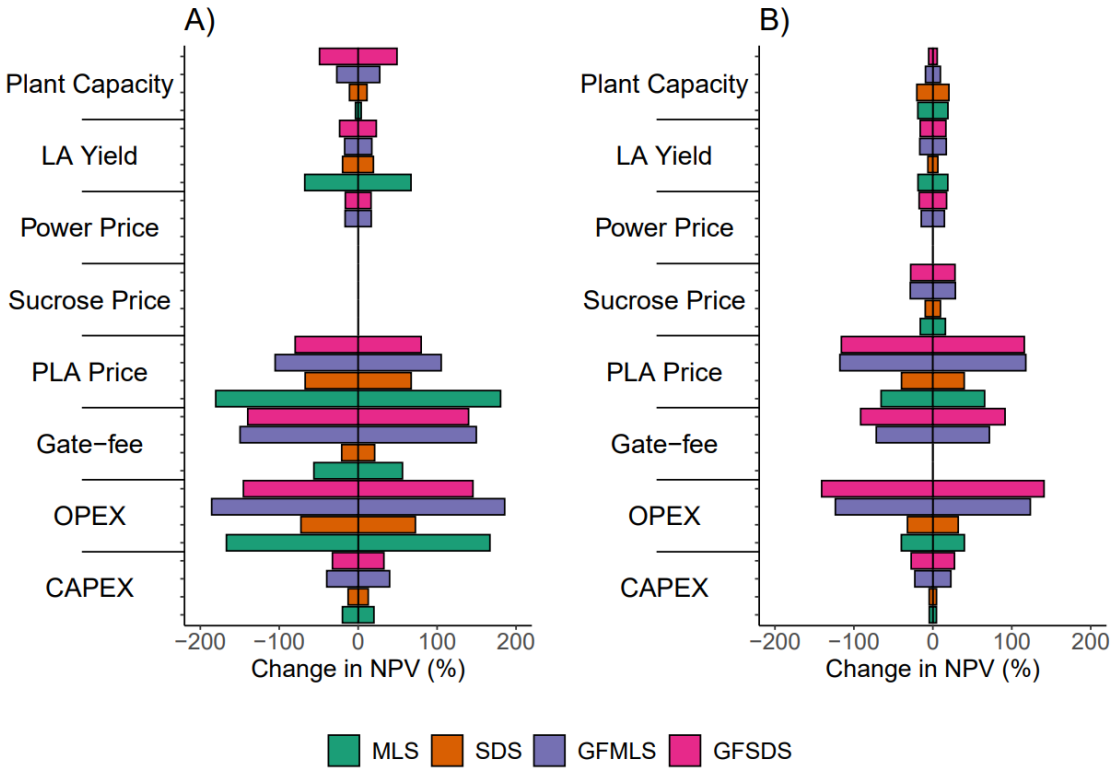


Figure 7.4: Sensitivity analysis (±25%) of PLA production scenarios for A) conventional fermentation (CNV), and B) sucrose and digestate fermentation (SDAF). MLS and SDS are Brownfield mainline and side-stream recovery, respectively, and GFMLS and GFSDS are Greenfield mainline and side-stream recovery, respectively. All scenarios were evaluated over a timeframe of 20 years.

7.4 CONCLUSION

The technoeconomic analysis demonstrated the integration of LA production into two-stage FW AD can lead to significant capital savings and boost to the revenue/financial benefits of existing AD facilities. Greenfield mainline extraction utilising sucrose and digestate added fermentation for LA production provided the highest net present value, while comparative Brownfield scenarios were more economically attractive. Integration of LA production into AD has the potential to eliminate the need for subsidies for Greenfield scenarios. PLA production within AD was found to not be financially feasible, yielding an NPV <0 for nearly all scenarios, and the forecasted lack of opportunities to increase PLA sale price to the point of economic feasibility was discussed. Overall, all financial metrics calculated in this Chapter showed that Brownfield integration of mainline LA recovery with sucrose and digestate to fermentation was the most economically attractive scenario. Furthermore, this same scenario showed the lowest sensitivity to all influencing factors that were varied, except for the price of LA. These results suggest that the integration of LA fermentation and recovery into two-stage FW AD is financially feasible and commercially attractive.

7.5 REFERENCES

Abdel-Rahman, M.A., Tashiro, Y., Sonomoto, K. 2013. Recent advances in lactic acid production by microbial fermentation processes. Biotechnology Advances, 31(6), 877-902.

Abedi, E., Hashemi, S.M.B. 2020. Lactic acid production – producing microorganisms and substrates sources-state of art. Heliyon, 6(10), e04974-e04974.

ABS. 2021. Wage Price Index, Australia, Vol. 2021, australian bureau of statistics.

Ahmad, A., Banat, F., Taher, H. 2020. A review on the lactic acid fermentation from low-cost renewable materials: Recent developments and challenges. Environmental Technology & Innovation, 20, 101138.

Alves De Oliveira, R., Alexandri, M., Komesu, A., Venus, J., Vaz Rossell, C.E., Maciel Filho, R. 2019. Current Advances in Separation and Purification of Second-Generation Lactic Acid. Separation & Purification Reviews, 1-17.

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R. 2018. Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. Biochemical Engineering Journal, 133, 219-239.

Banks, C.J., Chesshire, M., Heaven, S., Arnold, R. 2011. Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance. Bioresource Technology, 102(2), 612-620.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

BIRMAN, J., BURDLOFF, J., DE PEUFEILHOUX, H., ERBS, G., FENIOU, M., LUCILLE, P.-L. 2021. Biomethane: potential and cost in 2050.

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum: Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

Carlu, E., Truong, T., Kundevski, M. 2019. Biogas opportunities for Australia.

Cucchiella, F., D'Adamo, I. 2016. Technical and economic analysis of biomethane: A focus on the role of subsidies. Energy Conversion and Management, 119, 338-351.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Edwards, J., Othman, M., Burn, S. 2015. A review of policy drivers and barriers for the use of anaerobic digestion in Europe, the United States and Australia. Renewable and Sustainable Energy Reviews, 52, 815-828.

Efe, Ç., van der Wielen, L.A.M., Straathof, A.J.J. 2013. Techno-economic analysis of succinic acid production using adsorption from fermentation medium. Biomass and Bioenergy, 56, 479-492.

Felder, R.M., Rousseau, R.W. 2000. Elementary principles of chemical processes. 3rd ed. John Wiley, New York.

Gao, S., Lei, X., Ruan, W., Zhao, M. 2021. Synergetic enhancement of methane production and system resilience during anaerobic digestion of food waste in ammonia-tolerant anaerobic sludge system. Environmental science and pollution research international, 28(17), 21851-21861.

Garrison, R.H., Noreen, E.W. 2000. Managerial Accounting. 9th ed. Jeffrey J Shelstad.

Ghosh, R., Bhattacherjee, S. 2013. A review study on anaerobic digesters with an Insight to biogas production. International Journal of Engineering Science Invention, 2(3), 8-17.

Grand View Research. 2021. Lactic Acid Market Size Worth $5.02 Billion By 2028 | CAGR: 8.0%.

Holliger, C., Fruteau de Laclos, H., Hack, G. 2017. Methane Production of Full-Scale Anaerobic Digestion Plants Calculated from Substrate's Biomethane Potentials Compares Well with the One Measured On-Site. Frontiers in Energy Research, 5(12).

Kim, K.I., Kim, W.K., Seo, D.K., Yoo, I.S., Kim, E.K., Yoon, H.H. 2003. Production of Lactic Acid from Food Wastes. in: Biotechnology for Fuels and Chemicals:

The Twenty-Fourth Symposium, (Eds.) B.H. Davison, J.W. Lee, M. Finkelstein, J.D. McMillan, Humana Press. Totowa, NJ, pp. 637-647.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2018. Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly(lactic acid) production. Journal of Cleaner Production, 181, 72-87.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2016. Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with Lactobacillus casei Shirota. Bioresource Technology, 217, 129-136.

Madhavan Nampoothiri, K., Nair, N.R., John, R.P. 2010. An overview of the recent developments in polylactide (PLA) research. Bioresource Technology, 101(22), 8493-8501.

Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. Journal of Cleaner Production, 143, 615-623.

Pleissner, D., Neu, A.-K., Mehlmann, K., Schneider, R., Puerta-Quintero, G.I., Venus, J. 2016. Fermentative lactic acid production from coffee pulp hydrolysate using Bacillus coagulans at laboratory and pilot scales. Bioresource Technology, 218, 167-173.

RBA. 2021. Historical Data - Exchange rates, Vol. 2021.

Serna-Maza, A., Heaven, S., Banks, C.J. 2015. Biogas stripping of ammonia from fresh digestate from a food waste digester. Bioresource Technology, 190, 66-75.

Surendra, K.C., Sawatdeenarunat, C., Shrestha, S., Sung, S., Khanal, S. 2015. Anaerobic Digestion-Based Biorefinery for Bioenergy and Biobased Products. Industrial Biotechnology, 11(2), 103-112.

Tait, S., Harris, P.W., McCabe, B.K. 2021. Biogas recovery by anaerobic digestion of Australian agro-industry waste: A review. Journal of Cleaner Production, 299, 126876.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y. 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management, 52, 278-285.

Towler, G.P., Sinnott, R.K. 2013. Chemical engineering design: principles, practice, and economics of plant and process design. 2nd ed. Butterworth-Heinemann, Boston, MA.

Tribe, M., Alpine, R.L.W. 1986. Scale economies and the "0.6 rule". Engineering Costs and Production Economics, 10, 271-278.

Wang, Q., Wang, X., Wang, X., Ma, H., Ren, N. 2005. Bioconversion of Kitchen Garbage to Lactic Acid by Two Wild Strains of Lactobacillus Species. Journal of Environmental Science and Health, Part A, 40(10), 1951-1962.

Wang, Q., Yang, L., Feng, K., Li, H., Deng, Z., Liu, J. 2021. Promote lactic acid production from food waste fermentation using biogas slurry recirculation. Bioresour Technol, 337, 125393.

Zhang, C., Yang, H.-Q., Wu, D.-J. 2019. Study on the reuse of anaerobic digestion effluent in lactic acid production. Journal of Cleaner Production, 239, 118028.

Zhang, W., Li, X., He, Y., Xu, X., Chen, H., Zhang, A., Liu, Y., Xue, G., Makinia, J. 2020a. Ammonia amendment promotes high rate lactate production and recovery from semi-continuous food waste fermentation. Bioresource Technology, 302, 122881.

Zhang, W., Xu, X., Yu, P., Zuo, P., He, Y., Chen, H., Liu, Y., Xue, G., Li, X., Alvarez, P.J.J. 2020b. Ammonium Enhances Food Waste Fermentation to High-Value Optically Active l-Lactic acid. ACS Sustainable Chemistry & Engineering, 8(1), 669-677.

CHAPTER 8 GENERAL DISCUSSION

Foreword

Chapter 8 provides a summary of the initial research problem and major findings of each experimental chapter (Chapter 3-7). Furthermore, this chapter discusses the results of each of these chapters in the context of the LA-AD biorefinery.

8.1 SUMMARY OF RESEARCH PROBLEM

Anaerobic digestion (AD) is a mature technology capable of recycling food waste (FW) for the recovery of nutrients and production of renewable power (Edwards et al., 2015). However, due to the high cost of construction, operation, and management the economic feasibility of the technology is often questionable (Kim et al., 2016). For this reason commercial AD projects are often reliant on government subsidies or policy incentives for profitability (Cucchiella & D'Adamo, 2016). However, recent literature has suggested integration of lactic acid (LA) production technologies into AD can significantly increase the revenue generated from FW processing (Demichelis et al., 2018; Demichelis et al., 2017; Kim et al., 2016), even being proposed to eliminate the need for government support (Bastidas-Oyanedel & Schmidt, 2018). However, literature has not explored the feasibility of integrating LA production into existing two-stage FW AD. Prior to merging LA production with AD, the influence LA fermentation and recovery may have on the operation and performance of the existing AD facility should be carefully considered. Factors which should be carefully considered would include, but are not limited to, 1) feedstock receival and sorting, 2) reagent addition during fermentation and recovery, 3) feasibility of recovery from complex media, and 4) feasibility of utilising the residues from fermentation within AD. Therefore, this PhD thesis focused on the integration of LA production into existing two-stage AD through, 1) exploring the natural LA production capacity at a commercial facility, 2) optimising environmental conditions for LA production, 3) increasing the production of LA through sucrose supplementation and digestate recirculation, 4) exploring the feasibility of LA to be recovered from complex commercially produced broth and its impact on downstream AD, and 5) exploring the technoeconomic feasibility of integrating LA production technologies into existing two-stage AD facilities.

8.2 SUMMARY AND DISCUSSION OF FINDINGS

The following section provides a summary of the major findings of each chapter and discusses these findings in the context of the research problem.

8.2.1 Chapter 3 - Monitoring an Industrial Facility

In chapter 3 an industrial pre-fermenter at a commercial AD facility was monitored to assess LA production performance. For this the feed composition, environmental conditions, and organic acid concentration (including LA), were minored for the waste

homogenisation vessel and pre-fermenter to understand how the standard operation of commercial AD facility influenced LA production.

Overall, the AD feedstock was subject to the availability of each waste stream and, as standard operation aimed to maximise waste received, the resulting feedstock composition and receival rate varied weekly. As a result, the hydraulic retention time, while relatively short, varied considerably (1-3.5 days). However, the organic loading rate remained relatively stable (12 ± 2 $kg_{VS} \cdot m^{-3} \cdot day^{-1}$), which could be due to the to the nature of the various feedstocks received and feed stability provided by the waste homogenisation vessel. During the period for which the waste homogenisation vessel was offline, the variability in the feed rate significantly influenced the LA concentration, leading to large daily variations (see Fig. 3.3), which were stabilised following its reintroduction (from 35 days). An LA-AD biorefinery is anticipated to be subject to similar feedstock uncertainties and variations in availability, and the results of Chapter 3 suggest simple homogenisation of the waste can effectively provide a feedstock buffer which can aid in stabilising daily LA production.

In addition to the limited control of the feedstock composition and feed rate, the pre-fermenter operated without control of environmental conditions, allowing temperature and pH to fluctuate with the surrounding environment and feedstock composition. As a result, the pre-fermenter operated at warm ambient temperatures (24-35 ^{o}C), and low pH (3.45 ± 0.03). These conditions, along with the short hydraulic retention time and organic loading rate, naturally promoted LA formation and the selective dominance of *Lactobacillus*, a bacterium closely associated with LA production (Kim et al., 2016; Tang et al., 2016). Furthermore, fermentation at these conditions naturally promoted the formation of LA at the mixed FW AD facility, achieving a promising average concentration of 22 $g_{LA} \cdot L^{-1}$. Furthermore, while LA production did vary over the monitoring period, LA production was surprisingly stable considering the varying feed rate, feed composition, and varying environmental conditions. In the context of the LA-AD biorefinery, these results are promising and suggest minimal operational changes would be required at an existing AD facility to produce LA from a mixed FW feedstock, albeit LA production would likely benefit from feedstock composition and supply stabilisation, and process optimisation and control. Even so, the concentrations achieved suggest integration of LA production into existing AD is technically feasible.

8.2.2 Chapter 4 – Optimisation of fermentation conditions

Although the results of Chapter 3 were promising and suggested LA production at a commercial FW AD facility is technically feasible, fermentation was likely limited due to the inhibitory and fluctuating process conditions. Optimisation and control of important parameters, such as pH and temperature, would likely improve and stabilise LA production. Therefore, Chapter 4 explored the effects of targeted control of pH (uncontrolled vs. controlled pH 4-6) and temperature (35-60 ^{o}C) on LA fermentation from synthetic FW. Furthermore, the study utilised an inoculum obtained from the industrial pre-fermenter monitored in Chapter 3 to promote LA formation.

Overall, the results of the optimisation trials in Chapter 4 outlined the inoculum effectively promoted LA formation while pH and temperature control were effective process control levers which could regulated fermentation and selectively target LA as an end product. Uncontrolled pH conditions favoured the growth of *Lactobacillus* and production of LA, similar to the industrial facility explored in Chapter 3. However, fermentation was limited due to product inhibition which was exacerbated by the acidic conditions. Optimisation of conditions and control at pH 6.0 and 50 ^{o}C maximised the LA yield at 0.56 $g_{LA} \cdot g_{VS}^{-1}$, approximately double that at uncontrolled conditions. Furthermore, optimal conditions selectively promoted the growth of LA producers, reduced the production of alternate organic acids, and eliminated observable consumption of LA. These results are promising for future LA-AD biorefinery concepts, indicating LA production at a commercial FW AD facility can be enhanced with simple optimisation of pH and temperature. Furthermore, the fermentation process can be effectively controlled by the process handles of pH and temperature, allowing the fermentation outcomes to be directly controlled by operator intervention.

Control of the operational pH and temperature are practical and realistic control measures to be implemented within future LA-AD biorefinery concepts, and it is anticipated future biorefineries will implement such controls to improve fermentation efficiency and productivity. However, application of any control method should consider implications to downstream recovery and AD. For example, while alkaline pH values have been suggested to further improve LA fermentation (Chapter 4), an LA-AD biorefinery should utilise such values with caution. Alkaline conditions would not only elevate operational costs associated with chemical pH correction during fermentation, and possibly prior to

recovery (depending on the recovery method utilised) but may adversely affect downstream AD, which would require equimolar acid addition to neutralise remaining alkalinity within the fermentation residues. Furthermore, the elevated salinity of the extraction residues may negatively impact AD performance, if inhibitory concentrations were reached (Zhang et al., 2017), and could limit the use of digestate within agriculture. Digestate is already a burden to AD (Turnley et al., 2016) and care should be taken to ensure the end of life outcomes for the AD effluent are not hindered or further restricted when integrating additional biorefinery technologies.

8.2.3 Chapter 5 – Effect of sucrose addition and digestate recirculation

In Chapter 4, LA production was improved through targeted optimisation and control of operational parameters (pH and temperature). While the trials conducted improved the LA yield on FW, the LA concentration is still a major challenge for commercial LA production. Therefore, to further improve the LA yield and to increase the LA concentration, the results of Chapter 4 were utilised in Chapter 5, which aimed to assess the impacted of sucrose supplementation and digestate recirculation on fermentation performance. For this, the effect of sucrose addition (0-150 $g \cdot L^{-1}$) and nitrogen supplementation (0-400 $mg_N \cdot L^{-1}$) using either NH_4Cl or digestate on LA fermentation was explored using a bi-factorial experimental design.

The results of Chapter 5 were promising, indicating nitrogen supplementation could effectively improve the LA yield, with digestate yielding further improvements, while sucrose addition could effectively improve the LA concentration. Furthermore, digestate increased microbial diversity and the production of alternate organic acids, sucrose effectively drove fermentation back towards LA and promoted the selective growth of *Lactobacillus*. Digestate is generally identified as a cost-burden for AD due to its bulky nature and additional costs required for transport, land spreading, and storage (Turnley et al., 2016). However, the results of Chapter 5 show digestate can play an integral role in LA fermentation, providing essential nutrients to improve LA production. Furthermore, digestate recirculation provides the LA-AD biorefinery an opportunity to yield cost savings related to freshwater consumption by supplementing a fraction of the process water with digestate.

While sucrose improved LA production, its use would logically increase operational costs associated with fermentation. However, due to the relatively high value of LA (2.18

AUD·$kg_{60wt\%\ LA}^{-1}$; Chapter 5) and low value of sucrose (0.28 AUD·kg^{-1}; Chapter 5), the cost of sucrose supplementation is easily overcome by the additional LA produced, with only 0.13 $kg_{60wt\%\ LA}$·$kg_{sucrose}^{-1}$ required to overcome the additional cost of sucrose. While sucrose was not fully utilised during fermentation, with higher concentrations reducing total yields on sucrose, further optimisation or utilisation of an alternative LAB could improve LA yields. Even so, it is anticipated a fraction of the added sucrose will remain within the broth following fermentation and recovery. An integrated LA-AD biorefinery provides a second opportunity for unfermented sucrose to value-add to the process. Depending on the extraction method utilised, a large fraction of the unfermented sucrose may remain within the extraction residues, which if used as dilution water for AD, could aid biogas production. While the increased production of power provides an opportunity to improve revenue generated from power sales, it is anticipated that a larger economic benefit may be yielded from offsetting costs associated with LA separation and recovery which are known to be energy intensive (Din et al., 2021).

8.2.4 Chapter 6 - Effect of lactic acid recovery on Anaerobic Digestion

Although the results of Chapters 3-5 indicated LA could be effectively produced from FW and enhanced with sucrose and digestate supplementation, LA would need to be efficiently recovered for its economical production. For this, LA should be efficiently extracted while having minimal impacts on downstream AD. Therefore, LA was recovered using an ion exchange resin (BA765), not previously utilised for LA, while the solid and liquid extraction residues were utilised within biochemical methane potential (BMP) tests to elucidate the impacts of LA recovery on downstream AD. Ion exchange was selected as the recovery method for this study for two primary reasons. Firstly, ion exchange is a robust technology and has a history of being used within the biotechnological industry (da Silva & Miranda, 2013). Secondly, utilising ion exchange for LA recovery separates the LA containing broth from harsh chemicals required for LA recovery, allowing the solid and liquid extraction residues to be utilised within AD without prior treatment.

The results of Chapter 6 outlined the BA765 resin had a high capacity for LA, yielding a maximum capacity of 0.211 g_{LA}·g_{resin}^{-1}, which was competitive with similar resins. Moreover, optimal adsorption occurred at conditions which favoured upstream fermentation at uncontrolled pH (pH ~3.8; Chapter 3), which would allow the natural

depression of the pH, due to the production of LA, within the optimal range for adsorption (pH 2-4). While such a low pH would inhibit the continued production of LA (see Chapter 3), they favour *in-situ* extraction. *In-situ* recovery processes have been shown to improve LA fermentation and reduce the need for pH correction via alkaline addition (Ataei & Vasheghani-Farahani, 2008; Boonmee et al., 2016; Zhang et al., 2018). Within an LA-AD biorefinery, *in-situ* recovery could not only reduce operational costs associated with pH correction, but may benefit downstream AD by reducing added alkalinity, and therefore, salinity of the extraction residues. However, it should be noted implementation of *in-situ* fermentation with ion exchange would require pre-treatment of the FW to solubilise the solids, as inclusion of the solids during fermentation would vastly complicate the recovery of the resin for recovery of LA.

Following LA recovery, the solid and liquid extraction residues may be utilised within AD for methane production. The results of Chapter 6 indicated the extraction residues retained the majority of their methane potential with only a 21% reduction in methane yield being estimated following LA recovery. At an LA-AD biorefinery, a portion of the substrate within FW will be utilised for LA production, and logically the recovery of LA would reduce the organic loading to the downstream digesters. However, the results of Chapter 6 show, while there is a reduction in methane yield, the production of LA outweighed the loss in methane in terms of relative value, suggesting the overall LA-AD biorefinery concept improves the economic feasibility of FW biorefining.

8.2.5 Chapter 7 – Technoeconomic feasibility of integrating LA production into AD

In Chapters 3-6 LA production from FWs was explored, optimised, and enhanced. The results reported in these chapters are encouraging, suggesting the LA-AD biorefinery is technically feasible. However, it is also necessary to assess the economic feasibility of the suggested biorefinery to understand the economic feasibility of integrating LA production and recovery into AD. Therefore, Chapter 7 explored the technoeconomic feasibility of integrating LA production into AD. For this, the technoeconomic analysis conducted by Demichelis et al. (2018) was utilised to estimated capital and operational costs for LA extraction and purification systems for LA as well as estimate costs for AD equipment (for Greenfield scenarios). Furthermore, to assess the feasibility of further processing LA to PLA, the technoeconomic analysis conducted by Kwan et al. (2018) was utilised to estimate the cost of PLA synthesis.

The results of Chapter 7 suggested the LA-AD biorefinery was highly profitable for both Brownfield (integration of LA production into existing AD facilities) and Greenfield scenarios, yielding high NPVs and low simple payback periods. Furthermore, while sole AD required a minimum gate-fee of 61.60 AUD·tonne$_{FW}^{-1}$ to break even, Greenfield LA-AD biorefineries did not require gate-fees for feasibility, instead remining profitable from LA sales. Sole FW AD is known to be reliant on government subsidies or gate-fees to remain profitable (Bastidas-Oyanedel & Schmidt, 2018; Cucchiella & D'Adamo, 2016; Edwards et al., 2015; Kim et al., 2016), however, the results of Chapter 7 suggest integration of LA production eliminates the need for government support, instead being supported by commercial LA sales. This is promising for future LA-AD biorefineries, indicating development of these facilities is not restricted by government policies allowing the development in areas where sole FW AD is not profitable.

Although Greenfield LA-AD biorefineries yielded the highest NPVs, Brownfield scenarios were more profitable, having a higher cost benefit ratio (CBA), return on investment (ROI), internal rate of return (IRR), and a lower payback period due to the capital cost savings resulting from existing AD infrastructure. Compared to mainline recovery, side-stream fermentation performed worse in all economic indicators assessed due to the lower production rate of LA and higher capital costs due to the requirement for a second fermentation unit. However, such a system would provide an opportunity to further optimise fermentation, potentially improving the LA yield and productivity beyond mainline recovery (Chapter 7). Furthermore, future biorefineries may evolve the LA-AD biorefinery concept, implementing multiple parallel processes to utilise different waste streams within various processes. Such a process could utilise different waste streams for the production of different biomass derived chemicals, fuels, and/or materials.

Integrating sucrose supplementation and digestate recirculation further improved the economic feasibility of the LA-AD biorefinery, increasing the LA production rate, improving scenario NPVs, and lowering payback periods. As mentioned, digestate is generally burden to many AD facilities but can be a valuable input for LA fermentation, boosting the production of LA (Chapter 5), while providing an opportunity to reduce freshwater consumption.

PLA production scenarios were generally infeasible primarily due to substantial increase in capital and operational costs required for PLA synthesis. Furthermore, while PLA is a

higher value product compared to LA, for PLA to compete with fossil-base plastics the price must reduce significantly from that utilised in Chapter 7 (2.97 AUD.kg^{-1}). While integrating PLA production into AD was identified as infeasible, AD can play an important role during PLA synthesis, providing a method of disposal for fermentation residues and delivering renewable energy to meet the demands of processing equipment.

8.3 FUTURE OF THE FOOD WASTE BIOREFINERY

Continual growth in the generation of FW has received global attention in recent years, prompting the development of technologies aimed at providing alternative end of life outcomes for this waste stream. Biorefineries will play a major role in the recycling of FWs to high value bio-products and reducing the environmental impact associated with its production and disposal. AD is one of the simplest and oldest biorefinery technologies utilised for waste treatment, environmental protection, conversion of low-value material to high-value material, renewable power generation, generation of heat, and production of advanced gaseous biofuels (Fagerström et al., 2018). Clearly the AD technology has an important role to play in the circular economy.

However, while simple biorefinery approaches, like AD, provide an environmental benefit for FW recycling, especially when compared to landfilling, they are economically restricted by the low value of the products they produce. The results of this thesis show integration of additional biorefinery technologies can yield higher value biorefineries, generating multiple high value products from FW processing. Furthermore, literature has shown LA isn't the only products which can be co-produced with biogas and digestate from two-stage FW AD, with production of alternate high-value fuels, chemicals, and materials also being integrated into AD (Bastidas-Oyanedel & Schmidt, 2018; Moraes et al., 2014; Sawatdeenarunat et al., 2016). Future biorefineries could further evolve the LA-AD biorefinery concept, integrating additional biorefinery technologies to receive additional waste streams and/or increase the value recovered from FWs. The future of the FW biorefinery is promising and will likely develop rapidly over the coming decades. Biorefineries are integral in the circular bio-economy, and continued development could yield a high value FW biorefinery capable of rivelling modern oil refineries for their production of a wide variety of high value products.

Overall, this thesis aimed to advance the FW biorefinery concept through the integration of LA production with AD. Notably, through addressing the research questions stated in

Chapter 1, the investigations conducted in this thesis identified key research points which should be considered by future FW biorefinery concepts. Firstly, the commercial FW context is highly variable with substrate subject to variability based on availability. Secondly, process optimisation and control are essential for improving fermentation efficiency, stability, and productivity, and therefore, economics of fermentation. Thirdly, LA recovery from highly complex FW matrix is technically feasible, but careful consideration is required for downstream processing to ensure no toxic or inhibitory compounds are introduced into the extraction residues which could harm AD performance or restrict the use of digestate within agriculture. Fourth, the use of relatively low-value materials (i.e. sucrose, digestate, and FW) can effectively promote the formation of relatively high-value LA and are required to reduce the cost of the final LA product. Finally, the technoeconomic feasibility of the LA-AD biorefinery is dependent on the interaction between all previously mentioned research points, effecting the LA yield, concentration, productivity, and cost and complexity of downstream processing, and simultaneously influencing the performance of downstream AD. Future FW biorefinery concepts should carefully consider the interplay between upstream and downstream processes to ensure efficient and economical use of the FW substrate.

8.4 REFERENCES

Ataei, S.A., Vasheghani-Farahani, E. 2008. In situ separation of lactic acid from fermentation broth using ion exchange resins. Journal of Industrial Microbiology & Biotechnology, 35(11), 1229.

Bastidas-Oyanedel, J.-R., Schmidt, J. 2018. Increasing Profits in Food Waste Biorefinery—A Techno-Economic Analysis. Energies, 11(6), 1551.

Boonmee, M., Cotano, O., Amnuaypanich, S., Grisadanurak, N. 2016. Improved Lactic Acid Production by In Situ Removal of Lactic Acid During Fermentation and a Proposed Scheme for Its Recovery. Arabian Journal for Science and Engineering, 41(6), 2067-2075.

Cucchiella, F., D'Adamo, I. 2016. Technical and economic analysis of biomethane: A focus on the role of subsidies. Energy Conversion and Management, 119, 338-351.

da Silva, A.H., Miranda, E.A. 2013. Adsorption/Desorption of Organic Acids onto Different Adsorbents for Their Recovery from Fermentation Broths. Journal of Chemical & Engineering Data, 58(6), 1454-1463.

Demichelis, F., Fiore, S., Pleissner, D., Venus, J. 2018. Technical and economic assessment of food waste valorization through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J. 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresource Technology, 241, 508-516.

Din, N.A.S., Lim, S.J., Maskat, M.Y., Mutalib, S.A., Zaini, N.A.M. 2021. Lactic acid separation and recovery from fermentation broth by ion-exchange resin: A review. Bioresources and Bioprocessing, 8(1), 1-23.

Edwards, J., Othman, M., Burn, S. 2015. A review of policy drivers and barriers for the use of anaerobic digestion in Europe, the United States and Australia. Renewable and Sustainable Energy Reviews, 52, 815-828.

Fagerström, A., Seadi, T.A., Trasi, S., Briseid, T. 2018. The role of Anaerobic Digestion and Biogas in the Circular Economy.

Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H. 2016. More value from food waste: Lactic acid and biogas recovery. Water Research, 96, 208-216.

Kwan, T.H., Hu, Y., Lin, C.S.K. 2018. Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly(lactic acid) production. Journal of Cleaner Production, 181, 72-87.

Moraes, B.S., Junqueira, T.L., Pavanello, L.G., Cavalett, O., Mantelatto, P.E., Bonomi, A., Zaiat, M. 2014. Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: Profit or expense? Applied Energy, 113, 825-835.

Sawatdeenarunat, C., Nguyen, D., Surendra, K.C., Shrestha, S., Rajendran, K., Oechsner, H., Xie, L., Khanal, S.K. 2016. Anaerobic biorefinery: Current status, challenges, and opportunities. Bioresource Technology, 215, 304-313.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y. 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management, 52, 278-285.

Turnley, D., Hopwood, L., Burns, C., Maio, D.D. 2016. Assessment of digestate drying as an eligible heat use in the Renewable Heat Incentive.

Zhang, Y., Alam, M.A., Kong, X., Wang, Z., Li, L., Sun, Y., Yuan, Z. 2017. Effect of salinity on the microbial community and performance on anaerobic digestion of marine macroalgae: Effect of salinity on anaerobic digestion of marine macroalgae. Journal of chemical technology and biotechnology (1986), 92(9), 2392-2399.

Zhang, Y., Qian, Z., Liu, P., Liu, L., Zheng, Z., Ouyang, J. 2018. Efficient in situ separation and production of l-lactic acid by Bacillus coagulans using weak basic anion-exchange resin. Bioprocess and Biosystems Engineering, 41(2), 205-212.

CHAPTER 9 CONCLUDING REMARKS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Foreword

Each chapter presented outcomes which were integral in determining the feasibility of integrating LA production into existing FW AD facilities and developing the LA-AD biorefinery concept. The following chapter concludes the thesis, presenting the overall conclusions obtained from the project and outlines recommendations for future research.

9.1 CONCLUSIONS

The inherently low value of traditional products from FW AD (i.e. biogas and digestate), and high costs of construction, operation, and management, mean that commercial AD projects often exhibit marginal economic feasibility without government subsidy or policy incentives. Integration of biorefinery technologies for LA production into AD could increase the revenue generated from FW processing and reduce the reliance on such government support. Therefore, the primary aim of this research project was to explore the feasibility of integrating, optimisation, and fundamental understanding of LA production into existing two-stage FW AD facilities by answering the research questions listed in Chapter 1 and repeated in below.

1. *How does the operation of a commercial two-stage anaerobic digestion facility impact lactic acid fermentation?*
2. *What is the lactic acid production capacity of the commercially produced anaerobic fermentation inoculum? Can lactic acid production be enhanced with carbohydrate and nitrogen addition?*
3. *Can lactic acid be recovered from real fermentation broth and what are the effects on downstream anaerobic digestion performance?*
4. *What is the technoeconomic feasibility of integrating lactic acid fermentation and production into existing two-stage anaerobic digestion?*

Accordingly, the primary conclusions from each chapter are presented below.

From the results of the monitoring study in Chapter 3, it was concluded that:

- The typical commercial FW context is highly variable and complex
- Substrate availability is dependent on market forces
- Standard operational procedures of the commercial two-stage AD facility naturally promoted LA formation at elevated concentrations
- Waste homogenisation provided stability to LA fermentation

From the study of pH and temperature for optimisation of LA fermentation in Chapter 4, it was concluded that:

- pH and temperature control were effective process levers to improve LA yield and target a narrower product spectrum with higher LA purity and provide operator level control of fermentation outcomes

- Optimisation doubled the yield of LA compared to uncontrolled conditions
- The competitive conversion of LA into butyrate was important, and was eliminated by pH and temperature control
- LA bacteria remained dominant at optimised conditions which correlated with optimal LA production

From the study of sucrose supplementation and digestate recirculation to increase LA fermentation in Chapter 5, the following conclusions were drawn:

- Nitrogen supplementation promoted LA formation and increased the LA yield
- Digestate further increased the yield of LA, but increased microbial diversity and production of competitor organic acids
- Sucrose increased the LA concentration, but only up to a certain level of added sucrose, with LA yield on sucrose progressively declining with progressively higher sucrose doses
- Sucrose addition controlled the impact that digestate had on fermentation by selectively promoting the growth of LA producers
- Additional operational costs introduced by sucrose were overcome by the extra LA produced
- Digestate recirculation can reduce operational costs by reducing freshwater consumption

In Chapter 6, the feasibility of LA recovery from complex media and the impact of recovery on downstream AD was assessed. It was concluded that:

- LA recovery from highly complex fermentation media is technically feasible
- Impurities within the fermentation broth lowered recovery efficiency, but LA was still selectively extracted
- The solid and liquid extraction residues can be effectively utilised for methane production
- LA recovery reduced the overall methane yield on FW by 21%, but increased the value generated from FW biorefining

Finally, by assessing the technoeconomic feasibility of the LA-AD biorefinery in Chapter 7, it was identified that:

- LA production vastly improved the economics of FW AD

- Brownfield scenarios were the most economically feasible, due to significant savings from existing AD infrastructure capital
- Economics of Greenfield LA-AD biorefinery scenarios were sensitive to gate-fees, but did not require gate-fees for economic feasibility
- Implementing sucrose supplementation and digestate recirculation improved LA production by 84%
- PLA production scenarios were largely unfeasible due to additional capital and operational costs associated with PLA synthesis, and limited increase in product value from LA to PLA

To conclude, by answering the research questions stated in Chapter 1, this thesis aimed to improve the economic feasibility of AD, reduce reliance on government support, and further the development of the FW LA-AD biorefinery. Overall, the results of this thesis identified LA production within typical two-stage AD designs was technically and economically feasible, with realistic control measures and simple operational changes to AD significantly improving the production of LA and economic performance of the LA-AD biorefinery. Moreover, LA production effectively reduced the reliance on government support for AD feasibility, yielding a high-value biorefinery concept. With the ever-growing production of FW with an increasing global population, the LA-AD biorefinery can be an effective method to manage FW while recovering value and displacing fossil-fuel-derived chemicals and materials.

9.2 RECOMMENDATIONS

While the results from this research project indicated that the LA-AD biorefinery is technically feasible and potentially highly profitable, additional work is required to further develop and improve the concept. Therefore, it is recommended that future work investigate the following:

9.2.1 Upstream waste homogenisation

In Chapter 3, it was outlined that the commercial FW context was highly complex, and the waste homogenisation tank (Blending tank) provided some stability to the LA fermentation of the complex FW media. However, the mechanism for this is unclear. It is recommended that future laboratory-based work consider the following:

- Assess the influence of waste homogenisation on LA fermentation

- Determine the impact of changing waste composition on fermentation performance and product spectrum, and the role homogenisation plays in reducing its impact
- Outline methods to mitigate the influence of changing waste composition on LA production, especially seasonal variations
- Assess the feasibility of using waste pre-treatment (e.g. thermal or ultrasonication) for homogenisation and sterilisation and subsequent LA production

9.2.2 Engineered inoculum

While the naturally developed and adapted inoculum aided LA fermentation in Chapters 4 and 5, performance was similar to literature studies using broad microbial seed communities. Moreover, LA yield on sucrose remained low. This should be addressed by future work considering the following:

- Utilise operational and environmental conditions to selectively develop an engineered inoculum for LA production from various diverse seed sources, and assess convergence and adapted performance
- Combine the inoculum with bacteria known to utilise carbohydrates within FW and sucrose and assess performance
- Assess performance of the inoculum with different FW feedstocks and develop optimum FW mixtures

9.2.3 Continuous LA-AD biorefinery

In Chapter 6, the solid and liquid extraction residues were identified as suitable substrates for methane production. However, as microbial communities adapt, and or acclimate to the variable composition of the FW, it is unknown how long-term operation of an LA-AD biorefinery may influence the AD microbial community and resulting process stability. Therefore, future laboratory work should assess:

- Continuous production of LA from a complex, and variable FW substrate
- Continuous operation of AD utilising extraction residues from LA recovery
- Identify accumulation of any toxic or inhibitory components introduced from LA fermentation or LA recovery

- Assess the development of the microbial community within AD

9.2.4 Technoeconomic feasibility

Finally, while the technoeconomic assessment conducted in Chapter 7 outlined the LA-AD biorefinery is economically feasible, future studies should:

- Conduct an in-depth analysis to reduce economic uncertainty
- Explore different LA recovery options
- Explore the overall benefits of utilising AD to supply power and heat for operational equipment
- Explore PLA production and identify the minimum requirements needed for economic feasibility

SUPPLEMENTARY MATERIAL

Appendix A: MONITORING AN INDUSTRIAL FACILITY

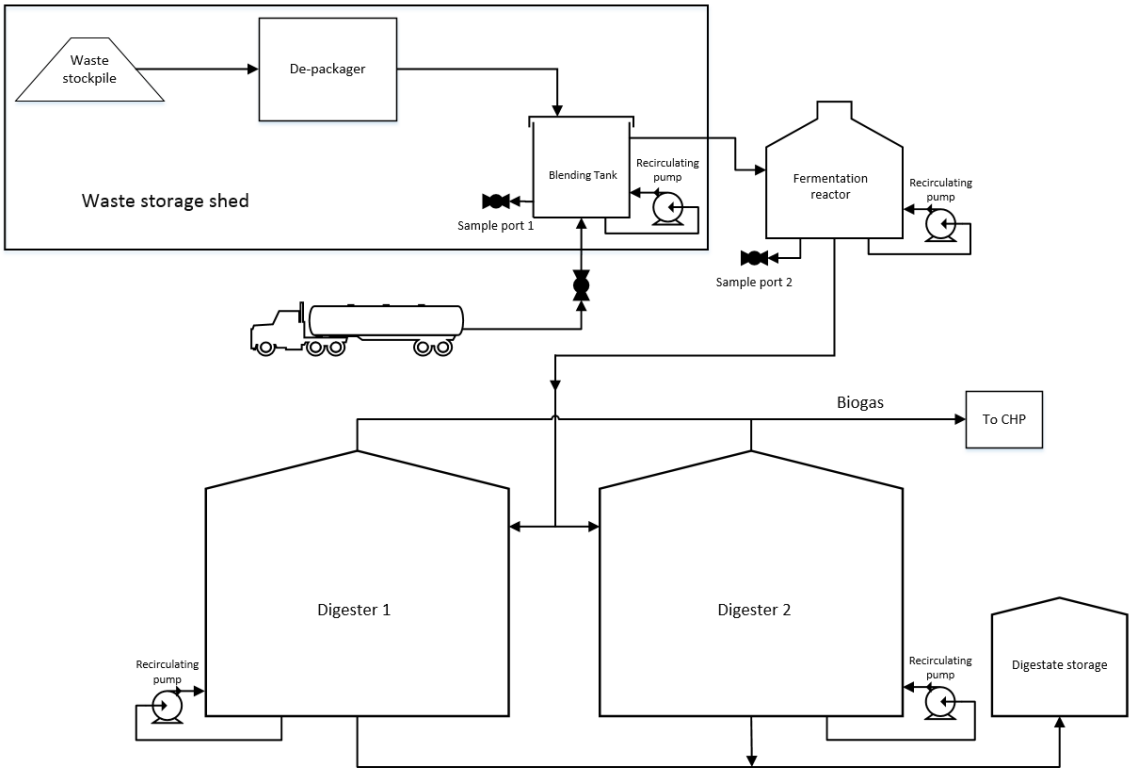


Figure A1: Simplified plant-wide layout of the full-scale AD plant understudy

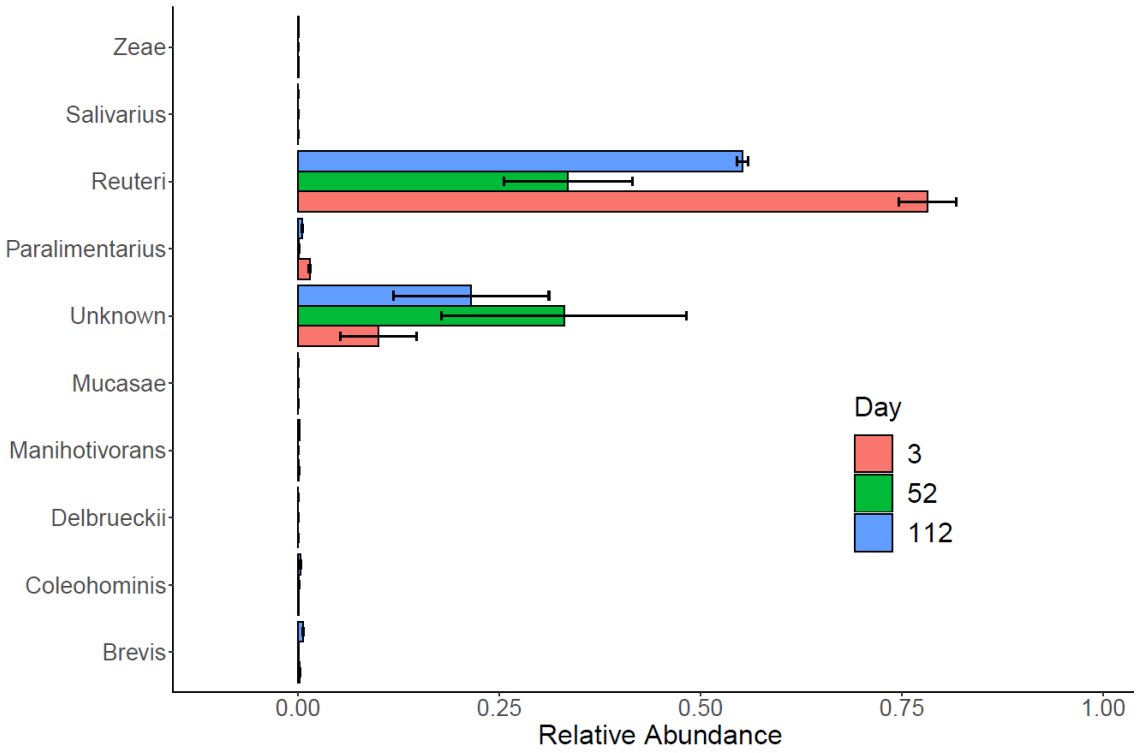


Figure A2: Relative abundance of *Lactobacillus* species. Microbial community analysis at the genus level identified *Lactobacillus* was the dominant genus with the relative abundance of other bacteria being < 0.22%. Values are presented as the mean of triplicates ± the standard error.

Table A1: Average weekly waste composition of the AD facility. Milk and milk paste were combined under Milk waste, and WFS1 and liquid waste were combined under FW.

Waste	Percent of total waste
Bleaching Earth	4.1%
Brewery liquid wastes	7.5%
FW	51.1%
Grain processing waste	2.0%
Milk waste	6.9%
Restaurant scraps	4.5%
Spent grain	0.1%
Soft drink	17.1%
Unknown	6.7
Total	100.0%

Table A2: Detailed composition of some of the waste materials received by the AD facility understudy

Sample ID	TS%	VS%	Lactic acid ($g.L^{-1}$)	Succinic acid ($g.L^{-1}$)	Acetic acid ($g.L^{-1}$)	Propionic acid ($g.L^{-1}$)	Butyric acid ($g.L^{-1}$)
Bulk soft drink waste	5.39	5.26	1.27	-	1.17	-	0.00
Food waste (Type 1)	20.31	17.81	1.91	0.66	1.41	0.82	0.75
Food waste (Type 2)	32.88	29.21	7.74	0.60	1.20	0.99	0.80
Food waste (Type 3)	22.90	20.73	10.79	0.82	1.86	-	0.73
Food waste (Type 4)	22.64	16.07	5.74	1.15	2.05	-	0.90
Sugary liquid mix	45.77	36.07	1.90	0.87	0.91	0.66	-
Brewery Liquid waste	4.46	1.48	1.44	2.08	2.01	2.99	-
Reject beer waste	5.75	4.92	4.05	1.78	1.20	1.16	-
Liquid waste	3.20	1.88	12.06	1.09	2.75	0.74	2.18
Brewery liquid waste	3.95	2.69	0.79	1.68	1.06	0.73	-
Milk waste	15.03	13.88	7.22	0.69	1.97	0.83	0.87
Bottled reject milk	9.30	7.85	0.60	1.62			-
Compostable waste	13.47	12.24	2.28	1.02	1.19	1.36	-

Appendix B: FERMENTATION OPTIMISATION

Methods

Taxonomy analysis

Sequence data was processed using Mothur version 1.46.1 using a slightly modified standard operating procedure (Schloss et al., 2009). Sequences were removed if they did not meet the following quality control: barcode miss match = 1, primer mismatch = 2, ambiguous base calls = 0, minimum quality score Q > 6 or > 25 depending on if forward and reverse reads contained any missing bases, maximum homopolymers length = 8, and maximum length (311) of base pairs per amplicon. Following quality control, retained sequences were pre-clustered to remove any PCR-based bias and singletons. Chimeric sequences were identified and removed using chimera.vsearch() and remove.seqs() in Mothur. Sequences were aligned with the Silva database (Release 132) and assigned to operational taxonomic units (OTU- based taxonomic analysis) based on 97 % similarity.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis (PICRUSt)

Sequence data for PICRUSt analysis were processed using Mothur version 1.46.1 as described above. Processed sequences were separately aligned with the Greengenes database (gg_13_5) and assigned to operational taxonomic units (OTU- based taxonomic analysis) based on 97 % similarity.

To determine the dominance of various functional genes, a PICRUSt analysis was performed using the Huttenhower Lab Galaxy server (Harvard, Massachusetts) with the Greengenes OTU identification codes (Langille et al., 2013). To assess the accuracy of the PICRUSt predictions, the weighted Nearest Sequenced Taxon Index (NSTI) was calculated for each sample. The weighted NSTI describes the average branch length that separates each OTU in the sample from the referenced genome weighted by the relative abundance of the genome in the sample. NSTI values for this study ranged from 0.05 to 0.14 with an average of 0.10 ± 0.024 s.d.. Lower NSTI values are associated with higher similarity between the reference genome database and the sample genome. The average weighted NSTI for this study is similar to those for environmental communities and lower

than the 0.15 threshold used to indicate similarity with the reference genome database (Langille et al., 2013; Louvado et al., 2020). All genes were identified via the KEGG database (KEGG, 2022).

Data analysis and statistical methods

Canonical Correspondence Analysis (CCA) was used to identify the impact of pH and temperature on the abundance of genes related to the production of LA, acetic acid, succinic acid, propionic acid, and butyric acid (See Supplemental material). The concentrations of the organic acids measured at day 5 (when the DNA sequencing was conducted) were used as the response variable in the analysis. Modelling was conducted using the Vegan Package in R (R Development Core Team, 2022). Estimation of the maximum LA production rate was determined base on a linear regression of the LA production curve during the linear zone, as previously described (Buhlmann et al., 2018).

References

Buhlmann, C.H., Mickan, B.S., Jenkins, S.N., Tait, S., Kahandawala, T.K.A., Bahri, P.A. 2018. Ammonia stress on a resilient mesophilic anaerobic inoculum: Methane production, microbial community, and putative metabolic pathways. Bioresource Technology, 275, 70-77.

KEGG. 2022. Kyoto Encyclopedia of Genes and Genomes.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology, 31(9), 814-821.

Louvado, A., Coelho, F.J.R.C., Palma, M., Tavares, L.C., Ozorio, R.O.A., Magnoni, L., Viegas, I., Gomes, N.C.M. 2020. Effect of glycerol feed-supplementation on seabass metabolism and gut microbiota. Applied microbiology and biotechnology, 104(19), 8439-8453.

R Development Core Team. 2022. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F. 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology, 75(23), 7537-7541.

Figures

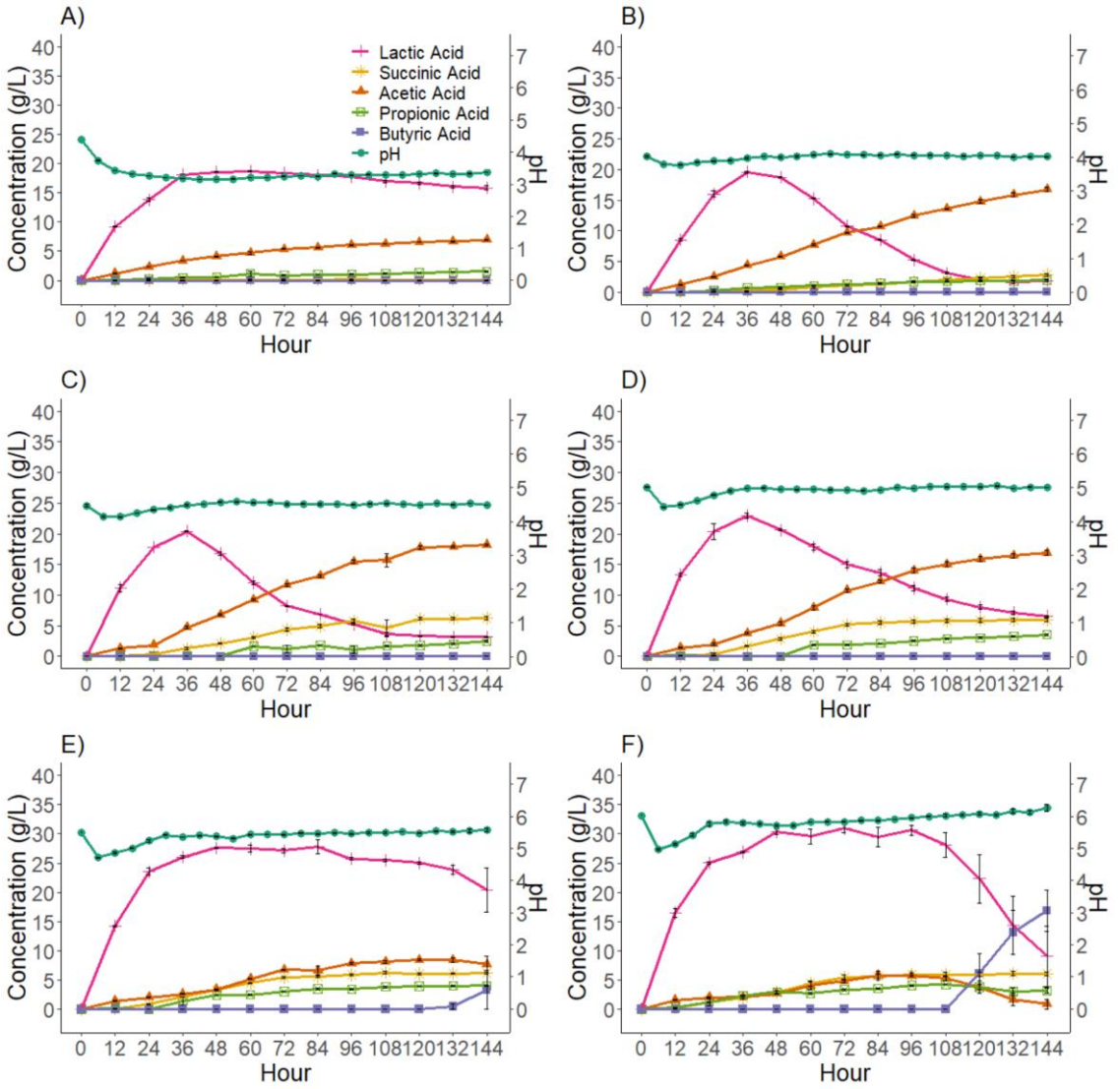


Figure. B1: Concentration of measured VFAs during the fermentation of FW at 35 ºC and pH A) uncontrolled, B) 4.0, C) 4.5, D) 5.0, E) 5.5, and F) 6.0.

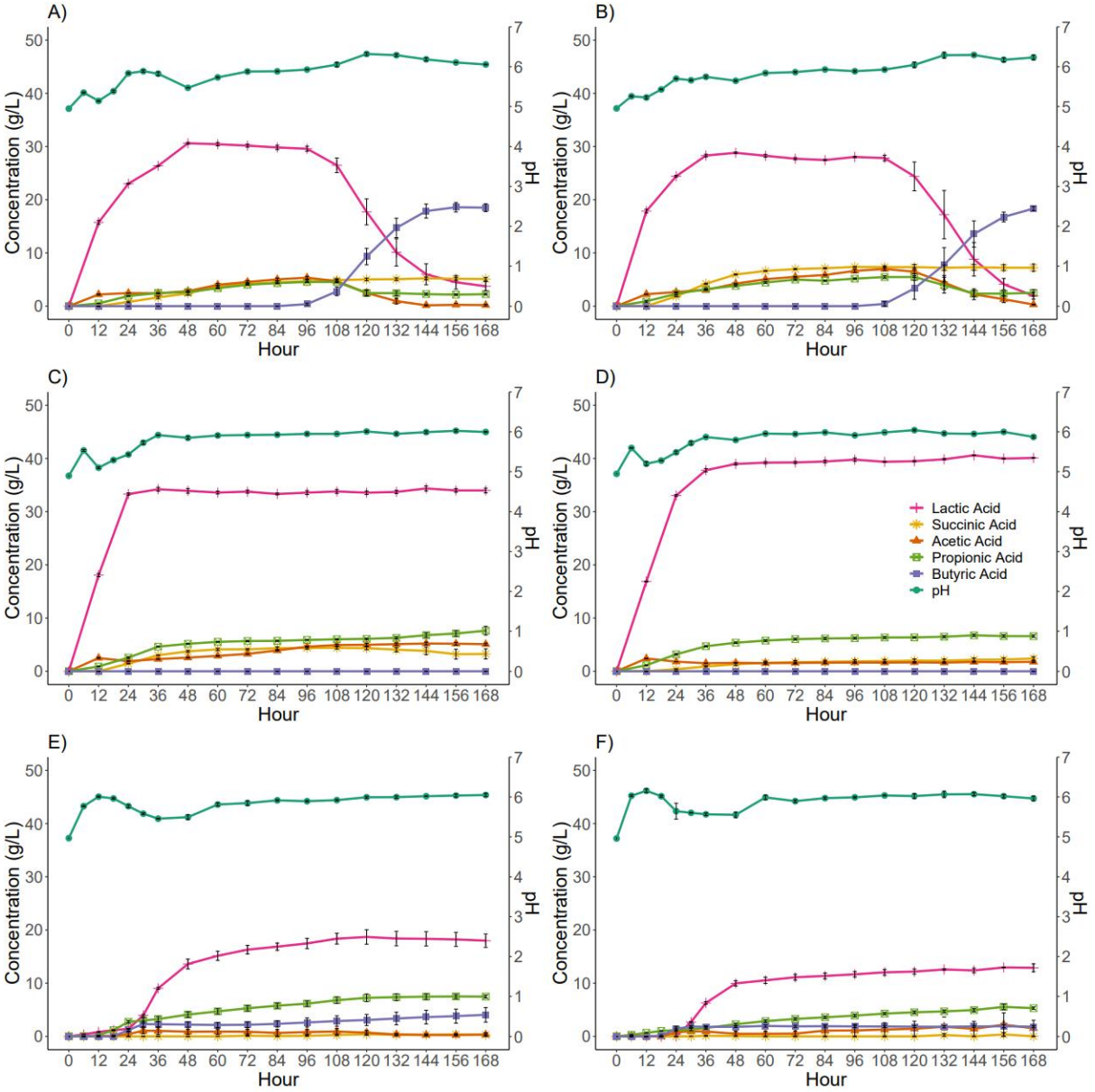


Figure. B2: Concentration of measured organic acids produced by fermentation of FW at pH 6.0 and at A) 35 ºC, B) 40 ºC, C) 45 ºC, D) 50 ºC, E) 55 ºC, and F) 60 ºC.

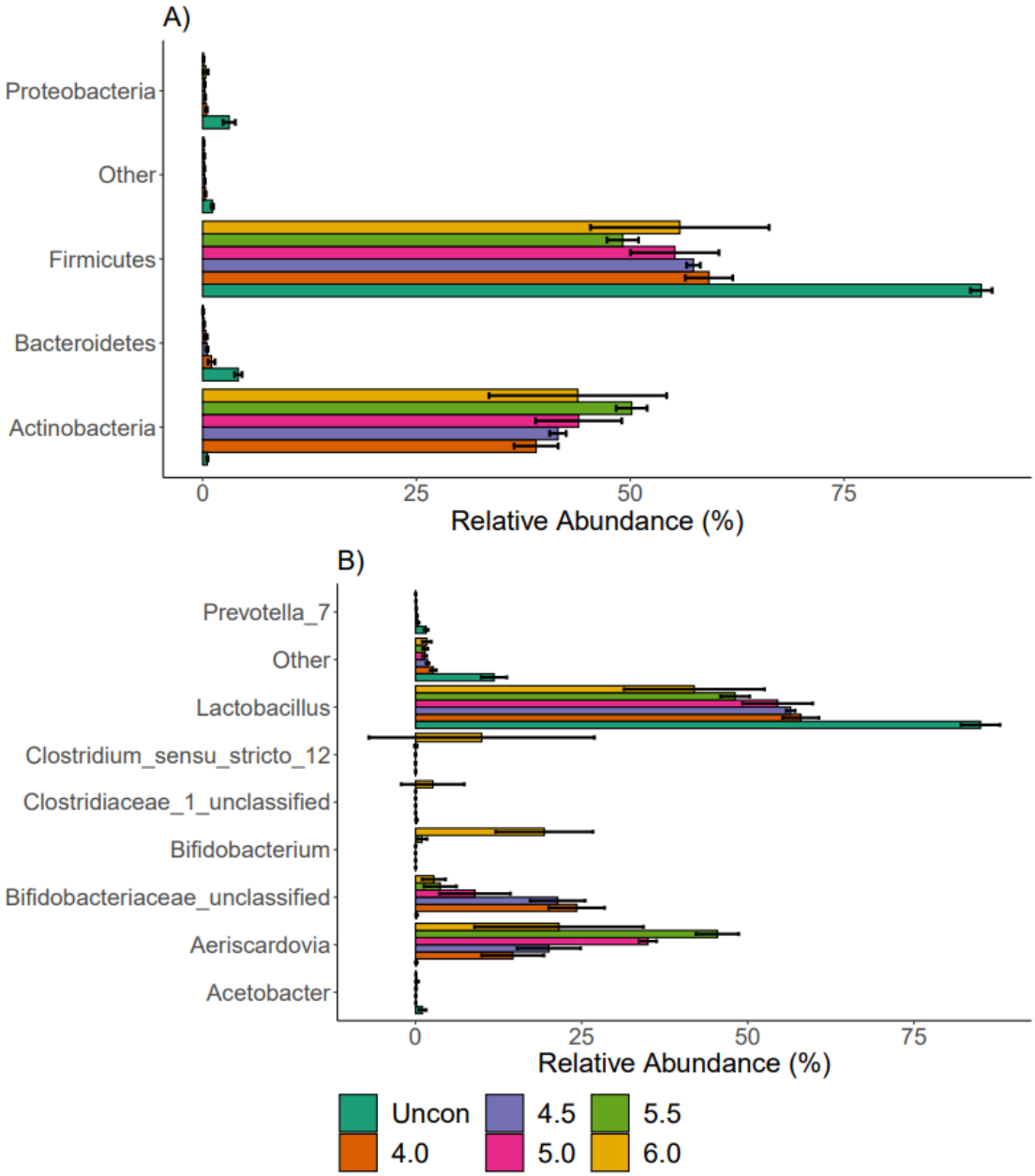


Figure. B3: Effect of pH on the microbial relative abundance (>1%) at A) Phyla, and B) Genus levels. Error bars represent the 95% confidence interval.

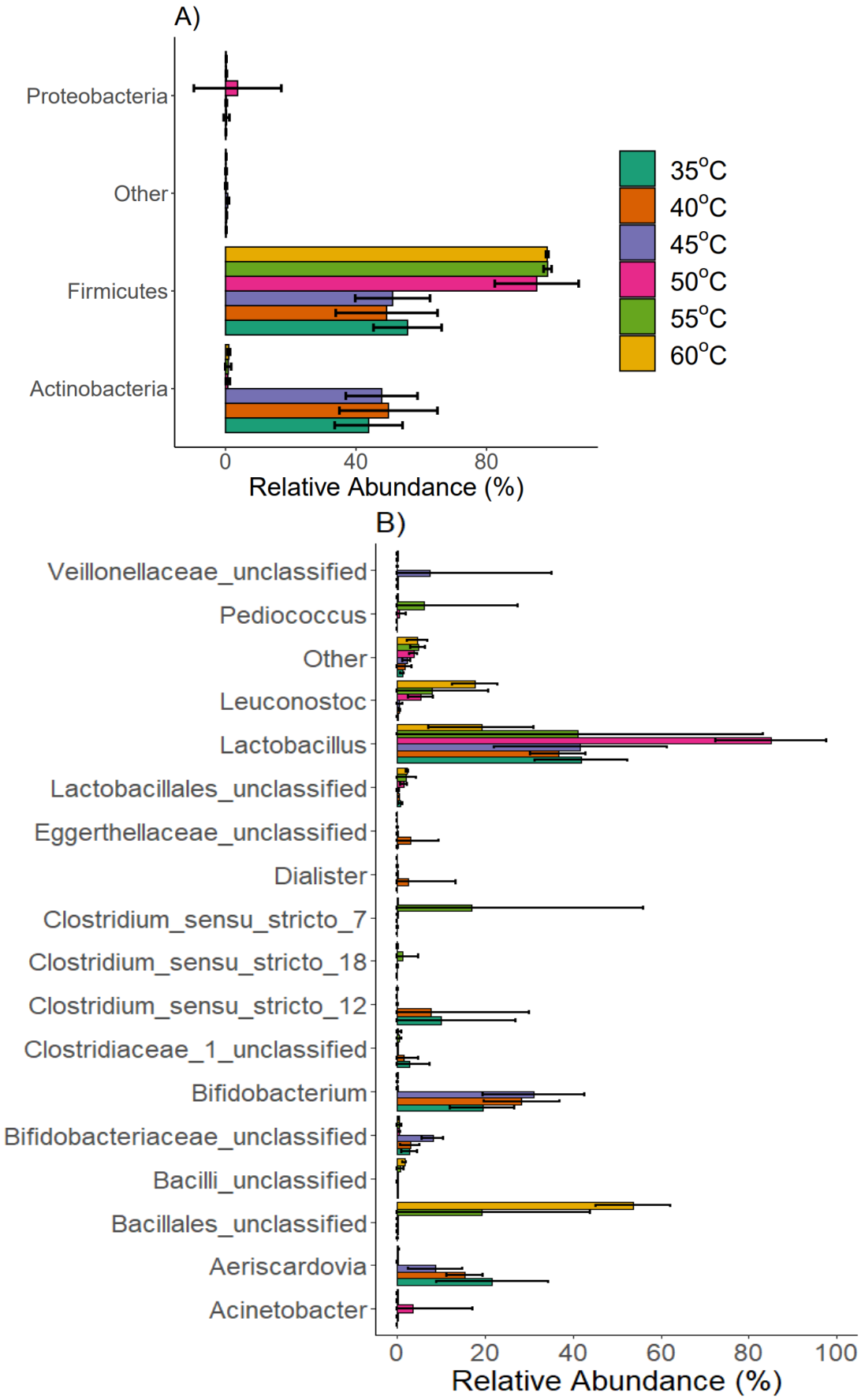


Figure. B4: Effect of Temperature on the microbial relative abundance (>1%) at A) Phyla, and B) Genus levels. Error bars represent the 95% confidence interval.

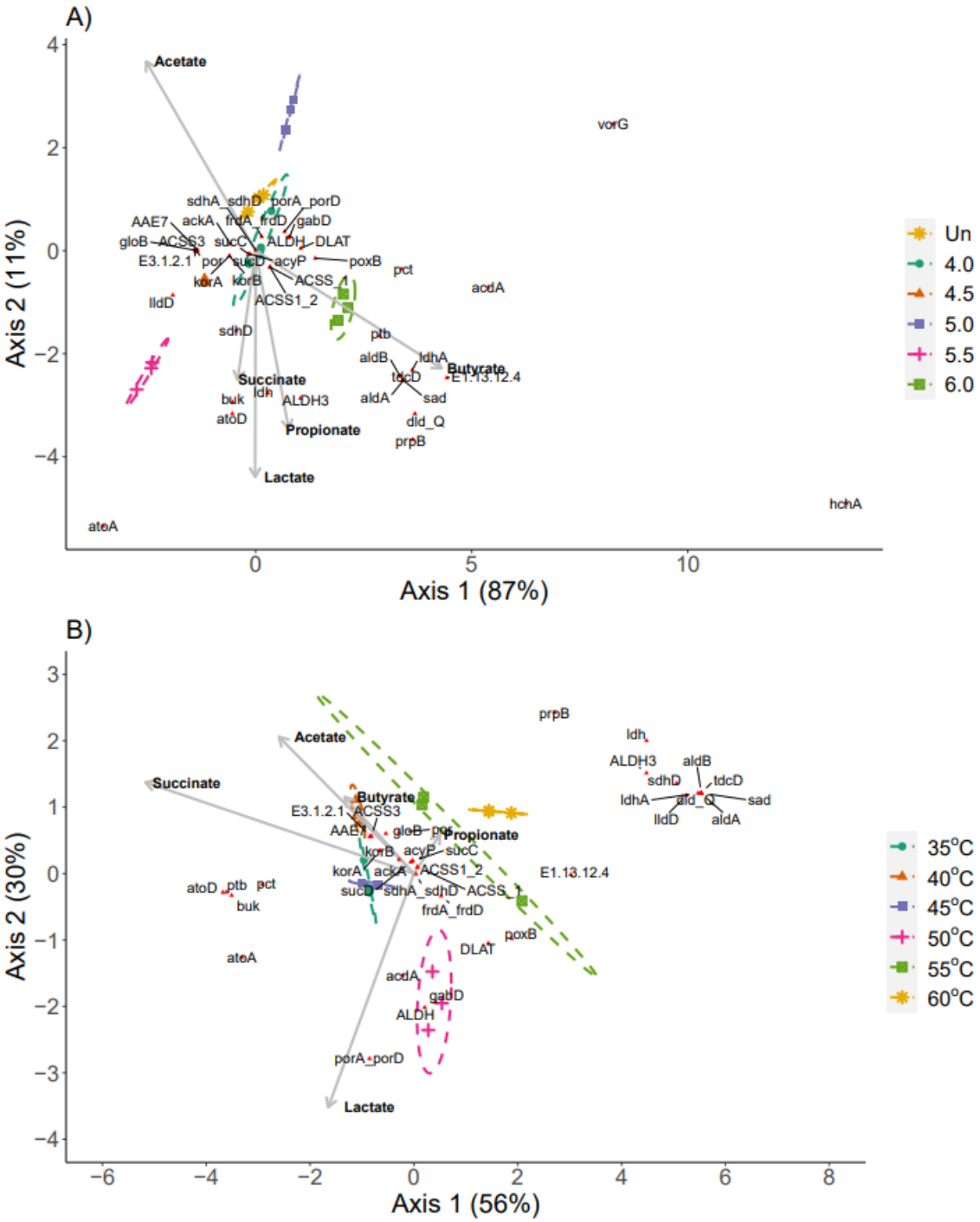


Figure. B5: Canonical correspondence analysis (CCA) to show the effect of A) pH, and B) temperature on the relative abundance of acid producing genes. Specific genes were identified from the pyruvate, galactose, fructose and mannose, propionate, and butanoate, metabolisms as well as the citrate cycle, and glycolysis pathways. Ellipses represent 95% occupancy spaces for different conditions.

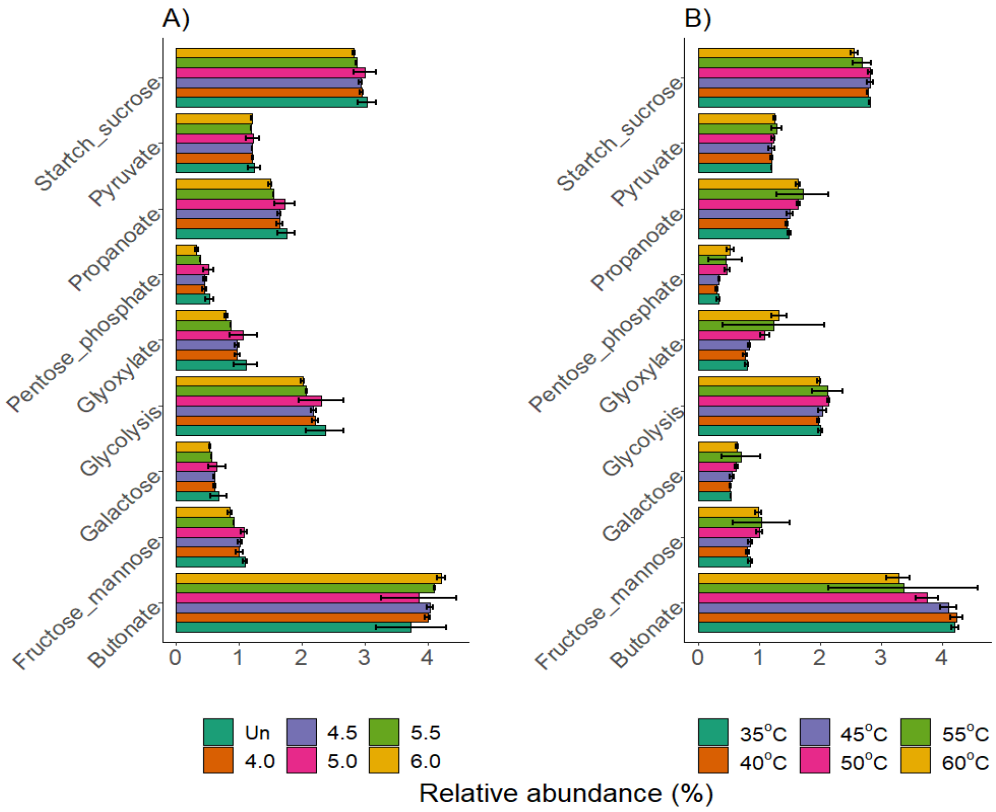


Figure. B6: Relative abundance of genes associated with different carbon utilisation pathways at different operational A) pH and B) temperature. Values presented as the mean of triplicates ± the 95 % confidence interval.

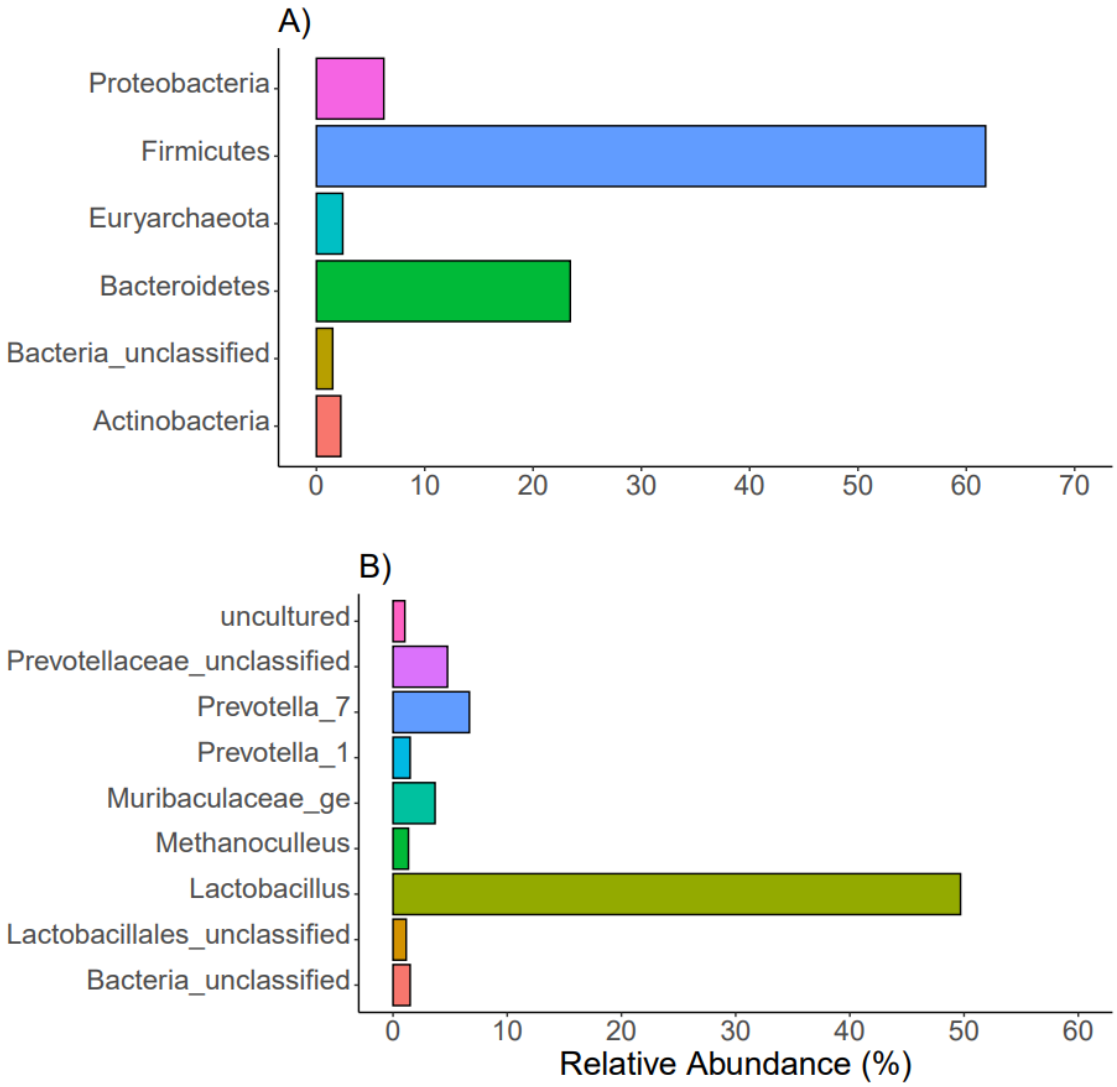


Figure. B7: Relative abundance of bacteria and archaea within the inoculum (>1%) at A) Phylum, and B) Genus level.

Tables

Table B1: Synthetic food waste composition (Capson-Tojo et al., 2017)

Ingredient	Proportion (% wet basis)
Apples	25.9
Lettuce	25.9
Potato	25.9
Pasta (Cooked)	1.2
Rice (Cooked)	1.2
Flour	1.2
Cereals	1.2
Bread	6.2
Chicken	4.1
Beef	4.1
Cheese	1.9
Biscuits	1.5

Table B2: Maximum LA production rate at varying pH and temperatures. Calculated by linear regression on the linear zone of the LA production curve.

pH	Temp	Linear zone (hour)	Adj. R^2	LA production rate ($g_{LA} \cdot kg_{VS}^{-1} \cdot h^{-1}$)
Uncontrolled	35	**0-36**	0.96	6.85 ± 0.99
4		**0-36**	0.97	7.73 ± 0.98
4.5		**0-24**	0.97	10.93 ± 1.74
5		**0-24**	0.95	11.85 ± 2.32
5.5		**0-24**	0.98	13.7 ± 1.62
6		**0-24**	0.96	14.57 ± 2.5
6	40	**0-24**	0.92	12.8 ± 3.08
	45	**0-24**	1.00	17.46 ± 0.84
	50	**6-30**	0.96	20.86 ± 2.52
	55	**24-36**	0.95	11.6 ± 2.17
	60	**24-36**	0.98	9.56 ± 1.05

Appendix C: ENHANCING LACTIC ACID FERMENTATION

Figures:

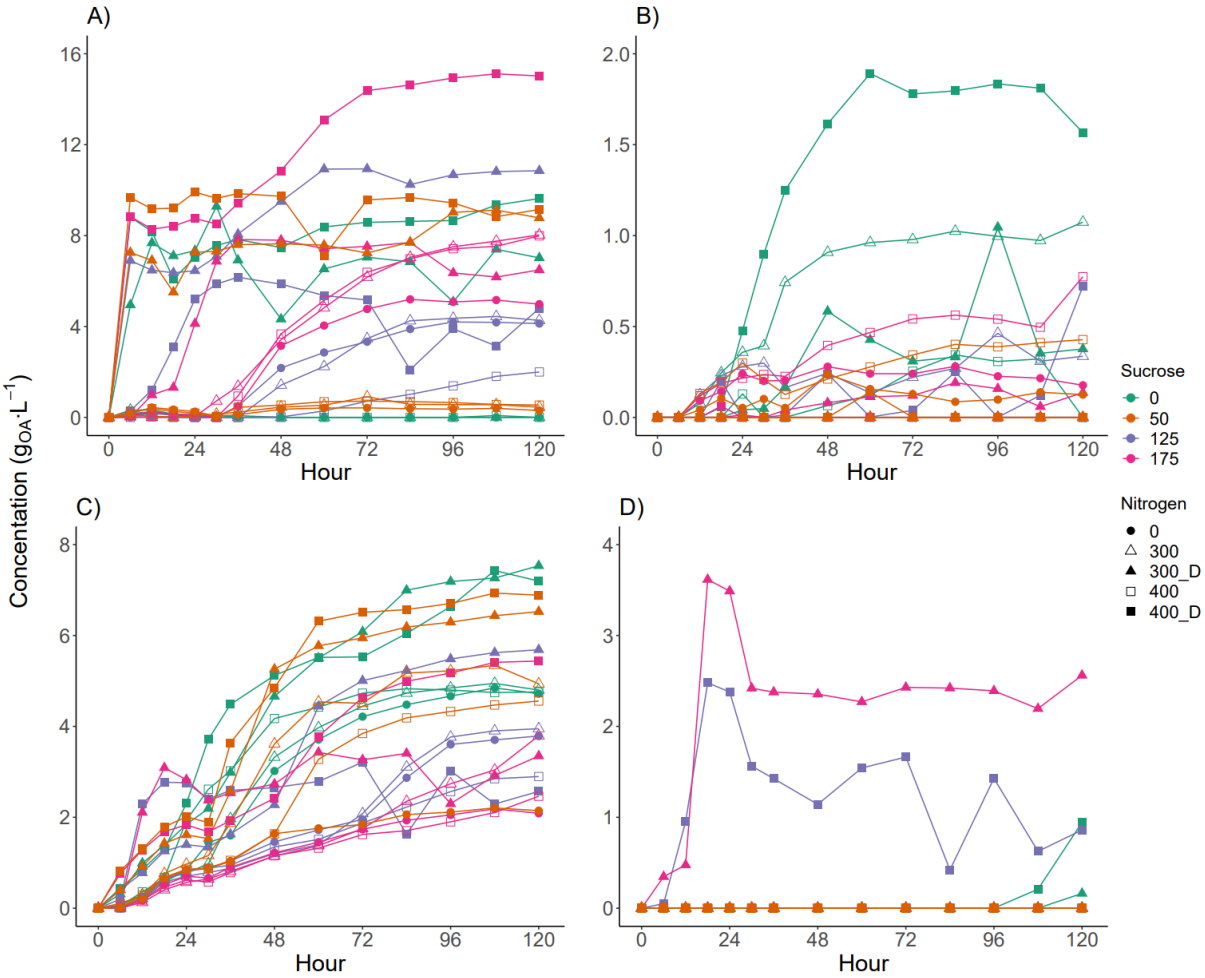


Figure C1: Production of A) Acetic acid, B) Succinic Acid, C) Propionic Acid, and D) Butyric Acid at varying sucrose and nitrogen dosages with and without digestate. Values are presented as the mean of triplicates. Error bars are removed to improve visualisation.

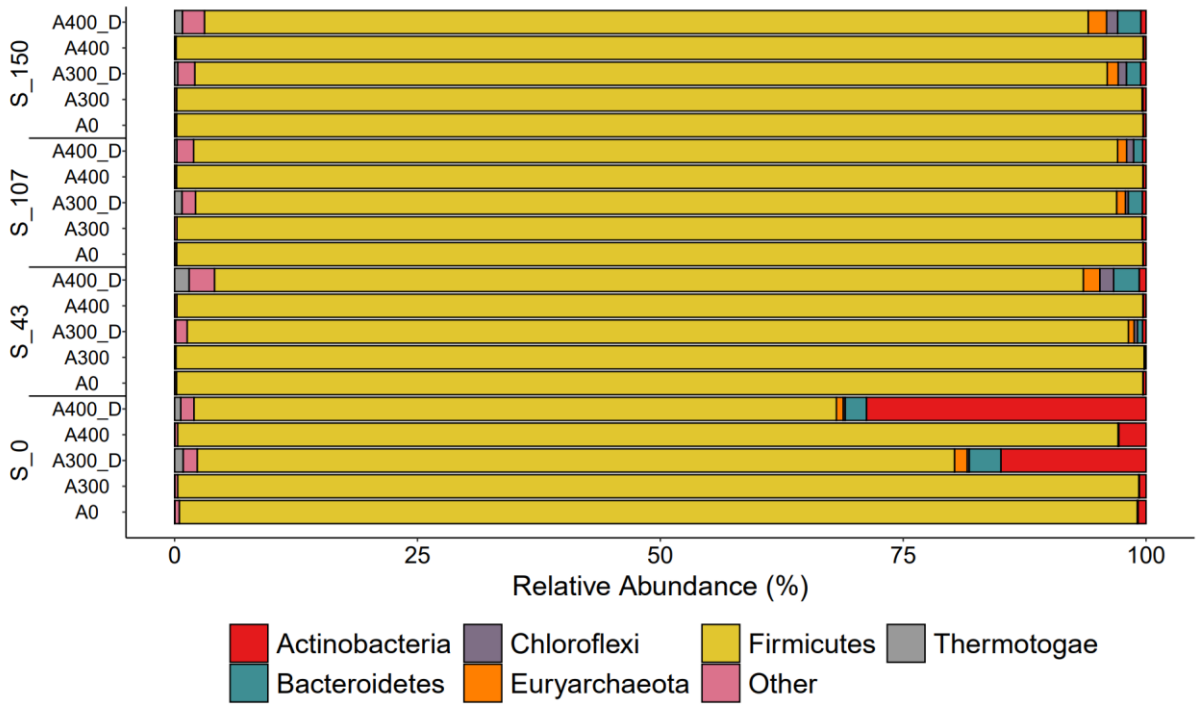


Figure C2: Relative abundance of microbial phyla (>1%) following 5-days of FW fermentation supplemented with sucrose and N (NH4Cl or digestate). Sucrose level is denoted by an "S" followed by the initial supplement concentration, while supplemented N is denoted by an "A" and N added as digestate is denoted by "D".

Tables

Table C1: Synthetic food waste composition (Capson-Tojo et al., 2017)

Ingredient	Proportion (% wet basis)
Apples	25.9
Lettuce	25.9
Potato	25.9
Pasta (Cooked)	1.2
Rice (Cooked)	1.2
Flour	1.2
Cereals	1.2
Bread	6.2
Chicken	4.1
Beef	4.1
Cheese	1.9
Biscuits	1.5

Table C2: Composition of the synthetic food waste, inoculum, and digestate utilised within this study. Analyses were conducted by ARL (Welshpool, Western Australia)

	Synthetic Food waste	Inoculum	Digestate	HCC [a]
Potassium - Total (mg/L)	870	840	900	871.79
Magnesium - Total (mg/L)	59	130	110	72.48
Calcium - Total (mg/L)	140	1,500	1,600	468.38
Sodium - Total (mg/L)	280	940	1,000	441.03
Sulphur - Total (mg/L)	150	240	120	153.33
Iron - Total (mg/L)	2.3	110	93	24.68
Manganese - Total (mg/L)	1.5	5.1	5	2.32
Copper - Total (mg/L)	0.38	1.3	1.4	0.61
Cobalt - Total (mg/L)	0.005	0.082	0.071	0.02
Zinc - Total (mg/L)	2.9	9.3	10	4.48
Nickel - Total (mg/L)	0.02	0.22	0.17	0.06
Total Nitrogen (mg/L)	3,000	3,000	3,400	3,058.12
Total Phosphorus (mg/L)	110	290	370	163.16
Chloride (mg/L)	1,100	1,800	1,900	1,276.07
Ammonia-N (mg/L)	130	570	2,500	511.97
NOx-N (mg/L)	<1	<1	14	2.03
Nitrate-N (mg/L)	<1	<1	14	2.03

a: Highest Calculated combined Concentration (HCC) during lactic acid fermentation (i.e. with 400 $mg_N \cdot L^{-1}$ as digestate)

Table C3: Factorial design for the FW fermentation experiments with sucrose and digestate supplementation

Block*	Sucrose	NH_4Cl	Digestate	Normalised Sucrose	Normalised N dosage
1	0	0	0	0.00	0.00
1	107	0	0	0.71	0.00
1	43	300	0	0.29	0.75
1	150	300	0	1.00	0.75
1	0	400	0	0.00	1.00
1	107	400	0	0.71	1.00
2	43	0	0	0.29	0.00
2	150	0	0	1.00	0.00
2	0	300	0	0.00	0.75
2	107	300	0	0.71	0.75
2	3	400	0	0.02	1.00
2	150	400	0	1.00	1.00
3	0	0	300	0.00	0.75
3	10	0	300	0.07	0.75
3	107	0	300	0.71	0.75
3	0	0	400	0.00	1.00
3	43	0	400	0.29	1.00
3	150	0	400	1.00	1.00
4	150	0	300	1.00	0.75
4	107	0	400	0.71	1.00

*Blocks 1-4 were performed in time sequence

Table C4: Organic acid concentration ($g \cdot L^{-1}$) RSM model output and selectivity. Errors (±) display the 95% confidence interval

Block	Sucrose	N	Source[a]	Lactic	Succinic	Acetic	Propionic	Butyric	Selectivity ($gLA_{COD}/TCOD$)[b]
1	0	0	0	27.34(±3.44)	0.18(±0.26)	1.08(±1.63)	3.37(±0.89)	-0.22(±0.37)	83.0%
2	0	300	0	26.28(±3.19)	0.7(±0.23)	-0.71(±1.4)	4.14(±0.74)	0.3(±0.4)	80.7%
3	0	300	1	27.29(±2.84)	0.88(±0.27)	5.35(±1.58)	5.17(±0.65)	-0.12(±0.42)	67.4%
1	0	400	0	32.55(±3.1)	0.38(±0.24)	1.48(±1.42)	4.39(±0.72)	-0.22(±0.37)	81.0%
3	0	400	1	28.59(±2.84)	1.21(±0.27)	6.87(±1.58)	5.92(±0.7)	-0.12(±0.42)	64.0%
2	50	0	0	44.44(±3.36)	0.15(±0.24)	-0.5(±1.71)	2.5(±0.71)	0.28(±0.3)	92.4%
1	50	300	0	53.32(±2.48)	-0.07(±0.18)	1.89(±1.17)	5.24(±0.63)	-0.24(±0.29)	85.8%
3	50	300	1	49.36(±2.48)	0.18(±0.22)	5.86(±1.53)	5.14(±0.6)	0.16(±0.29)	78.5%
2	50	400	0	49.65(±3.02)	0.35(±0.21)	-0.11(±1.52)	3.14(±0.67)	0.28(±0.3)	90.7%
3	50	400	1	50.67(±2.48)	0.51(±0.22)	7.38(±1.53)	5.79(±0.61)	0.16(±0.29)	75.7%
1	125	0	0	62.96(±3.36)	-0.3(±0.24)	4.25(±1.6)	3.54(±0.71)	-0.28(±0.3)	88.1%
2	125	300	0	61.89(±2.48)	0.22(±0.18)	2.45(±1.12)	3.58(±0.63)	0.24(±0.29)	88.4%
3	125	300	1	62.91(±2.59)	-0.34(±0.23)	8.52(±1.5)	4.61(±0.61)	0.59(±0.32)	80.0%
1	125	400	0	68.17(±3.02)	-0.09(±0.21)	4.65(±1.39)	3.59(±0.67)	-0.28(±0.3)	88.2%
4	125	400	1	59.24(±3.04)	0.36(±0.25)	7.94(±1.76)	3.99(±0.7)	1.11(±0.36)	79.0%
2	175	0	0	53.98(±3.44)	0.37(±0.26)	5.2(±1.76)	2.02(±0.89)	0.22(±0.37)	86.1%
1	175	300	0	62.86(±3.19)	0.15(±0.23)	7.59(±1.63)	4.02(±0.74)	-0.3(±0.4)	83.0%
4	175	300	1	53.93(±3.23)	0.02(±0.28)	9.46(±1.9)	2.8(±0.72)	1.4(±0.44)	77.3%
2	175	400	0	59.19(±3.1)	0.57(±0.24)	5.6(±1.57)	1.68(±0.72)	0.22(±0.37)	87.0%
3	175	400	1	60.2(±3.05)	-0.01(±0.29)	13.08(±1.84)	4.34(±0.73)	0.87(±0.46)	74.4%

a. Nitrogen source, 0 = NH_4Cl, 1 = Digestate, b. calculated based on measured VFAs.

Table C5: Microbial community RSM model parameters with associated 95% confidence intervals.

Genus	Intercept	R_B	β_S	β_N	β_{NS}	β_{S2}	β_{N2}	β_{S_N}	β_{S_NS}	β_{N_NS}	Adj.R^2
Allisonella	0.13 (±0.21)	-	-1.28 (±0.73) ***	0.00 (±0.21)	1.75 (±0.82) ***	1.28 (±0.69) ***	-	-	-1.11 (±0.37) ***	-0.99 (±0.92) *	0.61
Bacillales unclassified	1.97 (±0.42) ***	-	-3.67 (±1.28) ***	-1.58 (±0.55) ***	-0.41 (±0.41)	1.5 (±1.11) **	-	1.89 (±0.87) ***	0.50 (±0.65)	-	0.56
Bifidobacterium	2.33 (±3.34)	-	-21.01 (±11.77) ***	0.42 (±3.45)	3.72 (±13.3)	20.41 (±11.14) ***	-	-	-16.12 (±6.03) ***	10.29 (±14.79)	0.57
Clostridiaceae 1 unclassified	-0.40 (±0.44)	0.32 (±0.23) **	-0.01 (±0.35)	0.07 (±0.32)	-0.75 (±1.27)	-	-	-	-0.58 (±0.56) *	1.00 (±1.36)	0.29
CSS_15	7.20 (±5.58) *	-1.93 (±2.67)	-29.95 (±13.45) ***	15.83 (±5.78) ***	-9.10 (±5.84) **	28.47 (±11.65) ***	-	-20.17 (±9.19) ***	16.07 (±7.00) ***	-	0.64
CSS_18	0.64 (±1.37)	-	-6.24 (±4.84) *	0.00 (±1.42)	11.01 (±5.47) ***	6.24 (±4.58) **	-	-	-5.37 (±2.48) ***	-7.56 (±6.08) *	0.47
CSS_7	0.61 (±1.79)	-	-5.73 (±6.30)	0.00 (±1.85)	-4.22 (±7.12)	5.73 (±5.96)	-	-	-5.02 (±3.23) **	10.25 (±7.91) *	0.37
Defluviitoga	-0.31 (±0.31) *	0.21 (±0.17) *	-	0.00 (±0.24)	-0.68 (±0.95)	-	-	-		1.10 (±1.04) *	0.54
DTU014_ge	-0.06 (±0.12)	-	0.61 (±0.44) **	0.00 (±0.13)	-0.35 (±0.49)	-0.61 (±0.41) **	-	-	0.23 (±0.22) *	0.99 (±0.55) ***	0.81
Lactobacillaceae unclassified	0.75 (±0.61) *	0.32 (±0.33)	-	-2.69 (±1.77) **	-0.72 (±0.68) *	-	1.78 (±1.73) *	-	-	-	0.33

Genus	Intercept	R_B	β_S	β_N	β_{NS}	β_{S2}	$\beta_{N\,2}$	β_{S_N}	β_{S_NS}	β_{N_NS}	Adj.R^2
Lactobacillales unclassified	1.17 (±0.23) ***	-0.21 (±0.12) **	-1.55 (±0.55) ***	-0.05 (±0.16)	-0.08 (±0.65)	1.11 (±0.52) ***	-	-	0.25 (±0.29)	0.80 (±0.69) *	0.66
Lactobacillus	77.44 (±11.78) ***	-3.93 (±5.58)	77.58 (±28.16) ***	31.01 (±29.71) *	-15.37 (±12.24) *	-66.97 (±24.38) ***	-39.65 (±27.5) **	18.82 (±19.23)	14.91 (±14.66) *	-	0.72
Leuconostoc	3.83 (±0.62) ***	-	-5.75 (±2.23) ***	0.61 (±0.63)	-1.79 (±0.74) ***	2.89 (±2.11) **	-	-	2.37 (±1.14) ***		0.56
Olsenella	0.10 (±0.28)	-	-0.93 (±0.99)	0 (±0.29)	-1.00 (±1.12)	0.93 (±0.94)	-	-	-0.79 (±0.51) **	1.87 (±1.25) **	0.33
Pediococcus	4.23 (±5.28)	3.3 (±2.88) *	-	-20.97 (±15.41) **	-6.53 (±5.88) *	-	12.79 (±15.08)	-	-	-	0.80
Proteiniphilum	-0.31 (±0.44)	0.29 (±0.25) *	-0.99 (±1.15)	-	1.69 (±0.53) ***	0.94 (±1.09)	-	-	-1.63 (±0.60) ***	-	0.32
Streptococcus	0.28 (±1.1)	-	-2.68 (±3.89)	0.00 (±1.14)	-1.00 (±4.4)	2.67 (±3.68)	-	-	-3.26 (±1.99) **	5.24 (±4.89) *	0.77
Syntrophaceticus	0.00 (±0.18)	-	0.60 (±0.66)	-0.01 (±0.20)	-0.07 (±0.75)	-0.60 (±0.64)	-	-	-	1.15 (±0.85) **	0.43

***=(P<0.001), **=(P<0.01), *=(P<0.05).

Table C6: Functional gene RSM model parameters with associated 95% confidence intervals. The relative abundance of genes were scaled by 1000 (Section 2.6 in MS).

Gene	Intercept	R_B	β_S	β_N	β_{NS}	β_{S2}	$\beta_{N\,2}$	β_{S_N}	β_{S_NS}	β_{N_NS}	Adj.R^2
aldA	5.22 (±1.08) ***	-	-8.48 (±3.51) ***	2.17 (±1.32) **	-1.06 (±1.31)	4.69 (±3.15) **	-	-1.64 (±1.81)	3.61 (±1.70) ***	-1.51 (±1.39) *	0.62
AOC3	2.64 (±0.54) ***	-	-4.34 (±1.74) ***	1.08 (±0.66) **	-0.59 (±0.65)	2.42 (±1.56) **	-	-0.80 (±0.90)	1.84 (±0.84) ***	-0.75 (±0.69) *	0.62
dld_Qu	2.64 (±0.53) ***	-	-4.36 (±1.72) ***	1.07 (±0.65) **	-0.63 (±0.64)	2.45 (±1.55) **	-	-0.80 (±0.89)	1.83 (±0.83) ***	-0.77 (±0.68) *	0.63
E2.3.1.8	-0.08 (±0.46)	0.24 (±0.24) *	-0.95 (±1.12)	-0.01 (±0.32)	0.51 (±0.57)	0.76 (±1.05)	-	-	-0.69 (±0.58) *	0.39 (±0.47)	0.54
fucO	0.09 (±0.21)	-	-0.67 (±0.95)	-	0.44 (±0.3) **	0.66 (±0.9)	-	-	-0.54 (±0.48) *	-	0.15
ghrA	5.59 (±1.10) ***	-	-9.44 (±3.57) ***	2.18 (±1.35) **	-1.10 (±1.33)	5.43 (±3.20) **	-	-1.69 (±1.84)	3.72 (±1.73) ***	-1.50 (±1.41) *	0.63
ghrB	8.47 (±1.70) ***	-	-16.45 (±6.07) ***	0.75 (±1.75)	-3.84 (±2.37) **	10.33 (±5.71) ***	-	-	7.17 (±3.07) ***	-2.12 (±2.52)	0.49
gldA	22.81 (±5.23) ***	8.43 (±3.07) ***	-7.53 (±3.80) ***	-23.22 (±20.48) *	-10.88 (±6.22) ***	-	20.19 (±20.87)	-	-	-	0.48
GLO1	5.57 (±1.15) ***	-	-9.61 (±4.22) ***	2.53 (±1.41) ***	1.99 (±0.8) ***	6.19 (±3.88) **	-	-2.53 (±2.23) *	-	-	0.69

Gene	Intercept	R_B	β_S	β_N	β_{NS}	β_{S2}	$\beta_{N\,2}$	β_{S_N}	β_{S_NS}	β_{N_NS}	Adj.R^2
gloB	43.19 (±10.45) ***	16.87 (±6.13) ***	-13.08 (±7.60) **	-46.86 (±40.9) *	-21.75 (±12.42) ***	-	40.36 (±41.69)	-	-	-	0.48
hchA	0.00 (±0.01) *	0.00 (±0.01) ***	0.00 (±0.01) **	-	-	0.00 (±0.01) **	-	-	-	-	0.23
K15024	0.00 (±0.72)	-	0.00 (±0.87)	0.00 (±0.79)	1.73 (±1.07) **	-	-	-	-2.03 (±1.38) **	0.79 (±1.14)	0.34
LDH	0.05 (±0.49)	-	-0.03 (±0.6)	0.01 (±0.54)	1.39 (±0.73) ***	-	-	-	-1.00 (±0.95) *	0.59 (±0.78)	0.46
ldhA	2.66 (±0.60) ***	-	-4.20 (±1.97) ***	1.05 (±0.74) **	-0.13 (±0.73)	2.24 (±1.77) *	-	-0.74 (±1.02)	1.74 (±0.95) ***	-0.67 (±0.78)	0.57
MAO	63.39 (±6.33) ***	-6.8 (±3.26) ***	23.01 (±16.03) **	18.34 (±21.53)	12 (±7.12) **	-11.75 (±14.35)	-19.82 (±21.52)	6.88 (±8.36)	-8.2 (±7.85) *	-	0.55
pct	0.01 (±0.28)	-	0.00 (±0.44)	-	0.97 (±0.44) ***	-	-	-	-1.03 (±0.70) **	-	0.28
pta	24.95 (±5.09) ***	-7.95 (±2.99) ***	7.19 (±3.70) ***	22.85 (±19.92) *	9.96 (±6.05) **	-	-19.83 (±20.31)	-	-	-	0.48
yqhD	22.84 (±5.22) ***	8.47 (±3.06) ***	-7.40 (±3.79) ***	-23.36 (±20.41) *	-11.44 (±6.2) ***	-	20.21 (±20.81)	-	-	-	0.48

***=(P<0.001), **=(P<0.01), *=(P<0.05).

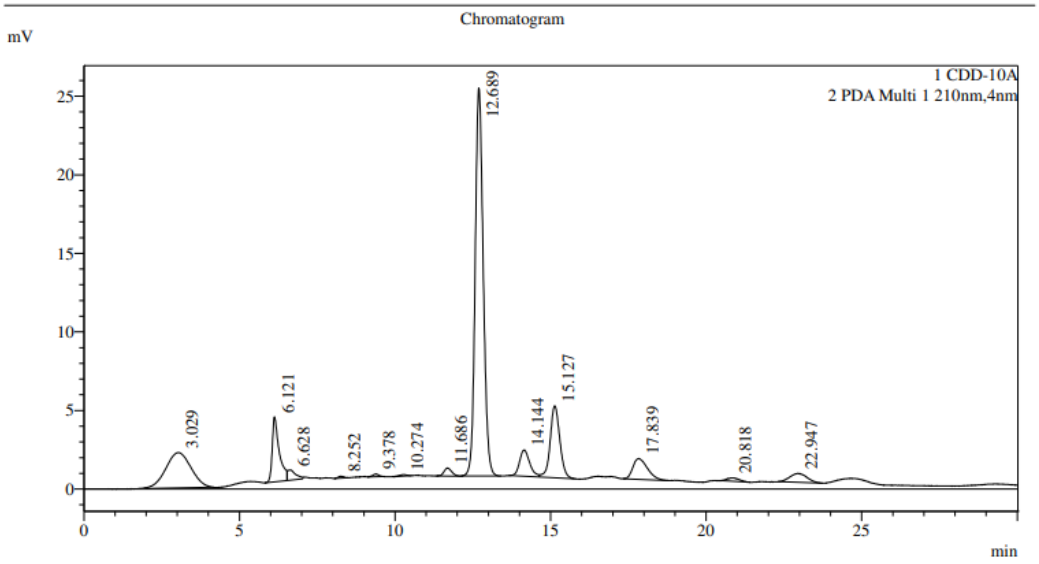
Appendix D: LACTIC ACID RECOVEY AND DOWNSTREAM EFFECTS

Figures

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: Broth_No_extract		
Sample ID	:		
Method Filename	: Lactic acid Column Fermentation samples.lcm		
Batch Filename	: Lactic Acid Run (HPX 87H).lcb		
Vial #	: 1-22	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 20/12/2020 1:30:52 PM	Acquired by	: System Administrator
Date Processed	: 23/09/2021 6:35:34 AM	Processed by	: System Administrator



Peak Table

CDD-10A

Peak#	Ret. Time	Area	Height	Name	Resolution(USP)	Noise
Total						

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Name	Resolution(USP)	Noise
1	3.029	126428	2242		--	33.14
2	6.121	64325	4129		3.259	33.14
3	6.628	11882	649		0.328	33.14
4	8.252	1401	116		1.034	33.14
5	9.378	2304	173		2.784	33.14
6	10.274	1505	87		2.030	33.14
7	11.686	8714	502	Succinic Acid	2.884	33.14
8	12.689	445630	24684	Lactic Acid	2.108	33.14
9	14.144	38024	1676		2.714	33.14
10	15.127	98517	4579	Acetic Acid	1.707	33.14
11	17.839	42876	1317	Propionic acid	3.808	33.14
12	20.818	5909	213	Butyric acid	3.703	33.14
13	22.947	21697	567		2.399	33.14
Total		869212	40933			

Figure D1: Example generated report from the HPLC showing the various unidentified peaks when analysing real world fermentation broth.

Tables

Table D1: Properties of BA765 anion exchange resin used to extract LA.

Resin	Matrix	Skeleton	Basicity	Functional Group	Ionic Form	Capacity (mmol·ml^{-1})	Operational pH range	Operating temperature
BA765	Gel	Acrylic Acid	Weak Base	R-N(R2)H_2O/ Tertiary amine	Free Amine	≥1.6	pH < 7	< 60ºC

Table D2: Fitted kinetic model parameters for the adsorption of LA onto the BA765 Resin at 36 oC and pH of 2.5

Kinetic Model	R^2	Parameter	Value	units
Pseudo-first order	0.984	qe	222.3 ± 8.7	mg·g^{-1}
		K_1	0.076 ± 0.016	min^{-1}
Pseudo-second order	0.963	qe	231.2 ± 14.9	mg·g^{-1}
		K_2	0.00056 ± 0.00024	g·mg^{-1}·min^{-1}
Intra-particle diffusion	~0*	k_{id}	3.2 ± 2.9	mg·g^{-1}·min$^{-1/2}$
		C	138 ± 51	mg·g^{-1}

*Significant fit parameters were obtained for the intra-particle diffusion model using non-linear regression analysis, but it was not possible to calculate an R^2 value for this model due to the very poor extent of fit.

Table D3: Fitted parameter values of equilibrium isotherms for the adsorption of LA onto BA765 resin. Parameters for the Redlich-Peterson at pH 3.5 could not be estimated.

Model	pH	R^2	Isotherm Parameters	Value	Units
Langmuir	2.5	0.97	Q_0	0.211 ± 0.019	g·g^{-1}
			b	0.874 ±0.461	L·g_{LA}^{-1}
Freundlich	2.5	0.86	K_F	0.095 ±0.035	g·g^{-1}
			n	4.697 ±2.339	-
Redlich-Peterson	2.5	0.97	K_R	0.208 ±0.201	L·g_{resin}^{-1}
			a_R	1.105 ±1.642	L·g_{LA}^{-1}
			g	0.969 ±0.152	-
Langmuir	3.5	0.98	Q_0	0.247 ±0.032	g·g^{-1}
			b	0.075 ±0.034	L·g_{LA}^{-1}
Freundlich	3.5	0.89	K_F	0.035 ±0.024	g·g^{-1}
			n	2.279 ±0.988	-

Appendix E: TECHNOECONOMICS

Table E.1: Average percent composition of the waste received by the model anaerobic digestion facility.

Waste	Percent of total waste
Bleaching Earth	4.10%
Brewery liquid wastes	7.50%
FW	51.10%
Grain processing waste	2.00%
Milk waste	6.90%
Restaurant scraps	4.50%
Spent grain	0.10%
Soft drink	17.10%
Unknown	6.70%
Total	100.00%