

Full-scale UASB for pre-treatment and energy valorization of high-load wastewater

Saleh Abuzir



The photo on the cover shows two EGSB (R2S) reactors and an anaerobic digester at the Bronzolo Wastewater Treatment Plant in northern Italy.

Photo taken by Saleh Abuzir.

Full-scale UASB for pre-treatment and energy valorization of high-load wastewater

Saleh Abuzir



Finanziato dall'Unione Europea
NextGenerationEU

"Progetto Finanziato dall'Unione europea – NextGenerationEU"



**Università
di Brescia**

DIPARTIMENTO DI INgegNERIA CIVILE, ARCHITETTURA, TERRITORIO, AMBIENTE E DI
MATEMATICA

DOTTORATO DI RICERCA IN
INGEGNERIA CIVILE, AMBIENTALE, DELLA COOPERAZIONE INTERNAZIONALE E DI
MATEMATICA

settore scientifico disciplinare

Tecnologie e processi per l'ambiente e l'agricoltura – 6

CICLO
XXXVIII

TITOLO TESI

Full-scale UASB for pre-treatment and energy valorization of high-load wastewater

NOME DEL DOTTORANDO

Saleh Abuzir

NOME DEL Primo SUPERVISORE

Giorgio Bertanza, Università degli Studi di Brescia

NOME DEL CO-SUPERVISORE

Marta Domini, Università degli Studi di Brescia

Gianluca Simion, Eco-Center

Acknowledgments

When I look back on the past three years, I realize how enjoyable and how challenging they have been: a journey full of ups and downs, and one that helped me grow both professionally and personally. Along the way, I met many people who supported me in different ways. Without them, I would not have been able to complete this thesis, and I am deeply grateful to you all.

First, I would like to deeply thank my supervisors, **Prof. Giorgio Bertanza** and **Dr. Marta Domini**, for your continuous guidance, support, and encouragement. Thank you for listening to me and showing genuine care, especially during the war. I learned a great deal from both of you, and I am particularly grateful for the freedom you gave me to explore this research field, develop my ideas, and grow with confidence. I would also like to thank **Gianluca Simion** for supporting my research ideas and for being not only a collaborator but also a friend. Thank you for showing me the beauty of South Tyrol and for all the memorable conversations and discussions we shared. Thank you to **João Paulo Bassin** for the interesting and fruitful discussions we had, which helped me plan my experiments. Thank you to the **PhD office (Fabiana Farro and Mehwish Shahbaz)** at the university of Brescia for supporting me and for the interesting conversations we had.

My sincere thanks go to **Hamse Kjerstadius** for giving me the opportunity to explore the world of anaerobic digestion in greater depth through my internship at NSVA and Lund University. I truly enjoyed our discussions (especially the scientific ones), and I always felt we shared a similar way of thinking. Before this experience, I had never run experiments entirely on my own; yet, even from a distance, you guided me clearly and patiently. I still remember how challenging it was to write my first paper, and I appreciate the time and support you invested in it. Thank you as well for your continued support after the internship and for your valuable advice. I am also grateful to **Åsa Davidsson** for hosting me at Lund University and for the many interesting discussions and kind support. I appreciate the opportunity to meet such wonderful people in the Department of Process and Life Science Engineering. Thanks to **Sybrand Metz** and **Ignatius Ariffin** for the interesting discussion we had on their blackwater and food waste co-digestion system and for providing me with their operating data.

Thank you to my colleagues at the Eco-Center—**Mario Brando**, **Daniele Pellegrini**, **Elia Betta**, and **Hans Peter**—for your help with the laboratory work, the pilot plant, for helping me to improve my Italian, for the interesting discussions we had, and for helping me with my PhD research. Thank you to **Glauco Menin** for guiding me through the BMP experiments; I could not have carried them out without your support. I also thank **Christian Ebner** and **Anna Arthofer** for helping with my experiments and for hosting me at the University of Innsbruck.

Thank you to **Victoria Outram** for your help with the HPLC. Without your support, I would not have been able to use the instrument, and I truly appreciated your patience and the conversations we shared. I truly appreciate the discussion we had. Thank you to **Salar Haghighatafshar** for all the interesting conversations we had, it was really great meeting you and learning from your experience. Thank you to **Ashley Hall** for being such a kind colleague and for helping me, especially on my first day in the department. I also enjoyed our discussions and wish

you all the best with your MBBR reactors and your PhD journey. Thank you to **Borbála Erdei** and **Per Falås** for helping me prepare for the experiments. Thank you to **Michael Cimbritz** for the wastewater book, and for the memorable session during the course you taught. Thank you to **Malgorzata Szopińska** for our interesting discussions and for the unforgettable ice trip (especially the moment I fell off the bike). Thank you to **Mahla Bagheri** for the engaging conversations we had. Thank you to **Julius Klemens Lorek**, my gym bro, for all the time we spent together. Thank you to **Madeleine Larsson** and **Karin Tonderski** for the opportunity to attend the Biogas solutions course and for our discussions. Thank you to **Miriam van Eckert** for your guidance and our valuable conversations. Thank you to **Melesse Eshetu Moges** for our discussions on blackwater; it was a pleasure learning from your experience.

Of course, none of this would have been possible without my family and without their love and care. Thank you, Dad, Mum, and my brothers, for your constant support, belief in me, and encouragement throughout this journey. Dad, thank you for being my inspiration, without you I would not be writing these words. You were the one who encouraged me to pursue a PhD.

Finally, thank you to **Maftuna Gapparova** for being a wonderful friend and for the conversations that genuinely changed the way I viewed the PhD journey. Thank you, **Jacobo Ortiz**, for being a good friend and for the bike ride we had, it was my first time hitting 90 km. Your cycling skills inspired me. Thank you to **Abdelrahim Moumen** for being a great support and for all your advice. Thank you **Kassem Sabeh** for being a great friend and flat mate and for all the moments we shared together. Thank you to **Mahum Khan** for being a great support and for all the discussions we had and for sharing your experiences. Thank you **Rula Abu Hilal** for the interesting discussions we had, I do appreciate your passion for self- and professional growth, keep it up. Thank you to **Amin Mohsenifar** for being a kind flat mate, for our bike rides to the beach, and for doing Ramadan together. Thank you as well to all the friends I met along the way, your support, moments, and conversations had a positive impact on me, and I am grateful to have learned from your experiences.

~Beautiful things happen when they are not expected to happen.

List of abbreviations

Abbreviation	Meaning
AD	Anaerobic digestion
Alk	Alkalinity
AMPTS	Automatic Methane Potential Test System
BMP	Biochemical methane potential
BW	Blackwater (source-separated blackwater)
Ca	Calcium
CFD	Computational fluid dynamics
CH ₄	Methane
COD	Chemical oxygen demand
COD _{in}	Influent COD
COD _{removed}	Removed COD
COD _{tot} or COD _{total}	Total COD
CSTR	Continuous stirred-tank reactor
DoA	Degree of acidification
EGSB	Expanded granular sludge blanket (reactor)
F/M	Food-to-microorganism ratio
FJPWW	Fruit juice processing wastewater
FW	Food waste
GLS	Gas-liquid-solid (separator)
HAc	Acetic acid (reference for VFAs: 'as HAc')
HPLC	High-performance liquid chromatography
HRT	Hydraulic retention time
N	Nitrogen
N ₂	Nitrogen gas
NaOH	Sodium hydroxide
NH ₄ ⁺ -N	Ammonium nitrogen

Nm ³	Normal cubic meter (gas volume at reference conditions)
NmL	Normal milliliter (gas volume at reference conditions)
OLR	Organic loading rate
P	Phosphorus
PE	Population equivalent
PO ₄ ⁻³ -P	Phosphate phosphorus
PS	Primary sludge
R2S	High-rate methanogenic reactor (used for the EGSB/R2S stage)
sCOD	Soluble COD
S/I	Substrate-to-inoculum ratio
SMA	Specific methanogenic activity
SRT	Solids retention time
STP	Standard temperature and pressure
TN	Total nitrogen
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solids
TVFA	Total volatile fatty acids
UASB	Upflow anaerobic sludge blanket (reactor)
UASB _{BW}	UASB reactor treating blackwater (system label)
VFA or VFAs	Volatile fatty acid(s)
VFA/Alk	Volatile fatty acids to alkalinity ratio
VS	Volatile solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

List of publications

This Thesis is based on the following papers and publications:

- I. Abuzir, S., Gren, L., Domini, M., Bertanza, G., Davidsson, Å., & Kjerstadius, H. (2025). Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden. *Cleaner Water*, 4, 100082. <https://doi.org/10.1016/j.clwat.2025.100082>.
- II. Abuzir, S.; Simion, G.; Domini, M.; Bertanza, G. Performance Evaluation of a Full-Scale Two-Stage Anaerobic Digestion System Treating Fruit Juice Processing Wastewater. Submitted to the journal of Water Process Engineering.
- III. Abuzir, S.; Simion, G.; Domini, M.; Bertanza, G. Impact of co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production. Submitted to the journal of Chemical Engineering.

Author's contributions:

- I. Participated in experimental planning, conducted the experiment, performed the data analyses, discussed the interpretation of the results with the co-authors, and served as the main writer of the manuscript.
- II. Planned the study and experimental design, performed the data analyses, discussed the interpretation of the results with the co-authors, and served as the main author of the manuscript.
- III. Planned and conducted the experiments, performed the data analyses, discussed the interpretation of the results with the co-authors, and served as the main author of the manuscript.

Summary in English

Rapid urbanization, industrial growth, and increasing food production are increasing the generation of municipal and agro-industrial wastewaters. Addressing these challenges require treatment approaches that are effective, enabling recovery of energy and nutrients alongside organic matter removal. This thesis focuses on two contrasting high-strength wastewater streams: source-separated blackwater (BW) and fruit juice processing wastewater (FJPWW). Source-separated BW is toilet-derived wastewater collected separately from other domestic wastewater streams and consists mainly of feces, urine, flush water, and toilet paper. FJPWW is an agro-industrial effluent generated during fruit washing, pressing, and juice production, and is typically characterized by readily biodegradable organic matter and low pH. Although both streams are suitable for anaerobic treatment, they differ in composition, treatment challenges, and resource-recovery potential.

Anaerobic digestion (AD) can convert organic matter in wastewater into methane-rich biogas while retaining nutrients such as nitrogen and phosphorus in streams that may be further processed for recovery. High-rate anaerobic reactors, including upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) systems, are particularly relevant for concentrated wastewaters because they retain active biomass and enable organic matter conversion at relatively short hydraulic retention times. However, studies on full-scale applications remain limited for some waste streams, particularly source-separated BW and full-scale two-stage AD systems treating highly biodegradable agro-industrial wastewater.

The overall aim of this thesis was to evaluate the anaerobic treatment of source-separated BW and FJPWW in high-rate anaerobic systems, with an emphasis on full-scale performance, process stability, methane production, operating challenges, and opportunities for process optimization. Three study contexts were investigated: (i) a full-scale UASB reactor treating source-separated BW in Helsingborg, Sweden; (ii) a full-scale two-stage AD system treating FJPWW in Bronzolo, Italy, consisting of homogenization/acidification followed by two EGSB (R2S) reactors; and (iii) batch acidification and biochemical methane potential (BMP) tests assessing the co-processing of FJPWW with primary sludge (PS). Together, these studies address the need for improved knowledge on anaerobic treatment of high-strength wastewater streams and on the operational factors affecting high-rate AD performance.

In **Chapter 1**, the thesis establishes the need for sustainable and effective wastewater management technologies, such as AD, to address the challenges associated with rapid urbanization and the increasing generation of municipal and industrial wastewaters, including source-separated BW and FJPWW. It identifies BW as a wastewater stream rich in organic matter and nutrients, making it suitable for energy recovery as biogas, mainly methane, and for fertilizer production. It also presents FJPWW as a substrate with high concentrations of readily biodegradable organic matter, supporting its potential for energy recovery through anaerobic treatment. However, both BW and FJPWW remain insufficiently studied as substrates for biogas production.

Against this background, the thesis introduces three study contexts: (i) a full-scale upflow anaerobic sludge blanket (UASB) reactor treating source-separated BW in Helsingborg, Sweden;

(ii) a full-scale two-stage anaerobic digestion plant treating FJPWW in Bronzolo, Italy, comprising homogenization/acidification followed by two expanded granular sludge bed (EGSB/R2S) reactors; and (iii) batch acidification and biochemical methane potential (BMP) tests to assess the co-processing of FJPWW with primary sludge. Together, these contexts support sustainable management of high-strength wastewater streams and strengthen the evidence base on high-rate (UASB and EGSB) systems for biogas production and nutrient recovery. The thesis also proposes operational actions to improve understanding of system performance and enhance process efficiency, with emphasis on full-scale operation, process optimization, and co-digestion.

Based on the research needs mentioned in Chapter 1, in **Chapter 2**, the thesis provides the conceptual and technical foundation needed for subsequent research work. In Chapter 2, the thesis reviews the main biochemical steps of anaerobic digestion and the differences between single-stage and two-stage anaerobic digestion configurations. It summarizes the operational parameters that affect process stability and efficiency, including organic loading rate (OLR), hydraulic and solids retention times (HRT and SRT), pH control, and the dynamics of alkalinity and VFAs, as well as substrate composition. It also describes co-digestion as an efficient optimization strategy to balance nutrients, buffering capacity, and biodegradability. Moreover, it introduces key analytical tools used in anaerobic digestion research, including biochemical methane potential (BMP) tests and specific methanogenic activity (SMA) measurements. Finally, it synthesizes evidence on UASB and EGSB performance and highlights the limited number of full-scale evaluations of BW digestion in UASB reactors and of two-stage full-scale systems treating highly biodegradable wastewaters, thereby defining the knowledge gaps that motivate the experimental chapter.

In **Chapter 3**, the thesis outlines the experimental and analytical approach used to assess AD of high-strength wastewater streams, with a primary focus on source-separated BW and FJPWW. Source-separated BW collected from vacuum toilets in Oceanhamnen, Sweden, was evaluated in a full-scale UASB reactor, whereas FJPWW from Bronzolo, Italy, was investigated in full-scale and pilot-scale two-stage anaerobic digestion systems. The FJPWW work also included batch co-digestion tests with primary sludge and continuous operation of a two-stage pilot AD system. Long-term operational performance was assessed using pseudo-steady-state periods of at least 14 days and was complemented by biochemical methane potential (BMP) assays to determine methane potential and biodegradability, specific methanogenic activity (SMA) tests to quantify methanogenic activity, and batch acidogenic fermentation experiments to investigate the effects of combining fruit juice processing wastewater with primary sludge. It further discusses the main limitations, including sludge washout and variable effluent COD/TSS in the blackwater UASB system, restricted energy-balance and microbial data, incomplete monitoring of biomass and alkalinity in the FJPWW system, the absence of sludge settleability and CFD analyses, and limited gas measurements during acidogenic fermentation. Overall, the methodology establishes an integrated full-scale and batch-scale framework for evaluating anaerobic digestion performance, substrate biodegradability, process stability, and potential optimization strategies.

In **Chapter 4**, the thesis assesses the performance of a full-scale UASB (50 m³) reactor treating vacuum-toilet source-separated BW from the Oceanhamnen district in Helsingborg, Sweden.

The BW UASB reactor served an actual population equivalent (PE) of 885 which is expected to increase to 2000 in the near future. Moreover, the thesis highlights the performance of a full-scale UASB (41 m³) reactor co-digesting source separated BW with food waste (FW) in Sneek, the Netherlands. The BW UASB reactor was monitored for 130 weeks at 35°C under two operational phases with different organic loading rates due to increase in the number of the inhabitants in the city district. The highest overall removal was achieved in Phase 1: operation at an average OLR of 0.95 kg COD m⁻³ d⁻¹ resulted in 88% COD removal and a methanization of 0.77 COD-CH₄ kg⁻¹ COD_{in}, with a methane yield of 0.307 Nm³ CH₄ kg⁻¹ COD_{removed}. When the average OLR was increased to 1.5 kg COD m⁻³ d⁻¹, COD removal declined to 72%, which was attributed to sludge washout and the associated increase in effluent COD/TSS variability; however, methanization remained high at 0.71 kg COD-CH₄ kg⁻¹ COD_{in}, with a methane yield of 0.304 Nm³ CH₄ kg⁻¹ COD_{removed}. The COD mass balance showed that 69% of influent COD was converted to methane, 28% was discharged in the effluent, and 3% was associated with removed sludge. Nutrient mass balances indicated that the decanted effluent contained 92% of N and 82% of P, mainly as soluble NH₄⁺-N and PO₄³⁻-P, whereas removed sludge accounted for 2% of N and 18% of P. Overall, these results indicate that high methane conversion can be maintained at higher loading, although sludge washout reduces effluent quality, suggesting that post-treatment may be required to meet discharge requirements under certain operating regimes.

The co-digestion of source-separated BW with FW showed that intermittent operation at varying temperatures (22.5°C in winter and 35°C in summer) resulted in occasionally lower yet comparable COD_{total} removal efficiency compared to BW mono-digestion. Increasing the temperature to 35°C improved the degree of methanisation from 0.60 to 0.68 kg CH₄-COD kg⁻¹ COD_{in}, methanisation in UASB_{BW+FW} was still 11–24 % lower than the maximum achieved in BW mono-digestion (0.77 kg CH₄-COD kg⁻¹ COD_{in}), highlighting the reduced efficiency of co-digestion under the tested conditions of uncontrolled mixing ratios of BW to FW. The degree of methanisation may have been impacted by biomass washout or microbial imbalances caused by unregulated BW to FW mixing ratios, with optimal ratios reported as 1:1 or 1:2.

In **Chapter 5**, the thesis assesses a full-scale two-stage AD system treating FJPWW at the Bronzolo wastewater treatment plant (Northern Italy) based on long-term operational data spanning 6 years. In the first stage, a homogenization tank produced a VFA-rich intermediate, characterized by a VFA/sCOD of 38–41% and a degree of acidification of 21–25%. Over the periods evaluated, operation at different temperatures (16–26°C) had no significant influence on either COD removal or the degree of acidification. The second stage, consisting of two parallel high-rate EGSB/R2S reactors, achieved effective overall performance where COD_{total} declined from 2902 to 349 mg COD L⁻¹ (88% removal), accompanied by an effective VFA removal (VFA as HAc from 1082 to 69.2 mg HAc L⁻¹). Stable operation was reflected by a VFA/Alk ratio of 0.07. The methane yield reached 0.277 Nm³ CH₄ kg⁻¹ COD_{removed}, and the average methane content was 80%. Although COD conversion was effective, TSS removal remained low (6.2%); this outcome was discussed as potentially associated with solids or sludge washout, highlighting that conversion performance and effluent solids quality may diverge and underscoring the need for dedicated solids management and post-treatment where required.

In **Chapter 6**, the thesis discusses the effect of co-fermentation and co-digestion of FJPWW with PS on VFA generation and methane yield which was assessed using controlled batch acidogenic fermentation (100 hours) followed by BMP testing at 35°C (terminated after 120 hours). The evaluated mixing conditions were FJPWW alone and FJPWW:PS ratios of 97:3 and 90:10 (vol/vol). Peak acidification occurred within approximately 24 h, reaching maximum DoA values of 33% for FJPWW, 31% for 97:3, and 15% for 90:10, with corresponding peak VFA yields of 0.89, 0.86, and 0.45 g net TVFA-COD gVS⁻¹, respectively. Methane yields were similar for FJPWW alone and 97:3 (approximately 504 NmL CH₄ gVS⁻¹), whereas a lower yield was observed at 90:10 (483.7 NmL CH₄ gVS⁻¹). Under the tested conditions and ratios, the addition of primary sludge did not enhance either VFA production or methane yield. The experimental setup also revealed an important methodological limitation: gas production was not monitored during the acidogenic co-fermentation phase, which constrained the interpretation of COD pathways.

In **Chapter 7**, the thesis integrates the findings and shows that interpreting full-scale performance is strengthened by jointly considering (i) conversion-based indicators (methanisation, methane yield, VFA removal), (ii) effluent quality and solids behavior, and (iii) targeted activity testing (SMA). Across the full-scale systems, stable methane conversion was observed alongside residual effluent COD and/or limited solids removal, demonstrating that process “success” cannot be judged using biological conversion metrics alone when solids washout is the main driver of effluent variability.

In **Chapter 8**, the thesis presents the main conclusions and future research needs. For the full-scale BW UASB system, the highest COD removal and methanization were achieved at the lower OLR. At higher OLR, COD removal decreased mainly due to sludge washout, although methane conversion remained high. Mass balances showed that most influent COD was converted to methane, while most nitrogen and phosphorus remained in the liquid effluent as NH₄⁺-N and PO₄³⁻-P, indicating that nutrient recovery should mainly target the liquid stream.

For the full-scale two-stage FJPWW system, the first stage produced a VFA-rich substrate, and temperature variation within the evaluated range did not significantly affect VFA production. The second stage achieved effective COD and VFA removal and methane production, despite limited TSS removal. In the FJPWW and PS co-fermentation/co-digestion experiments, PS addition did not improve VFA production or methane yield under the tested conditions, and the higher PS fraction slightly reduced methane yield.

Future work should focus on improving process understanding and operational control. For BW UASB digestion, priorities include optimizing sludge removal, investigating hydrolytic microbial communities, characterizing residual soluble COD, and extending the energy balance. For the FJPWW system, further work should monitor biomass and F/M in the first stage, assess granule settleability and solids behavior in the EGSB/R2S reactors, apply CFD, and evaluate alternatives for nutrient and alkalinity management. For co-digestion, future studies should test broader PS sources, mixing ratios, S:I ratios, and pH conditions, while improving BMP scale and measuring gas production during acidogenic fermentation.

Summary in Italian

La rapida urbanizzazione, la crescita industriale e l'aumento della produzione alimentare stanno incrementando la generazione di acque reflue municipali e agro-industriali. Affrontare queste sfide richiede approcci di trattamento efficaci, in grado di consentire il recupero di energia e nutrienti insieme alla rimozione della sostanza organica. Questa tesi si concentra su due differenti flussi di acque reflue ad alta concentrazione: la blackwater separata alla fonte (BW) e le acque reflue derivanti dalla lavorazione dei succhi di frutta (FJPWW). La BW separata alla fonte è costituita dalle acque reflue provenienti dai servizi igienici, raccolte separatamente dagli altri reflui domestici, e comprende principalmente feci, urina, acqua di scarico e carta igienica. La FJPWW è un effluente agro-industriale generato durante il lavaggio della frutta, la spremitura e la produzione di succhi, ed è tipicamente caratterizzata da sostanza organica facilmente biodegradabile e basso pH. Sebbene entrambi i flussi siano adatti al trattamento anaerobico, differiscono per composizione, problematiche di trattamento e potenziale di recupero delle risorse.

La digestione anaerobica (AD) può convertire la sostanza organica presente nelle acque reflue in biogas ricco di metano, trattenendo al contempo nutrienti come azoto e fosforo in correnti che possono essere ulteriormente trattate per il recupero. I reattori anaerobici ad alta velocità, inclusi gli upflow anaerobic sludge blanket (UASB) e gli expanded granular sludge bed (EGSB), sono particolarmente rilevanti per reflui concentrati poiché trattengono biomassa attiva e consentono la conversione della sostanza organica a tempi di ritenzione idraulica relativamente brevi. Tuttavia, gli studi sulle applicazioni a scala reale rimangono limitati per alcuni flussi di reflui, in particolare per la BW separata alla fonte e per i sistemi AD a due stadi a scala reale che trattano reflui agro-industriali altamente biodegradabili.

L'obiettivo generale di questa tesi è stato valutare il trattamento anaerobico della BW separata alla fonte e della FJPWW in sistemi anaerobici ad alta velocità, con particolare attenzione alle prestazioni a scala reale, alla stabilità del processo, alla produzione di metano, alle problematiche operative e alle opportunità di ottimizzazione del processo. Sono stati investigati tre contesti di studio: (i) un reattore UASB a scala reale che tratta BW separata alla fonte a Helsingborg, Svezia; (ii) un sistema AD a due stadi a scala reale che tratta FJPWW a Bronzolo, Italia, costituito da omogeneizzazione/acidificazione seguita da due reattori EGSB (R2S); e (iii) test batch di acidificazione e di potenziale biochimico di metano (BMP) per valutare il co-trattamento della FJPWW con fanghi primari (PS). Nel complesso, questi studi rispondono alla necessità di migliorare le conoscenze sul trattamento anaerobico di reflui ad alta concentrazione e sui fattori operativi che influenzano le prestazioni dei sistemi AD ad alta velocità.

Nel Capitolo 1, la tesi evidenzia la necessità di tecnologie sostenibili ed efficaci per la gestione delle acque reflue, come la digestione anaerobica, al fine di affrontare le sfide associate alla rapida urbanizzazione e alla crescente generazione di reflui municipali e industriali, inclusi la BW separata alla fonte e la FJPWW. La BW viene identificata come un flusso di acque reflue ricco di sostanza organica e nutrienti, rendendola adatta al recupero energetico sotto forma di biogas, principalmente metano, e alla produzione di fertilizzanti. La FJPWW viene inoltre presentata come un substrato con elevate concentrazioni di sostanza organica facilmente

biodegradabile, a supporto del suo potenziale per il recupero energetico tramite trattamento anaerobico. Tuttavia, sia la BW sia la FJPWW rimangono ancora insufficientemente studiate come substrati per la produzione di biogas.

In questo contesto, la tesi introduce tre ambiti di studio: (i) un reattore upflow anaerobic sludge blanket (UASB) a scala reale che tratta BW separata alla fonte a Helsingborg, Svezia; (ii) un impianto di digestione anaerobica a due stadi a scala reale che tratta FJPWW a Bronzolo, Italia, comprendente omogeneizzazione/acidificazione seguita da due reattori expanded granular sludge bed (EGSB/R2S); e (iii) test batch di acidificazione e di potenziale biochimico di metano (BMP) per valutare il co-trattamento della FJPWW con fanghi primari. Nel complesso, questi contesti supportano la gestione sostenibile di flussi di acque reflue ad alta concentrazione e rafforzano la base di conoscenze sui sistemi ad alta velocità (UASB ed EGSB) per la produzione di biogas e il recupero dei nutrienti. La tesi propone inoltre azioni operative volte a migliorare la comprensione delle prestazioni dei sistemi e ad aumentare l'efficienza del processo, con particolare attenzione al funzionamento a scala reale, all'ottimizzazione del processo e alla co-digestione.

Sulla base delle esigenze di ricerca menzionate nel Capitolo 1, nel Capitolo 2 la tesi fornisce le basi concettuali e tecniche necessarie per il successivo lavoro di ricerca. Nel Capitolo 2, la tesi esamina le principali fasi biochimiche della digestione anaerobica e le differenze tra le configurazioni di digestione anaerobica a singolo stadio e a due stadi. Riassume i parametri operativi che influenzano la stabilità e l'efficienza del processo, tra cui il carico organico volumetrico (OLR), i tempi di ritenzione idraulica e dei solidi (HRT e SRT), il controllo del pH, la dinamica dell'alcalinità e degli acidi grassi volatili (VFA), nonché la composizione del substrato. Descrive inoltre la co-digestione come un'efficace strategia di ottimizzazione per bilanciare nutrienti, capacità tampone e biodegradabilità. Inoltre, introduce i principali strumenti analitici utilizzati nella ricerca sulla digestione anaerobica, inclusi i test di potenziale biochimico di metano (BMP) e le misurazioni dell'attività metanogenica specifica (SMA). Infine, sintetizza le evidenze sulle prestazioni dei sistemi UASB ed EGSB ed evidenzia il numero limitato di valutazioni a scala reale della digestione della BW in reattori UASB e dei sistemi a due stadi a scala reale che trattano reflui altamente biodegradabili, definendo così le lacune conoscitive che motivano il capitolo sperimentale.

Nel Capitolo 3, la tesi delinea l'approccio sperimentale e analitico utilizzato per valutare la digestione anaerobica di flussi di acque reflue ad alta concentrazione, con particolare attenzione alla BW separata alla fonte e alla FJPWW. La BW separata alla fonte, raccolta da toilette a vuoto a Oceanhamnen, in Svezia, è stata valutata in un reattore UASB a scala reale, mentre la FJPWW proveniente da Bronzolo, in Italia, è stata studiata in sistemi di digestione anaerobica a due stadi a scala reale e pilota. Il lavoro sulla FJPWW ha incluso anche test batch di co-digestione con fanghi primari e il funzionamento continuo di un sistema pilota di digestione anaerobica a due stadi. Le prestazioni operative a lungo termine sono state valutate utilizzando periodi di pseudo-stato stazionario di almeno 14 giorni e sono state integrate da saggi di potenziale biochimico di metano (BMP) per determinare il potenziale di metano e la biodegradabilità, test di attività metanogenica specifica (SMA) per quantificare l'attività metanogenica, ed esperimenti batch di fermentazione acidogenica per investigare gli effetti della combinazione delle acque reflue derivanti dalla lavorazione dei succhi di frutta con fanghi

primari. Vengono inoltre discusse le principali limitazioni, tra cui il washout dei fanghi e la variabilità di COD/TSS nell'effluente del sistema UASB per blackwater, i dati limitati sul bilancio energetico e microbiologici, il monitoraggio incompleto della biomassa e dell'alcalinità nel sistema FJPWW, l'assenza di analisi sulla sedimentabilità dei fanghi e di simulazioni CFD, nonché le limitate misurazioni del gas durante la fermentazione acidogenica. Nel complesso, la metodologia definisce un quadro integrato a scala reale e batch per valutare le prestazioni della digestione anaerobica, la biodegradabilità del substrato, la stabilità del processo e le potenziali strategie di ottimizzazione.

Nel Capitolo 4, la tesi valuta le prestazioni di un reattore UASB a scala reale (50 m³) che tratta BW separata alla fonte proveniente da toilette a vuoto nel distretto di Oceanhamnen a Helsingborg, in Svezia. Il reattore UASB per BW serviva un equivalente di popolazione reale (PE) pari a 885, che si prevede aumenterà a 2000 nel prossimo futuro. Inoltre, la tesi evidenzia le prestazioni di un reattore UASB a scala reale (41 m³) che co-digerisce BW separata alla fonte con rifiuti alimentari (FW) a Sneek, nei Paesi Bassi. Il reattore UASB per BW è stato monitorato per 130 settimane a 35°C in due fasi operative caratterizzate da differenti carichi organici volumetrici, dovuti all'aumento del numero di abitanti del distretto urbano. La massima rimozione complessiva è stata raggiunta nella Fase 1: il funzionamento con un OLR medio di 0,95 kg COD m⁻³ d⁻¹ ha portato a una rimozione del COD pari all'88% e a un grado di metanizzazione di 0,77 kg COD-CH₄ kg⁻¹ COD_{in}, con una resa di metano pari a 0,307 Nm³ CH₄ kg⁻¹ COD_{removed}. Quando l'OLR medio è stato aumentato a 1,5 kg COD m⁻³ d⁻¹, la rimozione del COD è diminuita al 72%, fenomeno attribuito al washout dei fanghi e al conseguente aumento della variabilità di COD/TSS nell'effluente; tuttavia, il grado di metanizzazione è rimasto elevato, pari a 0,71 kg COD-CH₄ kg⁻¹ COD_{in}, con una resa di metano di 0,304 Nm³ CH₄ kg⁻¹ COD_{removed}. Il bilancio di massa del COD ha mostrato che il 69% del COD influente è stato convertito in metano, il 28% è stato scaricato nell'effluente e il 3% era associato ai fanghi rimossi. I bilanci di massa dei nutrienti hanno indicato che l'effluente decantato conteneva il 92% dell'azoto e l'82% del fosforo, principalmente sotto forma di NH₄⁺-N e PO₄³⁻-P solubili, mentre i fanghi rimossi rappresentavano il 2% dell'azoto e il 18% del fosforo. Nel complesso, questi risultati indicano che un'elevata conversione in metano può essere mantenuta anche a carichi più elevati, sebbene il washout dei fanghi riduca la qualità dell'effluente, suggerendo che potrebbe essere necessario un post-trattamento per soddisfare i requisiti di scarico in determinati regimi operativi.

La co-digestione della BW separata alla fonte con FW ha mostrato che il funzionamento intermittente a temperature variabili (22,5°C in inverno e 35°C in estate) ha determinato un'efficienza di rimozione del COD_{totale} occasionalmente inferiore ma comunque comparabile rispetto alla mono-digestione della BW. L'aumento della temperatura a 35°C ha migliorato il grado di metanizzazione da 0,60 a 0,68 kg CH₄-COD kg⁻¹ COD_{in}; tuttavia, la metanizzazione nel sistema UASBBW+FW è rimasta inferiore dell'11–24% rispetto al massimo raggiunto nella mono-digestione della BW (0,77 kg CH₄-COD kg⁻¹ COD_{in}), evidenziando la ridotta efficienza della co-digestione nelle condizioni sperimentali caratterizzate da rapporti di miscelazione BW/FW non controllati. Il grado di metanizzazione potrebbe essere stato influenzato dal washout della biomassa o da squilibri microbici causati da rapporti BW/FW non regolati, mentre in letteratura sono riportati come ottimali rapporti pari a 1:1 o 1:2.

Nel Capitolo 5, la tesi valuta un sistema AD a due stadi a scala reale che tratta FJPWW presso l'impianto di trattamento delle acque reflue di Bronzolo (Italia settentrionale), sulla base di dati operativi a lungo termine raccolti nell'arco di 6 anni. Nel primo stadio, un serbatoio di omogeneizzazione ha prodotto un intermedio ricco di VFA, caratterizzato da un rapporto VFA/sCOD del 38–41% e da un grado di acidificazione del 21–25%. Nei periodi valutati, il funzionamento a differenti temperature (16–26°C) non ha avuto un'influenza significativa né sulla rimozione del COD né sul grado di acidificazione. Il secondo stadio, costituito da due reattori paralleli ad alta velocità EGSB/R2S, ha raggiunto prestazioni complessive efficaci, con una riduzione del COD totale da 2902 a 349 mg COD L⁻¹ (88% di rimozione), accompagnata da un'efficace rimozione dei VFA (VFA come HAc da 1082 a 69,2 mg HAc L⁻¹). La stabilità operativa è stata riflessa da un rapporto VFA/Alk pari a 0,07. La resa di metano ha raggiunto 0,277 Nm³ CH₄ kg⁻¹ COD removed e il contenuto medio di metano è stato dell'80%. Sebbene la conversione del COD sia risultata efficace, la rimozione dei TSS è rimasta bassa (6,2%); questo risultato è stato associato potenzialmente alla presenza di solidi o al washout dei fanghi, evidenziando come le prestazioni di conversione e la qualità dei solidi nell'effluente possano divergere e sottolineando la necessità di una gestione dedicata dei solidi e di eventuali trattamenti successivi.

Nel Capitolo 6, la tesi discute l'effetto della co-fermentazione e della co-digestione della FJPWW con fanghi primari (PS) sulla generazione di VFA e sulla resa di metano, valutato mediante fermentazione acidogenica batch controllata (100 ore), seguita da test BMP a 35°C (terminati dopo 120 ore). Le condizioni di miscelazione valutate comprendevano FJPWW da sola e rapporti FJPWW pari a 97:3 e 90:10 (vol/vol). Il picco di acidificazione si è verificato entro circa 24 ore, raggiungendo valori massimi di DoA del 33% per la FJPWW, del 31% per il rapporto 97:3 e del 15% per il rapporto 90:10, con corrispondenti rese massime di VFA pari rispettivamente a 0,89, 0,86 e 0,45 g net TVFA-COD gVS⁻¹. Le rese di metano sono risultate simili per la FJPWW da sola e per il rapporto 97:3 (circa 504 NmL CH₄ gVS⁻¹), mentre una resa inferiore è stata osservata per il rapporto 90:10 (483,7 NmL CH₄ gVS⁻¹). Nelle condizioni e nei rapporti testati, l'aggiunta di fanghi primari non ha migliorato né la produzione di VFA né la resa di metano. L'impostazione sperimentale ha inoltre evidenziato un'importante limitazione metodologica: la produzione di gas non è stata monitorata durante la fase di co-fermentazione acidogenica, limitando così l'interpretazione dei percorsi del COD.

Nel Capitolo 7, la tesi integra i risultati ottenuti e dimostra che l'interpretazione delle prestazioni a scala reale risulta rafforzata considerando congiuntamente: (i) indicatori basati sulla conversione (metanizzazione, resa di metano, rimozione dei VFA), (ii) qualità dell'effluente e comportamento dei solidi, e (iii) test mirati di attività (SMA). Nei sistemi a scala reale è stata osservata una conversione stabile del metano insieme alla presenza di COD residuo nell'effluente e/o a una limitata rimozione dei solidi, dimostrando che il “successo” del processo non può essere valutato esclusivamente sulla base di metriche di conversione biologica quando il washout dei solidi rappresenta il principale fattore responsabile della variabilità dell'effluente.

Nel Capitolo 8, la tesi presenta le principali conclusioni e le future necessità di ricerca. Per il sistema UASB a scala reale trattante BW, la massima rimozione del COD e il più elevato grado di metanizzazione sono stati ottenuti al più basso OLR. A valori più elevati di OLR, la

rimozione del COD è diminuita principalmente a causa del washout dei fanghi, sebbene la conversione in metano sia rimasta elevata. I bilanci di massa hanno mostrato che la maggior parte del COD influente è stata convertita in metano, mentre la maggior parte dell'azoto e del fosforo è rimasta nell'effluente liquido sotto forma di $\text{NH}_4^+\text{-N}$ e $\text{PO}_4^{3-}\text{-P}$, indicando che il recupero dei nutrienti dovrebbe concentrarsi principalmente sulla fase liquida.

Per il sistema FJPWW a due stadi a scala reale, il primo stadio ha prodotto un substrato ricco di VFA e la variazione della temperatura nell'intervallo valutato non ha influenzato significativamente la produzione di VFA. Il secondo stadio ha ottenuto un'efficace rimozione del COD e dei VFA e una buona produzione di metano, nonostante la limitata rimozione dei TSS. Negli esperimenti di co-fermentazione/co-digestione tra FJPWW e PS, l'aggiunta di fanghi primari non ha migliorato né la produzione di VFA né la resa di metano nelle condizioni testate, mentre una maggiore frazione di PS ha leggermente ridotto la resa di metano.

Le attività future dovrebbero concentrarsi sul miglioramento della comprensione dei processi e del controllo operativo. Per la digestione della BW in reattori UASB, le priorità includono l'ottimizzazione della rimozione dei fanghi, l'indagine delle comunità microbiche idrolitiche, la caratterizzazione del COD solubile residuo e l'estensione del bilancio energetico. Per il sistema FJPWW, ulteriori studi dovrebbero monitorare la biomassa e il rapporto F/M nel primo stadio, valutare la sedimentabilità dei granuli e il comportamento dei solidi nei reattori EGSB/R2S, applicare simulazioni CFD e valutare alternative per la gestione dei nutrienti e dell'alcalinità. Per la co-digestione, studi futuri dovrebbero testare una gamma più ampia di fonti di fanghi primari, rapporti di miscelazione, rapporti S e condizioni di pH, migliorando al contempo la scala dei test BMP e monitorando la produzione di gas durante la fermentazione acidogenica.

Table of Contents

Acknowledgments	II
List of abbreviations.....	IV
List of publications.....	VI
Summary in English.....	VII
Summary in Italian	XI
List of Figures for the Main Chapters	XVIII
List of Figures for the Supplementary Information	XIX
List of Tables for the Main Chapters.....	XX
List of Tables for the Supplementary Information	XXI
Chapter 1. Introduction.....	3
1.1 . Background.....	3
1.2. Research Motivation and Problem Context:	6
1.3. Research objectives	6
1.4. Structure of the thesis	6
Chapter 2. Literature Review	10
2.1. Fundamentals of Anaerobic Digestion.....	10
2.2. Key factors affecting the Anaerobic Digestion process	11
2.3. Anaerobic Reactor Technologies Studied in the Thesis	15
2.4. State-of-the-Art in Source-Separated Blackwater Treatment via AD	18
2.5. Anaerobic Digestion of Highly Biodegradable Wastewater from the beverage industry (Fruit Juice Processing Wastewater)	20
2.6. Principles and Applications of Co-digestion	20
2.8. Knowledge Gaps Addressed by This Thesis.....	22
Chapter 3. Methodology	26
3.1. Case studies	26
3.2. Analytical methods.....	27
3.3. Limitations of the studies	27
Chapter 4. Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden	31
4.1. Abstract	31
4.2. Introduction.....	31
4.3. Methods.....	32
4.4. Results and discussion	36
4.5. Conclusions.....	48
Chapter 5. Performance Evaluation of a Full-Scale Two-Stage Anaerobic Digestion System Treating Fruit Juice Processing Wastewater.....	51

5.1. Abstract.....	51
5.2. Introduction.....	51
5.3. Methods and Materials.....	53
5.4. Results and Discussion	57
5.5. Conclusions.....	67
Chapter 6. Impact of co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production.....	71
6.1. Abstract.....	71
6.2. Introduction.....	71
6.3. Methods and Materials.....	74
6.4. Results and Discussion	77
6.5. Conclusions.....	86
Chapter 7. Synthesis and Concluding Discussion	89
7.1. Synthesis of Key Findings.....	89
7.2. Scientific Contributions of the Thesis	92
Chapter 8. Conclusions and recommendations for future research	95
8.1. Overall Conclusions	95
8.2. Recommendations for Future Research	96
References	100
Supplementary Information	124
Appendix A. Supplementary material for chapter 4.....	124
Appendix B. Supplementary material for Chapter 5	128
Appendix C. Supplementary material for Chapter 6	139
Appendix D. Operation of a pilot two-stage anaerobic digestion system	140
D.1. The Aim	140
D.2. Methods and Materials	140
D.3. Operational challenges and insights from running the pilot-scale two-stage anaerobic digestion system.....	142
Appendix E. Experimental photos	144

List of Figures for the Main Chapters

Fig. 2. 1. Stages of anaerobic digestion (AD). Adapted from Tho & Thanh (2025).	10
Fig. 2.2. Relationship between substrate degradation rate and hydraulic retention time in anaerobic digestion (AD) processes for different substrates. Adapted from Akunna (2018).	14
Fig. 2.3. UASB reactor process scheme. Adopted from Mainardis et al. (2020).	16
Fig. 2.4. Full-scale UASB reactor treating BW.	16
Fig. 2.5. EGSB reactor process scheme. Adopted from Chen et al. (2020).	17
Fig. 2.6. Full-scale EGSB reactors (highlighted in red) treating FJPWW.	17
Fig. 2.7. Conceptual framework for technological resource recovery with an emphasis on water and nutrient recovery. Adapted from Bisschops et al. (2019).	19
Fig. 4.1.A) Full-scale UASB treating BW at RecoLab, Helsingborg B) Schematic of the BW treatment process and sampling points for mass balance calculations. Green dots indicate grab samples, green dots with red outlines indicate flow-proportional samples, and blue triangles indicate flow-measurement points. Sampling valves for settleable solids and suspended solids measurements at each meter of the digester are indicated.	34
Fig. 4.2. Variations in the influent flow rates and the organic loading rates (OLR) of UASB _{BW} during the two phases of operation (Phase 1 = OLR lower than 0.95 kg COD m ⁻³ d ⁻¹ and Phase 2 = OLR around 1.5 kg COD m ⁻³ d ⁻¹).	39
Fig. 4.3. Variations in settleable solids at 5 m height, and in effluent concentrations of COD _{total} and TSS of UASB _{BW} (Phase 1 = OLR lower than 0.95 kg COD m ⁻³ d ⁻¹ and Phase 2 = OLR around 1.5 kg COD m ⁻³ d ⁻¹).	41
Fig. 4.4. Accumulated biogas yield versus accumulated influent COD load in the UASB _{BW}	43
Fig. 4.5. Mass balances for wet mass, COD _{total} , Total Nitrogen (N), and Total Phosphorus (P). The mass balances are expressed as percentages relative to the influent mass.	44
Fig. 4.6. Soluble COD (sCOD) concentrations, total suspended solids (TSS) concentrations (average values and standard deviation), and the sCOD equivalent of volatile fatty acid (VFA) concentrations measured along the height of the UASB _{BW} reactor.	47
Fig. 5.1. A) Full-scale homogenization tank and filtration unit (highlighted in red), and full-scale EGSB/R2S reactors (highlighted in blue). B) Schematic drawing of the fruit juice processing wastewater (FJPWW) treatment process.	55
Fig. 5.2. Impact of OLR on daily methane production in the EGSB/R2S reactors.	64
Fig. 5.3. Methane yield in the EGSB/R2S reactors. The blue solid curve shows the measured cumulative methane production plotted against the cumulative COD removed. The orange dashed line represents the linear regression used to estimate the methane yield from the slope of the relationship.	65
Fig. 5.4. Specific methanogenic activity test (SMA) of granular sludge.	66
Fig. 6.1. A) Schematic of the fermentation experiment. B) Co-digestion of FJPWW and PS BMP test using the OxiTop®-IDS AN 6 system.	75
Fig. 6.2. COD profile during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.	77
Fig. 6.3. Concentrations of acetic (A), propionic acid (B), butyric (C), Iso-valeric (D), Valeric (E), and total VFAs (TVFAs) (F) during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.	79
Fig. 6.4. pH profile (A), and alkalinity profile (B) during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.	80
Fig. 6.5. VFA composition at the end of the acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.	82
Fig. 6.6. DoA profile (A), and VFA yield profile (B) during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.	83
Fig. 6.7. BMP of FJPWW and its co-digestion with PS at different vol./vol. ratios, expressed as methane yield per VS mass.	85

List of Figures for the Supplementary Information

Fig. A.1. Blackwater System overview.	124
Fig. B.1. Temporal dynamics of COD, VFA, and pH in the first-stage homogenization tank of the two-stage anaerobic digestion plant. A) Influent and effluent COD concentrations. B) Effluent VFA concentrations expressed as HAc. C) Influent and effluent pH.	128
Fig. B.2. Temporal variation in the temperature of the R2S reactors.	129
Fig. B.3. Temporal variation in influent and effluent pH in the R2S reactors.	129
Fig. B.4. Temporal variation in influent and effluent COD concentrations in the R2S reactors.	130
Fig. B.5. Temporal variation in influent and effluent COD concentrations in the R2S reactors.	130
Fig. B.6. Temporal variation in daily methane production from the R2S reactors.	131
Fig. B.7. Relationship between influent total COD and VFA production in the homogenization tank under different operating temperatures.	131
Fig. B.8. Relationship between temperature and VFA production in the homogenization tank.	132
Fig. B.9. Relationship between pH and VFA production in the homogenization tank under different operating temperatures.	132
Fig. B.10. Relationship between hydraulic retention time (HRT) and VFA production in the homogenization tank under different operating temperatures.	133
Fig. B.11. Relationship between influent total COD and degree of acidification (DoA) in the homogenization tank under different operating temperatures.	133
Fig. B.12. Relationship between pH and degree of acidification (DoA) in the homogenization tank under different operating temperatures.	134
Fig. B.13. Relationship between HRT and degree of acidification (DoA) in the homogenization tank under different operating temperatures.	134
Fig. B.14. Relationship between COD:N ratio and degree of acidification (DoA) in the homogenization tank under different operating temperatures.	135
Fig. B.15. Relationship between organic loading rate (OLR) and daily methane production in the methanogenic reactors at different biomass masses.	135
Fig. B.16. Relationship between volumetric organic loading rate (OLR) and daily methane production in the methanogenic reactors at different biomass masses.	136
Fig. B.17. Relationship between hydraulic retention time (HRT) and daily biogas production in the methanogenic reactors at different biomass masses.	136
Fig. B.18. Relationship between temperature and daily biogas production in the methanogenic reactors at different biomass masses.	137
Fig. B.19. Relationship between daily removed COD mass and daily methane production in the methanogenic reactors at different biomass masses.	137
Fig. D.1. Pilot-scale two-stage AD system.	140
Fig. D.2. Schematic of the pilot-scale two-stage AD system.	140
Fig. E.1. Sampling from the full-scale BW UASB.	144
Fig. E.2. SMA test for BW samples.	144
Fig. E.3. Pressure measurement during the SMA test on BW samples.	145
Fig. E.4. HPLC.	145
Fig. E.5. FJPWW and PS co-fermentation test preparation.	146

List of Tables for the Main Chapters

Table 4. 1. BW influent characteristics and effluent quality of UASB systems (average values and standard deviations).	38
Table 4.2. Performance of UASB systems in BW: biogas yield, degree of methanization, and methane production rate with organic loading rates (average values with standard deviations).....	41
Table 4.3. COD _{total} mass balance in anaerobic digestion of BW: average values expressed as percentages relative to influent COD _{total}	45
Table 4.4. Average values of SMA of sludge from BW UASB systems.....	46
Table 5.1. FJPWW influent characteristics and effluent quality of the homogenization tank during the monitoring period 2019-2026: average values of the main parameters (standard deviations); n = number of data points used to calculate the average and standard deviation.....	59
Table 5.2. Performance of the homogenization tank: average values of the main parameters (standard deviations).	61
Table 5.3. Influent characteristics and effluent quality of methanogenic reactors (EGSB/R2S) treating highly biodegradable wastewater for biogas production during the monitoring period 2019-2026: average values of the main parameters (standard deviations); n = number of data points used to calculate the average and standard deviation.	62
Table 5.4. Performance of bioreactors treating highly biodegradable wastewater: organic loading rates and methane yield (average values with standard deviations).	65
Table 5.5. SMA values for different types of sludge.	66
Table 6. 1. Influent and Effluent Characteristics of Substrates Used in the Acidogenic Fermentation Experiment (average values, and standard deviations in brackets).	78

List of Tables for the Supplementary Information

Table A1. Population Equivalent (PE) Estimation for RecoLab Based on Influent Loads and Flow (Using Jönsson et al., 2005; Adapted from Gren, 2024).	124
Table A2. Comparison of Default Per-Capita Loads and Present Work Values for Estimating Population Equivalent (PE) and Flow per PE (Jönsson et al., 2005; Adapted from Gren, 2024)	125
Table A3. Weekly Mass Balance of Wet Mass, COD, Nitrogen, and Phosphorus: Average Values of the Main Parameters (Standard Deviations); n = Number of Data Points Used to Calculate the Average and Standard Deviation (Adapted from Gren, 2024).	125
Table A4. Weekly Mass Balance Normalized to per-COD (72.6 gCOD pe ⁻¹ d ⁻¹): average values of the main parameters (Adapted from Gren, 2024).	125
Table A5. Influent Characteristics Normalized to per-COD (72.6 g COD pe ⁻¹ d ⁻¹): average values of the main parameters (Adapted from Gren, 2024).	126
Table A6. BW UASB energy calculations: average values of the main parameters (standard deviations); n = number of data points used to calculate the average and standard deviation.	126
Table A7. Operational parameters applied to UASB systems treating BW: average values of the main parameters (standard deviations).	127
Table B1. Operating conditions of the homogenization tank during 2019-2016 (average values and standard deviations).	138
Table B2. Operating conditions of the EGSR/R2S reactors during 2019-2026 (average values and standard deviations).	138
Table C1. Characteristics of Raw Substrates Used in the Fermentation and Co-fermentation Experiment. (average values and standard deviations).	138
Table D1. Fruit juice processing wastewater influent characteristics.	139
Table D2. Operating conditions of the homogenization tank.	141
Table D3. Operating conditions for the biogas production reactor.	141



Chapter 1

Introduction

Chapter 1. Introduction

1.1. Background

The urgent need for sustainable wastewater management is driven by rapid urbanization, industrial growth, and increasing food production, all of which contribute to rising volumes of municipal and agro-industrial wastewater such as source-separated blackwater (BW) and fruit juice processing wastewaters (FJPWW) (Abdel-Shafy et al., 2009; Abuzir et al., 2025; Cremones et al., 2021; Cuff et al., 2018; El-Khateeb et al., 2023). Addressing these challenges requires effective and innovative solutions that not only biologically treat wastewater efficiently but also recover valuable resources such as energy, water, and nutrients. Anaerobic digestion (AD) has emerged as a promising biological technology in this context, offering the dual benefits of organic matter removal and renewable biogas production from a wide range of waste streams (Zheng & Li, 2024).

Energy in the form of biogas, along with fertilizers, could be produced from municipal and agro-industrial wastewaters. Among the various types of wastewaters, BW and FJPWW stand out as promising but underexplored substrates for biogas production in full-scale AD applications, mainly because of their high organic matter content and their nutrient content. PS is also relevant in this context, as it is a readily available municipal wastewater treatment by-product with substantial organic matter content. Its co-digestion with FJPWW could be interesting because PS may increase the alkalinity and nutrients concentrations for FJPWW and therefore potentially higher overall methane potential of the feed, while FJPWW may contribute readily biodegradable soluble COD that supports acidification and subsequent methanogenesis.

BW is a separately collected fraction within source-separation systems, originating from toilets and characterized by high concentrations of carbon, suspended solids, and nutrients (e.g., nitrogen and phosphorus) (Gao et al., 2019; Hernandez Leal et al., 2007; Otterpohl et al., 1999; Skambraks et al., 2017; Wang et al., 2023). When vacuum toilets are used, BW can be captured with minimal volume of water (approximately one liter per flush), which supports its suitability for biogas production via AD and nutrient recovery (Gao et al., 2019b; Kjerstadius et al., 2015; Otterpohl et al., 2004). Nutrient recovery from BW after AD can be achieved via processes such as struvite precipitation (Xu et al., 2023) and ammonia stripping (Kujawa-Roeleveld et al., 2006), enabling subsequent use as fertilizers (Desmidt et al., 2015; Moges et al., 2020).

FJPWW is an agro-industrial effluent produced during fruit washing, pressing, and juice manufacturing, and is generally characterized by a high concentration of readily biodegradable organic matter (375-124,800 mg COD L⁻¹) and is typically characterised by low pH and low alkalinity, along with a high carbon-to-nitrogen (C:N) ratio that can cause nutrient imbalances in the AD process (Dold & Ekama, 1981; Ozbas et al., 2006; Tyagi et al., 2019). In addition, substantial variability in flow volume, combined with these intrinsic properties, further complicates treatment (Austerman-Haun & Rosenwinkel, 1997; Chen et al., 2019; Dold & Ekama, 1981; Ozbas et al., 2006; Valta et al., 2015; Zerrouki et al., 2021). Despite these challenges, the high organic content of FJPWW makes it a suitable feedstock for generating biogas as a clean and environmentally friendly energy source via AD (Mateescu et al., 2008; Zerrouki et al., 2021).

AD is a complex process involving several stages, each carried out by different groups of microorganisms. These microbes work together to convert organic matter into biogas, which consists mainly of methane (CH₄) and carbon dioxide (CO₂), as well as a nutrient-rich digestate that can be used as fertilizer (Appels et al., 2008; Joshi & Visvanathan, 2019; Zheng & Li, 2024). The process includes four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In hydrolysis, complex polymers are broken down into simpler monomers. Acidogenesis then transforms these monomers into volatile fatty acids and alcohols. During acetogenesis, these intermediates are further converted into acetate, hydrogen, and carbon dioxide. Finally, methanogenesis produces methane, completing the process (De et al., 2019; Khanh et al., 2021; Pan et al., 2021; Paul et al., 2022; Wang et al., 2020).

AD of wastewater can be performed in a single stage or two stages. In single-stage AD, all phases (i.e. hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occur in one reactor. The two-stage process separates these phases to optimize conditions for specific microbes, improving efficiency (Ferrera et al., 2022). The first stage supports hydrolytic and acidogenic bacteria at pH 5 to 6 with shorter retention times (Ketheesan et al., 2015), while the second stage favors methanogens at pH 6 to 8 with longer retention times (Ferrera et al., 2022). This approach is especially useful for high-sugar wastewaters like apple juice wastewater, which can cause instability and acidification in single-stage systems (Feng et al., 2020; Mari et al., 2020).

The efficiency and stability of AD depend on several key operational parameters (Chapter 2). These include temperature, pH, substrate composition (with particular attention to the carbon to nitrogen ratio i.e. C/N), hydraulic and sludge retention times (HRT and SRT), volatile fatty acids to alkalinity ratio (VFA/ALK), food to microorganism ratio (F/M), and the organic loading rate (OLR). The balance of carbohydrates, proteins, and lipids in the substrate directly affects both biogas yield and the overall robustness of the process (Batstone et al., 2002; Rasapoor et al., 2020).

A variety of reactor configurations have been developed for AD. Continuously stirred tank reactors (CSTRs) are among the most commonly used configurations, particularly for sludge, manure, and other feedstocks with relatively high suspended solids content (Shah et al., 2024). However, CSTRs are generally less suitable for high-rate treatment of soluble or low-solids wastewaters because the hydraulic retention time (HRT) and solids retention time (SRT) are closely linked, which limits biomass retention at short HRTs. For such applications, high-rate anaerobic systems that retain active biomass are often more appropriate under suitable wastewater and operating conditions. Examples include the upflow anaerobic sludge blanket (UASB) reactor and the expanded granular sludge blanket (EGSB) reactor, with the latter representing a development of the UASB concept through higher upflow velocities and enhanced contact between wastewater and granular biomass (Abuzir et al., 2025; Cuff et al., 2018) (Chapter 2). UASB reactor is being widely adopted for both municipal, industrial, and agro-industrial applications (Chong et al., 2012). UASB reactors promote efficient contact between wastewater and granular sludge, enabling high organic loading rates and short hydraulic retention times while retaining slow-growing methanogens (Ouyang et al., 2018; Van Lier et al., 2009). Advanced designs, such as the EGSB reactor, further enhance loading capacity and operational stability (Chen et al., 2020).

EGSB reactors (Chapter 2) are the most widely implemented configuration for industrial wastewater treatment, accounting for approximately 90% of these systems globally (van Lier et al., 2015). Similar to UASB systems, their performance relies on the formation of dense granular biomass without the need for added support media. This granulation is promoted by upflow-induced turbulence, which improves biomass–substrate interactions and allows operation at high OLR with shorter HRT than conventional digesters (Cruz-Salomón et al., 2019). Although UASB and EGSB reactors rely on the same underlying biological processes, EGSB systems are run at markedly higher upflow velocity (V_{up}), typically 4–10 m h⁻¹ compared with 0.5–1.0 m h⁻¹ in UASB. Operating at this elevated V_{up} enhances hydraulic mixing and strengthens mass transfer.

Although mono-digestion is widely implemented, it is associated with several well recognized drawbacks, including operational instability (Li et al., 2018), limited year-round supply of some substrates (Mata-Alvarez et al., 2014), and lower biogas/methane yields (Zahan et al., 2018). In particular, highly biodegradable organic waste streams (e.g., FJPWW) can promote a rapid accumulation of volatile fatty acids (VFA) to high concentrations within digesters, which can inhibit methanogens (Li et al., 2018). Several of these challenges can be mitigated through operational interventions, including pH adjustment (via alkali supplementation) (Zapata Martínez et al., 2019), retention time control (Vanwonterghem et al., 2015), intermittent feeding (Bonk et al., 2018), micro-aeration (Nguyen et al., 2019), and the implementation of a two-stage AD system (Rajendran et al., 2020). Nevertheless, applying these measures typically adds to operational complexity and increases costs.

Anaerobic co-digestion (Chapters 2 and 6) can alleviate several drawbacks of mono-digestion by treating two or more feedstocks in a single process (Karki et al., 2021). Its main benefits are greater operational stability and increased methane production, which are commonly attributed to synergistic effects fostering a more diverse microbial community (Mata-Alvarez et al., 2014), improving nutrient balance through an appropriate carbon-to-nitrogen (C/N) ratio and trace-element supplementation (Xie et al., 2018), and improve AD process stability through improved buffering. In this context, combining rapidly biodegradable substrates, such as fruit FJPWW or food waste (FW), with substrates that degrade more slowly or provide greater buffering capacity, such as PS, can help balance acid production with methanogenic conversion. This balance is important because substrates rich in readily biodegradable organic matter may lead to rapid VFA accumulation and acidification when the acidogenic rate exceeds the capacity of the methanogenic community. Therefore, co-digestion can be used not only to increase methane production, but also to balance degradation kinetics, nutrient availability, and buffering capacity within the anaerobic system (Bolzonella et al., 2006; Elsayed et al., 2020; Paranjpe & Saxena, 2023; Tyagi et al., 2019).

Despite these advances, the application and studies of full-scale UASB and EGSB reactors to challenging substrates like BW and FJPWW remains limited. These wastewaters present unique operational challenges, including high variability in composition, potential for acidification, and nutrient imbalances, which can compromise process stability and biogas yields. Addressing these gaps is essential for realizing the full potential of AD in sustainable wastewater management and renewable energy recovery.

1.2. Research Motivation and Problem Context:

While UASB reactors have demonstrated successful treatment of BW at laboratory and pilot scales, their application at full scale remains limited (Chapter 3). Moreover, although full-scale EGSB reactors have been investigated for highly biodegradable wastewaters, in-depth evaluations of full-scale two-stage AD systems remain scarce. In particular, practical guidance on on-site monitoring of key operational parameters to better interpret two-stage AD performance and support process optimization is still limited (Chapter 4). Finally, research on co-digestion strategies for FJPWW at laboratory or pilot scales remains insufficient, despite the operational challenges associated with its low alkalinity and low nutrient content, together with its high carbon content that can promote rapid acidification during AD (Chapter 5).

Given the global imperative to improve resource recovery from wastewater and the limited research on advanced AD systems for these specific substrates, there is a need to investigate the performance, and optimization of UASB and EGSB reactors for BW and FJPWW treatment.

1.3. Research objectives

The overarching aim of this thesis is to **advance the understanding and application of high-rate AD UASB systems for treating BW and FJPWW, to propose operational actions to improve understanding of system performance and to enhance the performance of such systems**, with emphasis on full-scale operation, process optimization, and co-digestion. Specifically, this research seeks to:

1. Conduct an overall performance evaluation of full-scale anaerobic digesters, with proposed operational actions to improve understanding of system performance and enhance the performance of such systems.
2. Evaluate the co-digestion potential of FJPWW and PS to enhance overall AD process performance.

1.4. Structure of the thesis

This thesis consists of seven chapters, of which the present **Chapter 1** is the Introduction.

Chapter 2 presents a literature review on AD fundamentals, anaerobic reactors used for AD, AD of BW, AD of highly biodegradable wastewater, and co-digestion.

Chapter 3 provides an overview of the case studies, substrates, analytical methods, and main methodological limitations included in the thesis. The case studies are first introduced together with their substrates, reactor configurations, study scales, and links to the research objectives. The following sections then summarize the analytical methods and limitations relevant to interpreting the results. Detailed methodological descriptions are provided in the corresponding study chapters.

Chapter 4 presents a study on a novel full-scale UASB reactor treating BW. This work has been published as a paper entitled “Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden (*Cleaner Water*, 4, 100082.). The research was conducted at Lund University and NSVA in Helsingborg, Sweden.

Chapter 5 focuses on the AD of FJPWW in a two-stage AD system consisted of homogenization tank and two full-scale EGSB reactors at the Bronzolo treatment plant in Italy. This work is ready for submission to a peer-reviewed journal as a paper entitled “Performance Evaluation of a Full-Scale Two-Stage Anaerobic Digestion System Treating Fruit Juice Processing Wastewater”. The research was conducted at Bronzolo treatment plant and the university of Brescia, Italy.

Chapter 6 investigates the potential of co-fermenting and co-digesting FJPWW with PS to improve the VFA production and methane production and overall anaerobic digestion performance. This work is ready for submission to a peer-reviewed journal as a paper entitled “co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production” (ref....). The research was conducted at Bronzolo treatment plant and the university of Brescia, Italy.

Chapter 7 provides an overall discussion of the key findings from Chapters 3, 4, and 5. It connects the insights gained from treating different waste streams, such as BW and FJPWW, using high-rate AD systems, and highlights the scientific contributions of the thesis. This chapter also discusses the limitations of the studies and explores their practical and environmental implications.

Chapter 8 summarizes the main conclusions and offers suggestions for future research.



Chapter 2

Literature Review

Chapter 2. Literature Review

2.1. Fundamentals of Anaerobic Digestion

Anaerobic digestion (AD) consists of four metabolic stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Fig. 2.1). During hydrolysis (solubilization), extracellular enzymes depolymerize high molecular weight substrates (lipids, carbohydrates, and proteins) into soluble organic compounds, notably fatty acids, sugars, and amino acids (Van et al., 2020). These products are subsequently fermented during the acidogenesis stage, where acidogenic microorganisms transform them into volatile fatty acids (VFAs) (Chozhavendhan et al., 2023). In the third stage (acetogenesis), acetogens convert most VFAs into acetic acid (CH_3COOH), hydrogen (H_2), and carbon dioxide (CO_2) (Srisowmeya et al., 2020). In the final stage (methanogenesis), methane (CH_4) is produced by the methanogens. Methane is produced mainly via two routes: hydrogenotrophic and acetoclastic methanogenesis. In acetoclastic methanogenesis, acetotrophic methanogens convert carboxylic acid into methane and carbon dioxide, whereas in hydrogenotrophic methanogenesis, hydrogenotrophic methanogens produce methane from carbon dioxide and hydrogen (Srisowmeya et al., 2020; Van et al., 2020).

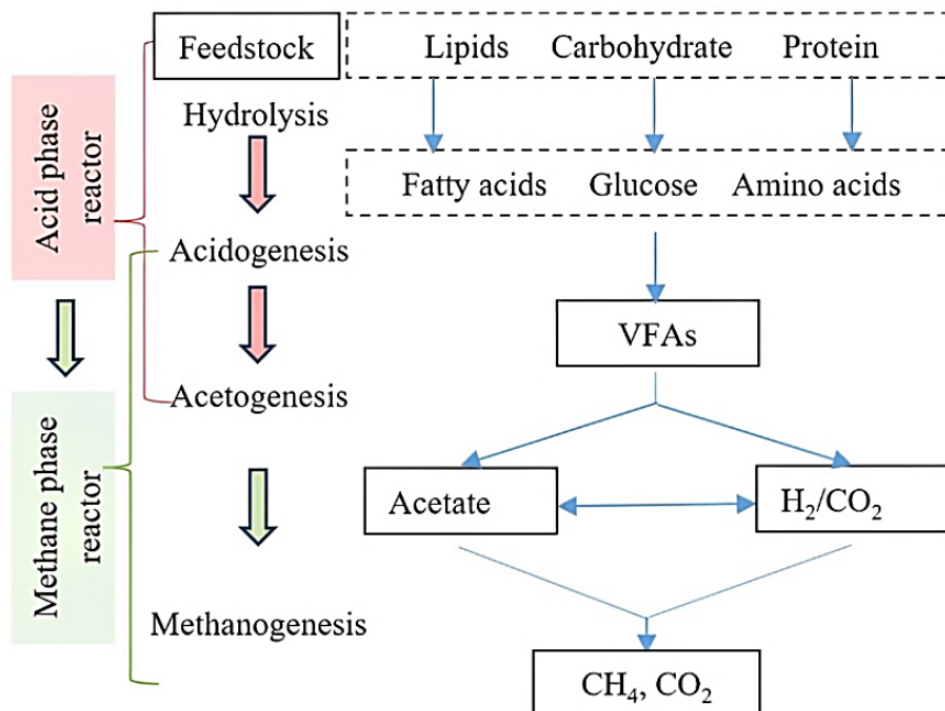


Fig. 2. 1. Stages of anaerobic digestion (AD). Adapted from Tho & Thanh (2025).

AD may be implemented as a single-stage or a two-stage process or multiple stages. In a single-stage configuration, all microbial conversions occur in one reactor. By contrast, two-stage AD separates the phases so that operating conditions can be aligned with the requirements of each microbial group, thereby improving efficiency (Holl et al., 2022; Simeonov et al., 2025; Srisowmeya et al., 2020). This separation is driven by intrinsic differences in growth kinetics and process optima. Microorganisms involved in hydrolysis and acidogenesis exhibit higher growth rates (0.05 to 1.79 h^{-1}) and generally prefer mildly acidic conditions (Xu et al., 2012). Conversely, microorganisms responsible for the methane production step (methanogens) have a slower growth rate (0.008 to 0.173 h^{-1}) and perform best under a slightly alkali environment

(Xu et al., 2012). In addition, acidogenic bacteria are less affected by changes in environmental factors such as temperature and pH (Venkiteshwaran et al., 2015).

In two-stage AD, the first reactor is operated to favor hydrolytic and acidogenic bacteria (pH 5–6, short hydraulic retention times), generating VFAs along with CO₂ and H₂ (Fuentes et al., 2021). The second reactor is then maintained to support methanogens (pH 6–8, longer retention times), where acetic acid is converted to methane (Fuentes et al., 2021; Simeonov et al., 2025; Srisowmeya et al., 2020). Compared with single-stage operation, this arrangement typically provides greater stability and increased tolerance to organic loads, and it may facilitate recovery of valuable byproducts (Fuentes et al., 2021). Phase separation also strengthens pH control, supporting higher biogas yields and improved substrate degradation (Fuentes et al., 2021; Perera et al., 2012). For instance, in food waste digestion, two-stage systems have been reported to reach COD removal efficiencies 35% higher than single-stage systems (Parra-Orobio et al., 2021).

Two-stage AD may be especially appropriate for high-sugar wastewaters such as FJPWW, which can destabilize single-stage systems (Feng et al., 2020). Reported outcomes include acidification degrees of 54–89%, and methane yields of 0.25–0.37 m³ kg⁻¹ COD_{removed} suggesting effective performance of two-stage AD systems (Bergland et al., 2010; Carvalheira et al., 2018; Mateus et al., 2020).

2.2. Key factors affecting the Anaerobic Digestion process

The AD process is influenced by several substrate-related, microbial, and operational parameters, including the food-to-microorganism ratio (F/M), pH, volatile fatty acid-to-alkalinity ratio, temperature, substrate characteristics, organic loading rate (OLR), hydraulic retention time (HRT), solids retention time (SRT), and carbon-to-nitrogen ratio (C/N). This section discusses the effects of these parameters on overall AD performance.

2.2.1. Food to Microorganism (F/M) ratio

F/M ratio represents the capacity of the microbiota to utilize the available substrate (Tho & Thanh, 2025). Maintaining the F/M ratio within an appropriate range is consistently identified as a primary determinant of bioreactor efficiency (Tho & Thanh, 2025). Food refers to the organic matter load, commonly quantified as total organic carbon, COD, or VS (Tho & Thanh, 2025). The microbial fraction is typically expressed as mixed liquor volatile suspended solids (MLVSS) for liquid feedstocks and as VS for solid feedstocks.

In wet single-stage AD (low TS content, TS < 10%), stable methane formation has been reported at low F/M values (< 0.4 g COD g⁻¹ VS d⁻¹) (Fuess et al., 2024). For dry single-stage AD (high TS content, TS > 15%), which is regarded as more robust than wet single-stage AD, suitable F/M values have likewise been reported in the low range of 0.13–0.17 g VS g⁻¹ VS d⁻¹ (Van et al., 2020). However, these values should not be directly generalized to high-rate anaerobic systems such as UASB and EGSB reactors, where biomass retention allows the SRT to be decoupled from the HRT and enables the AD process to be conducted at shorter HRTs and higher volumetric loading rates (Mainardis et al., 2020; van Lier et al., 2015). Therefore, the F/M ratio should be interpreted together with HRT and SRT: decreasing HRT at a given influent concentration increases the applied organic load, whereas increasing SRT or retained biomass lowers the effective F/M ratio and could improve process stability.

More recent work indicates that, when aiming for optimal fermentation activity, the acidogenic reactor should operate at F/M ratios between 4.0 - 6.0 g COD g⁻¹ VSS d⁻¹ (Fuess et al., 2024).

2.2.2. pH and VFA/alkalinity ratio

pH plays a crucial role in the oxidation reduction potential of the anaerobic process (Simeonov et al., 2025). Thus, it directly impacts the metabolic pathway (Simeonov et al., 2025; Cremonez et al., 2021).

The ideal pH range for the overall digestion process (systems without stage division) is close to neutrality (6.8-7.5), mainly due to the high sensitivity of methanogenic microorganisms to different pH conditions (Ağdağ & Sponza, 2007; Mao et al., 2015; Jain et al., 2015; Saratale et al., 2018). Nevertheless, the individual process stages have distinct pH optima. Hydrolysis can occur across a broad pH window of 4 - 11 (Zhang et al., 2009). For acidogenesis, the optimal pH for VFA production is 5.5 to 6.5 (Mao et al., 2015; Jiang et al., 2013; Lindner et al., 2015). In acetogenesis, the process is inhibited when pH < 3.8 (Moestedt et al., 2016). A mildly acidic environment (pH of 6.0 to 6.5) favors acetogenic activity, and acetogens are less affected by pH fluctuations in the incoming substrate under these conditions (Nayono, 2010). During methanogenesis, methane-forming bacteria are highly sensitive to pH fluctuations (Tho & Thanh, 2025). Although methanogenesis can occur between pH of 6.5-8.2, the optimal range is 7.0 to 7.2 (Mao et al., 2015). These pH requirements are one of the main operational reasons for using two-stage AD systems, as phase separation allows the acidogenic reactor or tank to be maintained under mildly acidic conditions that favor VFA formation, while the methanogenic reactor can be operated closer to neutrality to support methane production and process stability (Demirel & Yenigün, 2002; Wang et al., 2016; Zheng & Li, 2024).

The VFAs/alkalinity (VFA/ALK) ratio is a useful indicator of digester stability because it reflects the balance between acid accumulation and the system's buffering capacity. An increase in this ratio can indicate that VFA production exceeds consumption, which may precede pH decline, methanogenic inhibition, and process instability (Simeonov et al., 2025). Since the VFA/ALK ratio is influenced by operating conditions and substrate composition, it should be interpreted as an early-warning indicator of imbalance rather than as a stand-alone cause of inhibition. A VFA/ALK ratio of 0.1-0.25 is usually considered favorable without the risk of acidification (Khanal, 2008). An increase in the ratio beyond 0.3–0.4 indicates digester upset, and corrective measures are required (Khanal, 2008).

2.2.3. Temperature

Temperature is a key operational factor affecting the growth and activity of microbial communities involved in hydrolysis, acid formation, and biogas generation. On this basis, AD systems are typically categorized by operating temperature into psychrophilic (< 20°C), mesophilic (20–45°C), and thermophilic (55–70°C) regimes (Divya et al., 2015; Kumar et al., 2020).

Mesophilic operation is frequently used and preferred because this intermediate range generally promotes higher process stability and accommodates a wider diversity of active anaerobic microorganisms (Kainthola et al., 2019). Consequently, this temperature regime is widely used in applications where high methane production is desired (Cremonez et al., 2021).

By comparison, thermophilic conditions may enable higher organic loading rates and accelerate the early-stage hydrolysis of substrates, which can shorten hydraulic retention time (HRT) and enhance biogas production (Kainthola et al., 2019). Nevertheless, higher temperatures may

reduce the diversity of active microorganisms resulting in a more sensitive AD system, and the associated rapid degradation can increase the formation of toxic compounds that may impair or inhibit the development of methanogenic microorganisms (Kainthola et al., 2019).

2.2.4. Substrate characteristics

AD systems can be fed with a broad range of substrates, such as food waste, agricultural residues, industrial and municipal wastewater, sludge, source separated blackwater (BW), and highly biodegradable wastewater including FJPWW (Abuzir et al., 2025; Li et al., 2019). Because each biomass type comprises a distinct and heterogeneous set of organic and inorganic components, substrate selection can substantially affect digestion performance (Cremonez et al., 2021). As a result, biogas output is closely tied to substrate composition (Rasapoor et al., 2020).

In most biogas production systems, the primary organic constituents are carbohydrates, proteins, and lipids (Batstone et al., 2002). Of these, proteins are typically viewed as less favorable for biogas generation because their degradation may form toxic compounds and lead to inhibitory effects (Braun et al., 2003). Even so, when maintained at balanced levels, nitrogen availability remains necessary for cell generation, producing equivalent moles of ammonia nitrogen, in addition to bicarbonate alkalinity (Ma et al., 2018).

Carbohydrate-rich substrates, particularly those dominated by readily fermentable sugars, tend to degrade quickly and can support high biogas production over short timeframes (Cremonez et al., 2021). At the same time, rapid conversion to volatile acids can lower pH and compromise overall process performance (Cremonez et al., 2021). More broadly, carbohydrate-rich materials often exhibit low C/N ratios, which can hinder cell formation and decrease biogas production rates (Jain et al., 2015). For this reason, co-digestion is widely regarded as an effective strategy to balance substrate properties (Rasapoor et al., 2020).

Lipids represent one of the most energy-dense substrate fractions and are strongly associated with higher specific methane yields; accordingly, they can influence chemical oxygen demand recovery as this highly energetic gas (Srisowmeya et al., 2020; Suksong et al., 2015). However, lipid breakdown is generally slow in digesters and commonly requires pre-treatments, including enzyme-based approaches, to accelerate degradation (Cremonez et al., 2021).

2.2.5. Organic loading rate (OLR) and retention time (HRT and SRT)

Organic loading rate (OLR) quantifies the daily input of organic matter to a reactor per unit working volume and is commonly expressed as kg COD or kg VS m⁻³ d⁻¹ (Hartmann & Ahring, 2006; Tho & Thanh, 2025). Hydraulic retention time (HRT) refers to the period during which the substrate remains in the reactor, whereas solid retention time (SRT) describes how long microorganisms are maintained within the system (Tho & Thanh, 2025). The required HRT is closely linked to substrate biodegradability and degradation kinetics: slowly degradable substrates generally require longer retention times, whereas readily biodegradable substrates may be treated at shorter HRTs. Conversely, when HRT is constrained by reactor volume or flow rate, the achieved degree of degradation may vary because a shorter HRT reduces the contact time between the substrate and anaerobic biomass (Fig. 2.2). Reported OLRs also depend on substrate characteristics, operating conditions, and reactor design, and values up to 40 kg COD m⁻³ d⁻¹ in high-rate AD systems have been published (Corbari et al., 2019; Cruz-Salomón et al., 2019; Jijai et al., 2015; Jiraprasertwong et al., 2019).

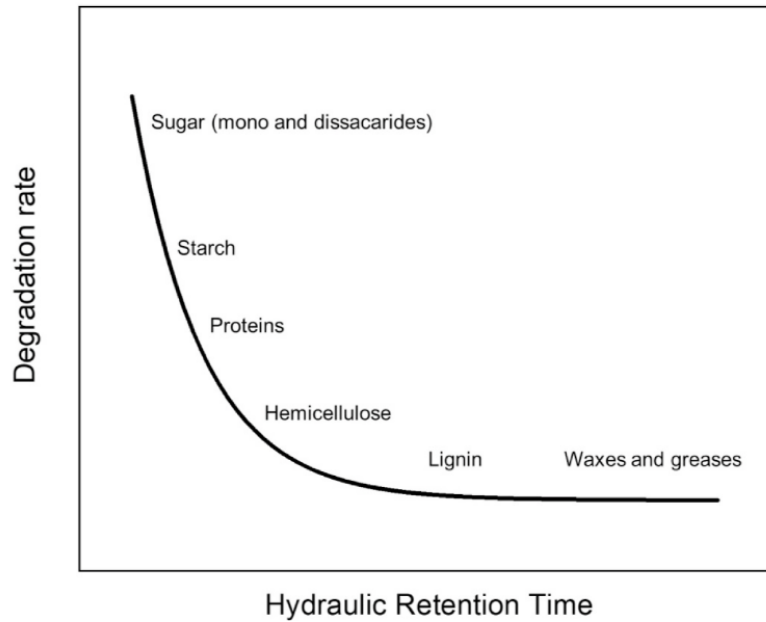


Fig. 2.2. Relationship between substrate degradation rate and hydraulic retention time in anaerobic digestion (AD) processes for different substrates. Adapted from Akunna (2018).

Because OLR and HRT are inversely related ($OLR = \text{kg COD or kg VS HRT}^{-1}$), reducing HRT increases OLR (Tho & Thanh, 2025). If OLR rises beyond a critical limit, VFAs can accumulate and cause pH to decrease (Tho & Thanh, 2025), which suppresses methanogenic activity and may halt biogas production (Zaher et al., 2007). Consequently, early signs of OLR overload include declining biogas generation and a drop in pH (Tho & Thanh, 2025). Across substrates and reactor types, reported HRTs range from more than 6 hours up to 85 days: highly biodegradable feeds generally allow shorter HRTs, whereas substrates with high solids require higher HRTs ($TS > 15\%$) (Tho & Thanh, 2025; Kongjan et al., 2013; Santos et al., 2017; da Silva et al., 2019; Arantes et al., 2020; Cremonez et al., 2021; Larsson et al., 2015).

Regarding SRT, optimizing biogas production typically requires ≥ 20 days under mesophilic conditions, while at thermophilic temperatures the recommended SRT is 7–15 days (Tho & Thanh, 2025; Paudel et al., 2017). However, the relationship between HRT and SRT depends strongly on reactor configuration. In completely stirred tank reactors (CSTRs) without biomass retention, SRT is generally close to HRT because microorganisms leave the reactor with the effluent. In contrast, high-rate anaerobic systems, such as UASB and EGSB reactors, retain biomass as granules or sludge beds, allowing SRT to be decoupled from HRT. This enables operation at shorter HRTs while maintaining sufficient active biomass for organic matter conversion and methane production (Tassew et al., 2019; Mainardis et al., 2020).

2.2.6. Carbon to nitrogen ratio (C:N)

Carbon contained in degradable organic structures is a key requirement for digestion because it serves as a direct precursor for methane formation, while nitrogen (derived mainly from proteins) is necessary for synthesizing the bacterial biomass that drives the process. For this reason, an appropriate balance between carbon and nitrogen is crucial for the AD process (Cremonez et al., 2021). Reported optimal C/N ratios typically fall between 20 and 35 (Weiland, 2006; Khalid et al., 2011). When the C/N ratio is high, inoculum renewal and the formation of new cells can become limiting; conversely, very low C/N ratios (i.e., elevated nitrogen as ammonium concentrations) may increase environmental toxicity to the microorganisms of interest

(Esposito et al., 2012; Rocamora et al., 2020). Additionally, nitrogen present as nitrite can markedly enhance substrate solubilization, hydrolysis, and organic acidification because of its redox properties (Cremonez et al., 2021).

2.3. Anaerobic Reactor Technologies Studied in the Thesis

Anaerobic digestion of waste and wastewater can be carried out in a range of bioreactors/digesters, with the choice affected by substrate characteristics and the targeted effluent quality. Widely applied configurations include Continuous Stirred-Tank Reactor (CSTR), Anaerobic Baffled Reactors (AnBR), Moving Bed Biofilm Reactors (MBBR), Up-flow Anaerobic Sludge Blanket Reactors (UASB), and the Expanded Granular Sludge Blanket (EGSB), which is an evolution of UASB reactors (Gianico et al., 2015; Barber & Stuckey, 1999; Morgan-Sagastume et al., 2024; Abuzir et al., 2025; Yan et al., 2021).

In this thesis, the emphasis was placed on high-rate anaerobic digesters, specifically UASB and EGSB for treating BW (Chapter 3) and FJPWW (Chapter 4). Interest in high-rate anaerobic digestion systems has grown in recent years because these systems combine high loading capacity with low sludge production (Chong et al., 2012). A key advantage is that biomass retention (SRT) is decoupled from HRT, enabling a substantial reduction in reactor volume relative to conventional systems (Latif et al., 2011).

2.3.1. Upflow anaerobic sludge blanket reactors (UASB)

The upflow anaerobic sludge blanket (UASB) reactor was first developed in the Netherlands in the 1970s, and its adoption expanded quickly because it was reported to perform exceptionally well across a range of biodegradable wastewater streams (Lim et al., 2014). One defining characteristic of the UASB configuration is the presence of granular sludge, which enables retention of highly active biomass with strong settling properties within the reactor (Latif et al., 2011), thereby enhancing sludge–effluent separation.

Fig. 2.3 and 2.4 illustrate a simplified schematic and a real-world example of a UASB reactor. In this system, wastewater is introduced at the reactor base and moves upward through the “sludge blanket,” which comprises a bed of granular sludge (Tauseef et al., 2013). This configuration promotes effective interaction and mixing between the influent substrate and the biomass, supporting rapid anaerobic degradation (Tauseef et al., 2013). UASB reactor performance is primarily determined by the granular sludge bed, which expands as wastewater rises vertically through the bed (Abbasi et al., 2012). Pollutant removal is achieved by microflora associated with the sludge granules; accordingly, biofilm quality and the degree of sludge–wastewater contact are critical determinants of UASB reactor success (Abbasi et al., 2012). Biogas produced during treatment further enhances mixing and contact, while a three-phase gas-liquid-solid separator in the upper section enables biogas collection and separates it from the liquid effluent and remaining sludge particles (Tauseef et al., 2013). Typical UASB geometrical and operational parameters include a height to diameter ratio of 0.2–0.5 and an up-flow velocity of 0.5–1.0 m h⁻¹ (Lim et al., 2014).

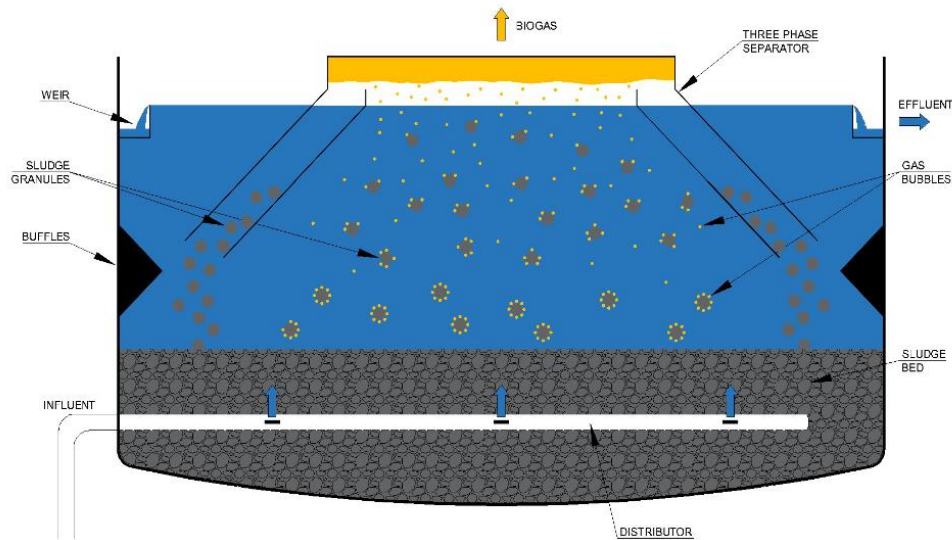


Fig. 2.3. UASB reactor process scheme. Adopted from Mainardis et al. (2020).

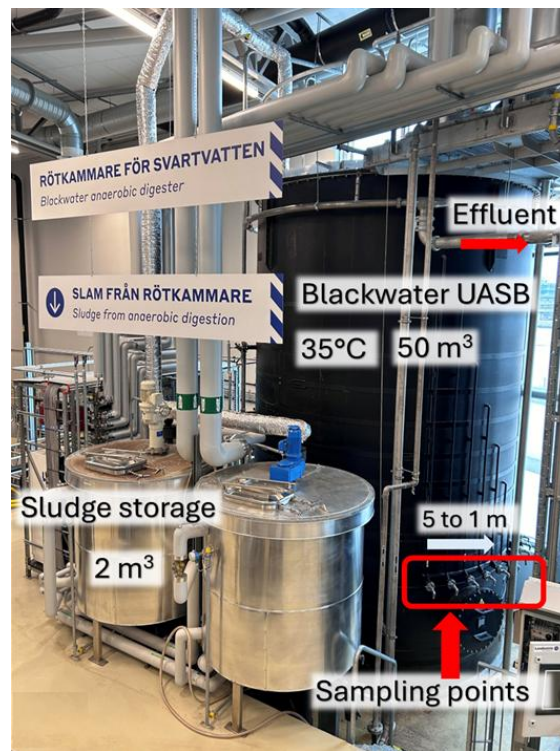


Fig. 2.4. Full-scale UASB reactor treating BW.

Compared with aerobic wastewater treatment, anaerobic treatment of wastewater in UASB reactors requires less energy, maintains high efficiency at higher organic loading rates, requires only limited micro- and macronutrients, and produces less sludge with improved dewaterability (Lim et al., 2014). Notably, only about 5–10% of the organic matter in wastewater is transferred to the sludge fraction (Lim et al., 2014). However, UASB treatment is also recognized to have a limited effect on nutrients (nitrogen and phosphorous) and on micro-pollutants removal (Vassalle et al., 2020).

2.3.2. Expanded granular sludge blanket reactors (EGSB)

Expanded anaerobic sludge blanket (EGSB) reactors are the predominant configuration applied in industrial wastewater treatment and represent about 90% of such systems worldwide (van Lier et al., 2015). Like UASB systems, they depend on the development of dense granular biomass without additional supports; this is driven by upflow turbulence, which promotes effective biomass–substrate contact and enables high OLR and lower HRT than conventional digesters (Cruz-Salomón et al., 2019). The EGSB reactors were developed in the mid-1980s by Biothane Anaerobic Technologies (now VEOLIA). The EGSB reactors are an advancement of the UASB concept. A simplified scheme and a real example of an EGSB reactor are presented in Fig. 2.5 and 2.6.

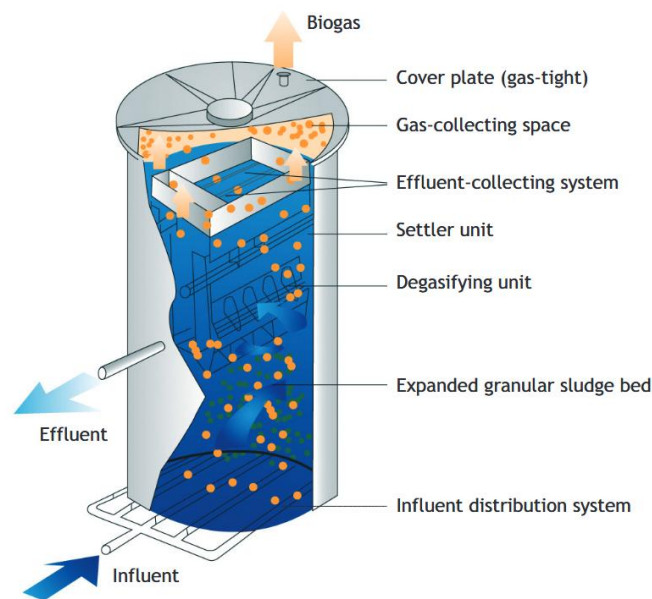


Fig. 2.5. EGSB reactor process scheme. Adopted from Chen et al. (2020).

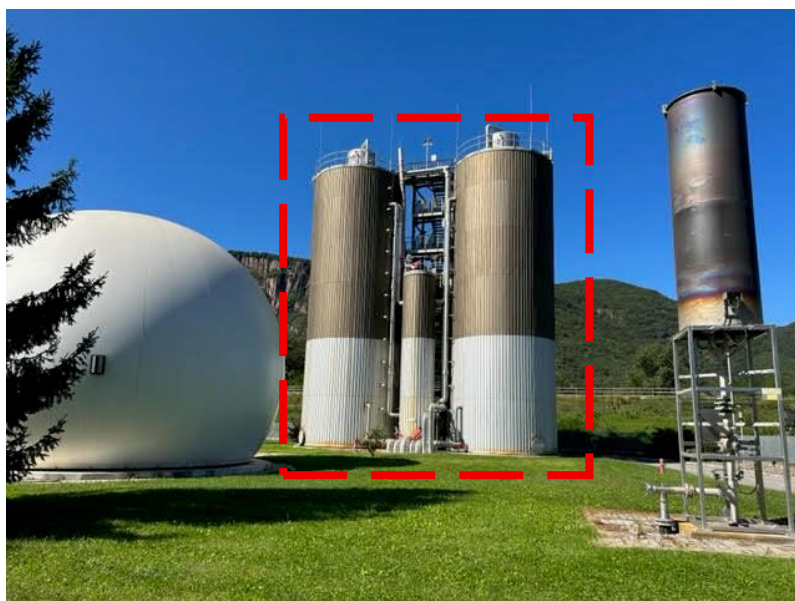


Fig. 2.6. Full-scale EGSB reactors (highlighted in red) treating FJPWW.

Although the two configurations share the same biological principles, the EGSB operates at substantially higher upflow velocity (V_{up}) (typically 4–10 m h⁻¹ versus 0.5–1.0 m h⁻¹ in UASB). This higher V_{up} increases mixing intensity and improves mass transfer. Moreover, a taller reactor column combined with an effluent recirculation system strengthens biomass–substrate contact, reduces dead zones and preferential flow paths, and supports more efficient use of the reactor volume (Lim and Kim, 2014). Under these conditions, highly compact and dense granular biomass can form and be stably retained along the entire reactor height in a stratified way, aided by the high height-to-diameter ratio. This, in turn, enables a strong decoupling between SRT and HRT and allows operation under high OLRs.

At full scale, EGSB technology has been implemented for carbohydrate-rich wastewater with relatively low suspended solids (SS) and moderate organic concentrations, commonly from the food industry (Yan et al., 2021a), distilleries (Lin et al., 2020), or pulp and paper production (Fykse et al., 2016), with chemical oxygen demand (COD) removal efficiencies of 80–90%. Nevertheless, diffusion limitations and the need to optimize conditions for improved microbial activity and biogas yield remain key challenges (Meng et al., 2021). In addition, the diversity of WW types treated introduces uncertainty regarding optimal operational conditions (Aragón-Barroso et al., 2026).

A central feature of EGSB operation is the spontaneous development of dense microbial granules, which support efficient pollutant degradation through syntrophic interactions across diverse communities. By providing a protective microenvironment, granules can enhance metabolic activity under harsh conditions, improve biomass retention, and help the system withstand fluctuations, thereby strengthening reactor stability and effluent quality (Show et al., 2020). Spatial stratification within granules is also observed: acidifying and hydrolytic bacteria predominate in the outer layers, whereas methanotrophs are concentrated in the core, facilitating syntrophic interactions and substrate transport. Despite these observations, granulation in EGSB reactors remains poorly understood (Lim & Kim, 2014; van Lier et al., 2015), largely because few studies have examined how operational variables influence biomass aggregation and microbial dynamics.

2.4. State-of-the-Art in Source-Separated Blackwater Treatment via AD

Conventional domestic wastewater management at the household and residential levels generally relies on gravity sewer networks that convey wastewater to centralized treatment facilities; however, this configuration can impose both technical and economic constraints that hinder efficient recovery of resources and energy (Gao et al., 2019a; Papa et al., 2017). As a potential solution, source-separation treatment concepts have been advanced as a viable strategy (Fig. 2.7) (Gao et al., 2019b; Huang et al., 2023; Kujawa-Roeleveld et al., 2006). In these systems, domestic wastewater is partitioned into blackwater (BW) from toilets and greywater (GW) from showers, laundry, and sinks, with food waste (FW) from kitchen disposers optionally incorporated (Skambraks et al., 2017). This separation is motivated by the contrasting compositions of the streams: BW typically carries concentrated carbon sources and nutrients, including nitrogen and phosphorus (Hernandez Leal et al., 2007; Otterpohl et al., 1999), while GW accounts for most of the wastewater volume but contains comparatively low nutrient loads (Oteng-Peprah et al., 2018). Accordingly, handling BW and GW independently can enhance the optimization of resource recovery (Wang et al., 2023).

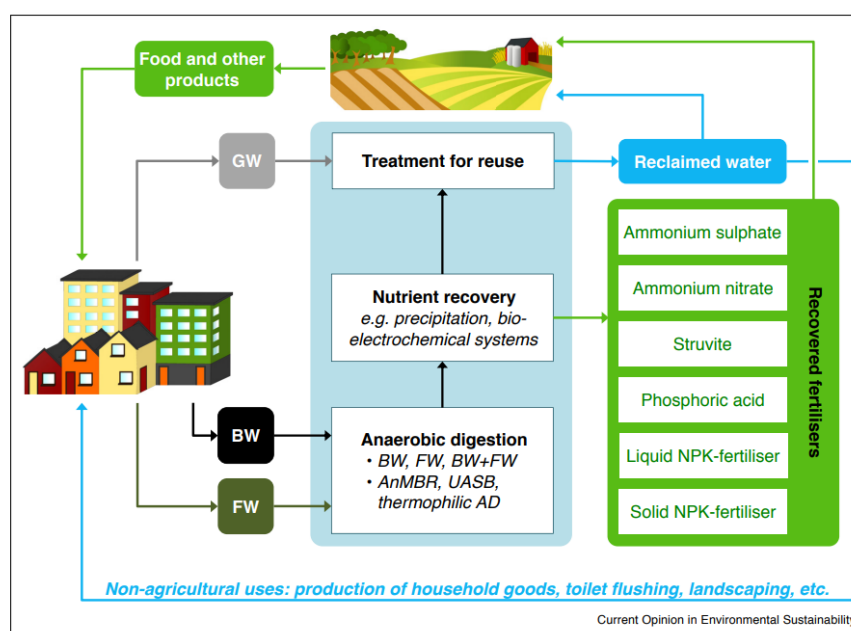


Fig. 2.7. Conceptual framework for technological resource recovery with an emphasis on water and nutrient recovery. Adapted from Bisschops et al. (2019).

With vacuum toilets, BW can be collected using very small flush volumes (approximately one liter per flush), which increases its suitability for anaerobic digestion (AD) and strengthens prospects for biogas generation (Gao et al., 2019b; Kjerstadius et al., 2015; Otterpohl et al., 2004). In parallel, nutrients in BW can be recovered through processes such as struvite precipitation (Xu et al., 2023) and ammonia stripping (Kujawa-Roeleveld et al., 2006), producing outputs that can be applied as fertilizers (Desmidt et al., 2015; Moges et al., 2020).

Findings from studies evaluating AD of source-separated BW in small-scale digesters, including CSTRs (Wendland et al., 2007), accumulation systems (Kujawa-Roeleveld et al., 2006), UASB-septic tanks (Kujawa-Roeleveld et al., 2006), and UASB reactors (De Graaff et al., 2010), collectively suggest encouraging performance. For instance, Wendland et al. (2007) reported 61% COD_{total} removal and a 60% degree of methanization (the amount of influent COD converted into methane during the AD process, expressed as kg CH₄-COD kg⁻¹COD_{in} and represented as a percentage (%)) in a mesophilic CSTR. Similarly, Kujawa-Roeleveld et al. (2006) recorded a 58% degree of methanization during AD of BW combined with kitchen refuse in a small-scale accumulation system operated at 20°C. Kujawa-Roeleveld et al. (2005) further assessed a lab-scale UASB-septic tank treating BW at 15°C and 25°C, achieving COD_{total} removals of 61% and 78% and associated degrees of methanization of 39% and 60%, respectively. De Graaff et al. (2010) finally showed effective BW treatment in a lab-scale flocculant sludge UASB reactor at 25°C, attaining 78% COD_{total} removal and a 54% degree of methanization. However, these studies also indicate important remaining challenges, including incomplete conversion of influent COD to methane, operation mainly at laboratory or pilot scale, and the high ammonium content of source-separated BW, which could inhibit methanogenesis if free ammonia accumulates under unfavorable pH and temperature conditions (De Graaff et al., 2010; Florentino et al., 2019; Kujawa-Roeleveld et al., 2006; Wen et al., 2022).

2.5. Anaerobic Digestion of Highly Biodegradable Wastewater from the beverage industry (Fruit Juice Processing Wastewater)

Highly biodegradable wastewater such as FJPWW is typically characterized by a high organic load (375-124,800 mg COD L⁻¹) and is often accompanied by low pH and low alkalinity. It also commonly exhibits a high carbon-to-nitrogen (C:N) ratio, which results in nutrient imbalances (Dold & Ekama, 1981; Ozbas et al., 2006; Tyagi et al., 2019). In combination with substantial fluctuations in flow volume, these properties increase the overall complexity of treatment (Austerman-Haun & Rosenwinkel, 1997; Chen et al., 2019; Dold & Ekama, 1981; Ozbas et al., 2006; Valta et al., 2015; Zerrouki et al., 2021). Nevertheless, because FJPWW contains high levels of biodegradable organics, it remains a suitable feedstock for biogas production as a clean and environmentally friendly energy source via biological processes such as anaerobic digestion (AD) (Mateescu et al., 2008; Zerrouki et al., 2021).

AD of highly biodegradable substrates from the beverage industry such as FJPWW has been evaluated across multiple bioreactor scales and has generally demonstrated stable and effective performance with respect to COD removal and methane generation. Austermann-Haun et al. (1997), for instance, reported COD removal of more than 80% during treatment of wastewater effluent from a fruit juice industry in a full-scale UASB reactor. Using lab-scale two-stage up-flow anaerobic sponge reactors (UASRs), El-Kamah et al. (2010) treated wastewater generated from the fruit juice industry and achieved COD removal of 91-70%. In addition, Cuff et al. (2018) treated wastewater generated from a soft drink industry in an EGSB, obtaining COD removal of more than 85% and a daily methane production of up to 1950 Nm³ CH₄ d⁻¹. Finally, Chun-Te Lin et al. (2020) treated wastewater in a whiskey distillery using EGSB and external circulation sludge bed (ECSB) reactors and achieved COD removals of 94.8-95.7%.

2.6. Principles and Applications of Co-digestion

Despite their widespread use, mono-digestion presents notable limitations, such as digester instability (Li et al., 2018), restricted year-round availability of certain substrates (Mata-Alvarez et al., 2014), and reduced biogas/methane yield (Zahan et al., 2018). For example, readily biodegradable organic waste streams (e.g., FJPWW) may trigger a rapid build-up of high concentrations of volatile fatty acids (VFA) in digesters, thereby inhibiting methanogens (Li et al., 2018). In contrast, high proteinaceous wastes (e.g., slaughterhouse waste and BW) can lead to the formation of toxic compounds, including ammonia (NH₃) (Mu et al., 2020). To some degree, these problems can be alleviated through operational measures such as pH adjustment (via alkali supplementation) (Zapata Martínez et al., 2019), retention time control (Vanwonterghem et al., 2015), intermittent feeding (Bonk et al., 2018), micro-aeration (Nguyen et al., 2019), and implementation of a two-stage AD system (Rajendran et al., 2020). However, adopting such measures increases both operational complexity and costs.

Anaerobic co-digestion provides a route to reduce the limitations of mono-digestion by digesting two or more feedstocks simultaneously (Karki et al., 2021). Key advantages include improved process stability and higher methane yield, driven by synergistic interactions such as support for a more diverse microbial community (Mata-Alvarez et al., 2014), better nutrient balance (proper carbon-to-nitrogen (C/N) ratio and supplementation of trace elements) (Xie et al., 2018), and enhanced buffering capacity (Bolzonella et al., 2006).

Typical co-feedstocks include food waste (Bong et al., 2018), sewage sludge (Mehariya et al., 2018; Bolzonella et al., 2006), and animal manure (Zahan et al., 2018). For example, Kamravamanesh et al. (2024) evaluated the potential of co-digesting BW with food waste (FW) reported a 38% increase in methane yield. Li et al. (2013) co-digested corn stover with chicken manure (3:1), which ensured 0.4 C:N for process stability and increased methane yield by 0.87 times. In addition, Pitk et al. (2013) co-digested slaughter-house waste (5% w/w) with sewage sludge and observed an increase in biomethane by 5.7 times. However, the selection and choice of co-substrates should not only be based on the improvement of the process performance, since it may affect the quality of the digestate and therefore influences its potential end use (Azarmanesh et al., 2023; Rao & Baral, 2011; Tormo, 2015). Consequently, the selection of co-substrates depends on the local treatment context, including substrate availability, regulatory conditions, contaminant content, and whether the digestate is intended for agricultural reuse (Catenacci et al., 2022; Delzeit & Kellner, 2013; Gopinath et al., 2021).

Although co-digestion can offer substantial benefits, antagonistic effects arising from an incompatible feedstock mixing ratio may result in organic overloading, acidification, and eventual system failure (Chow et al., 2020). Such risks can be reduced by characterizing the heterogeneous organic compounds present in digester feedstocks and by understanding their intrinsic biodegradability patterns (Hagos et al., 2017).

2.7. Methodological Approaches in AD Research

In this subsection, methodological approaches in AD research such as biochemical methane potential (BMP) and specific methanogenic activity (SMA) tests are described. These tests provide insights into substrate biodegradability, methane production potential, and the methanogenic activity of the biomass.

2.7.1. Biochemical methane potential (BMP)

BMP tests provide insights into the methane potential and the biodegradability of a substrate (Filer et al., 2019). In a BMP test, the substrate is combined with anaerobic biomass (suspended or attached), typically collected from an active digester (Filer et al., 2019). Incubation bottles are maintained at a constant temperature commonly in the range of 35°C to 55°C and continuously mixed for 30–60 days (Holliger et al., 2016; Strömberg et al., 2014). During incubation, anaerobic degradation of the substrate's organic matter produces methane and carbon dioxide (Filer et al., 2019). Methane production attributable to the substrate is determined by measuring methane formation and correcting for background gas formation using a blank; methane potential is then expressed per mass of volatile solids added or COD added (Filer et al., 2019). Biodegradability can also be reported by normalizing the cumulative methane yield to the theoretical cumulative methane production, calculated using the relationship 1 g COD = 0.35 mL CH₄ at standard temperature and pressure conditions (STP) (Angelidaki et al., 2004).

2.7.2. Specific methanogenic activity (SMA)

SMA quantifies the methane-producing capacity of anaerobic biomass (suspended or attached) for a given substrate (acetic acid is most common) and is measured at a concentration where substrate availability is not a limiting factor (Hussain & Dubey 2014; Remigi et al., 2006). The amount of active methanogenic biomass in an anaerobic reactor is a key determinant of

effective wastewater treatment performance (Hussain et al., 2017). SMA testing can also help define suitable operating conditions and serve as a performance indicator by improving insight into process stability and overall reactor function (Hussain et al., 2017). In addition, SMA tests support assessment of anaerobic sludge with respect to methanogenic activity and relative microbial levels under varying conditions (Hussain et al., 2017). Applying SMA enables estimation of the potential loading capacity of anaerobic treatment systems, thereby supporting selection of appropriate organic loading rates (Javed & Tare, 1999).

2.7.3. Opportunities and Challenges in Scaling anaerobic digestion (AD) Research AD research is performed across a range of experimental scales, each offering specific strengths and limitations. Laboratory-scale studies (or bench-scale studies) are especially useful for examining biodegradability, microbial responses, substrate interactions, inhibitory phenomena, and the effects of individual operating parameters under controlled conditions. Nevertheless, such experiments are often conducted under simplified and tightly regulated conditions, which can lead to discrepancies between the measured performance and the behaviour of larger systems operated under real-world conditions (Ilo et al., 2021; Wickham et al., 2019).

Pilot-scale studies serve as a crucial transitional stage between laboratory experiments and full-scale application. Unlike batch or bench-scale systems, pilot reactors are better able to reflect the effects of continuous feeding, hydrodynamic behaviour, mixing conditions, sludge retention, and operational variability. As a result, they provide a more representative basis for evaluating process stability, control needs, and scale-dependent design limitations. This is especially important in anaerobic digestion, where factors such as varying organic loads, mixing intensity, reactor configuration, and mass transfer can vary markedly with scale and substantially affect reactor performance (Singh et al., 2019; Wickham et al., 2019).

Full-scale studies are essential for evaluating the practical applicability of AD technologies under real operating conditions. At this scale, process performance is influenced not only by biological and physicochemical factors, but also by feedstock availability and variability, operational reliability, energy demand, maintenance, plant integration, and economic and regulatory constraints. Consequently, direct translation of laboratory or pilot results to full-scale practice is not always straightforward. The progression from laboratory to pilot and full scale should therefore be understood not simply as an increase in reactor volume, but as a transition toward greater operational complexity and higher technology readiness. Recognizing these scale-related differences is important for interpreting AD literature and for motivating the combined use of lab-, pilot-, and full-scale evidence in the present thesis (Elsayed et al., 2024; Nghiem et al., 2017).

2.8. Knowledge Gaps Addressed by This Thesis

AD has advanced substantially through laboratory- and pilot-scale studies; however, uncertainties remain when translating these outcomes to full-scale installations, particularly for high-strength wastewater such as BW and highly biodegradable wastewater such as FJPWW. Source-separated BW, for example, is typically characterized by a low C:N ratio, which can be addressed through co-digestion with source-separated food waste (FW) (Kamravamanesh & Koko, 2024). However, the full-scale potential of co-digesting source-separated BW with source-separated FW has not been sufficiently investigated.

FJPWW is highly biodegradable and may therefore promote volatile fatty acid (VFA) accumulation. One strategy to mitigate this risk is to separate the hydrolysis/acidogenesis and methanogenesis stages in a two-stage anaerobic digestion system (Zheng & Li, 2024). However, the optimization of full-scale two-stage anaerobic digestion for treating FJPWW has not been adequately explored. Key evidence gaps include: (i) for source-separated BW, a lack of studies on full-scale applications ; and (ii) for highly biodegradable wastewater such as FJPWW, a lack of studies on full-scale two-stage AD in R2S reactors, including recommendations for plant monitoring and operation, and on optimizing the overall two-stage AD system via co-digestion. Accordingly, this thesis addresses these gaps in Chapters 3, 4, and 5.



Chapter 3

Methodology

Chapter 3. Methodology

This chapter provides an overview of the case studies, substrates, analytical methods, and main methodological limitations included in the thesis. The case studies are first introduced together with their substrates, reactor configurations, study scales, and links to the research objectives. The following sections then summarize the analytical methods and limitations relevant to interpreting the results. Detailed methodological descriptions are provided in the corresponding study chapters.

3.1. Case studies

3.1.1. Case 1 – Source-separated blackwater treatment (BW)

Source-separated blackwater (BW) was collected from domestic and office buildings in the new city district of Oceanhamnen, Helsingborg, Sweden, using vacuum toilets that have been in operation since 2020. This substrate consisted of toilet-derived wastewater collected separately from other domestic wastewater streams. The BW was treated in a full-scale 50 m³ up-flow anaerobic sludge blanket reactor (UASB_{BW}) to evaluate the performance of full-scale anaerobic digestion for source-separated sanitation streams. This case study addressed Research Objective 1 and is presented in Chapter 4.

3.1.2. Case 2 – Co-digestion of source-separated blackwater (BW) and food waste (FW)

Source-separated BW was also evaluated in combination with food waste (FW) in a full-scale UASB reactor in Sneek, the Netherlands. In this case, BW and FW were co-digested in a 41 m³ UASB reactor (UASB_{BW+FW}) to assess the performance of a full-scale co-digestion system treating source-separated organic waste streams. This case study addressed Research Objective 1 and is included in Chapter 4 as a comparative full-scale system.

3.1.3. Case 3 – Fruit juice processing wastewater (FJPWW) treatment in a full-scale two-stage anaerobic digestion system

Fruit juice processing wastewater (FJPWW) was obtained from a fruit juice industry in Bronzolo, northern Italy. This wastewater originated from fruit processing activities and was treated in a full-scale two-stage anaerobic digestion system at the Bronzolo wastewater treatment plant. The system consisted of a homogenization/acidification tank of 3,500 m³ followed by two expanded granular sludge bed reactors of 750 m³ each. This case study evaluated the long-term performance of full-scale two-stage anaerobic digestion of FJPWW and addressed Research Objective 1. The results are presented in Chapter 5.

3.1.4. Case 4 – Co-digestion of fruit juice processing wastewater (FJPWW) and primary sludge (PS) in a pilot-scale two-stage anaerobic digestion system

FJPWW from Bronzolo was also investigated together with primary sludge (PS) obtained from the primary settler at the municipal wastewater treatment plant in Bronzolo. This case study evaluated the effect of adding PS to FJPWW on the overall anaerobic digestion process. The work included batch acidogenic fermentation and biochemical methane potential tests, and was

further supported by operation of a pilot-scale two-stage anaerobic digestion system. This case study addressed Research Objective 2 and is presented in Chapter 6 and Appendix D.

3.2. Analytical methods

3.2.1. Data analysis

Long-term operational data from the studies on anaerobic digestion of source-separated BW (Chapter 4) and two-stage anaerobic digestion of fruit juice processing wastewater (Chapter 5) were analysed to evaluate the performance of each system separately. This data analysis was used for process performance evaluation and corresponded to Research Objective 1 outlined in Chapter 1. Process performance was assessed based on pseudo-steady-state periods, which were defined as stable operating intervals lasting at least 14 consecutive days. For each selected period, the mean and standard deviation were calculated from the operational dataset (Chapters 4 and 5).

3.2.2. Biochemical methane potential (BMP)

BMP tests were conducted to evaluate the methane potential and biodegradability of mixtures of primary sludge and fruit juice processing wastewater at different volume-to-volume ratios (Chapter 6). The BMP results addressed Research Objective 2 outlined in Chapter 1.

3.2.3. Determination of volatile fatty acids (VFAs) using HPLC

VFA determination was conducted to quantify individual volatile fatty acid components, including acetic, propionic, valeric, butyric, iso-valeric, and iso-butyric acids, in samples from the BW and FJPWW anaerobic digestion systems described in Chapters 4 and 6. The resulting VFA concentrations addressed Research Objective 1 outlined in Chapter 1.

3.2.4. Specific Methanogenic Activity Test (SMA)

SMA tests (Fig. E2 and E6) were conducted to evaluate the methanogenic activity of flocculent sludge from a full-scale UASB reactor treating source-separated BW (Chapter 4) and granular sludge from a full-scale EGSB reactor treating fruit juice processing wastewater (Chapter 5). The SMA results contributed to Research Objective 1, which focused on evaluating full-scale process performance, as outlined in Chapter 1.

3.2.4. Batch acidogenic fermentation and co-fermentation tests

To address the overarching aim of this thesis and Research Objective 2 outlined in Chapter 1, which focused on evaluating the impact of adding primary sludge to fruit juice processing wastewater on the overall anaerobic digestion process, acidogenic fermentation experiments were performed at the Bronzolo WWTP using 1 L Duran® glass bottles. The experiments were conducted for 100 hours using three substrate mixtures with different volume-to-volume ratios of fruit juice processing wastewater and primary sludge (Chapter 6).

3.3. Limitations of the studies

Several limitations of this thesis are related to the use of full-scale systems, where operating conditions, wastewater composition, sampling frequency, and data availability cannot be controlled to the same extent as in laboratory-scale experiments. However, these full-scale systems

provide valuable real-world insight into anaerobic digestion performance under practical operating conditions.

In the BW UASB system (Chapter 4), operation during the higher OLR phase was affected by sludge washout and fluctuating effluent COD/TSS, which limits the interpretation of performance based solely on biological conversion metrics. COD removal, therefore, cannot be used as a direct indicator of microbial conversion, because increased solids losses elevated effluent COD even when methane conversion remained high. In addition, the energy balance was constrained by limited data coverage (20 weeks), and the methane content during the SMA tests was not directly measured but was assumed based on the methane content observed in the full-scale system. Another limitation is that the sludge samples from the co-digestion system treating source-separated BW and food waste (FW) were not as fresh as those obtained from the source-separated BW system, which may have influenced the comparability of the SMA results. Furthermore, the remaining soluble COD in the effluent warrants further investigation regarding its composition and biodegradability. Microbial community analyses were also absent, which could have supported the interpretation of the hydrolysis step. Collectively, these constraints reduce the extent to which the full-scale outcomes can be generalized across sites and highlight the need for more detailed characterization of the residual effluent fraction.

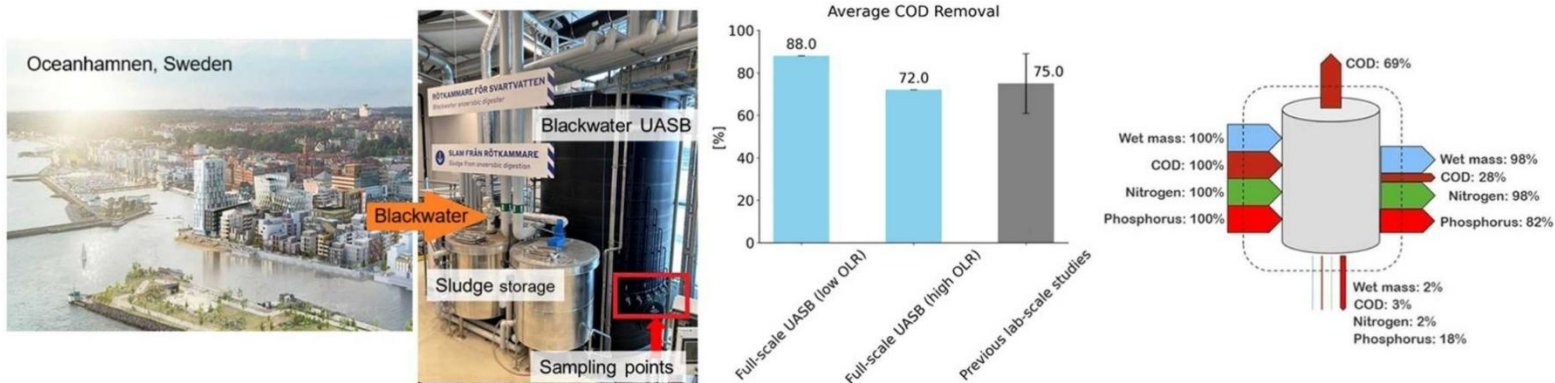
For the full-scale two-stage FJPWW system (Chapter 5), the biomass quantity (VS) in the first stage (hydrolysis/acidogenesis) was not monitored, which limits interpretation of the temperature-insensitive performance and prevents assessment of whether operational factors such as F/M influenced acidification outcomes. Although the influent alkalinity range was described, the underlying data were not presented (“data not shown”) and was not frequently measured, underscoring the need for frequent alkalinity measurements given their importance for AD stability. Finally, while the second stage (acetogenesis/methanogenesis) achieved high COD and VFA removal, the need for post-treatment and the low TSS removal indicate that the dataset primarily demonstrates anaerobic conversion performance rather than complete treatment to discharge standards. In addition, information on granular sludge settleability in the methanogenic reactors and CFD analyses were not provided; these could have supported interpretation of the low TSS removal and the observed sludge washout.

Regarding the co-fermentation/co-digestion experiments (Chapter 6), gas production during the acidogenic phase was not measured, which prevents confirming whether the observed COD variations were linked to H_2/CO_2 production or to methanogenic activity. In addition, the BMP tests used a low working volume (200 mL) due to instrument pressure constraints, which may have influenced methane measurement accuracy. As a result, mechanistic resolution and precision are reduced, even though the comparative outcome across mixing ratios remains clear within the experimental design.



Chapter 4

Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden



This chapter was published as:

Abuzir, S., Gren, L., Domini, M., Bertanza, G., Davidsson, Å., & Kjerstadius, H. (2025). Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden. *Cleaner Water*, 4, 100082.

<https://doi.org/10.1016/j.clwat.2025.100082>

Chapter 4. Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden

4.1. Abstract

The growing global population has increased the demand for circular sanitation solutions that enhance energy and nutrient recovery. Anaerobic digestion (AD) of blackwater (BW) offers a promising solution, but its application in full-scale UASB reactors remains unexplored. This study evaluated the performance of a full-scale UASB (50 m³) treating BW collected from a new city district (Oceanhamnen) located in Helsingborg, Sweden, over 130 weeks, applying two different organic loading rates (OLRs), focusing on COD removal, biogas production, and mass balances for COD, nitrogen, and phosphorus, and compared its performance with previous lab-scale studies. The full-scale UASB achieved its highest COD removal efficiency (88%) and degree of methanization (0.77 kg COD-CH₄ kg⁻¹ COD_{in}) during the low organic loading rate (OLR) phase. In the higher OLR phase, COD removal dropped to 72% due to sludge washout, although the degree of methanization remained high (0.71 kg COD-CH₄ kg⁻¹ COD_{in}). Mass balance results showed 69% of influent COD was converted into methane, while 28% remained in the effluent, likely due to sludge washout. The decanted effluent contained 92% of N and 82% of P reflecting the low sludge production. Compared with lab-scale studies, the full-scale UASB achieved comparable COD removal and biogas yields despite challenges like sludge washout during the higher OLR. The results confirm that the full-scale application of blackwater treatment is suitable for circular sanitation solutions. Further research is needed to optimize solids retention and reduce effluent COD for sustainable full-scale operations, as well as optimizing the energy balance calculations.

4.2. Introduction

The growing global population has increased the demand for circular sanitation solutions that enhance energy, water, and nutrient recovery (Abdel-Shafy et al., 2009). Traditionally, domestic wastewater is collected via gravity sewer pipelines and treated in centralized plants, which presents technical and economic challenges for resource and energy recovery (Gao et al., 2019a; Papa et al., 2017). Source separation treatment systems offer a promising alternative (Gao et al., 2019b; Huang et al., 2023; Kujawa-Roeleveld et al., 2006). These systems separate domestic wastewater into blackwater (BW) from toilets and greywater (GW) from showers, laundry, and sinks, with optional food waste (FW) from kitchen disposers (Skambraks et al., 2017). BW, rich in carbon sources and nutrients like nitrogen and phosphorus (Hernandez Leal et al., 2007; Otterpohl et al., 1999), and GW, high in volume but low in nutrients (Oteng-Peprah et al., 2018), can be treated separately for optimal resource recovery (Wang et al., 2023). Using vacuum toilets, BW can be collected with a minimal amount of water (approximately one liter per flush), making it suitable for anaerobic digestion (AD) and increasing biogas production potential (Gao et al., 2019b; Kjerstadius et al., 2015; Otterpohl et al., 2004). Nutrients from BW can be recovered through processes such as struvite precipitation (Xu et al., 2023) and ammonia stripping (Kujawa-Roeleveld et al., 2006) for use as fertilizers (Desmidt et al., 2015; Moges et al., 2020). Research on AD treatment of source-separated BW in small-scale digesters, such as continuously stirred tank reactors (CSTRs) (Wendland et al., 2007), accumulation

systems (Kujawa-Roeleveld et al., 2006), UASB-septic tanks (Kujawa-Roeleveld et al., 2006), and up-flow anaerobic sludge blankets (UASB) (De Graaff et al., 2010), has shown promising results. For instance, Wendland et al., (2007) achieved 61% COD_{total} removal and 60% degree of methanization (the amount of influent COD converted into methane during the AD process, expressed as kg CH₄-COD kg⁻¹ COD_{in} and represented as a percentage (%)) in a CSTR operating under mesophilic conditions for 413 days. Similarly, Kujawa-Roeleveld et al., (2006) reported a 58% degree of methanization during the AD of BW and kitchen refuse in a small-scale accumulation system operating at 20°C for 105 days. In another study by Kujawa-Roeleveld et al., (2005), a lab-scale UASB-septic tank treating BW at 15°C and 25°C was investigated over 315 days and achieved COD_{total} removals of 61% and 78%, with corresponding degrees of methanization of 39% and 60%. De Graaff et al., (2010) successfully treated BW in a lab-scale flocculant sludge UASB reactor at 25°C, achieving a 78% COD_{total} removal and 54% degree of methanization over 951 days.

The feasibility of the AD of BW has thus been demonstrated in small-scale digesters with promising COD_{total} removal and biogas production, but full-scale studies are lacking. Furthermore, many lab-scale digesters were not tested over extended periods at mesophilic temperatures or at higher organic loading rates (OLRs) (>1.0 kg COD_{total} m⁻³ d⁻¹), limiting applicability to full-scale systems. Further research on mass balances for COD_{total}, nitrogen, and phosphorus in full-scale digesters is needed to validate the suitability of AD of BW for producing an effluent suitable for nutrient recovery processes (Bisschops et al., 2019).

Given the limited research on the full-scale AD of BW, it is essential to evaluate whether full-scale results align with findings from earlier lab-scale studies. This study assessed the performance of the first full-scale engineering case of a novel UASB reactor for the treatment of source-separated BW in an urban setting under a cold Nordic climate. The assessment focused on key parameters such as influent characteristics, biogas production, total COD removal, and the mass balances of COD, nitrogen (N), and phosphorus (P). These results were compared with those of previous lab-scale studies. Furthermore, the study analyzed the performance differences between mono-digestion of BW and co-digestion with food waste (FW) in full-scale UASB reactors to identify potential differences to mono-digestion of BW.

4.3. Methods

4.3.1. Site description and plant operation

Source-separated BW was treated in a full-scale 50 m³ UASB reactor (UASB_{BW}) in Helsingborg, Sweden. The BW from vacuum toilets was collected from domestic and office buildings in the new city district of Oceanhamnen, which has been in operation since 2020. The BW was transported via vacuum pipes to a local pumping station, after which it was pumped through a pressurized sewer to a local treatment plant (RecoLab), where the BW was treated (Fig. 4.1A).

In the treatment process, BW was led into a 10 m³ collection tank without any pretreatment or screening (Fig. 4.1B) and then pumped into a UASB_{BW} with an active volume of 50 m³ (Fig. 4.1A and 1B). The UASB_{BW} was equipped with six sampling points distributed from the bottom up to a height of 5 meters (Fig. 4.1A and 1B). The UASB_{BW} separated the sludge at the bottom and the decanted effluent water from the top. UASB_{BW} was continuously operated at 35°C using the city district heating, over a total period of 130 weeks, divided into two phases

with different OLRs ; Phase 1 (average OLR = $0.95 \text{ kg COD m}^{-3} \text{ d}^{-1}$) lasted 56 weeks, and Phase 2 (average OLR = $1.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$) lasted 74 weeks (Table A7, Supplementary Information). The UASB_{BW} feeding occurred in 12-minute cycles, with 2 minutes of feeding at a flow rate of $3 \text{ m}^3 \text{ h}^{-1}$, followed by 10 minutes without feeding. This cycle was repeated for 18-20 consecutive hours per day. This intermittent feeding strategy was the same for the two phases and was designed to optimize the utilization of BW over a 24-hour period, thereby extending the effective feeding duration. Additionally, it is anticipated that the intermittent feeding approach minimizes its impact on both the biomass and effluent quality, due to the relatively low average feeding rate employed. The mixer inside the digester was run for 5 minutes every 120 minutes to break foam if needed.

The biomass in UASB_{BW} consisted of flocculent sludge, seeded with 5 m^3 of sludge obtained from a municipal wastewater treatment plant with a total solids concentration (TS) of 5%. In order to control the solids retention time (SRT), excess sludge was removed from the bottom of the reactor (800-1500 L per removal, occurring twice per week); the targeted SRT was 75 days in Phase 1 and 35 days in Phase 2. The sludge was discharged into a 2 m^3 sludge tank and subsequently directed to the wastewater treatment plant of Öresundsverket in Helsingborg (Fig. 4.1B). The acclimatization of the sludge occurred throughout the entire operational period of 130 weeks.

Source-separated BW was co-digested with FW in a full-scale 41 m^3 UASB reactor (UASB_{BW+FW}) in Noorderhoek, Netherlands. The mixing ratios of BW and FW were not specified. Both waste streams were collected via a vacuum sewer system from 207 residential units, including houses and apartments, equipped with vacuum toilets and kitchen grinders. The reactor contained flocculent sludge as the anaerobic biomass. Due to limitations in the heating system, the reactor was operated at 22°C during winter and 35°C in summer, despite the intended target temperature of 35°C . The UASB_{BW+FW} was in operation for 82 weeks (Table A7, Supplementary Information) with an average OLR of $0.8 \text{ kg COD m}^{-3} \cdot \text{d}^{-1}$.

Decentralized treatment plants, such as the one investigated in this study, typically receive low flows (Table A7, Supplementary Information) compared to municipal wastewater treatment plants, which often handle flows exceeding $1000 \text{ m}^3 \text{ d}^{-1}$.

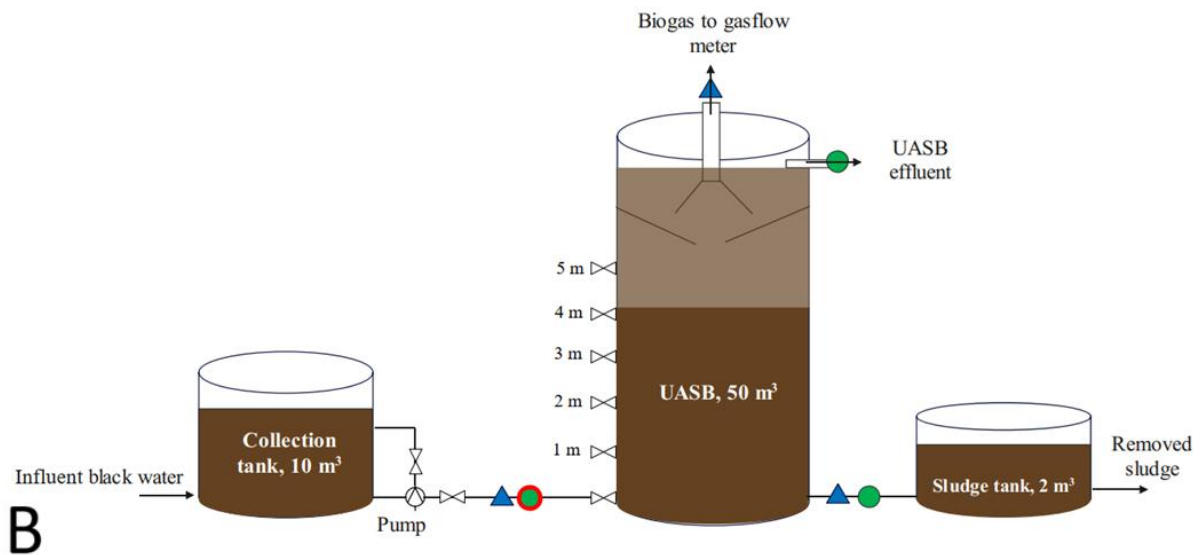
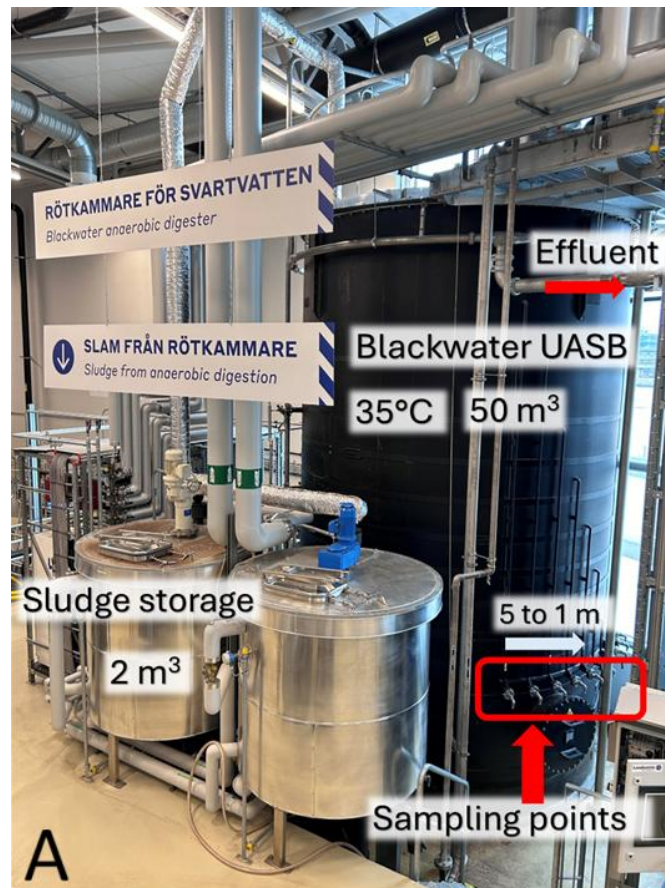


Fig. 4.1.A) Full-scale UASB treating BW at RecoLab, Helsingborg B) Schematic of the BW treatment process and sampling points for mass balance calculations. Green dots indicate grab samples, green dots with red outlines indicate flow-proportional samples, and blue triangles indicate flow-measurement points. Sampling valves for settleable solids and suspended solids measurements at each meter of the digester are indicated.

4.3.2. Sampling and analysis

Weekly flow-proportional 24-h influent samples and grab samples of UASB_{BW} effluent were collected for chemical analysis and analyzed using Hach Lange kits, measuring chemical oxygen demand (COD) (LCK514), total and volatile suspended solids (TSS and VSS) (SS-EN 872:2005), total nitrogen (TN) (LCK138, 238 and 338), ammonium (NH₄⁺-N) (LCK302), total phosphorus (TP) (LCK 348, 349 and 350), phosphate (PO₄³⁻-P) (LCK350), and alkalinity. Spectrophotometric analysis was performed using a Hach Lange spectrophotometer (DR 2800) on unfiltered samples for COD, TN, and TP and on both unfiltered and centrifuged samples for PO₄³⁻-P, NH₄⁺-N, and soluble COD (sCOD). Monthly grab samples from the sludge were analyzed for COD (ISO 15705:2002), TSS and VSS (SS-EN 12880:2000), TN (SS-EN ISO 20236:2021), NH₄⁺-N (ISO 15923-1:2013 B), and TP (SS-EN ISO 15681-2:2018, SS-EN ISO 54321:2021).

Biogas volume was measured using a drum-type flow meter (Ritter TG20-5) positioned at the top of the UASB_{BW} at 20°C and an overpressure of 65 mbar. The methane (CH₄) concentration in the biogas (% vol./vol.) was determined using an online sensor (INCA4003).

Samples for volatile fatty acids (VFA) were centrifuged at 4000 rpm for 15 minutes and filtered through 0.45 µm syringe filters (Sartorius Minisart RC 25). Specific VFAs were measured using high-performance liquid chromatography (HPLC), as described by Ossiansson et al., (2023), with the following modifications: the operating temperature was maintained at 70°C, the flow rate was adjusted to 0.5 mL min⁻¹, and an Aminex HPX-87H column was employed.

The settleable solids in sludge from different UASB_{BW} heights were measured weekly using a 1-liter graduated cylinder and 30 min settling time, following the Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

4.3.3. Batch test for specific methanogenic activity (SMA)

Specific Methanogenic Activity (SMA) was employed to assess the potential of anaerobic sludge for biogas (methane) production by quantifying the conversion rate of acetate to methane, as demonstrated in previous studies, including Hussain & Dubey (2017). This conversion rate is influenced by several factors such as temperature, microbial community composition, and physicochemical parameters (Donoso-Bravo et al., 2009; Kettunen & Rintala, 1997; Souto et al., 2010).

The SMA for acetotrophic methanogens was quantified utilizing the same apparatus as Kjerstad et al., (2016), employing the methodology described by Collins et al., (2003), modified through the use of the anaerobic buffer solution described by Gonzalez-Gil et al., (2002) and micro- and macronutrients addition in accordance with Shelton & Tiedje, (1984). The UASB_{BW} sludge samples were degassed for 48 h at 35°C and subsequently introduced into 120 mL glass vials with a total liquid volume (i.e., sludge, anaerobic buffer solution, and 3 M acetic acid substrate) of 60 mL and an initial concentration of 8 g VSS L⁻¹. The vials were prepared in triplicates, evaluating 10 different initial acetic acid concentrations (ranging from 5 to 50 mM) and blanks. The vials were flushed with nitrogen gas (N₂) and incubated at 180 rpm, as per Conklin et al., (2008), at 35°C. The accumulated biogas production was measured at 8-hour

intervals for 3 days using a pressure transducer (Collins et al., 2003) to calculate the initial rates of gas production and generate SMA curves. The methane content was not measured during the SMA experiment but was assumed to be equivalent to the average from the full-scale measurements.

4.3.4. The solids retention time

The solids retention time (SRT) in the UASB was calculated once a week using Equation (1):

$$SRT = \frac{\sum(V_{\text{reactor } 1-5} \times C_{s \text{ } 1-5})}{Q_{\text{eff}} \times C_{\text{eff}} + V_{\text{wasted}} \times C_{\text{wasted}}} \quad (1)$$

Where $V_{\text{reactor } 1-5}$ is the volume of the UASB reactor segment between 1-5m height (m^3), $C_{s \text{ } 1-5}$ is the corresponding solid concentrations within each UASB reactor segment (mg TSS L^{-1}). Due to the uneven sludge distribution within the UASB reactor, the solids concentrations vary across the five segments. Therefore, an average concentration ($C_{s \text{ } 1-5}$) was calculated. Q_{eff} is the effluent flow rate from the UASB ($\text{m}^3 \text{ d}^{-1}$), and C_{eff} is the solid concentration in the decanted effluent (mg TSS L^{-1}). Additionally, V_{wasted} refers to the daily volume of wasted sludge ($\text{m}^3 \text{ d}^{-1}$), while C_{wasted} is the solids concentration in the wasted sludge (mg TSS L^{-1}).

4.3.5. Mass balances

The mass balances for wet mass, $\text{COD}_{\text{total}}$, N, and P were calculated weekly using flow measurements and chemical analyses (Fig. 4.1B). The mean and standard deviation were computed individually using data from the year 2023, i.e., weeks 79-130 from the start of the UASB_{BW} operation. Any weekly value that deviated by more than two standard deviations from the average was considered an outlier and was excluded from the mass balance calculations. The mass balance was calculated using a Microsoft Excel spreadsheet, which is included in Supplementary Material (Table A3).

4.4. Results and discussion

4.4.1. Influent characteristics

Table 4.1 summarizes influent BW characteristics in the full-scale UASB_{BW} and compares them with previous lab-scale studies and a full-scale UASB system co-digesting BW and kitchen grinder FW (UASB_{BW+FW}). The measured data (influent and effluent characteristics) in Table 4.1 regarding UASB_{BW} were collected from the day UASB_{BW} was started. The average influent $\text{COD}_{\text{total}}$ concentration in UASB_{BW} was 8.6 g COD L^{-1} , aligning with previous lab-scale study values ranging from 9.2 to $12.3 \text{ g COD L}^{-1}$ (Table 4.1). The influent sCOD was $2.47 \text{ mg COD L}^{-1}$ which was comparable to the sCOD measured in the previous lab-scale (De Graaff et al., 2010; Kujawa-Roeleveld et al., 2005; Gao et al., 2019a) indicating that the BW biodegradability ($\text{sCOD}/\text{COD}_{\text{total}}$) is consistent. In this study, the VSS/TSS ratio was 0.94, indicating a high proportion of organic matter and suggesting significant biodegradability and high suitability for biogas production. This finding aligns closely with the VSS/TSS ratio of 0.95 reported by Gao et al., (2019a).

The alkalinity of the influent BW in this study ($5.5 \text{ g HCO}_3^- \text{ L}^{-1}$) was comparable to the value reported by De Graaff et al., (2010), indicating that BW provides sufficient alkalinity for AD. The influent pH to the UASB_{BW} remained stable at 8.7, consistent with previous lab-scale

studies using vacuum BW (De Graaff et al., 2010; Kujawa-Roeleveld et al., 2005; Gao et al., 2019a), suggesting that the high pH could potentially result in a high pH (around 8) in the effluent suitable for struvite precipitation.

The concentrations of TN (1.4 g N L^{-1}) and TP (0.12 g P L^{-1}) measured in the BW from this study were comparable to those reported in previous lab-scale studies (De Graaff et al., 2010; Kujawa-Roeleveld et al., 2005) (Table 4.1). These findings indicate that BW is nutrient-rich and has good potential for nutrient recovery (Wang et al., 2023). The influent BW FA concentration was as high as 0.65 g N L^{-1} , which could potentially cause instability in the AD BW system. This high FA concentration may be attributed to the influent BW pH of 8.7. Similarly, De Graaff et al., (2010) reported a high FA concentration of 0.48 g N L^{-1} , which led to temporary inhibition.

The influent characteristics of BW in UASB_{BW}, as shown in Table 4.1, are similar to those reported in previous laboratory-scale studies. This similarity is noteworthy as it indicates that BW characteristics from vacuum toilets remain relatively consistent, even when comparing data from different countries.

Table 4. 1.BW influent characteristics and effluent quality of UASB systems (average values and standard deviations).

Influent									Effluent								
Parameter	Unit	Full-scale UASB _{BW} (this study)	Full-scale UASB _{BW+FW} (this study)	Lab-scale UASB ^a	Lab-scale UASB-septic tank ^b		Lab-scale UASB ^c	Lab-scale UASB ^d	Full-scale UASB _{BW} (this study)		Full-scale UASB _{BW+FW} (this study)		Lab-scale UASB ^a	Lab-scale UASB-septic tank ^b		Lab-scale UASB ^c	Lab-scale UASB ^d
					UASB _{a1}	UASB _{a2}			Phase 1 (OLR<1.5)	Phase 2 (OLR=1.5)	22.5°C	35°C		UASB _{a1}	UASB _{a2}		
COD _{total}	[g COD L ⁻¹]	8.59 (1.85) (n=92)	9.16 (2.2) (n=51)	9.8 (2.6)	9.5 (6.46)	12.3 (7.78)	11 (0.9)	9.49 (0.33)	1.05 (0.18)	2.24 (1.2)	2.24 (0.95)	2.24 (0.95)	2.4 (0.84)	3.7 (0.46)	2.73 (0.49)	1.6 (0.37)	1.19 (0.08)
sCOD	[g COD L ⁻¹]	2.47 (0.58) (n=70)	-	3.4 (0.47)	1.43 (0.48)	2.0 (1.2)	3.48 (0.2)	-	-	-	-	-	1.5 (0.39)	2.09 (0.45)	1.38 (0.19)	0.8 (0.14)	-
COD _{total} re- moval	[%]	-	-	-	-	-	-	-	88 (2.2)	72.1 (15.4)	76.2 (10)	76.2 (10)	78	61	78	83.5 (3.8)	89 (2)
TSS	[g TSS L ⁻¹]	4.08 (0.8) (n=92)	-	-	-	-	4.93 (0.73)	-	0.18 (0.08)	0.7 (0.55)	-	-	-	0.96	-	0.19 (0.26)	-
VSS	[g VSS L ⁻¹]	3.82 (0.7) (n=84)	-	-	-	-	4.68 (0.73)	-	0.18 (0.01)	0.65 (0.55)	-	-	-	0.83	-	0.16 (0.23)	-
TN	[g N L ⁻¹]	1.43 (0.34) (n=86)	1.28 (0.14) (n=35)	1.9 (0.19)	1.0 (0.13)	1.44 (0.25)	-	-	1.7 (0.65)	1.5 (0.36)	1.36 (0.14)	1.36 (0.14)	1.8 (0.22)	-	1.2	-	-
NH ₄ ⁺ -N	[g N L ⁻¹]	1.13 (0.31) (n=84)	-	1.4 (0.15)	0.71 (0.1)	1.04 (0.1)	1.14 (0.05)	-	1.4 (0.4)	1.26 (0.27)	0.9 (0.06)	0.9 (0.06)	1.5 (0.19)	-	1.07	1.22 (0.07)	-
Free ammo- nia (FA)*	[g N L ⁻¹]	0.65 (0.31)	-	0.48	-	-	0.15 (0.09)	-	0.06 (0.02)	0.03 (0.02)	0.01 (0.0)	0.03 (0.02)	0.03	-	-	0.11 (0.04)	0.12 (0.01)
TP	[g P L ⁻¹]	0.12 (0.02) (n=89)	0.11 (0.11) (n=33)	0.22 (0.07)	0.11 (0.06)	0.11 (0.06)	0.11 (0.03)	-	0.10 (0.01)	0.11 (0.02)	0.12 (0.01)	0.12 (0.01)	0.13 (0.01)	0.06	0.06	0.09 (0.035)	-
PO ₄ ⁻³ -P	[g P L ⁻¹]	0.052 (0.01) (n=93)	-	0.08 (0.01)	0.03 (0.02)	0.03 (0.02)	0.03 (0.01)	-	0.09 (0.01)	0.09 (0.01)	-	-	0.09 (0.01)	0.05	0.05	0.03 (0.01)	-
pH	[-]	8.7 (0.2) (n=93)	7.82 (0.25) (n=17)	8.8 (0.22)	8.8 (0.23)	8.8 (0.23)	8.05 (0.24)	8.7 (0.1)	7.5 (0.1)	7.5 (0.1)	7.5 (0.15)	7.5 (0.15)	7.6 (0.13)	-	-	7.87 (0.19)	7.9 (0.2)
VFA	[g COD L ⁻¹]	-	1.42 (0.22) (n=33)	1.5 (0.48)	0.5 (0.37)	0.5 (0.37)	~1.5	-	-	-	0.82 (0.3)	0.82 (0.3)	0.36 (0.3)	1.0	-	0.07 (0.03)	0.04 (0.0)
Alkalinity	[g HCO ₃ ⁻¹ L ⁻¹]	5.5 (0.24) (n=57)	-	6.09 (1.88)	-	-	-	-	-	-	-	-	-	-	-	-	-

a: De Graaff et al., (2010), b: Kujawa-Roeleveld et al., (2005), c: Gao et al., (2019a), d: Gao et al., (2019c), *: Calculated value.

4.4.2. Operational Phases and Organic Loading Rate Dynamics

Fig. 4.2 illustrates the variations in the OLR and inflow to UASB_{BW} over the operational period. The UASB_{BW} was operated continuously for 130 weeks, which were divided into two distinct phases based on the applied OLR (Fig. 4.2). The OLR in this study was not controlled as all the BW from the housing districts must be treated. In Phase 1 (weeks 0–46), increased BW inflow from the housing district gradually raised the OLR from 0.5 to 1.5 kg COD m⁻³ d⁻¹, averaging 0.95 kg COD m⁻³ d⁻¹. In Phase 2 (weeks 47–130), the OLR increased to 1.7 kg COD m⁻³ d⁻¹ (average sludge loading rate of 0.25 kg COD kgVSS⁻¹ d⁻¹) by week 57, after which feeding was suspended for 5 weeks to remove floating sludge from the top of UASB_{BW}. The OLR then averaged 1.5 kg COD m⁻³ d⁻¹ until week 100, with variability between 0.7 - 2.5 kg COD m⁻³ d⁻¹. The average influent COD_{total} concentration remained constant at 8.6 g COD L⁻¹ throughout both phases.

It is important to note that although the OLR in Phase 2 (OLR = 1.5 kg COD m⁻³ d⁻¹) was higher than in some of the systems studied previously (Table 4.1), even higher OLRs, such as 15.7 kg COD m⁻³ d⁻¹, have been reported in the literature for blackwater treatment (Zhang et al., 2022). This suggests that there may be potential for even higher OLRs in full-scale systems. However, during Phase 2 of the present study, increased issues with sludge washout were observed, which must be addressed before higher OLRs can be applied in full-scale systems.

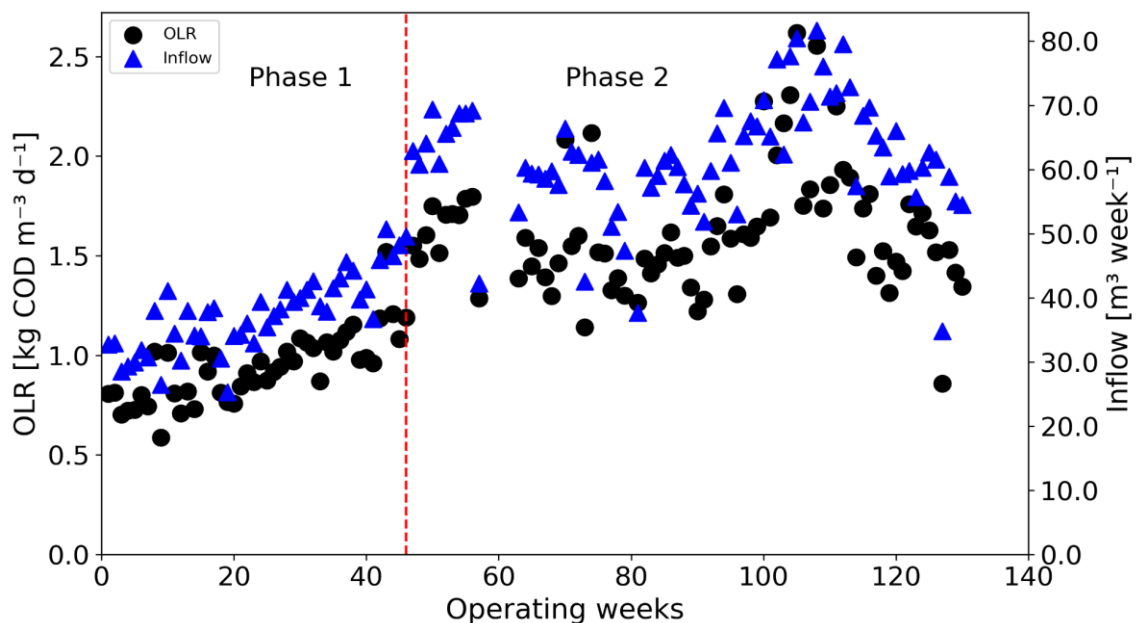


Fig. 4.2. Variations in the influent flow rates and the organic loading rates (OLR) of UASB_{BW} during the two phases of operation (Phase 1 = OLR lower than 0.95 kg COD m⁻³ d⁻¹ and Phase 2 = OLR around 1.5 kg COD m⁻³ d⁻¹).

4.4.3. COD_{total} and TSS removal

During phase 1 (OLR < 1.5 kg COD m⁻³ d⁻¹) UASB_{BW} achieved the highest COD_{total} removal efficiency (88%) during operation, comparable to Gao et al., (2019c) (HRT = 12 days, OLR = 0.8 kg COD m⁻³ d⁻¹) under comparable OLR, and exceeding some earlier studies with 61% to

89% COD removal (De Graaff et al., 2010; Gao et al., 2019a; Kujawa-Roeleveld et al., 2005) (Table 4.1). During Phase 1, the effluent COD_{total} and TSS concentrations were stable and low (1.05 g COD L⁻¹ and 0.18 g TSS L⁻¹) (Table 4.1), with settleable solids at 4 ± 5 mL L⁻¹ (Fig. 4.3), indicating effective COD removal and conversion into biogas.

Transitioning into Phase 2 (OLR = 1.5 kg COD m⁻³ d⁻¹), effluent concentrations of COD_{total} (1–7.6 g COD L⁻¹) and TSS (0.18–7.7 g TSS L⁻¹) fluctuated, with COD_{total} removal efficiency dropping from 88% to 72% (Table 4.1), likely due to sludge washout. These variations aligned with inflow and OLR changes (Fig. 4.2). Similarly, Gao et al., (2019a) (OLR = 1.6 kg COD m⁻³ d⁻¹) reported increased effluent COD_{total} concentrations (1.2 to 1.6 g COD L⁻¹), TSS (0.14 to 0.2 g TSS L⁻¹), and reduced efficiency from 87% to 83.5% as OLR rose from 0.8 to 1.6 kg COD m⁻³ d⁻¹ (Table 4.1). Despite the drop in the COD_{total} removal efficiency, the 72% COD_{total} removal efficiency remained within the reported range of 61% to 89% (Table 4.1). Sludge washout patterns were also noted by De Graaff et al. (2010) at 1.0 kg COD m⁻³ d⁻¹ during the first weeks of operation, while Kujawa-Roeleveld et al. (2005) observed no washout at lower OLRs (0.3–0.4 kg COD m⁻³ d⁻¹). Wu et al., (2020) observed sludge washout in granular sludge treating low-strength starch wastewater (OLR = 4 kg COD m⁻³ d⁻¹), attributing it to a significant increase in the protein-to-polysaccharide ratio within the tightly-bound extracellular polymeric substances (EPS) of the granular sludge; however, it should be noted that the sludge used in the present study was flocculant, not granular. The effluent from the UASB_{BW}, particularly in Phase 2, requires post-treatment to remove the remaining COD and TSS.

During Phase 2, the variations in effluent COD_{total}, TSS, and settleable solids suggest the need to consider factors such as pH and upflow velocity. With pH (7.5) and upflow velocity (0.29 m h⁻¹) within recommended ranges (Grady et al., 2011; Mariraj Mohan & Swathi, 2022), their impact on effluent quality was minimal. The structure of EPS and Long-term microbial changes may explain these issues, which were absent in the current study and in short-term lab studies. The observed variations and reduced COD_{total} removal efficiency underscore the challenges in operating full-scale UASB reactors at higher OLRs, warranting further research to enhance effluent quality.

Overall, sludge washout during the higher OLR phase (Phase 2) increased the effluent COD_{total} and TSS concentrations, settleable solids at 5m, and reduced the COD_{total} removal efficiency, although the latter remained comparable to the previous lab-scale results (Table 4.1).

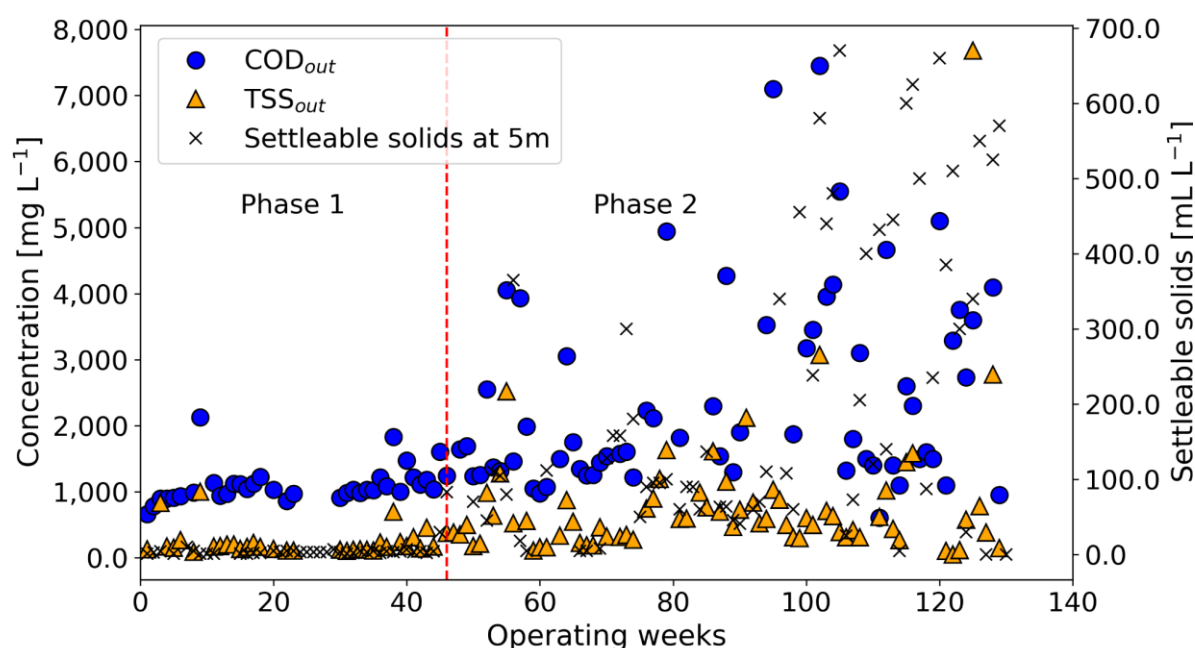


Fig. 4.3. Variations in settleable solids at 5 m height, and in effluent concentrations of $\text{COD}_{\text{total}}$ and TSS of UASB_{BW} (Phase 1 = OLR lower than $0.95 \text{ kgCOD m}^{-3}\text{d}^{-1}$ and Phase 2 = OLR around $1.5 \text{ kgCOD m}^{-3}\text{d}^{-1}$).

4.4.4. Biogas Production

Table 4.2 summarizes biogas production from UASB_{BW}, UASB_{BW+FW}, and previous lab-scale UASB systems.

Table 4.2. Performance of UASB systems in BW: biogas yield, degree of methanization, and methane production rate with organic loading rates (average values with standard deviations).

Parameter	Unit	Full-scale UASB _{BW} (this study)		Full-scale UASB _{BW+FW} (this study)		Lab- scale UASB ^a	Lab-scale UASB-septic tank ^b		Lab- scale UASB ^c	Lab- scale UASB ^d
		Phase 1 (OLR<1.5)	Phase 2 (OLR=1.5)	22.5°C	35°C		UASB _{st1}	UASB _{st2}	Phase IV	Phase I
Methane yield	[Nm ³ CH ₄ kg COD _{in} ⁻¹]	0.27	0.24	0.21	0.24	0.19	0.14	0.21	0.14	-
Degree of Methanization	[kg COD- CH ₄ kg ⁻¹ COD _{in}]	0.77	0.71	0.60	0.68	0.54	0.39	0.60	~0.4 ⁺	0.44
Daily methane production rate	[m ³ CH ₄ m ⁻³ reactor d ⁻¹]	0.25 (0.02)	0.39 (0.08)	0.18 (0.07)	0.2 (0.03)	0.21*	0.02*	0.04*	0.14- 0.36 ⁺	0.68
OLR	[kg COD m ⁻³ d ⁻¹]	0.95 (0.2)	1.5 (0.45)	0.79 (0.2)	0.79 (0.2)	1.0	0.3-0.4	0.3-0.4	1.64	4.10

a: De Graaff et al., (2010), b: Kujawa-Roeleveld et al., (2005), c: Gao et al., (2019a), d: Gao et al., (2021), *: Calculated value, +: estimated.

The methane production rate increased from $0.25 \text{ m}^3 \text{ CH}_4 \text{ m}^{-3} \text{ reactor d}^{-1}$ in Phase 1 (OLR < $1.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$) to $0.39 \text{ m}^3 \text{ CH}_4 \text{ m}^{-3} \text{ reactor d}^{-1}$ in Phase 2 (OLR = $1.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$), driven by higher biodegradable organic matter availability at higher OLR (Gómez et al., 2011). The

methane production rate in Phase 2 was 8% to 95% higher than the UASB_{BW+FW} system and previous lab-scale studies (De Graaff et al., 2010; Kujawa-Roeleveld et al., 2005) (Table 4.2). Despite a slightly higher OLR (1.6 kg COD m⁻³ d⁻¹) in Gao et al., (2019a), the longer operational period in the present study (130 vs. ~7 weeks) could have contributed to the higher methane production rate (Table 4.2) by allowing microbial adaptation. However, the methane production rate was 37% to 57% lower than that reported by Gao et al., (2021) possibly due to the higher OLR in their study (4.1 kg COD m⁻³ d⁻¹), indicating that higher OLRs could result in higher methane production rate due to greater availability of easily biodegradable organic matter (Gao et al., 2021; Gómez et al., 2011; Huang et al., 2021).

The results for methane production per COD removed and for the degree of methanization provide additional insights into the efficiency of biodegradable matter conversion into methane under varying OLRs. In this study, the methane production per COD_{removed} for both phases was comparable: 0.304 Nm³ CH₄ kg⁻¹ COD_{removed} in phase 1 and 0.307 Nm³ CH₄ kg⁻¹ COD_{removed} in phase 2. These values were approximately 86% of the standard methane production of 0.35 Nm³CH₄ kg⁻¹ COD_{removed} under standard temperature and pressure (STP) conditions (20°C and 1 atm) indicating high conversion of COD into methane. The degree of methanization decreased from 0.77 to 0.71 kg CH₄-COD kg⁻¹ COD_{in} as the OLR increased from <1.5 to 1.5 kg COD m⁻³ d⁻¹ (Fig. 4.4, Table 4.2) may be due to sludge washout caused by higher methane production rates entraining biomass with gas bubbles, subsequently reducing the SRT from an average value of 75 to 56 days (Table A7, Supplementary Information), a trend also noted by Gao et al. (2019b). Despite this reduction in the degree of methanization at high OLR, the degree of methanization in this study was 4% to 49% higher than the 0.39 to 0.68 kg CH₄-COD kg⁻¹ COD_{in} observed in UASB_{BW+FW} and previous lab-scale studies (Table 4.2), showing that operating the full-scale UASB at an average SRT of 56 days still achieved high biogas yields compared to longer SRTs (>75 days) in earlier lab-scale research.

Furthermore, the extended operational period (130 weeks) likely allowed sufficient microbial adaptation, possibly contributing to high degrees of methanization, demonstrating that a full-scale UASB digester can achieve high biogas production. The high degree of methanization in both phases was comparable to the previous batch biomethane potential (BMP) tests (0.71 kg CH₄-COD kg⁻¹ COD_{in}) (Kjerstadius et al., 2015), which was consistent with the range of 0.34 to 0.85 kg CH₄-COD kg⁻¹ COD_{in} reported in previous studies (Gao et al., 2019a, Gao et al., 2020; Zhang et al., 2019), suggesting effective methanogenesis capacity.

Overall, a higher OLR resulted in a higher methane production rate. The methanization of BW in both phases was higher than previous lab-scale results indicating high methanogenic activity.

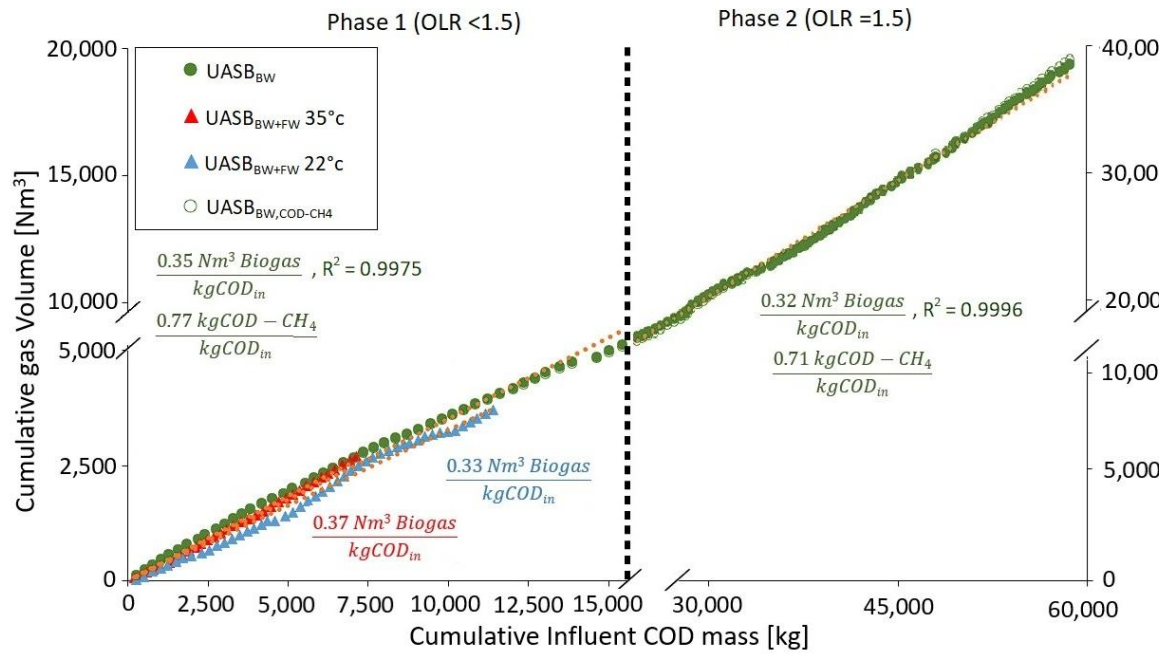
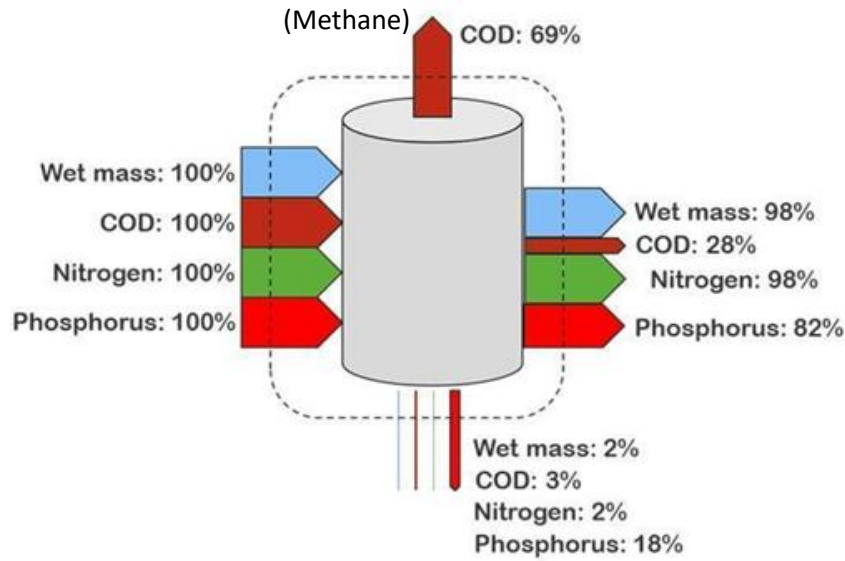


Fig. 4.4. Accumulated biogas yield versus accumulated influent COD load in the UASB_{BW}.

4.4.5. Mass Balances

Fig. 4.5 summarizes the mass balances of wet mass, COD, N, and P. For the wet mass, only 2% of the sludge was removed, reflecting the very small amount of removal needed to control the SRT.



Parameter	Unit	Inflow			Biogas			Sludge			Decanted effluent			Sum out-flows	% out-flow/in-flow
		n	average	stdev	n	average	stdev	n	average	stdev	n	average	stdev		
Wet mass	[kg Week ⁻¹]	50	63,334	8,394	-	-	-	26	1,023	153	-	62,311	8,41	63,334	100
COD _{total}	[kg Week ⁻¹]	47	533	97	4	406	95	10	20	8	36	166	80	592	109
Nitrogen	[kg Week ⁻¹]	90	82.6	12	-	-	-	10	1.7	0.60	32	87	20	88.7	108
Phosphorus	[kg Week ⁻¹]	47	7.85	1.61	-	-	-	10	1.47	0.48	33	6.8	1.23	8.27	108

Fig. 4.5. Mass balances for wet mass, COD_{total}, Total Nitrogen (N), and Total Phosphorus (P). The mass balances are expressed as percentages relative to the influent mass.

The COD mass balance showed an equilibrium between inflows and outflows (Fig. 4.5 and Table 4.3), with 69% of the influent COD_{total} converted into methane, surpassing the 43% to 59% range reported in previous studies (Fig. 4.5 and Table 4.3). This high efficiency is perhaps due to the 35°C operating temperature, compared to the 25°C used by De Graaff et al., (2010). Additionally, this study's lower OLR of 1.5 kg COD m⁻³ d⁻¹, compared to the higher OLRs of 0.3 to 10 kg COD m⁻³ d⁻¹ used by Gao et al., (2019a, 2020), likely contributed to the increased degree of methanization. Low sludge production also influenced the COD_{total} retained in the sludge, which was 3%, far lower than the 15% to 43% observed in other studies. This discrepancy was also highlighted by the higher suspended solids in the effluent, accounting for 30% of the effluent COD_{total} in the present study, compared to 13% to 17% reported by Gao et al. (2019a, 2020) and 29% by de Graaff et al. (2010).

Table 4.3. COD_{total} mass balance in anaerobic digestion of BW: average values expressed as percentages relative to influent COD_{total}.

Study	Average Out-flows/Inflows	COD _{total} in the effluent	COD _{total} converted into methane	COD _{total} in the removed sludge
	[%]	[%]	[%]	[%]
Full-scale UASB _{BW} (this study)	100	28	69	3
De Graaff et al., (2010)	96	27	54	15
Gao et al., (2019a)	92-114	13	43	43
Gao et al., (2020)	101-105	16.4-17.6	59-62	26-29

Regarding the N and P mass balances, the results showed that 98% of N was present as free ammonium nitrogen and 82% of P was present as soluble phosphate (Fig. 4.5), primarily in the liquid effluent, presenting opportunities for recovery via processes such as ammonia stripping or struvite precipitation. The removed sludge only contained 2% of N and 18% of P, which was lower than previously reported values (e.g., 33% to 46% P and 0% to 8% N by Kujawa-Roeleveld & Weijawa, 2012); this is likely an effect of the sludge washout via the decanted effluent, which had suspended solids concentrations between 500 and 2500 mg SS L⁻¹, and caused the need for low sludge removal to maintain the SRT. The remaining P in the effluent could be recovered as struvite in future processes (Ueno et al., 2001). However, high suspended solids would have to be combined with filtration systems, as demonstrated by Kaykhahi et al., (2025).

In addition, the BW vacuum flush volume per connected person equivalent (PE) was calculated using the default values for COD, P and N found in Jönsson et al., (2005) (Table A2, Supplementary Information). The resulting BW flush volume ranged from 8 to 11 L PE⁻¹ day⁻¹, which is higher than the 6 L PE⁻¹ day⁻¹ assumed in previous studies (Kjerstadius et al., 2017). These differences in flush volumes should be considered in future studies of BW vacuum systems.

For a better understanding of the performance of UASB_{BW}, an energy calculation was conducted for UASB_{BW} using limited data covering 20 weeks (details provided in Table A6 in the Supplementary Information). This analysis was constrained by the absence of insulation on the UASB_{BW} system and the lack of data for the whole operational period (130 weeks). The results, as presented in the supplementary information, indicate a positive and beneficial primary energy calculation. However, future research should focus on conducting optimized energy balance calculations over extended periods. Overall, the mass balances for wet mass, COD, N, and P indicated the effective functioning of the system in terms of biogas production, COD_{total} removal, and nutrients in the effluent. However, the contrasting values compared to other studies on similar systems call for more research to confirm the present findings as being representative of full-scale blackwater systems.

4.4.6. SMA and trend of VFA through the UASB

The SMA (Table 4.4) and VFA trends throughout the digester (Fig. 4.6) provide additional insights into UASB_{BW} efficiency at higher OLR during Phase 2.

The SMA from BW mono-digestion in UASB_{BW} (185.6 NmL CH₄ g VSS⁻¹ d⁻¹) doubled that of UASB_{BW+FW} and surpassed previous laboratory results, despite not all studies focusing on BW (Table 4.4). This elevated SMA indicates that the reactor's biomass in Phase 2 showed high activity, especially among acetogenotrophic methanogens.

Table 4.4. Average values of SMA of sludge from BW UASB systems.

Study	Average Temperature	Average Specific methanogenic activity for acetotrophs (SMA _{ac})
	[°C]	[N mL CH ₄ g VSS ⁻¹ d ⁻¹]
Full-scale UASB _{BW} (this study)	35	185.6
Full-scale UASB _{BW+FW} (this study)	35	79.9
Gao et al., (2019a)	35	30-80.5
Zhang et al., (2022)	35	105
Gao et al., (2020)	35	70

The distribution of sCOD and VFA along the UASB_{BW} height offers insights into the methanogenic population's efficiency. Samples from two sampling campaigns on the same day (week 141) at an OLR of 1.5 kg COD m⁻³ d⁻¹ are shown in Fig. 4.6. The sCOD concentration decreased from 2400 mg sCOD L⁻¹ in the influent to 625 mg sCOD L⁻¹ in the effluent, indicating a decreasing trend throughout the digester. VFA, expressed as COD-equivalent, was only detected in the influent. This indicates two key observations: (i) a high level of methanogenic activity throughout the reactor, maintaining low VFA concentrations, and (ii) the presence of residual sCOD in the effluent despite the relatively low OLR and an HRT of 5.6 days, suggesting incomplete degradation of soluble organics. Compared with the lab-scale UASB operated by De Graaff et al. (2010) at 25 °C, which reported effluent sCOD concentrations of 0.9–1500 mg COD L⁻¹ and VFA concentrations of 140–360 mg COD L⁻¹, the full-scale UASB treating source-separated BW showed a lower sCOD concentration of 625 mg COD L⁻¹ and undetectable VFA concentrations, suggesting more complete VFA conversion at 35 °C and enhanced methanogenic activity. However, the persistent sCOD in this study suggests the need to investigate its composition and biodegradability. Similarly, Gao et al., (2019a, 2019c) reported low effluent VFA levels (70 and 40 mg COD L⁻¹) and comparable sCOD (800 mg COD L⁻¹) in lab-scale reactors at 35°C (Table 4.1).

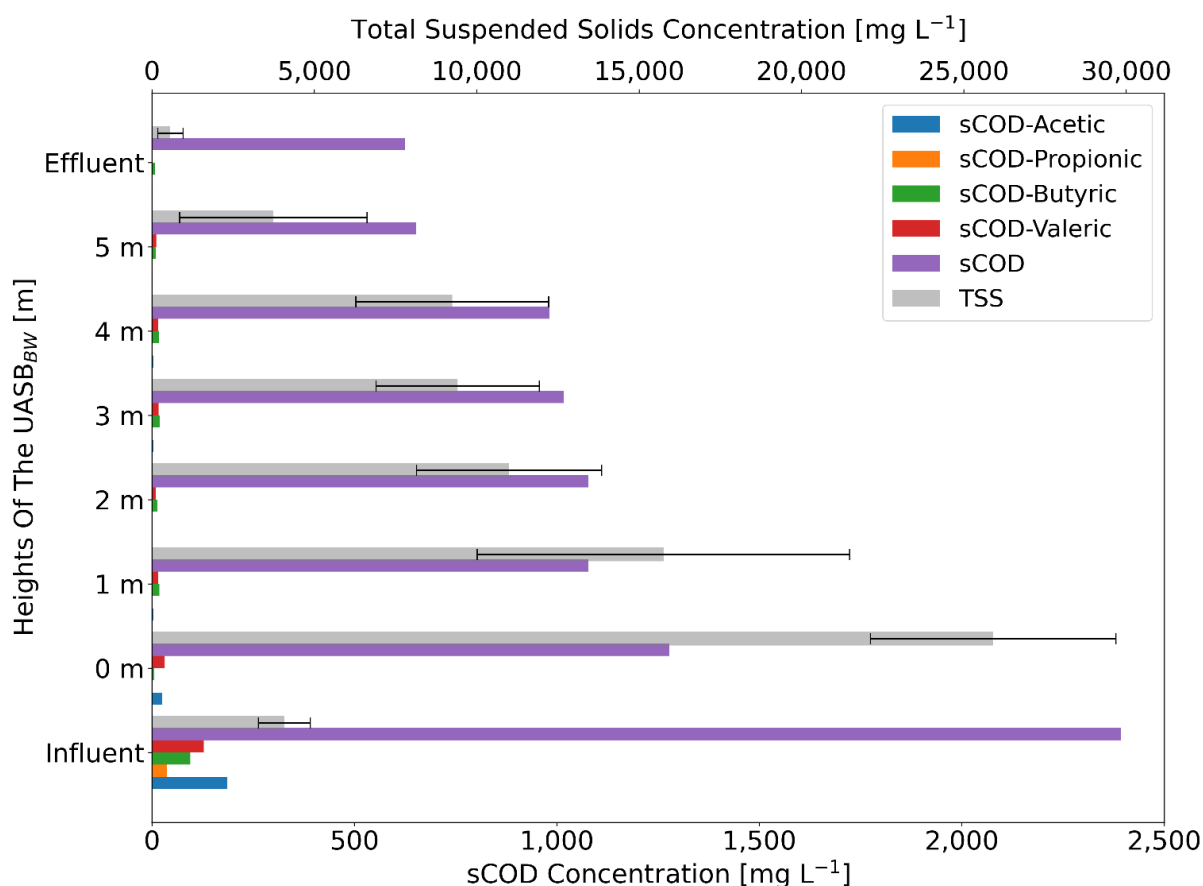


Fig. 4.6. Soluble COD (sCOD) concentrations, total suspended solids (TSS) concentrations (average values and standard deviation), and the sCOD equivalent of volatile fatty acid (VFA) concentrations measured along the height of the UASB_{BW} reactor.

4.4.7. Co-digestion of blackwater and food waste

A study on a full-scale (41 m³) UASB system co-digesting BW and FW (UASB_{BW+FW}) in Sneek (Netherlands) showed that intermittent operation at varying temperatures (22.5°C in winter and 35°C in summer) resulted in occasionally lower yet comparable COD_{total} removal efficiency compared to BW mono-digestion (Table 4.1). Increasing the temperature to 35°C improved the degree of methanization from 0.60 to 0.68 kg CH₄-COD kg⁻¹ COD_{in} (Table 4.2), aligning with findings from Seneesrisakul et al., (2018) and Luostarinen et al., (2007). Despite this improvement, methanization in UASB_{BW+FW} was still 11% to 24% lower than the maximum achieved in BW mono-digestion (0.77 kg CH₄-COD kg⁻¹ COD_{in}), highlighting the reduced efficiency of co-digestion under these conditions.

The SMA at 35°C for UASB_{BW+FW} (80 NmL CH₄ gVSS⁻¹ d⁻¹) was 57% lower than for UASB_{BW} alone (186 NmL CH₄ g VSS⁻¹ d⁻¹), indicating that co-digesting BW with FW did not enhance acetogenotrophic activity, unlike findings by Kamravamanesh & Kokko. (2024) who found it increased methane production.

The degree of methanization and SMA may have been impacted by biomass washout or microbial imbalances caused by unregulated BW to FW mixing ratios, with optimal ratios reported as 1:1 or 1:2 (Wang et al., 2024; Wen et al., 2024). Furthermore, the freshness of the sludge sample used in the SMA analysis might have also influenced the results. Although co-

digestion can enhance performance by providing diverse substrates, it may also induce microbial competition and reduce methanogenic efficiency. Despite its potential benefits, such improvements were not evident in this full-scale system.

4.5. Conclusions

The performance of a full-scale UASB reactor treating blackwater was evaluated over 130 weeks in terms of COD_{total} removal, biogas production, and mass balances for COD, phosphorus, and nitrogen to assess whether its performance aligned with previously reported lab-scale studies.

The results showed that the full-scale UASB reactor treating blackwater demonstrated comparable performance in terms of COD removal, biogas production, and influent characteristics to those observed in previous lab-scale experiments, confirming that anaerobic digestion of blackwater is a viable treatment solution for blackwater.

The full-scale UASB exhibited the highest COD_{total} removal efficiency (88%) and the highest degree of methanization (0.77 kg COD-CH₄ kg⁻¹ COD_{in}) during the lower OLR phase, with COD_{total} removal comparable to previous lab-scale studies and methanization exceeding previously reported results, indicating stable performance. In the higher OLR phase, COD_{total} removal dropped to 72% due to sludge washout, but the degree of methanization remained high.

The mass balance results showed that 69% of the influent COD_{total} was converted to methane. However, a large degree of the influent COD_{total} was also found in the effluent (28%), likely due to sludge washout, warranting further studies to optimize blackwater anaerobic digestion in full-scale applications. The decanted effluent contained 92% N and 82% P in mineralized forms (ammonium and phosphate), which is attributed to low sludge production (2% of wet mass), which is suitable for nutrient recovery.

Although the full-scale UASB treatment of blackwater showed promising results, further research is needed to optimize the full-scale system, particularly in terms of reducing sludge washout. This could include strategies such as sludge removal from different heights of the reactor, as well as investigations into the dynamics of the microbial communities. Furthermore, an energy balance should be performed to quantify the energy generated from blackwater treatment and the energy required to maintain the reactor at its operating temperature, which is particularly important to optimize given that the digester was not insulated.



Chapter 5

Performance Evaluation of a Full-Scale Two-Stage Anaerobic Digestion System Treating Fruit Juice Processing Wastewater

This chapter submitted to the journal of Water Process Engineering as:

Abuzir, S.; Simion, G.; Domini, M.; Bertanza, G. Performance Evaluation of a Full-Scale Two-Stage Anaerobic Digestion System Treating Fruit Juice Processing Wastewater.

Chapter 5. Performance Evaluation of a Full-Scale Two-Stage Anaerobic Digestion System Treating Fruit Juice Processing Wastewater

5.1. Abstract

Fruit juice processing wastewater (FJPWW) is rich in highly biodegradable organic matter but often exhibits low buffering capacity and nutrient imbalance, which can compromise anaerobic digestion stability. Two-stage anaerobic digestion can decouple hydrolysis/acidogenesis from acetogenesis/methanogenesis to improve robustness when treating readily fermentable wastewater. This study evaluated the long-term performance of a full-scale two-stage anaerobic digestion system treating FJPWW in terms of degree of acidification (DoA), COD removal, VFA and methane production, proposed practical recommendations for plant operation and future research needs, based on full-scale observations. The system comprised a homogenization tank (3500 m³) for hydrolysis/acidogenesis (16–26°C; HRT 29.1–33.3 hours; OLR 3.06–3.35 kg COD m⁻³ d⁻¹), followed by two parallel EGSB (R2S) reactors (750 m³ each; HRT 17 h; OLR 4.77 kg COD m⁻³ d⁻¹; 29.4°C). Performance was assessed under pseudo-steady state periods. The homogenization tank achieved 32–38% COD_{total} removal with DoA of 21–25% and effluent VFA of 898–1093 mg HAc L⁻¹. The R2S reactors reduced COD_{total} from 2902 to 349 mg COD L⁻¹ (88.1% COD removal) and decreased VFA from 1082 to 69.2 mg HAc L⁻¹ (VFA/Alk 0.07). Methane yield was 0.277 Nm³CH₄ kg⁻¹COD_{removed} with average production of 1763 (846) Nm³ CH₄ d⁻¹ (CH₄ content 80%); methane production increased from 566 to 4560 Nm³ d⁻¹ as OLR increased from 1.52 to 9.53 kg COD m⁻³ d⁻¹. Overall, the results supported full-scale two-stage digestion for stable FJPWW treatment and identified operational control strategies to maintain performance under variable loading such as measuring the settleability of granular biomass in the R2S reactors.

5.2. Introduction

Population growth has increased demand for fruit juice production, which in turn has generated larger quantities of fruit juice processing wastewater (FJPWW) and intensified public pressure on water management within the food and beverage industry (Alkaya & Demirer, 2015). This sector consumes substantial volumes of fresh water for operations such as cleaning, fermenting, washing, evaporating, and sterilizing, and much of this water is subsequently discharged as effluent. As a result, the effluent may contain high concentrations of cleaning agents, organics, suspended solids (TSS), and elevated chemical oxygen demand (COD), and it can exhibit substantial pH variations (Ait Hsine et al., 2005; Amuda & Amoo, 2007; Isla et al., 2013; Oktay et al., 2007).

FJPWW, an agro-industrial effluent produced during fruit washing, pressing, and juice manufacturing, typically contains a high organic load (375–124,800 mg COD L⁻¹) and is associated with low pH and low alkalinity, alongside a high carbon-to-nitrogen (C:N) ratio that leads to nutrient imbalances (Dold & Ekama, 1981; Ozbas et al., 2006; Tyagi et al., 2019). These characteristics, together with pronounced variability in flow volume, add further complexity to its treatment (Austerman-Haun & Rosenwinkel, 1997; Chen et al., 2019; Dold & Ekama, 1981; Ozbas et al., 2006;

Valta et al., 2015; Zerrouki et al., 2021). Nevertheless, because FJPWW is rich in organics, it remains a suitable substrate for the production of a clean and environmentally friendly energy source such as biogas through biological processes such as anaerobic digestion (AD) (Mateescu et al., 2008; Zerrouki et al., 2021).

AD proceeds through multiple stages in which distinct microbial consortia transform organic matter into biogas, primarily methane (CH_4) and carbon dioxide (CO_2) (Appels et al., 2008; Joshi & Visvanathan, 2019; Zheng & Li, 2024). This conversion is commonly described in four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During these stages, complex polymers are first depolymerized to monomers, which are subsequently converted into volatile fatty acids and alcohols; these intermediates are then transformed into acetate, hydrogen, and CO_2 , and finally into methane (Khan et al., 2021; Wang et al., 2018).

The performance and robustness of AD are governed by key operating conditions, such as temperature, pH, and the carbon to nitrogen (C:N) ratio (Odedina et al., 2017; Zheng & Li, 2024). Under conventional mesophilic AD operation, the overall microbial consortium is generally favored at temperatures of 20–45°C and around neutral pH (7.0) (Cremonez et al., 2021). However, these conditions refer to the consortium as a whole, since the preferred environmental conditions vary across the different stages of AD. For stable AD operation, an ideal C:N ratio is typically 20–30:1 (Laiq et al., 2019; Odedina et al., 2017).

AD can be operated as a single-stage, two-stage, or multi-stage system. Single-stage AD carries out all microbial conversions in one reactor, while two-stage AD separates the phases to better match conditions to each microbial group and improves efficiency (Holl et al., 2022; Simeonov et al., 2025; Srisowmeya et al., 2020). In two-stage AD, the first reactor favors hydrolytic and acidogenic bacteria (pH 5–6, short hydraulic retention times), producing volatile fatty acids (VFAs) as well as CO_2 and H_2 (Fuentes et al., 2021). The second reactor supports methanogens (pH 6–8, longer retention times), converting acetic acid into methane (Fuentes et al., 2021; Simeonov et al., 2025; Srisowmeya et al., 2020). This configuration is well-suited for high-sugar wastewaters such as FJPWW, which can destabilize single-stage systems (Feng et al., 2020). Methane contents of 70–81% and acidification degrees of 54–89% have been reported, indicating effective performance of two-stage AD systems (Bergland et al., 2010; Carvalheira et al., 2018; Mateus et al., 2020).

Relative to single-stage operation, two-stage AD offers higher stability, greater tolerance to organic loads, and enables recovery of valuable byproducts (Fuentes et al., 2021). Phase separation improves pH control, supporting higher biogas yields and enhanced substrate degradation (Fuentes et al., 2021; Perera et al., 2012). For example, two-stage systems can achieve COD removal efficiencies 35% higher than single-stage systems in food waste digestion (Parra-Orobio et al., 2021).

AD of highly biodegradable substrates similar to FJPWW has been studied at different bioreactor scales and has shown stable and effective performance in terms of COD removal and methane production. For instance, Austermann-Haun et al. (1997) reported a COD removal of more than 80% when treating wastewater effluent from a fruit juice industry using a full-scale UASB reactor. El-Kamah et al. (2010) treated wastewater generated from the fruit juice industry in lab-scale two-stage up-flow anaerobic sponge reactors (UASRs) and achieved a COD removal of 70–91%. Cuff

et al. (2018) treated wastewater generated from a soft drink industry in an expanded granular sludge bed (EGSB) and achieved COD removal of more than 85% and a daily methane production of up to 1950 Nm³ CH₄ d⁻¹. Chun-Te Lin et al. (2020) treated wastewater in a whiskey distillery in EGSB and external circulation sludge bed (ECSB) reactors and achieved COD removals of 94.8-95.7%.

Although prior studies have reported stable performance in terms of COD removal and methane production, research on the performance evaluation of full-scale R2S reactors treating highly biodegradable wastewater remains limited. In addition, in-depth evaluation of overall two-stage AD processes treating highly biodegradable wastewater, including measurements of the microbial activity of granular biomass for methane production, is scarce. Practical guidance on plant operation and monitoring for two-stage AD plants is also limited. Therefore, the aim of this study is to update the literature by evaluating the performance of a full-scale two-stage AD process treating highly biodegradable wastewater, provide insight into the methane-producing activity of granular biomass, and propose plant operation and monitoring recommendations for two-stage AD plants, while identifying future research needs to further optimize such systems.

5.3. Methods and Materials

5.3.1. Substrate

Fruit juice processing wastewater (FJPWW) was obtained from a nearby fruit juice industry in Bronzolo, Northern Italy, and treated in a two-stage AD system at the Bronzolo municipal wastewater treatment plant (MWWTP). The physio-chemical characteristics are provided in Table 4.1 in the results and discussion section.

Dewatering sludge liquors, obtained from the dewatering of digestate from an anaerobic digester at the MWWTP of Bronzolo receiving municipal and industrial wastewater, were used for nutrient supplementation as soluble ammonium and phosphate (NH₄⁺-N and PO₄⁻³-P, respectively), and for alkalinity supplementation. The anaerobic digester treated primary sludge, secondary sludge, waste activated sludge, fats, FJPWW, and the effluents from two high-rate EGSB (R2S) reactors treating FJPWW. The average concentrations of NH₄⁺-N and PO₄⁻³-P were 1080 mg N L⁻¹ and 72.8 mg P L⁻¹, respectively

5.3.2. Site description and plant operation

FJPWW was treated in a two-stage AD system (Fig 5.1A and B). The wastewater first entered a homogenization tank (3500 m³), where hydrolysis and acidogenesis (the first stage of AD) occurred at different temperature ranging between 16-26°C depending on the season (no external heating), average HRT ranging between 29.1 and 33.3 hours, and average organic loading rate (OLR) ranging between 3.06 and 3.35 kg COD m⁻³ d⁻¹ (Fig. 5.1A and B, and Table B1, Supplementary Information). During this stage, nutrients (nitrogen and phosphorus) were added in the form of urea, and NH₄⁺-N and PO₄⁻³-P were supplemented by de-watering sludge liquors to achieve a COD:N:P ratio close to 350:5:1 (de Lemos Chernicharo, 2007). Moreover, NaOH was dosed in the homogenization tank to adjust the pH of the treated wastewater. The hydrolyzed and acidified wastewater then passed through two parallel belt filters, after having added a Fe-based

coagulant and, in some periods, polyelectrolyte to remove solids (Fig. 5.1A and B). After filtration, the filtered, hydrolyzed, and acidified wastewater was pumped to a re-circulation tank (30 m³). Prior to pumping from the re-circulation tank to the two high-rate reactors EGSB reactors (R2S), NaOH was added to adjust pH. The wastewater was then pumped to two R2S reactors with an active volume of 750 m³ each, operated at an average HRT of 17 hours, average OLR of 4.77 kg COD m⁻³ d⁻¹, and average temperature of 29.4°C (Table B2, Supplementary Information), where acetogenesis and methanogenesis occurred (Fig. 5.1A and B). The two-stage AD system has been in operation since 2012; however, data were recorded in the treatment's plant digital database since 2019. Therefore, the Results and Discussion section regarding the performance of the two-stage AD plant is based on operational data from 2019 to 2026.

Biogas and methane were measured using ultrasonic flowmeters at a pressure of ca. 25 mbar.

The biomass in the homogenization tank was flocculant sludge (TS and VS not measured). The homogenization tank was not seeded with any sludge; but, the sludge formed naturally within a few days. The biomass in the R2S reactors was granular sludge. The R2S reactors were inoculated with granular sludge from other anaerobic plants treating paper and pulp wastewater in central Europe.

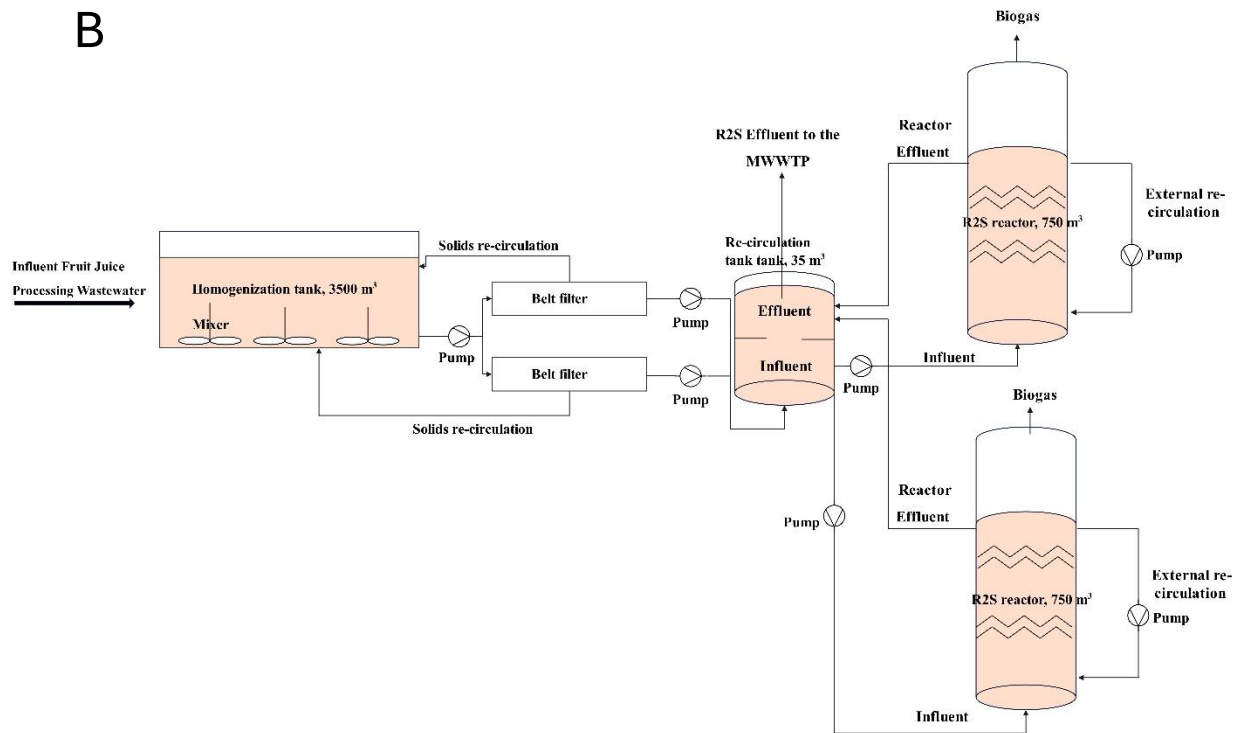
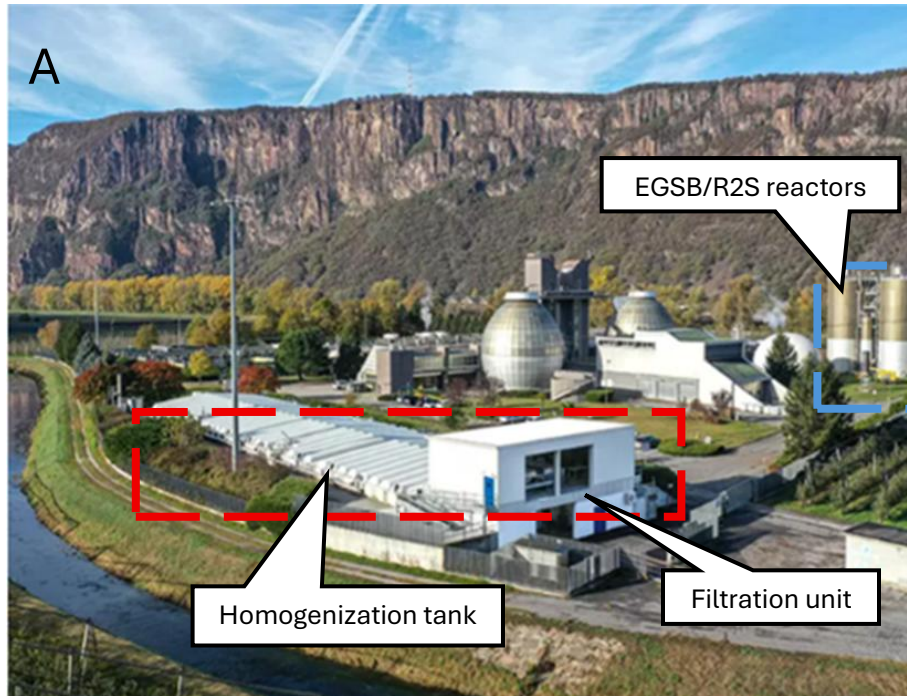


Fig. 5.1. A) Full-scale homogenization tank and filtration unit (highlighted in red), and full-scale EGSB/R2S reactors (highlighted in blue). B) Schematic drawing of the fruit juice processing wastewater (FJPWW) treatment process.

5.3.3. Analytical methods

FJPWW was chemically analyzed twice a week using 24-hours samples for total and volatile solids (TS, VS; APHA, 2005), total and soluble COD (LCK014), alkalinity (LCK362), volatile fatty acids (VFA) in the form of acetic acid (LCK365), total nitrogen (N_{tot} ; LCK338), ammonium (NH_4^+ -N; LCK302, LCK303), total phosphorus (P_{tot} ; LCK348), and phosphate (PO_4^{3-} -P; LCK348). Spectrophotometric measurements were performed using a Hach Lange DR 3900.

5.3.4. Batch test for specific methanogenic activity (SMA)

Specific methanogenic activity (SMA) was used to evaluate the methane-production potential of anaerobic sludge/granules by quantifying the acetate-to-methane conversion rate (Abuzir et al., 2025; Hussain & Dubey, 2017). This rate is influenced by temperature, microbial community composition, and physicochemical parameters (Donoso-Bravo et al., 2009; Kettunen & Rintala, 1997; Souto et al., 2010).

SMA batch experiments were conducted in triplicate using sodium acetate (2000 mg COD L^{-1}) (Lee et al., 2025) and blanks under mesophilic conditions (37°C) with an Automatic Methane Potential Test System (AMPTS, BPC Instruments, Lund, Sweden). For nutrient supplementation, an anaerobic mineral medium was prepared according to Shelton & Tiedje (1984). Granular sludge samples were collected from the R2S reactors during a single sampling event in 2025, with sampling performed at different heights (i.e., 4.7, 7, 9, and 11 m), mixed, and added to SMA bottles (working volume of 400 mL) at a substrate-to-inoculum ratio of 0.5 (g COD:g VSS). The bottles were flushed with N_2 to ensure anaerobic conditions and incubated in a 37°C water bath.

To quantify methane production, the biogas was passed through a 3 M NaOH solution containing thymolphthalein indicator to absorb CO_2 (Lee et al., 2024), and methane production was recorded automatically. The test lasted 100 hours and was terminated when methane production decreased to <1% of the cumulative methane produced.

5.3.5. Calculations

The performance calculations for the homogenization tank and the R2S reactors (degree of acidification, COD removal efficiency, and methane yield) were based on pseudo-steady state conditions. Pseudo-steady state periods were defined as stable operating intervals lasting at least 14 consecutive days. For each selected period, the mean and standard deviation were calculated from the operational dataset (2019–2026) using a Microsoft Excel spreadsheet. Data points deviating by more than two standard deviations from the period mean were treated as outliers and excluded from further calculations (Abuzir et al., 2025; Bertanza et al., 2022).

The degree of acidification (DoA) is commonly used as an indicator of the fermentation process (Ramos-Suarez et al., 2021) and was calculated as the ratio of total VFA produced to the initial total COD ($\text{COD}_{\text{total, initial}}$), as shown in Equation 1.

$$\text{DoA [\%]} = \frac{\text{g TVFA-COD}}{\text{g COD}_{\text{total, initial}}} \quad (1)$$

where TVFA-COD represents the equivalent mass of VFA expressed as COD (in grams), and $\text{COD}_{\text{total, initial}}$ represents the mass of total COD (in grams) at the start of the experiment.

The COD removal efficiency provides insights into the AD performance in degrading COD and was calculated as shown in Equation 2.

$$\text{COD removal efficiency [\%]} = \frac{\text{kg COD}_{\text{total, influent}} - \text{kg COD}_{\text{total, effluent}}}{\text{kg COD}_{\text{total, influent}}} \quad (2)$$

where $\text{kg COD}_{\text{total, influent}}$ represents the mass of total influent COD (in kg), and $\text{COD}_{\text{total, effluent}}$ represents the mass of total effluent COD (in kg).

The methane yield provides insights into the conversion of removed COD to methane and was calculated as shown in Equation 3.

$$\text{Methane yield [Nm}^3 \text{ CH}_4 \text{ kg COD}_{\text{total, removed}}] = \frac{\text{Nm}^3 \text{ CH}_4 \text{ Cumulative methane production}}{\text{kg COD}_{\text{total, removed}}} \quad (3)$$

Where the cumulative methane production is expressed as $\text{Nm}^3 \text{ CH}_4$, and $\text{COD}_{\text{total, removed}}$ represents the mass of total removed COD (in kg).

5.4. Results and Discussion

The Results and Discussion section presents the overall performance of the two-stage AD plant based on operational data collected from 2019 to 2026. It first evaluates hydrolysis and acidogenesis in the homogenization tank, as discussed in Sections 5.4.1 and 5.4.2. It then examines the subsequent acetogenesis and methanogenesis in the R2S reactors, as described in Sections 5.4.3 and 5.4.4. Finally, it presents the results of the SMA experiments, which were conducted to assess the activity of granular biomass collected during a single sampling event in 2025.

5.4.1. Influent Characteristics

The average $\text{COD}_{\text{total}}$ concentration of FJPWW received by the anaerobic treatment plant in Bronzolo ranged between 3775 and 4045 mg COD L^{-1} (Table 5.1 and Fig. B.1A, Supplementary Information), depending on the seasonal production of the fruit juice (FJ). This $\text{COD}_{\text{total}}$ range indicates that FJPWW is characterized by a high organic matter content, making it suitable for AD and biogas production (Chen et al., 2019; Ozbas et al., 2006; Zerrouki et al., 2021). However, FJPWW is also low in alkalinity and has high carbon to nitrogen ratios, which may cause nutrient imbalances and operational challenges during the AD process, indicating the need for alkalinity and nutrient supplementation during the hydrolysis and acidogenesis step (Chen et al., 2019; Ozbas et al., 2006; Zerrouki et al., 2021). The observed $\text{COD}_{\text{total}}$ range is within the range reported for similar substrates, including fruit juice industry, soft drink industry, citrus juice process wastewater, and beverage wastewater (668-32,855 mg COD_{total} L^{-1}) (Cuff et al., 2018; El-Kamah et al., 2010; Fuentes et al., 2021; Güneş Durak et al., 2023).

The influent FJPWW had a near-neutral to slightly alkaline pH (7.76–8.60; Table 5.1 and Fig. B.1B, Supplementary Information), placing it toward the upper end of the broad pH interval reported for beverage and juice-industry wastewaters (2.7–11.8) (El-Kamah et al., 2010; Fuentes et al., 2021; Güneş Durak et al., 2023; Xing et al., 2024). The neutral to slightly alkaline pH of FJPWW could be attributed to the cleaning and washing materials used by the industry (Imoobe et al., 2011).

Regarding TSS, the influent FJPWW contained 429–557 mg TSS L⁻¹ (Table 5.1), which supports the need for solids reduction prior to methanization in the R2S reactors. High-rate granular sludge systems such as EGSB reactors are generally better suited to wastewaters dominated by soluble COD (Lettinga et al., 2001). The influent wastewater was sent to the belt filters after being hydrolyzed and acidified for the reduction of TSS. Reported TSS concentrations in soft drink and citrus wastewaters, as well as other beverage effluents, span a wide interval (63–1442 mg TSS L⁻¹), and the influent in the present study falls within this broader context (Güneş Durak et al., 2023; Imoobe et al., 2011; Tyagi et al., 2019).

Alkalinity and macronutrients (such as nitrogen) are important parameters for the AD process (Simeonov et al., 2025). FJPWW is low in nitrogen (0.76–0.89 mg NH₄⁺-N, Table 5.1) and low in alkalinity (~50–200 mg CaCO₃ L⁻¹, data not shown because it was occasionally measured), which could negatively impact the AD process and indicate the need for alkalinity and nutrient additions. To address these deficiencies, dewatering sludge liquors and NaOH were dosed. Another approach to address alkalinity and nutrient deficiencies is co-digestion with substrates that are high in alkalinity and nitrogen, which can improve system stability and methane yield by encouraging a varied microbial population, improving nutrient balance, diluting hazardous chemicals, and increasing buffering capacity (Khanal, 2008; Liu et al., 2022).

Overall, FJPWW is suitable for biogas production; however, it is characterized by low alkalinity and a high C:N ratio, which requires the addition of alkalinity and nutrients during the AD process.

Table 5.1. FJPWW influent characteristics and effluent quality of the homogenization tank during the monitoring period 2019-2026: average values of the main parameters (standard deviations); n = number of data points used to calculate the average and standard deviation.

Influent to homogenization tank					Effluent from homogenization tank							
Operating temperature	COD _{total} [mg COD L ⁻¹]	pH [-]	TSS [mg TSS L ⁻¹]	NH ₄ ⁺ -N [mg N L ⁻¹]	COD _{total} [mg COD L ⁻¹]	sCOD [mg COD L ⁻¹]	pH [-]	TSS [mg TSS L ⁻¹]	NH ₄ ⁺ -N [mg N L ⁻¹]	PO ₄ ³⁻ -P [mg P L ⁻¹]	VFA [mg Hac L ⁻¹]	Alk [mg CaCO ₃ L ⁻¹]
16°C	3775 (877) (n=348)	8.39 (1.50) (n=338)	429 (156) (n=358)	0.76 (0.28) (n=59)	2915 (559) (n=116)	2758 (488) (n=106)	5.47 (0.80) (n=345)	320 (253) (n=94)	32 (24.5) (n=130)	2.25 (3.33) (n=116)	985.5 (348) (n=132)	177 (95) (n=22)
21°C	4045 (1077) (n=214)	7.76 (1.63) (n=338)	496 (186) (n=213)	0.75 (0.25) (n=52)	3309 (849) (n=62)	2720 (421) (n=55)	5.36 (0.75) (n=221)	370 (300) (n=58)	23.2 (19) (n=67)	2.26 (2.70) (n=67)	1093 (394) (n=73)	305 (123) (n=21)
26°C	3839 (1041) (n=141)	8.60 (1.70) (n=129)	557 (198) (n=141)	0.89 (0.44) (n=31)	2981 (852) (n=61)	2517 (547) (n=47)	5.38 (0.72) (n=144)	422 (367) (n=47)	26.6 (22.4) (n=61)	1.80 (2.42) (n=58)	898 (244) (n=60)	-

5.4.2. Homogenization Tank Performance

The performance of the homogenization tank in terms of total removal ($\text{COD}_{\text{total}}$) and the degree of acidification (DoA) at different operating temperatures is summarized in Table 5.2.

Higher temperatures had no significant impact on $\text{COD}_{\text{total}}$ removal. Average $\text{COD}_{\text{total}}$ removal was comparable across the three conditions (38%, 32%, and 35% at 16°C, 21°C, and 26°C, respectively; Table 5.2 and Fig. B1A, Supplementary Information). Operating conditions were also similar across temperatures, including average HRT (29.1, 32.6, and 33.3 hours; Table B1, Supplementary Information), average pH within the homogenization tank (5.47, 5.36, and 5.38; Table 5.1 and Fig. B1C), and average OLR (3.35, 3.18, and 3.06 kg COD $\text{m}^{-3} \text{d}^{-1}$; Table B1, Supplementary Information). Similarly, higher temperatures had no significant impact on DoA, with comparable average values of 23%, 25%, and 21% at 16°C, 21°C, and 26°C, respectively (Table 4.2).

Higher temperatures were expected to increase $\text{COD}_{\text{total}}$ removal efficiency and DoA due to faster AD reactions (Tho & Thanh, 2025; van Niel et al., 2003). The absence of a temperature effect suggests limited enhancement in FJPWW solubility, likely because FJPWW is already highly biodegradable (Chen et al., 2019; Rabii et al., 2019). It also suggests that other operational factors may have a stronger influence on COD removal and DoA, such as the food to microorganism ratio (F/M) (Tho & Thanh, 2025); however, biomass in terms of VS was not monitored in this study.

The observed $\text{COD}_{\text{total}}$ removal (32–38%; Table 5.2) falls within the range reported for hydrolysis–acidogenesis of comparable highly biodegradable wastewaters (7–47%) (Fuentes et al., 2021; Mari et al., 2020). The observed DoA (21–25%; Table 5.2) is of the same order as values reported across a range of wastewaters treated under acidogenic conditions (24–79%) (Liu et al., 2019; Tawfik & El-Kamah, 2012; Vergine et al., 2015; Yu et al., 2021).

The DoA observed in this study (21–25%) is not expected to negatively affect the methanogenic reactor, given that it is below the commonly recommended upper limit DoA of 50% (Alexiou et al., 1994). The relatively low COD removal in the hydrolysis and acidogenesis stage can be attributed to the conversion of substrate primarily into soluble intermediates such as VFA and alcohol, resulting in limited net reduction in COD concentration (Lu et al., 2019). It should also be noted that hydrolysis and acidogenesis may produce gaseous metabolites, particularly H_2 and CO_2 , in addition to soluble intermediates (Zheng & Li, 2024). Since these gaseous products were not measured in this study, their fate within the system and their contribution to the overall process performance remain outside the scope of the present analysis. The acetic acid and VFAs produced in this stage may affect the second stage, where methane production takes place; acetic acid accumulation above 2000 mg HAc L^{-1} was observed to negatively impact the overall stability of the system and the methanogenic step (Karthikeyan & Visvanathan, 2013).

The concentration of volatile fatty acids (VFAs) in the effluent of the homogenization tank and the pH within the homogenization tank are key parameters to track because they provide insights into the performance of the hydrolysis and acidogenesis process.

The average VFA concentration range was comparable across temperatures, indicating no temperature effect (985, 1093, and 898 mg HAc L^{-1} at 16°C, 21°C, and 26°C, respectively; Table 5.2 and

Fig. B.1B, Supplementary Information). The effluent sCOD of the homogenization tank contained similar VFA content (38-41%, Table 5.2), indicating effective hydrolysis and acidogenesis processes and suggesting that the effluent was rich in VFA for the methanogenesis process. The VFA/sCOD ratios in this study (38-41%, Table 5.2) were higher than those reported for a homogenization tank treating a relatively similar substrate by Austermann-Haun et al., (1997) (20-27%), which may be attributed to the higher HRT in this study (29-33 hours) compared with 13 hours in their study. Austermann-Haun et al. (1997) reported that a VFA/sCOD range of 20-27% was identical to operating a UASB for methane production from the fruit juice industry.

Regarding pH in the homogenization tank, pH varied only marginally with temperature (5.49, 5.36, and 5.38 at 16°C, 21°C, and 26°C, respectively; Table 5.1 and Fig. B.1C, Supplementary Information). This mildly acidic pH is typical of hydrolysis–acidogenesis (4.0–6.5) (Amo et al., 2015; Simeonov et al., 2025; Srisowmeya et al., 2020; Van et al., 2020).

Overall, higher temperatures had no significant impact on the hydrolysis and acidification process. The homogenization tank showed stable and comparable performance in terms of COD_{tot} removal and VFA production compared to previously reported studies.

Table 5.2. Performance of the homogenization tank: average values of the main parameters (standard deviations).

	COD_{total} removal efficiency [%]	DoA [%]	VFA/sCOD [%]
Operating temperature			
16°C	38 (23)	23 (12)	39 (16)
21°C	32 (10)	25 (9)	41 (15)
26°C	35 (19)	21 (9)	38 (10)

5.4.3. TSS and COD removal in the Methanogenic Reactors (R2S reactors)

The influent and effluent characteristics and COD_{tot} performance of the R2S reactors are summarized in Table 4.3 and compared with other reactors treating similar substrates.

Table 5.3. Influent characteristics and effluent quality of methanogenic reactors (EGSB/R2S) treating highly biodegradable wastewater for biogas production during the monitoring period 2019-2026: average values of the main parameters (standard deviations); n = number of data points used to calculate the average and standard deviation.

Influent to the R2S reactors						Effluent from the R2S reactors				
Parameter	This study (R2S)	Full-scale ECSB ^a	Full-scale UASB ^b	Full-scale EGSB ^c	Two Lab-scale UASRs ^d	This study (R2S)	Full-scale ECSB ^a	Full-scale UASB ^b	Full-scale EGSB ^c	Two Lab-scale UASRs ^d
COD_{tot} [mg COD L⁻¹]	2902 (547) (n=217)	9046.6 (2265)	820-2411	1999-2244	5157 (2897) (n=20)	349 (171) (n=220)	212-1099	-	310	2033
sCOD [mg COD L⁻¹]	2787 (510) (n=241)	7540-11,687	-	-	2,429 (401) (n=20)	191 (46.7) (n=220)	205-221	-	-	-
COD_{tot} removed [%]	-	-	-	-	-	88.1 (4.95) (n=220)	96 (1.3)	82-90	>85	61-70
pH [-]	5.69 (0.76) (n=581)	5.90-6.30	6.30	-	< 5.0	7.06 (0.20) (n=220)	7.34 (0.15)	6.8-6.9	6.5-7.5	7.40
TSS [mg TSS L⁻¹]	160 (112) (n=160)	-	-	-	323 (349) (n=20)	151 (115) (n=45)	-	-	-	~ 100
VFA [mg Hac L⁻¹]	1082 (367) (n=246)	170-581	-	234-434	-	69.2 (34.2) (n=45)	83.4	-	223	-
Alkalinity [mg CaCO₃ L⁻¹]	198 (138) (n=76)	344-2545	-	-	-	826 (46.4) (n=76)	-	-	-	-
NH₄⁺-N [mg N L⁻¹]	26.4 (19.7) (n=243)	195-249	-	-	-	26.9 (13.79) (n=57)	-	-	-	-
PO₄³⁻-P [mg P L⁻¹]	2.67 (3.04) (n=223)	-	-	-	-	4.69 (1.21) (n=59)	-	-	-	-
VFA/Alk [-]	-	-	-	-	-	0.07 (0.015) (n=43)	-	-	-	-

a: Chun-Te Lin et al. (2020), b: Austermann-Haun et al. (1997), c: Cuff et al. (2018), d: El-Kamah et al. (2010)

The average influent COD_{tot} concentration decreased from 2902 mg COD L⁻¹ in the influent to 349 mg COD L⁻¹ in the effluent (Table 5.3 and Fig. B.4, Supplementary Information), corresponding to an average COD removal efficiency of 88.1% (Table 5.3). This reduction indicates high COD removal and effective conversion into biogas in the R2S reactors. The high COD removal efficiency could be attributed to the biodegradability and solubility of the influent wastewater, indicated by the high sCOD/COD_{total} ratio in the effluent from the homogenization tank (2787/2902=96%) (Table 5.3). Similar treatment systems have reported COD removal efficiencies spanning 61–96%, placing the present performance toward the upper part of this range (Austermann-Haun et al., 1997; Chun-Te Lin et al., 2020; Cuff et al., 2018; El-Kamah et al., 2010). However, the treated wastewater requires further treatment for additional COD removal before discharge into a river.

Moreover, the R2S reactors were efficient in degrading VFAs and converting them into biogas. The average influent VFA concentration decreased from 1082 mg HAc L⁻¹ to 69.2 mg HAc L⁻¹ (Table 5.3 and Fig. B.5, Supplementary Information), indicating high degradation efficiency. In comparison, effluent VFA concentrations reported for related treatment systems were typically higher (83.4–223 mg HAc L⁻¹) (Chun-Te Lin et al., 2020; Cuff et al., 2018).

In contrast, the average TSS concentration only decreased slightly from 161 mg TSS L⁻¹ in the influent to 151 mg TSS L⁻¹ in the effluent (i.e., a decrease of 6.2%) (Table 5.3). This low TSS removal may be attributed to the washout of granules, flocculent biomass, and solids in the effluent, potentially due to high mixing in the R2S reactors associated with effluent recirculation. In the long term and at high OLRs, biomass washout could negatively impact the overall AD performance by disturbing the formation of granules and reducing treatment efficiency, potentially leading to AD process failure (Zhang et al., 2024; Aragón-Barroso et al., 2026). The TSS decrease observed in this study was lower than the approximately 69% TSS removal reported by El-Kamah et al. (2010) for fruit juice industry wastewater treated in lab-scale UASRs, despite their higher average OLR of 8.7 kg COD m⁻³ d⁻¹ compared with 4.78 kg COD m⁻³ d⁻¹ (Table B2, supplementary information) in this study.

To further assess R2S stability, the VFA/Alk ratio was considered and was 0.07 (Table 5.3), which was close to the recommended value of 0.1 for stable AD performance (Gong et al., 2020).

Overall, the R2S reactors were effective in degrading COD.

5.4.4. Biogas Production

Fig. 5.2 shows the impact of OLR on methane production in the R2S reactors. Higher OLR resulted in higher methane production (Fig. 5.2). The lowest OLR observed in this study was $1.52 \text{ kg COD m}^{-3} \text{ d}^{-1}$, corresponding to a methane production of $566 \text{ Nm}^3 \text{ d}^{-1}$ (average methane content of 80%) (Fig. 5.2). The highest OLR was $9.53 \text{ kg COD m}^{-3} \text{ d}^{-1}$, corresponding to a methane production of $4560 \text{ Nm}^3 \text{ d}^{-1}$ (average methane content of 80%) (Fig. 5.2). The increase in methane production with increasing OLR could be attributed to the higher input of highly biodegradable organic matter at higher loads. A similar trend was reported by Zhou et al. (2022) for high-solids AD of food waste (FW) in a horizontal flow reactor. Likewise, Pan et al. (2023) reported that methane production rates in bio electrochemical AD of brewery wastewater increased from $0.48 \text{ LCH}_4 \text{ L}^{-1} \text{ d}^{-1}$ (OLR of $2 \text{ g COD L}^{-1} \text{ d}^{-1}$) to $5.64 \text{ L CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ (OLR of $20 \text{ g COD L}^{-1} \text{ d}^{-1}$). However, it is expected that this increase may reach a point at which methane production decreases at higher OLR. At higher OLRs, process performance can be disturbed because larger influent volumes may shift physiochemical conditions and cause temporary inhibition of microbial activity (Cruz-Salomón et al., 2019).

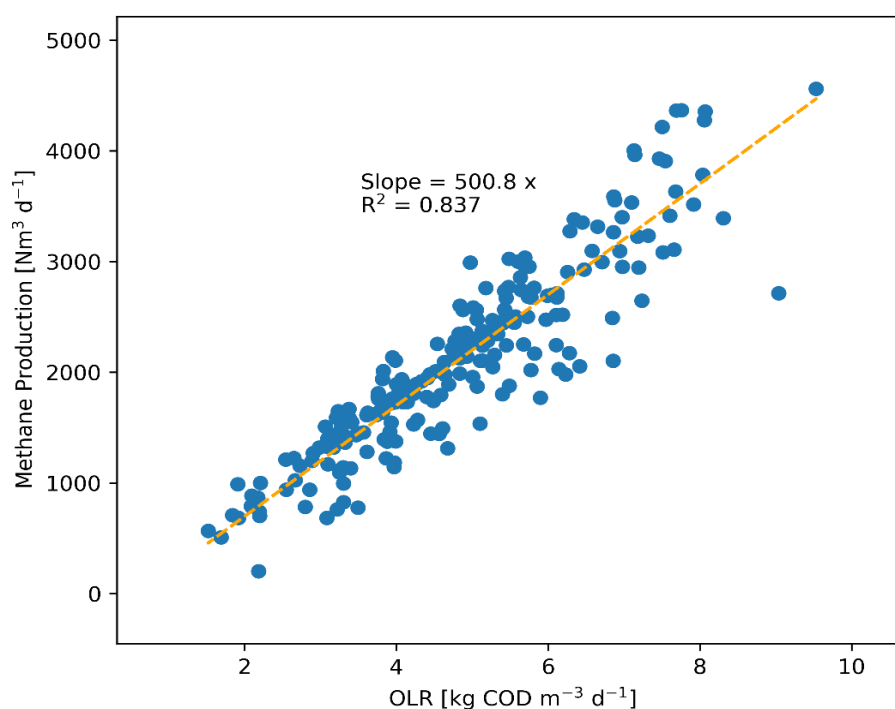


Fig. 5.2. Impact of OLR on daily methane production in the EGSB/R2S reactors.

Fig. 5.3 and Table 5.4 summarize the methane yield of the R2S reactors and compare them with previous studies on similar substrates. The methane yield observed in this study was $0.277 \text{ Nm}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ COD}_{\text{removed}}$ (Fig. 5.4 and Table 5.4), corresponding to 79% of the standard methane production of $0.35 \text{ Nm}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ COD}_{\text{removed}}$, indicating high conversion of COD into methane. The difference from the theoretical value may be explained by COD incorporation into biomass, the presence of slowly biodegradable or non-biodegradable substrate fractions, and possible methane losses not captured in the measured gas phase, such as dissolved methane in the effluent. In addition, the composition of fruit-processing wastewaters can vary over time and may include components of differing biodegradability, which can also contribute to

methane yields below the theoretical maximum. The methane yield obtained in this study was within the range reported in previous studies treating similar substrates at comparable OLRs, which showed values of 0.18–0.27 Nm³ CH₄ kg⁻¹ COD_{removed}.

Overall, the EGSB (R2S) reactors showed effective performance in converting VFAs and organic matter into methane.

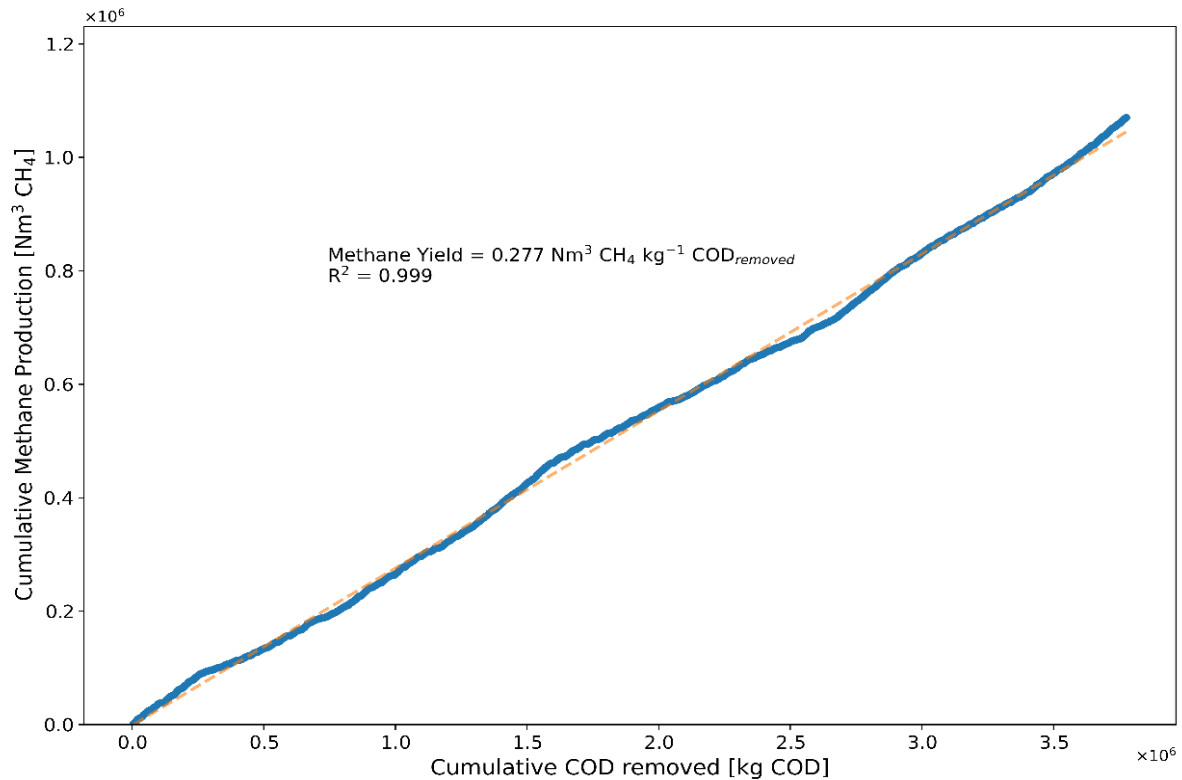


Fig. 5.3. Methane yield in the EGSB/R2S reactors. The blue solid curve shows the measured cumulative methane production plotted against the cumulative COD removed. The orange dashed line represents the linear regression used to estimate the methane yield from the slope of the relationship.

Table 5.4. Performance of bioreactors treating highly biodegradable wastewater: organic loading rates and methane yield (average values with standard deviations).

Parameter	Unit	This study	Lab-scale EGSB ^a	Lab-scale EGSB ^b
Average OLR	kg COD m ⁻³ d ⁻¹	4.77 (1.43)	5	2.21
Methane yield	Nm ³ CH ₄ kg ⁻¹ COD _{removed}	0.277	0.271	0.180

a: Xing et al. (2024), b: Rodrigues, et al. (2024).

5.4.5. Specific Methanogenic Activity (SMA)

SMA tests provide insights into the activity of granular or flocculant sludge in methane production. Fig. 5.4 shows the methane production profile during the SMA test, which lasted 92 hours. The SMA values obtained in this study, together with previously reported SMA values for granular and flocculant sludge from bioreactors at different scales, are presented in Table 5.5.

Methane production increased rapidly during the first 20 hours of the SMA test and then remained stable (Fig. 5.4). The SMA obtained in this study was 317 NmL CH₄ g⁻¹VSS d⁻¹ (Table 5.5), indicating high activity of the granular sludge in producing methane. This value is close to the upper end of SMA values commonly reported for sludges treating industrial wastewaters (105–350 NmL CH₄ g⁻¹ VSS d⁻¹) (Abuzir et al., 2025; Chen et al., 2020).

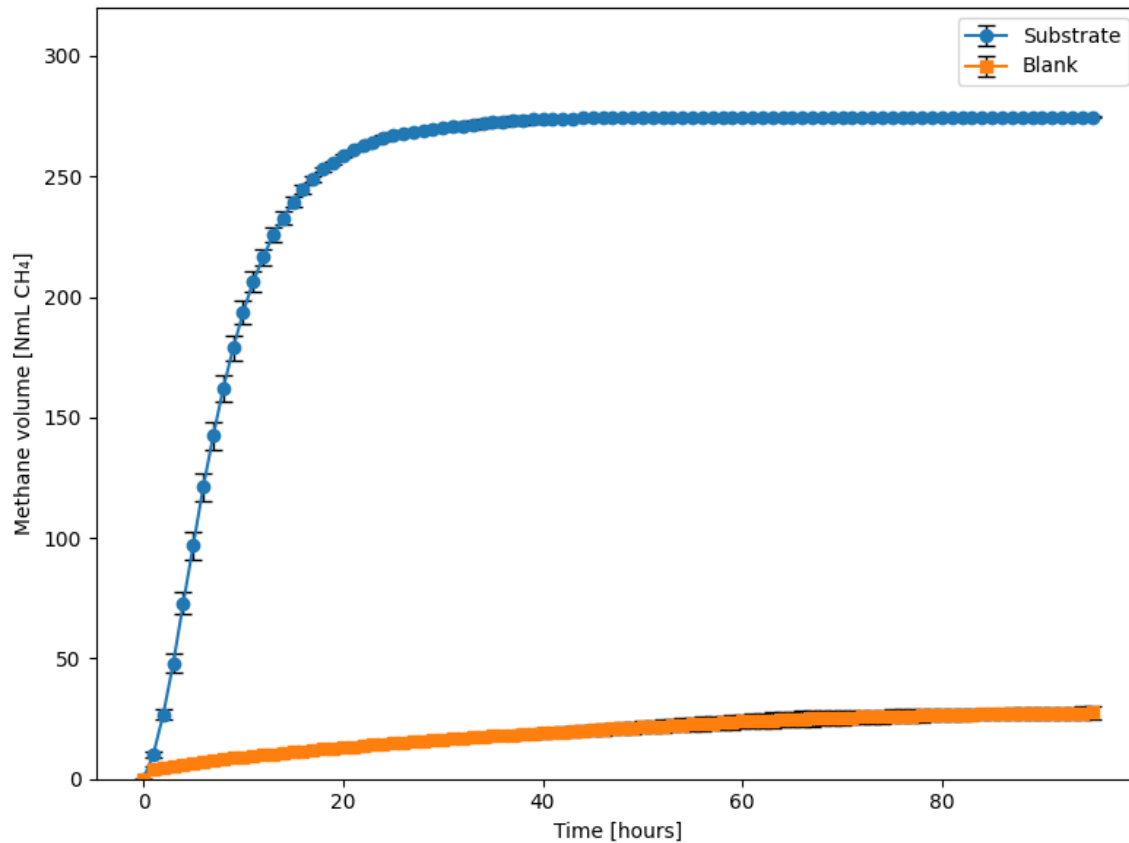


Fig. 5.4. Specific methanogenic activity test (SMA) of granular sludge.

Table 5.5.SMA values for different types of sludge.

SMA value	This study	Flocculant sludge ^a	Granular sludge ^b
NmLCH ₄ g ⁻¹ VSS d ⁻¹	317	185.6	105-350

a: Abuzir et al. (2025), b: Chen et al. (2020).

5.4.6. Suggestion for Plant Operation, Monitoring and Future Research

Plant operation and monitoring are essential for maintaining an effective and stable AD process. Parameters such as temperature, pH, OLR, VFA/ALK, and HRT are widely used as key performance indicators (KPI) (Aragón-Barroso et al., 2026; Cruz-Salomón et al., 2019; Tho & Thanh, 2025). However, as discussed in section 5.4.2, the impact of changing some of these parameters, such as temperature, was not significant, highlighting the need to monitor and measure other parameters such as F/M in the homogenization tank. This reflects what was noted by Tho & Thanh. (2025). The F/M ratio reflects the capacity of microbial communities to utilize the available substrate; therefore, maintaining it within an appropriate range is important for bioreactor efficiency (Tho & Thanh, 2025). F/M can be estimated by measuring the

VS (in grams) of the substrate and the biomass within the equalization tank at least once a month.

In the methanogenic reactor, monitoring granular sludge settleability and activity is important and may provide additional insight into granular sludge quality. For instance, in section 5.4.3, TSS removal was low (6.2%), indicating sludge/solids washout; therefore, monitoring granular sludge settleability could help assess granule stability. Measuring VS at different reactor heights provides insight into solid–liquid separation and microbial activity (Abuzir et al., 2025; Hussain & Dubey, 2017), where lower VS at the top indicates better settleability. Settleability can be assessed using the Imhoff cone test as described by Lin et al. (2019), while granule activity can be evaluated using SMA tests as described by Abuzir et al. (2025), Kjerstadius et al. (2016), and Lee et al. (2025).

Given the limited research on optimal operating and hydrodynamic conditions for EGSB reactors such as R2S treating real highly biodegradable wastewater such as FJPWW, future work could further characterize granule properties (e.g., diameter and mineral content) (Lin et al., 2019) and apply computational fluid dynamics (CFD) to optimize internal hydrodynamics and critical components, particularly GLS separators under high-rate conditions. Addressing these aspects could help explain the low TSS removal (6.2%) and sludge washout discussed earlier in section 3.3. For example, Pan et al. (2017) used validated 3D multiphase CFD to optimize the EGSB separator deflector angle, showing that a 40° configuration minimizes sludge washout by promoting vortex-driven sludge reflux under high upflow conditions. CFD-based approaches may also provide mechanistic insight into granulation by linking EGSB operating conditions to local flow fields, shear stresses, and particle interactions that govern granule stability, growth, and washout. Moreover, future research could focus on optimizing the first stage of the two-stage AD process, for example, by co-digesting with substrates that are high in alkalinity and nutrients (N and P), such as primary sludge or animal manure.

5.5. Conclusions

The performance of a full-scale two-stage anaerobic digestion system treating fruit juice processing wastewater (FJPWW) was evaluated over 6 years. The system comprised hydrolysis/acidogenesis in the homogenization tank and acetogenesis/methanogenesis in the R2S reactors. Performance was assessed based on VFA production, degree of acidification (DoA), COD_{total} removal, and biogas production, and was compared with previously reported lab-scale, pilot-scale, and full-scale studies on similar substrates. In addition, implications for plant operation, monitoring, and future research were discussed.

The full-scale acidification tank demonstrated comparable performance across operating temperatures, achieving COD_{tot} removal of 32–38% and conversion into VFAs (DoA of 21–25%). The full-scale R2S reactors also performed effectively, with COD_{tot} removal of 88% and biogas production of 1763 Nm³CH₄ d⁻¹ (average CH₄ content of 80%). Overall, the performance was comparable to previous lab-scale, pilot-scale, and full-scale studies on similar substrates, confirming that anaerobic digestion is a viable treatment solution for FJPWW.

Although the full-scale two-stage anaerobic digestion system treating FJPWW showed promising results, further research is needed to optimize the first stage, particularly with respect to

nutrient balance and alkalinity addition. Potential strategies include co-digestion with high-alkalinity, high-nitrogen substrates such as primary sludge or animal manure. In addition, monitoring the F/M ratio, especially in the first stage, is recommended to provide deeper insight into acidification performance. In the methanogenic reactor, granular sludge settleability should be assessed to better understand the risk of granular biomass washout which could lead to process failure. Future research could also apply computational fluid dynamics to optimize internal hydrodynamics in the methanogenic reactor, and conduct in-depth investigations of the microbial communities in both stages to further refine operating conditions for these microorganisms.



Chapter 6

Impact of co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production

This chapter is submitted to the journal of Chemical Engineering as:

Abuzir, S.; Simion, G.; Domini, M.; Bertanza, G. Impact of co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production.

Chapter 6. Impact of co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production

6.1. Abstract

Fruit juice processing wastewater (FJPWW) contains high concentrations of readily biodegradable COD and is therefore suitable for biogas production, but low buffering capacity and potential nutrient imbalance can accelerate acidification and destabilize anaerobic conversion. Co-digestion with substrates characterized by high buffering capacity and nutrient availability is a viable strategy to mitigate these limitations and support stable process. This study evaluated whether co-fermenting and co-digesting FJPWW with primary sludge (PS) improves VFA production and methane yield. Batch acidogenic fermentation was conducted for 100 hours in 1 L bottles using FJPWW alone and FJPWW+PS at 97:3 and 90:10 (vol./vol.), at a substrate-to-inoculum ratio of 2:1 g COD g VS⁻¹ and initial pH 6.0. After settling, supernatants were assessed in BMP assays (OxiTop®) at 35 °C for 120 hours, with a substrate-to-inoculum ratio of 0.25 gCOD gVS⁻¹ and initial pH adjusted to 7.0. During fermentation, COD_{tot} decreased from 6.47–7.62 to 4.70–6.16 g COD L⁻¹, corresponding to COD_{total} removal efficiencies of 27.4%, 29.4%, and 19.4% for FJPWW, 97:3, and 90:10, respectively. DoA peaked at 24 hours (33%, 31%, and 15%), alongside maximum VFA yields of 0.89, 0.86, and 0.45 g nTVFA-COD g VS⁻¹; final VFA profiles were acetate-dominated (51–57% of total VFAs). In BMP tests, methane yields were similar for FJPWW (504.3 NmL CH₄ g VS⁻¹) and 97:3 (504.5 NmL CH₄ g VS⁻¹), while 90:10 was lower (483.7 NmL CH₄ g VS⁻¹). Overall, PS at the tested ratios did not lead to a significant enhancement in either VFA accumulation or methane yield, however, co-fermentation with primary sludge improved the COD:N ratio of the substrate mixture. Further research is needed to investigate primary sludges from different municipal WWTPs, and evaluate the influence of controlled pH conditions.

6.2. Introduction

Rising demand for apple juice has led to increased production and larger volumes of fruit juice processing wastewater (FJPWW). If not properly managed, FJPWW's high organic carbon content can cause serious environmental problems, including pollution of ground and surface waters and risks to animal and human health (Foo et al., 2009; Khan et al., 2021; Uddin and Wright, 2023; Zerrouki et al., 2021).

FJPWW is characterized by high organic content (elevated chemical oxygen demand, i.e. COD), low pH, low alkalinity, and a high carbon-to-nitrogen (C:N) ratio. These characteristics can result in limited buffering capacity and nutrient imbalances, and treatment is further complicated by significant fluctuations in flow volume (Austerman-Haun and Rosenwinkel, 1997; Chen et al., 2019; Dold et al., 1980; Ozbas et al., 2006; Valta et al., 2015; Zerrouki et al., 2021). Owing to the generally high biodegradability of its organic matter, despite the wide variability in reported COD concentrations (375–124,800 mg COD L⁻¹), FJPWW can be treated using various technologies, including AD (Dold et al., 1987; Ozbas et al., 2006; Zerrouki et al., 2015). However, because FJPWW is highly biodegradable and has limited buffering capacity, rapid volatile fatty acids (VFA) production may occur and lead to a pH drop, potentially affecting

the stability of the AD system (Cremonese et al., 2022). AD enables the production of biogas, a clean and environmentally friendly energy source that can be converted into electricity and heat using combined heat and power generators, and upgraded to biomethane for use as a transportation fuel (Feiz & Kanda, 2024; Mateescu et al., 2008; Zerrouki et al., 2021).

AD is a multi-stage process in which different groups of microorganisms convert organic matter into biogas, mainly methane (CH_4) and carbon dioxide (CO_2) (Appels et al., 2008; Joshi and Visvanathan, 2019; Zheng & Li, 2024). The four main stages are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In sequence, complex polymers are broken down into monomers, then into volatile fatty acids and alcohols, followed by conversion to acetate, hydrogen, and CO_2 , and finally to methane (De et al., 2019; Khan et al., 2021; Pan et al., 2021; Paul et al., 2022; Wang et al., 2018).

The efficiency and stability of AD depend on several operational parameters, including temperature, pH, and the carbon to nitrogen (C:N) ratio (Odedina et al., 2017; Zheng & Li, 2024). Mesophilic temperatures between 20 and 45 degrees Celsius and a neutral pH optimize microbial activity (Cremonese et al., 2021; He et al., 2022; Kainthola et al., 2019). A C:N ratio of 20–30:1 is considered ideal for stable AD performance (Laiq et al., 2019; Odedina et al., 2017).

AD can be implemented using either single-stage or two-stage systems. In single-stage AD, all microbial processes occur within a single reactor, whereas two-stage AD separates these processes, allowing for the optimization of conditions for each microbial group and thereby enhancing overall efficiency (Ferrera et al., 2022). The first stage of two-stage AD favors hydrolytic and acidogenic bacteria (pH 5–6, short hydraulic retention times), resulting in the production of VFAs, CO_2 , and H_2 (Fuentes et al., 2021; Ketheesan et al., 2015). The second stage supports methanogens (pH 6–8, longer retention times), which convert acetic acid into methane (Ferrera et al., 2022; Fuentes et al., 2021). This configuration is particularly effective for treating high-sugar wastewaters, such as AJW, which can destabilize single-stage systems (Feng et al., 2020; Mari et al., 2020). Reported methane contents of 70–80.6% and acidification degrees of 54–89% (Bergland et al., 2010; Carnevalheira et al., 2018; Mateus et al., 2020) further demonstrate the strong performance of two-stage AD systems.

Two-stage AD provides greater stability, higher tolerance to organic loads, and enables recovery of valuable byproducts (Fuentes et al., 2021). Separating acidogenic and methanogenic phases allows for optimal pH control, boosting biogas yield and substrate degradation (Fuentes et al., 2021; Perera et al., 2012). For example, two-stage systems can achieve COD removal efficiencies 35% higher than single-stage systems in food waste digestion (Parra-Orobio et al., 2021).

A key challenge in treating carbohydrate-rich wastewater like FJPWW is rapid acidification and VFA accumulation, mainly due to a high C:N ratio, lack of trace elements, and low buffering capacity, especially during the acidogenic phase. High VFA levels lower pH, inhibit microbes, and reduce biogas production (Mishra et al., 2017; Rocha et al., 2023; Zheng & Li, 2024). Pre-treatment is often required, such as nutrient addition to improve the C:N ratio (Ozbas et al., 2006) or chemical buffering with agents like NaHCO_3 or NaOH (Feng et al., 2020; Rocha et al., 2024). However, chemical buffers like NaOH are costly for large-scale use and mainly suitable for laboratory research (Algapani et al., 2016). This challenge was also evident in parallel work on a full-scale two-stage anaerobic digestion system treating FJPWW in Bronzolo, Italy, where NaOH and dewatering sludge liquors from anaerobic digesters

digesting primary, secondary, and waste activated sludge from the municipal wastewater treatment plant were added to increase the buffering capacity to maintain a stable pH, and to increase the nutrient capacity for the AD process to occur. However, this approach increases operational costs, highlighting the need for alternative, cost-effective buffering and nutrient-balancing strategies.

An effective alternative is co-digestion, where FJPWW is combined with substrates that have a low C:N ratio and high buffering capacity, such as sewage sludge, primary sludge (PS), or animal manure (Baldi et al., 2019; Paranjpe et al., 2023; Paudel et al., 2017). However, the choice of co-substrate should not only be based on the process performance enhancement, as it may affect the quality of digestate and therefore influence its potential end use (Azarmanesh et al., 2023; Rao & Baral, 2011; Tormo, 2015). Consequently, the selection of co-substrates depends on the local treatment context, including substrate availability, regulatory conditions, contaminant content, and whether the digestate is intended for agricultural reuse (Catenacci et al., 2022; Delzeit & Kellner, 2013; Gopinath et al., 2021).

Co-digestion involves a simultaneous anaerobic treatment of two or more substrates (Rabi et al., 2019). Co-digestion could improve process stability and methane yield by creating synergistic conditions, including a more diverse microbial community, improved nutrient balance (proper C:N ratio and supplementation of trace elements) and greater buffering capacity (Bolzoni et al., 2006; Mata-Alvarez et al., 2014; Xie et al., 2018).

PS, generated during municipal wastewater treatment, is typically thickened to reduce water content before anaerobic digestion and is more biodegradable than secondary sludge (SS) (Azevedo et al., 2023; Elsayed et al., 2021; Perez-Esteban et al., 2022). PS is rich in organic matter and nutrients and has a low C:N ratio (Bouallagui et al., 2010; Silvestre et al., 2011). Co-digesting FJPWW with PS despite the relatively low methane potential of PS (132.4 NmL CH₄ g VS⁻¹) (Elsayed et al., 2016), could enhance VFA production and biogas yields by balancing the C:N ratio, improving the mineral nutrient profile, reducing toxic substances, increasing substrate degradation, and stabilizing the digestion process (Azevedo et al., 2023; Elsayed et al., 2019; Sosnowski et al., 2008). However, the suitability of PS as a co-substrate should not be assessed solely on process performance, as practical applicability also depends on substrate availability, transport distance, and integration with existing treatment infrastructure; moreover, the inclusion of sewage-derived materials may affect digestate management and regulatory constraints related to reuse (Azarmanesh et al., 2023; Catenacci et al., 2022; Ebrahimi et al., 2025). For example, Rakić et al. (2024) found that co-digesting food waste (FW) with PS at a 1:3 (volume to volume) ratio increased biogas production by 52% compared to mono-digestion of FW. Similarly, Elsayed et al. (2021) reported that co-digesting fruit and vegetable waste (FVW) with PS at a 50:50 (volatile solids) ratio resulted in a 5% increase in methane yield. However, there is a notable lack of studies investigating the co-digestion of FJPWW with PS to enhance methane production and improve acidogenesis in two-stage AD systems at batch, laboratory, or pilot scales.

Therefore, this study investigates the potential of co-digesting FJPWW with PS at varying volume-to-volume (vol./vol.) ratios, assessing its effects on VFA production and on methane yield, to better understand performance across both stages of the AD process.

6.3. Methods and Materials

6.3.1. Substrates and Inoculum

Fruit juice processing wastewater (FJPWW) was sourced from the Bronzolo wastewater treatment plant (WWTP) in Northern Italy, which treats this stream via a two-stage AD process followed by conventional municipal wastewater treatment (MWWTP). The Bronzolo WWTP has a treatment capacity of 342,000 population equivalent (PE) and has been in operation since 1996. It treats both municipal and industrial wastewater from Bolzano, Bronzolo, Laives, Nova Ponente, and Vadena, Northern Italy. Industrial wastewater undergoes pre-treatment (filtration, homogenization, and acidification) and is then treated in two anaerobic reactors producing biogas collected in a gasometer; the partially treated effluent is subsequently co-treated with municipal wastewater through conventional municipal treatment steps.

The FJPWW, generated by a nearby fruit juice factory, has a flow rate of 500–3,500 m³ d⁻¹. Physio-chemical characteristics are provided in Table C1 Supplementary Information). PS was obtained from the primary settler at the MWWTP of Bronzolo where the daily flow contribution of the industrial wastewater (FJPWW) entering the MWWTP was roughly 50%.

The inoculum for acidogenic fermentation was flocculent biomass from the Bronzolo WWTP homogenization tank (used for hydrolysis and acidogenesis), which receives FJPWW prior to methanization (HRT: 16–30 hours).

For biochemical methane potential (BMP) tests, the inoculum was granular sludge from various heights within an EGSB reactor treating filtered effluent from the homogenization tank at the same WWTP of Bronzolo. This granular biomass contained 92.4 g VS L⁻¹, and the EGSB reactor operated with an HRT of 12–24 hours.

6.3.2. Experimental set-up

The experiment was conducted in two parts to evaluate the impact of co-digesting FJPWW with different vol./vol. ratios of PS on VFA production and methane yield. The first part involved an acidogenic fermentation test (Figure 6.1A). The acidified substrates generated in this test were subsequently used as substrates for methane production in the second part, a Biochemical Methane Potential test (Figure 6.1B).

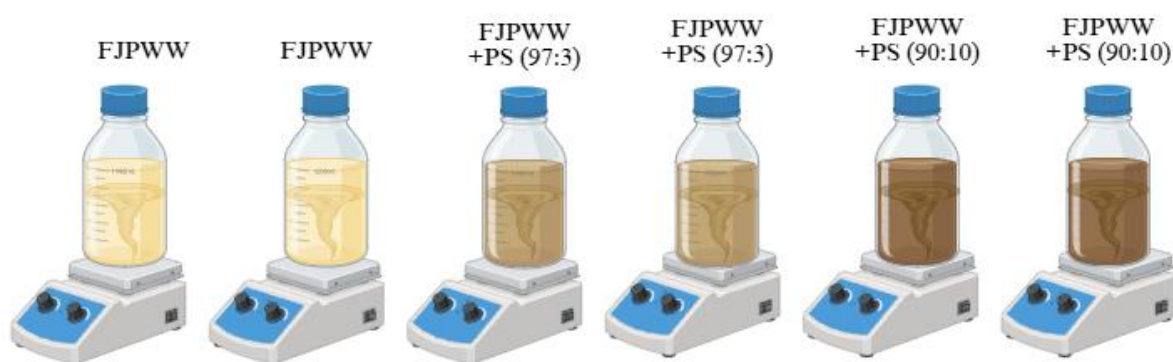




Fig. 6.1. A) Schematic of the fermentation experiment. B) Co-digestion of FJPWW and PS BMP test using the OxiTop®-IDS AN 6 system.

6.3.2.1. Acidogenic Co-fermentation Test

The acidogenic fermentation experiments were performed at the WWTP of Bronzolo using Duran® glass bottles with a capacity of 1 L for 100 hours. Three different substrate mixtures of varying vol./vol. ratios of FJPWW and PS were tested to evaluate the impact of co-fermenting FJPWW with PS on VFA production: one containing only FJPWW, one containing FJPWW co-fermented with PS at a vol./vol. ratio of 97:3 (8.26 g COD g⁻¹ COD), and one containing FJPWW co-fermented with PS at a vol./vol. ratio of 90:10 (2.30 g COD g⁻¹ COD),). The experiment was done in duplicates. The low PS volumes used were determined by the maximum availability of PS at the MWWTP in Bronzolo. All the substrates were collected as grab samples from their sources and stored at 4°C. The working volume of the experiment was 800 mL (including the substrate and the inoculum) with 200mL of head space. All bottles were closed with a cap with no gas measurements. The substrate-to-inoculum (S:I) ratio was maintained at 2:1 g COD g VS⁻¹, and the VS of the inoculum was 22.2 g VS L⁻¹ because S:I ratio of 2–4 g COD g VSS⁻¹ has been found to maximize VFA production (Gandhi et al., 2022; Lauzurique et al., 2022; Silva et al., 2013), and was a value selected based on previously reported ranges in the literature (Esfandiar et al., 2024; Guo et al., 2014; Tian et al., 2024).

Before the acidogenic fermentation experiment, FJPWW was supplemented with nutrients in the form of phosphoric acid (H₃PO₄; 0.3 mL) and with 0.5 g of urea. Nutrients were added at the start of the fermentation test to help maintain process stability and prevent process failure. Sodium hydrogen carbonate was added to all bottles to ensure that there is enough alkalinity for the test as suggested by Metcalf & Eddy, (2003).

All bottles were flushed with nitrogen gas (N₂) for two minutes to establish anaerobic conditions, and the contents were continuously gently stirred.

6.3.2.2. Biochemical methane potential (BMP)

Biochemical methane potential (BMP) tests were conducted to evaluate the effect of co-digesting FJPWW with PS on methane production at different vol./vol. mixing ratios. Three substrate configurations were tested: FJPWW alone, FJPWW co-digested with PS at a vol./vol. ratio of 97:3, and FJPWW co-digested with PS at a vol./vol. ratio of 90:10, each in duplicate, including blank controls. The experiments were performed using an OxiTop®-IDS AN 6 system.

The total operating volume of each bottle, including substrate and inoculum, was set to 200 mL. Higher operating volumes (400 and 500 mL) resulted in a rapid increase in internal pressure, exceeding the maximum allowable pressure of 500 hPa and necessitating frequent venting. The selected operating volume of 200 mL is also consistent with recommendations for homogeneous substrates (Holliger et al., 2016; Pearse et al., 2018). Substrates were obtained from the acidogenic fermentation tests after settling, and only the supernatant was used. The substrate-to-inoculum (S:I) ratio was maintained at 0.25 g COD gVS⁻¹, using granular biomass with a volatile solids concentration of 92.4 g VS L⁻¹.

The initial pH was adjusted to 7.0 using 37% hydrochloric acid and sodium hydrogen carbonate (EMSURE®). Sodium hydrogen carbonate was added to ensure an alkalinity above 1.5 g CaCO₃ L⁻¹, in accordance with BMP test guidelines (Holliger et al., 2016). Sodium hydroxide pellets (EMPLURA®) were used for CO₂ capture.

All bottles were flushed with N₂ for 2 min to establish anaerobic conditions, incubated at 35 °C and gently stirred to avoid damaging the granules. Gas volumes were normalized to standard conditions (0 °C, 1013 hPa). The tests were terminated after 120 h, when cumulative methane production had stabilized.

6.3.3. Analytical Methods

All substrates were chemically analyzed before and after acidogenic fermentation for total and volatile solids (TS, VS; APHA, 2005), total and soluble COD (LCK014), alkalinity (LCK362), total nitrogen (N_{tot}; LCK338), ammonium (NH₄⁺-N; LCK302, 303), total phosphorus (P_{tot}; LCK348), and phosphate (PO₄⁻³-P; LCK348). Spectrophotometric measurements were performed using a Hach Lange DR 3900.

Volatile fatty acid (VFA) concentrations and compositions were determined using a high-performance liquid chromatography (HPLC) system. The analysis employed 2 mM H₂SO₄ as the mobile phase (LC 20 AD pump), with samples maintained in a cooled autosampler (SIL-40C, 5°C). Separation was achieved using an AMINEX HPX-87H column as described by Abuzir et al. (2025), operated at 40°C and a flow rate of 0.7 mL min⁻¹. Detection was performed at a wavelength of 210 nm (SPD 20 AV), and data were processed using LabSolutions software.

6.3.4. Calculations

The yield of VFA is defined as the mass of net total VFA (nTVFA) produced per gram of organic solids in the substrate, as expressed in Equation 1:

$$\text{VFA yield} = \frac{g \text{ nTVFA-COD}}{g \text{ VS}_{\text{substrate}}} \quad (1)$$

where $g \text{ nVFA-COD}$ represents the net equivalent mass of VFA expressed as COD, and $g \text{ VS}_{\text{substrate}}$ represents the mass of volatile solids content of the substrate.

The degree of acidification (DoA) serves as an indicator of the fermentation process (Ramos-Suarez et al., 2021) and is calculated as the net amount of total VFA produced (i.e., the increase in VFA concentration relative to the initial value) divided by the initial total COD, as described by Vergine et al. (2015) and shown in Equation 2:

$$\text{DoA [\%]} = \frac{g \text{ nTVFA-COD}}{g \text{ COD}_{\text{tot, initial}}} \quad (2)$$

where $g \text{ nVFA-COD}$ represents the net equivalent mass of VFA expressed as COD, and $g \text{ COD}_{\text{tot, initial}}$ represents the mass of total COD at the start of the experiment.

6.4. Results and Discussion

6.4.1. COD profile

Batch tests were conducted using three volumetric ratios of FJPWW and PS to assess the effect of co-digesting FJPWW with PS on VFA and methane production.

Fig. 6.2 presents the temporal evolution of total and soluble chemical oxygen demand (COD_{tot} and sCOD) during the 100-hour acidification tests of FJPWW and its co-fermentation with PS at two mixing ratios (FJPWW+PS (97:3) and FJPWW+PS (90:10)).

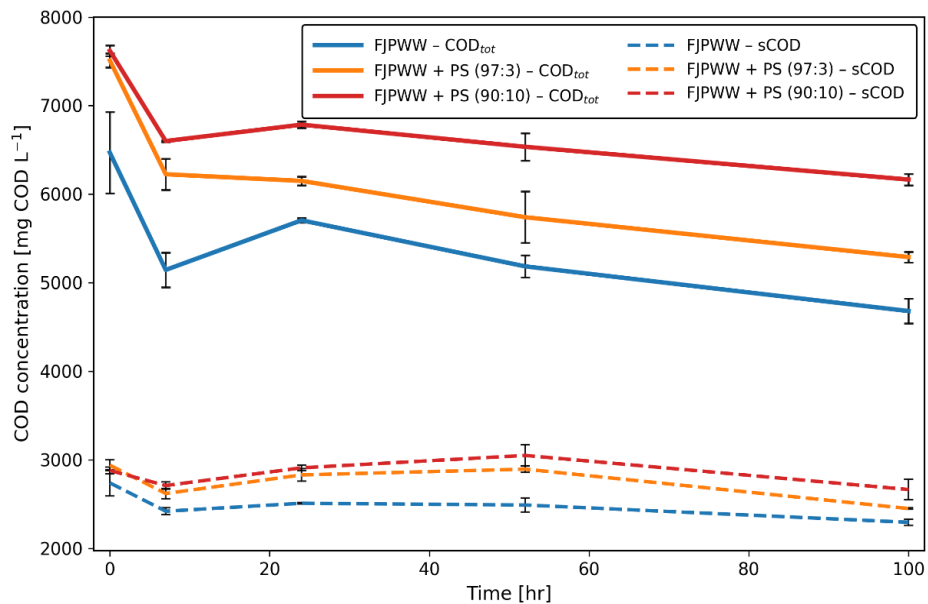


Fig. 6.2. COD profile during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.

The addition of PS increased COD_{tot} due to the supplementary organic load introduced with the sludge (Appels et al., 2008; Li et al., 2022). At the start of the experiment, the average COD_{tot} concentrations were 6470, 7510, and 7620 mg COD L^{-1} for only FJPWW, FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively (Fig. 6.2). These concentrations were higher than

the corresponding COD_{tot} values reported in Table C1 in the Supplementary Information (3504, 3804, and 4224 mg COD L⁻¹, respectively) because Table C1 (Supplementary Information) reports the COD_{tot} of the prepared feed mixtures (FJPWW and FJPWW+PS) before inoculation, whereas the COD_{tot} values in Fig. 6.2 and Table 6.1 were measured after inoculum addition. The inoculum introduced particulate organic matter, thereby increasing the measured COD_{tot} at time 0 hours.

Table 6. 1. Influent and Effluent Characteristics of Substrates Used in the Acidogenic Fermentation Experiment (average values, and standard deviations in brackets).

Parameter	Unit	At the start of the fermentation experiment			At the end of the fermentation experiment		
		FJPWW	FJPWW+PS (97:3)	FJPWW+PS (90:10)	FJPWW	FJPWW+PS (97:3)	FJPWW+PS (90:10)
COD _{tot}	[gCOD L ⁻¹]	6.47 (0.46)	7.51 (0.08)	7.62 (0.06)	4.70 (0.14)	5.30 (0.06)	6.16 (0.06)
sCOD	[gCOD L ⁻¹]	2.74 (0.15)	2.94 (0.06)	2.90 (0.04)	2.30 (0.03)	2.45 (0.01)	2.66 (0.1)
COD _{tot} re- moval	[%]	-	-	-	27.4	29.4	19.4
pH	[-]	6.0	6.0	6.0	7.51 (0.04)	7.51 (0.05)	7.35 (0.21)
VFA	[gCOD L ⁻¹]	1.83	1.78	2.4	2.55	3.13	2.86
Alkalinity	[gCaCO ₃ L ⁻¹]	0.90 (0.0)	0.90 (0.0)	0.91 (0.0)	0.81 (0.0)	0.79 (0.0)	0.81 (0.0)

For all substrates, both COD_{tot} and sCOD decreased rapidly within the first 7 hours. The most pronounced declines were observed for FJPWW and FJPWW+PS (97:3), which may be attributed to their higher initial solubility (85% and 76%, respectively) compared with the substrate containing a higher PS fraction (67% for FJPWW+PS (90:10)). This rapid reduction in both COD_{tot} and sCOD indicates fast hydrolysis and acidogenesis, likely driven by the high proportion of readily soluble organic compounds in FJPWW (Owusu-Agyeman et al., 2020; Rabii et al., 2019; Sun et al., 2021). Moreover, the concurrent decreases in COD_{tot} and sCOD are consistent with the conversion of carbohydrates, proteins, and lipids into VFAs and gaseous products (e.g., CO₂, H₂), together with other intermediates, mediated by hydrolytic and fermentative microorganisms (Chen et al., 2019; Di Maria et al., 2014; Laiq Ur Rehman et al., 2019; Sträuber et al., 2012).

After approximately 24 hours, sCOD remained relatively stable and comparable among the three substrates within the variability indicated by the error bars (2295, 2450, and 2665 mg COD L⁻¹ for FJPWW, FJPWW and FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively; Fig. 6.2), suggesting no further solubilization of particulate and complex organic matter. On the other hand, COD_{tot} exhibited a slight further decrease and subsequently remained relatively stable, with comparable values when considering standard deviations and error bars. Specifically, COD_{tot} declined from 5145, 6225, and 6600 to 4680, 5290, and 6165 mg COD L⁻¹ for FJPWW and FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively (Fig. 6.2). This modest decline in COD_{tot} may be attributed to the continued transformation of organic matter into gases such as CO₂, H₂, and methane (CH₄) (Chen et al., 2019; Di Maria et al., 2014; Laiq

Ur Rehman et al., 2019; Sträuber et al., 2012). However, gases were not measured during the experiment.

Regarding the COD_{tot} removal efficiencies, they ranged from 19.4% to 29.4% across the substrates (Table 6.1). The COD_{tot} and sCOD trends shown in Fig. 6.2, together with the COD_{tot} removal efficiencies reported in Table 5.1, are consistent with findings by Fuentes et al. (2021), who reported low COD removal during beverage wastewater fermentation. Similarly, Lu et al. (2019) noted that reductions in COD_{tot} may be limited under such conditions, as substrates are primarily converted into gases (H₂ and CO₂) and new biomass.

Overall, the first 7 hours were characterized by rapid hydrolysis and acidogenesis, resulting in pronounced decreases in both sCOD and COD_{tot}. Thereafter, both parameters stabilized, indicating limited additional conversion of organic matter under the tested conditions.

6.4.2. VFAs, pH and Alkalinity Profiles

Fig. 6.3A–F and 4A–B illustrate the temporal variations in the average concentrations of acetic, propionic, butyric, iso-valeric, and valeric acids, total volatile fatty acids (TVFAs), pH, and alkalinity during the 100-hour acidification test of FJPWW and its co-fermentation with PS at two mixing ratios (FJPWW+PS (97:3) and FJPWW+PS (90:10)).

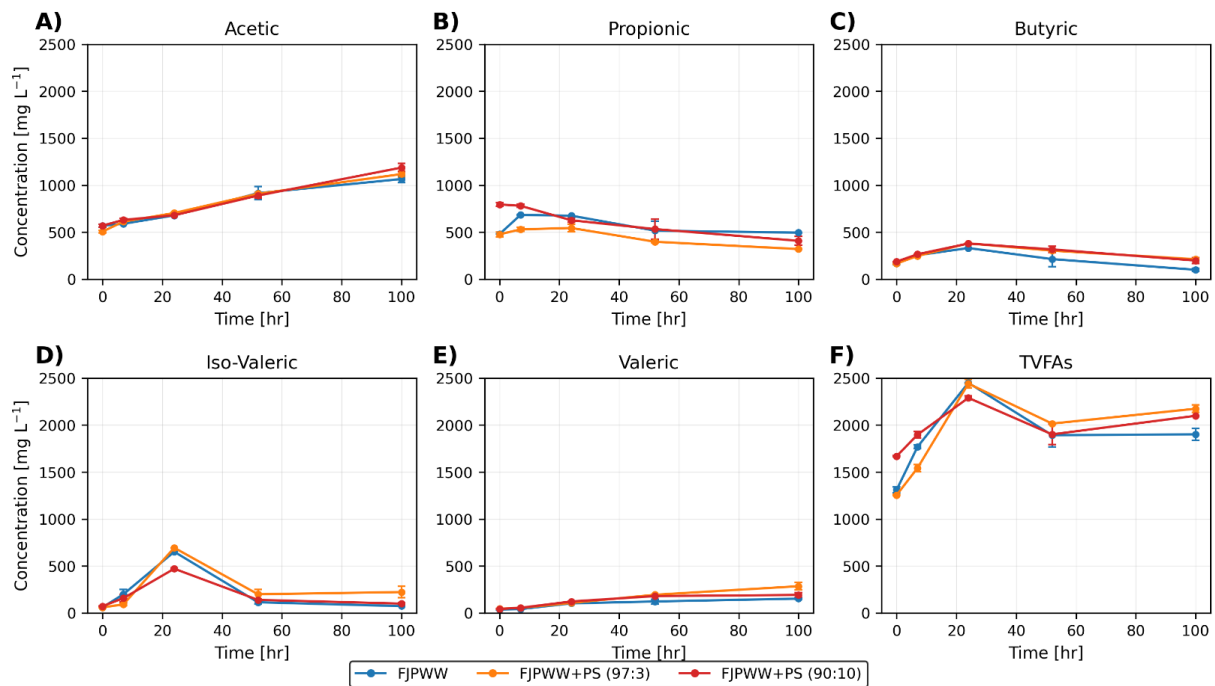


Fig. 6.3. Concentrations of acetic (A), propionic acid (B), butyric (C), Iso-valeric (D), Valeric (E), and total VFAs (TVFAs) (F) during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.

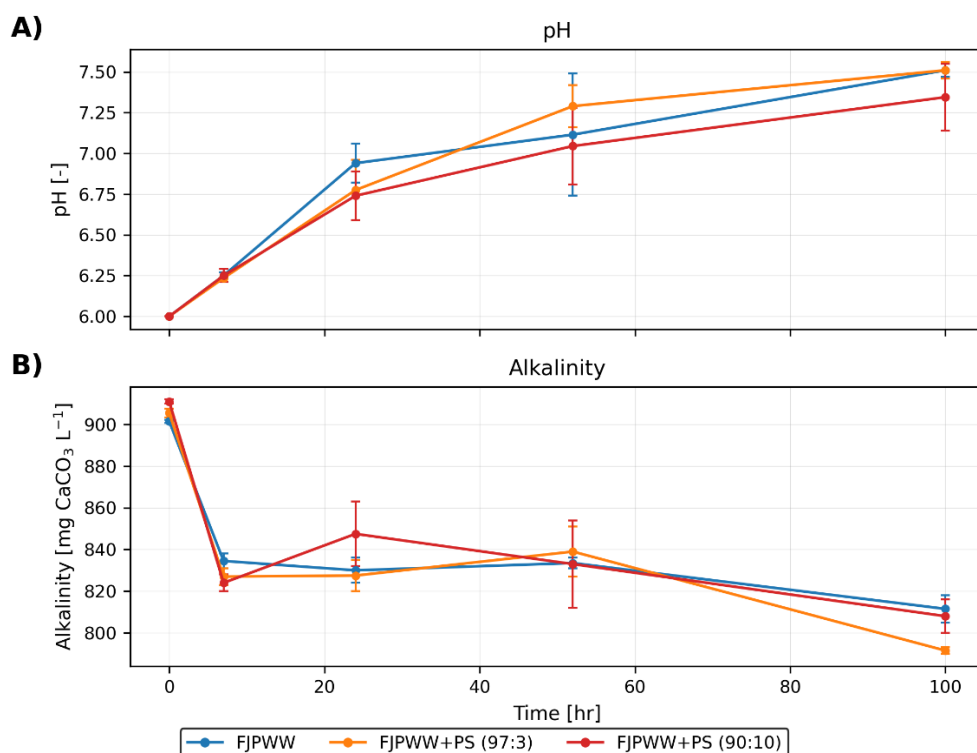


Fig. 6.4. pH profile (A), and alkalinity profile (B) during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.

Across all substrates, acetic acid, considered a favorable intermediate for subsequent methane production (Srisowmeya et al., 2020), increased consistently over time (Fig. 6.3A). Concentrations rose from 568, 506, and 570 mg HAc L⁻¹ to 1065, 1118, and 1187 mg HAc L⁻¹ for FJPWW, FJPWW +PS (97:3), and FJPWW+PS (90:10), respectively. This monotonic increase indicates effective acidogenesis with net acetate accumulation. However, potential conversion pathways involving VFAs, CO₂, and H₂ were not directly assessed in this study, as gases were not measured (Agrawal et al., 2023; Ramos-Suarez et al., 2021).

Iso-valeric acid reached peak concentrations within the first 24 hours (Fig. 6.3E), attaining 651, 694, and 471 mg L⁻¹ for FJPWW, FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively, before decreasing and subsequently stabilizing. A similar temporal pattern was observed for butyric acid, which increased during the initial 24 hours and then gradually declined (Fig. 6.3C). Propionic acid increased during the first 7 hours for FJPWW and FJPWW+PS (97:3), followed by a decrease and stabilization after 24 hours (Fig. 6.3B). In contrast, propionic acid in FJPWW+PS (90:10), declined throughout the experimental period (Fig. 6.3B). The observed reductions in iso-valeric, butyric, and propionic acids suggest further conversion and redistribution among fermentation products, potentially contributing to acetate accumulation (Agrawal et al., 2023; Ramos-Suarez et al., 2021). By comparison, valeric acid exhibited only a slight increase over the test duration (Fig. 6.3E).

TVFAs increased markedly during the first 24 hours, from 1310, 1543, and 1897 mg L⁻¹ to 2449, 2441, and 2289 mg L⁻¹ for FJPWW, FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively (Fig. 6.3F). Thereafter, TVFAs decreased and stabilized for FJPWW. This decline indicates limited net VFA accumulation during the later phase and may reflect further conversion of VFAs into other products; however, potential methanogenic activity cannot be

confirmed, as gas production was not measured. For the PS-amended substrates, TVFAs showed a slight additional increase, suggesting continued conversion of more slowly biodegradable fractions into VFAs. Nevertheless, the magnitude of this increase was limited (approximately 8% for FJPWW+PS (97:3), and 1.4% for FJPWW+PS (90:10), indicating that PS addition did not substantially enhance overall VFA accumulation. This limited effect may be related to the low PS volumes added and/or to the origin of the PS, which was obtained from a primary settler of a MWWTP where FJPWW contributed to 50% of the daily inflow, potentially diluting the composition of PS. A comparable late-stage increase in VFAs during co-fermentation of organic waste with sewage sludge was reported by Owusu-Agyeman et al. (2020).

The pH increased for all substrates from approximately 6.0 to 7.35–7.51 over the course of the experiment (Fig. 6.4A). Alkalinity decreased slightly within the first 7 hours, from 901, 905, and 911 to 834, 827, and 824 mg CaCO₃ L⁻¹ for FJPWW, FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively. Alkalinity then remained relatively stable until hour 52, followed by a further decrease to 811, 791, and 808 mg CaCO₃ L⁻¹, respectively (Fig. 6.4B). The increasing pH trend may be associated with the applied substrate-to-inoculum ratio (2 g COD g VS⁻¹), combined with system buffering capacity and the release of alkaline compounds (e.g., ammonia) during the biodegradation of nitrogen-containing substrates, as well as urea addition (Fang et al., 2019). A similar pH increase at a comparable substrate-to-inoculum ratio (1.6 g COD g VSS⁻¹) was reported by Vergine et al. (2015) during fermentation of synthetic soft drink wastewater.

In summary, the first 24 hours were characterized by rapid hydrolysis and acidogenesis, resulting in a pronounced increase in TVFAs. Thereafter, net TVFA accumulation was limited, indicating minimal additional conversion of organic matter into VFAs under the tested conditions. Overall, the addition of PS did not show a significant effect on VFA production compared with FJPWW alone.

6.4.3. The Impact of Co-fermenting FJPWW with PS on VFA Composition

Fig. 6.5 illustrates the VFA composition at the end of the 100-hour acidification test of FJPWW and its co-fermentation with PS at two mixing ratios (FJPWW+PS (97:3) and FJPWW+PS (90:10)).

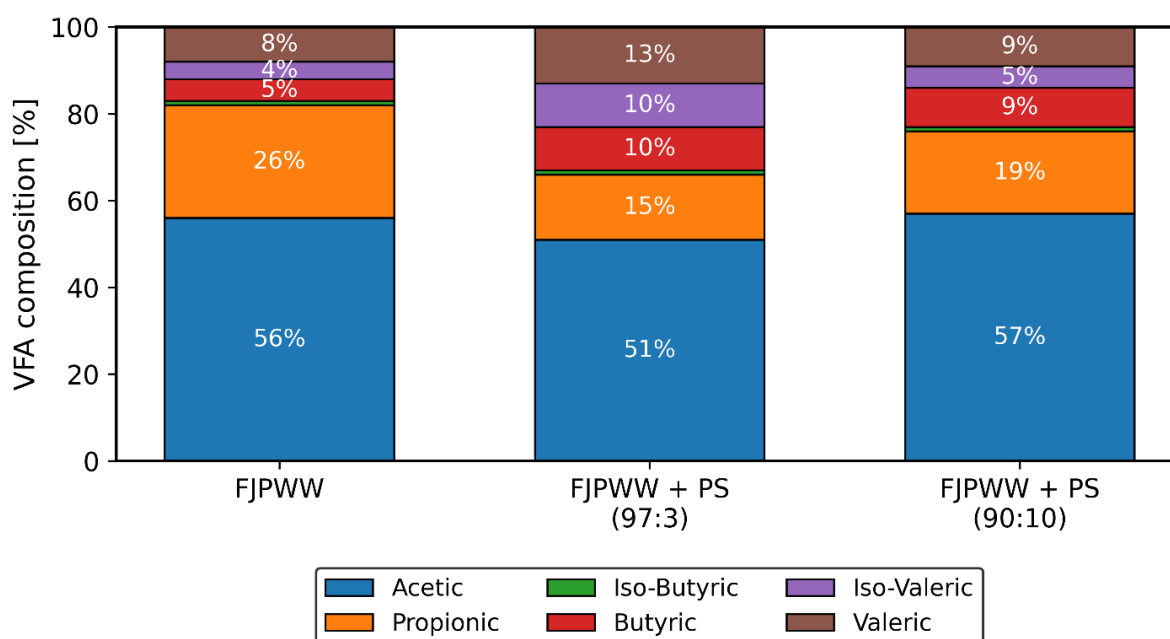


Fig. 6.5. VFA composition at the end of the acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.

No pronounced effect of PS addition on the overall VFA composition was observed at the end of the co-fermentation test (Fig. 6.5). Across all substrates, acetic acid was the dominant component, accounting for 51–57% of total VFAs, followed by propionic acid (15–26%), valeric acid (8–13%), and iso-valeric and butyric acids (4–10%) (Fig. 6.5). This distribution suggests that a broad spectrum of VFAs was produced under the tested experimental conditions. Although final acetic acid concentrations were comparable among all substrates (1065–1187 mg HAc L⁻¹; Fig. 6.3A), the relative share of acetic acid in FJPWW+PS (97:3) was 8–10% lower than in FJPWW+PS (90:10). This slight difference is attributable to higher relative contributions of iso-valeric, butyric, and valeric acids in the FJPWW+PS (97:3) mixture (Fig. 6.5). Variations in the relative proportions of individual VFAs are likely influenced by the specific metabolic pathways prevailing during fermentation (Banu et al., 2020).

The observed VFA distributions of acetic (51–57%), propionic (15–26%), butyric (4–10%), and valeric (8–13%) are consistent with values reported in previous studies using comparable substrates. Similar ranges have been reported for synthetic soft drink wastewater (approximately 25–69% acetic, 5–10% propionic, and 7–45% butyric acids) (Vergine et al., 2015), beverage wastewater (53%, 34%, 7%, and 6% for acetic, propionic, butyric, and valeric acids, respectively), sugarcane molasses (approximately 50% acetic acid) (Silva et al., 2013), and cassava wastewater (39–52% acetic, 16–24.6% propionic, 22.7–50.5% butyric, and 2.6–2.8% iso-valeric acids) (Sanchez-Ledesma et al., 2024).

Overall, no significant impact of co-fermenting JPWW with PS was observed at the end of the acidogenic co-fermentation test likely due to the low PS volumes added and/or the origin of the PS from a primary settler of a MWWTP where FJPWW contributed to 50% of the daily inflow, potentially diluting its composition.

6.4.4. The Impact of Co-fermenting FJPWW with PS on DoA and VFA Yield

The degree of acidification (DoA) and VFA yield were used to better assess the impact of co-fermenting different proportions of FJPWW and PS, as both indicators reflect fermentation performance and product quality (Garcia-Aguirre et al., 2017; Huang et al., 2018; Ramos-Suarez et al., 2021). While DoA reflects the extent of acidification, VFA yield (expressed as net VFAs-COD produced per unit of influent volatile solids (VS)) provides complementary information on substrate conversion efficiency.

Fig. 6.6A–B present the temporal evolution of DoA and VFA yield during the 100-hour acidification tests of FJPWW and its co-fermentation with PS at two mixing ratios (FJPWW+PS (97:3) and FJPWW+PS (90:10)).

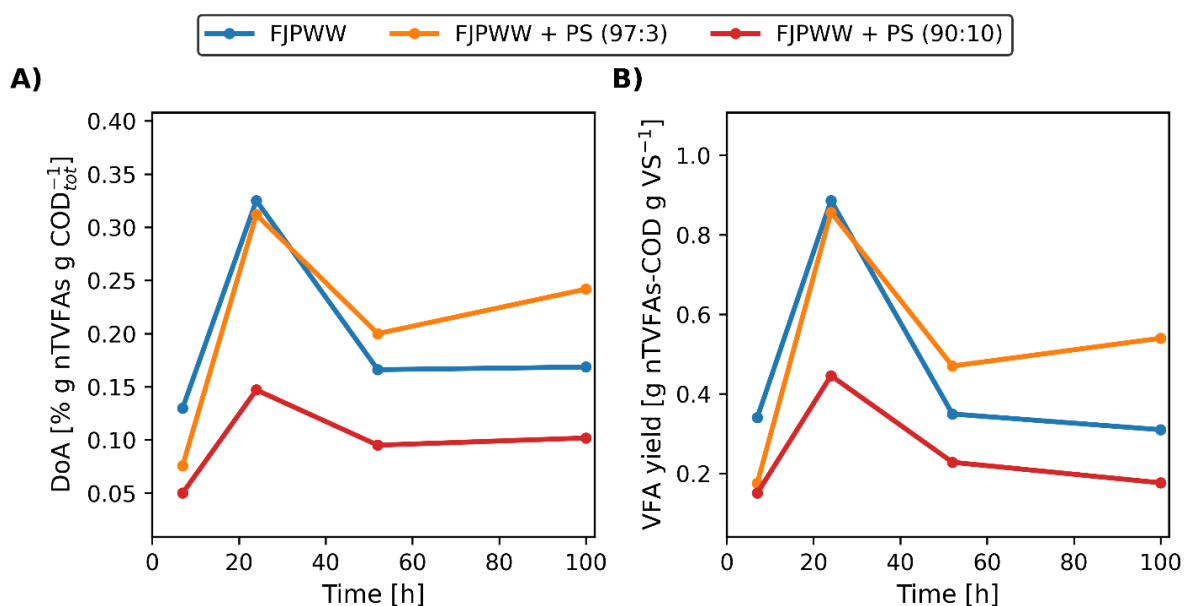


Fig. 6.6. DoA profile (A), and VFA yield profile (B) during acidogenic fermentation of FJPWW and FJPWW co-fermented with PS at varying vol./vol. ratios.

For all substrates, DoA increased sharply within the first 24 hours, reaching maximum values of 33%, 31%, and 15% for FJPWW, FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively (Fig. 6.6A). This rapid increase indicates efficient fermentation of readily biodegradable organic matter and overall good acidogenic performance. FJPWW+PS (97:3) exhibited comparable maximum DoA values (33% and 31%, respectively). In contrast, co-fermentation with a higher PS fraction (FJPWW+PS (90:10)) resulted in the lowest maximum DoA (15%), indicating that increasing PS content did not enhance acidification. This is an important observation, because TVFA concentration (Fig 6.3 A-F) and DoA describe different aspects of process performance. While TVFA concentration indicates the amount of acids accumulated, DoA reflects the efficiency of conversion of substrate COD into VFAs. As a result, DoA may reveal differences in acidification performance that are less evident when only VFA concentrations are considered. This occurred despite the fact that co-fermentation at FJPWW+PS (97:3) and FJPWW+PS (90:10) yielded COD:N:P ratios of 573.4:3.2:1 and 344:2.5:1, respectively, which are closer to the recommended ratio for fermentation (350:5:1) (de Lemos Chernicharo, 2007) compared with FJPWW alone (778:2.4:1). The reduced DoA observed for FJPWW+PS (90:10)

is likely associated with the introduction of fibrous and particulate matter from PS, which is less readily biodegradable (Azevedo et al., 2023; Hobson & Wheatley, 1993).

After 24 hours, DoA decreased to 17%, 24%, and 10% for FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively, and subsequently remained relatively stable. This trend indicates that acidification activity became limited beyond this point and suggests that a hydraulic retention time of approximately 24 hours is sufficient to achieve maximum VFA production under the tested conditions. Furthermore, the maximum DoA values observed at 24 hours (15–33%) (Fig. 6.6A) were slightly lower than those reported for synthetic soft drink wastewater (43.7–54.6%) (Vergine et al., 2015) and urban food waste fermentation (44–79%) (Yu et al., 2021). These differences are likely attributable to variations in substrate characteristics. Nevertheless, the obtained DoA values fall within the range generally considered favorable for effective acidogenesis (20–40%) (Lettinga & Hulshoff, 1991).

Regarding VFA yield, a trend similar to that observed for the degree of acidification was identified. All substrates exhibited a sharp increase within the first 24 hours, reaching maximum values of 0.89, 0.86, and 0.45 g nTVFA-COD g VS⁻¹ for FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively. These maxima indicate rapid fermentation of readily biodegradable organic matter and overall good acidogenic performance. FJPWW and 97% FJPWW + 3% PS showed comparable peak VFA yields (0.89 and 0.86 g nTVFA-COD g VS⁻¹, respectively). In contrast, co-fermentation with a higher PS fraction (FJPWW+PS (90:10)) resulted in the lowest VFA yield (0.45 g nTVFA-COD g VS⁻¹), suggesting that increasing PS content did not enhance VFA production. This lower yield is consistent with the reduced DoA (15%) observed for this substrate and is likely associated with the introduction of fibrous and particulate components from PS that are less readily biodegradable (Azevedo et al., 2023; Hobson & Wheatley, 1993).

After 24 hours, VFA yields declined to 0.30, 0.50, and 0.20 g nTVFA-COD g VS⁻¹ for FJPWW, FJPWW+PS (97:3), and FJPWW+PS (90:10), respectively, and subsequently remained relatively stable. This behavior indicates that acidogenic activity became limited beyond the initial 24 hours. The VFA yields obtained in this study (0.15–0.89 g nTVFA-COD g VS⁻¹) are comparable to those reported for food waste (0.48 g TVFAs-COD g VS⁻¹) (Khatami et al., 2021), co-fermentation of food waste with primary sludge (0.15 g TVFAs-COD g VS⁻¹) (Min et al., 2005), and cassava wastewater (approximately 0.18–0.25 g TVFAs-COD g VS⁻¹) (Sanchez-Ledesma et al., 2024), indicating stable acidogenic performance across the tested substrates.

Overall, co-fermenting FJPWW with PS at the investigated volumetric ratios did not result in a significant enhancement of VFA production. On the contrary, increasing the PS fraction led to lower DoA and VFA yields.

6.4.5. The impact of Co-digesting FJPWW with PS on Methane Production

Fig. 6.7 presents the average BMP results of duplicate tests, including standard deviations, expressed per gram of VS of substrate over a 120-hour incubation period for FJPWW and its co-digestion with PS at two mixing ratios (FJPWW+PS (97:3) and FJPWW+PS (90:10)). The test was terminated when methane production dropped below 1% of the cumulative methane yield, following the criterion proposed by Zaman et al. (2010).

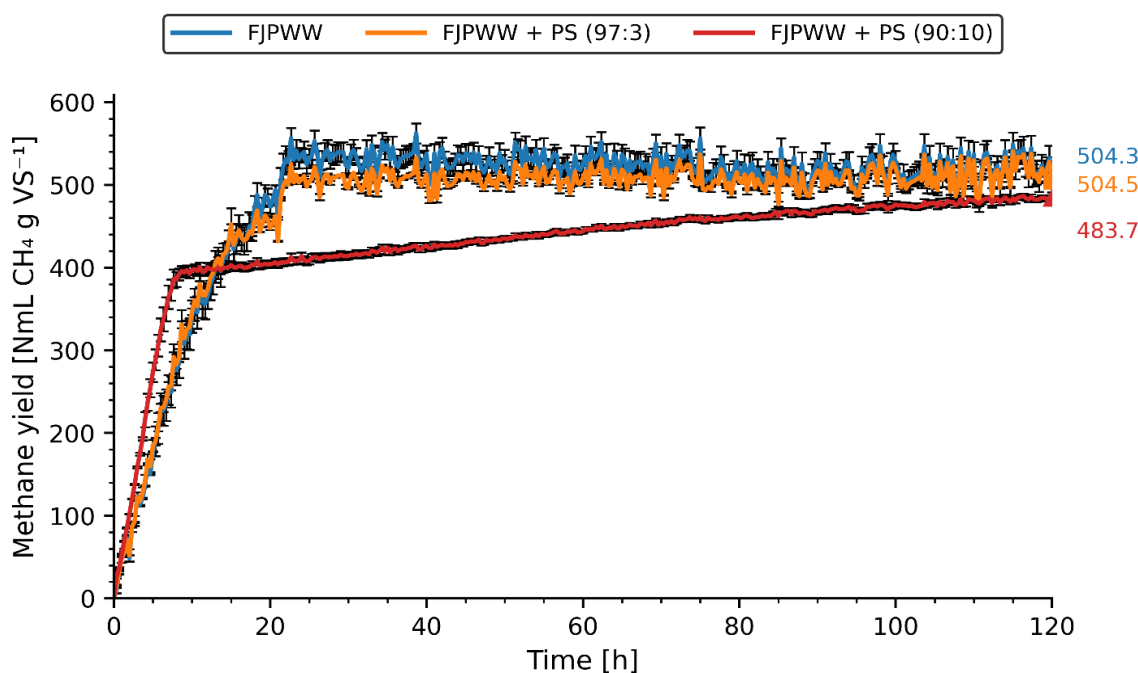


Fig. 6.7. BMP of FJPWW and its co-digestion with PS at different vol./vol. ratios, expressed as methane yield per VS mass.

Methane yield increased sharply within the first 24 hours for FJPWW and FJPWW+PS (97:3), whereas for the substrate with a higher PS content (FJPWW+PS (90:10)), a rapid increase was observed within the first 10 hours (Fig. 6.7). This pronounced early increase for all substrates indicates that readily fermentable sugars in FJPWW were efficiently degraded, resulting in high biogas potential (Cremonez et al., 2021). Co-digestion of FJPWW with PS did not lead to a significant enhancement in methane yield. After 24 hours, methane production stabilized and remained comparable for FJPWW+PS (97:3), reaching cumulative yields of 504.3 and 504.5 N mL CH₄ g VS⁻¹, respectively. Minor variations in methane yield observed after 24 hours may be partly attributed to the low substrate volume used in the BMP tests (200 mL), which could have influenced headspace pressure and gas measurement accuracy.

Increasing the PS fraction resulted in a slight reduction in methane yield (Fig. 6.7). For the substrate with the highest PS ratio (FJPWW+PS (90:10)), methane production increased more gradually and reached a lower maximum value of 483.7 N mL CH₄ g VS⁻¹ (Fig. 6.7). This behavior can be attributed to differences in the composition and biodegradability of the organic fractions present in the substrates (Owusu-Agyeman et al., 2020), as well as to the higher content of fibrous and particulate matter introduced with PS, which is less readily biodegradable (Azevedo et al., 2023; Hobson & Wheatley, 1993). In addition, PS has a substantially lower methane potential (132.4 N mL CH₄ g VS⁻¹) (Elsayed et al., 2016) compared with FJPWW (504.3 N mL CH₄ g VS⁻¹), which further explains the reduced methane yields observed with increasing PS addition.

The methane yields obtained in this study were lower than those reported for the co-digestion of food waste with mixed primary, secondary, and sewage sludge (595–899 NmL CH₄ g VS⁻¹) (Azarmanesh et al., 2020), likely reflecting differences in substrate composition and intrinsic methane potential. In the present study, FJPWW is a highly readily biodegradable substrate, which may have limited the scope for further increasing methane potential through PS addition.

in batch tests. Nevertheless, although the effect on methane yield was limited under the tested conditions, PS may still be relevant in continuous systems, where its contribution to nutrient supply, buffering capacity, and overall process stability could become more important over longer operating periods.

Overall, co-digesting FJPWW with PS did not result in a significant improvement in methane yield. On the contrary, increasing the proportion of PS in the substrate led to a slight reduction in methane production.

6.5. Conclusions

Batch acidogenic fermentation tests were conducted at an S:I ratio of 2 g COD g VS⁻¹ and an uncontrolled initial pH of 6. Biochemical methane potential (BMP) tests were then performed at a substrate-to-inoculum ratio of 0.25 g COD g VS⁻¹ and 35 °C to evaluate the effect of adding primary sludge from a municipal wastewater treatment plant (MWWTP) to fruit juice processing wastewater at different volumetric ratios. The objective was to assess the impact on VFA production and methane yield.

The results showed that the addition of primary sludge did not lead to a significant enhancement in either VFA production or methane yield. Increasing the proportion of primary sludge resulted in a reduction in the degree of acidification and VFA yield, as well as a slight decrease in methane production. However, co-fermentation with primary sludge improved the COD:N ratio of the substrate mixture.

To further elucidate the effects of co-fermentation and co-digestion of fruit juice processing wastewater with primary sludge, future studies should investigate primary sludges from MWWTPs with different proportions of industrial wastewater contributions, explore a wider range of substrate-to-inoculum ratios, and evaluate the influence of controlled pH conditions. In addition, continuous reactor studies should be conducted to assess whether primary sludge could provide longer-term benefits in terms of process stability, nutrient balance, and buffering capacity under prolonged operation.



Chapter 7

Overall Discussion

Chapter 7. Synthesis and Concluding Discussion

This chapter comprises three subsections. Section 7.1 synthesizes the key findings from **Chapters 4, 5, and 6**. Section 7.2 outlines the scientific contributions of the thesis as a whole.

7.1. Synthesis of Key Findings

This thesis studied and investigated AD across three operational contexts and waste types: (i) full-scale UASB treatment of source-separated black water (BW) and the co-digestion of BW with food waste (FW), (ii) full-scale two-stage digestion of FJPWW using a homogenization/acidification (hydrolysis/acidogenesis) tank followed by R2S reactors (acetogenesis/methanogenesis), and (iii) controlled batch and BMP tests evaluating whether co-fermenting and co-digesting FJPWW with PS at different vol./vol. ratios could increase VFA and methane production. Across the three studies, performance was primarily assessed based on COD removal and methane production.

In Chapter 4, the main focus was on the full-scale BW UASB which achieved efficient methane production under both OLR phases, with the best overall removal performance at the lower OLR (Phase 1). During Phase 1, the average OLR was $0.95 \text{ kg COD m}^{-3} \text{ d}^{-1}$, COD removal reached 88%, and the degree of methanization was $0.77 \text{ kg COD-CH}_4 \text{ kg}^{-1} \text{ COD}_{\text{in}}$. When the OLR increased to an average of $1.5 \text{ kg COD m}^{-3} \text{ d}^{-1}$ in Phase 2 (higher OLR), COD removal decreased to 72%. This decline was attributed to sludge washout, reflected by increased effluent COD and TSS. Despite reduced effluent quality, methanization remained high at $0.71 \text{ kg COD-CH}_4 \text{ kg}^{-1} \text{ COD}_{\text{in}}$, indicating that the biological capacity for methane production was sustained even as solids losses reduced apparent treatment performance.

This interpretation is supported by the COD mass balance during Phase 2 (higher OLR), which accounted for 69% of influent COD as COD converted to methane, while 28% was in effluent and 3% was removed with wasted sludge. Together, these fractions indicate that, at higher loading, solids washout largely explains the lower measured COD removal, even though methane production remained high. Nutrient mass balances further showed that nutrients were largely retained in the liquid phase, highlighting that the BW UASB system is suitable for nutrient recovery. Specifically, the decanted effluent contained 92% of N and 82% of P in soluble form, while the removed sludge accounted for only 2% of N and 18% of P. This distribution aligns with the small fraction of material leaving as wasted sludge and indicates that, in this configuration, nutrient management and recovery should mainly focus on the effluent stream.

Acetotrophic activity measurements reported an SMA of $185.6 \text{ NmL CH}_4 \text{ g VSS}^{-1} \text{ d}^{-1}$, supporting the conclusion that methane conversion remained high even when COD removal decreased due to washout-related effluent losses.

Co-digestion of BW with FW did not increase methane production, in contrast to Kamravanesh & Koko. (2024), who reported improved performance at a BW: FW VS ratio of 0.41, and Wang et al. (2020), who applied a kitchen waste:BW ratio of 1:1 g VS g⁻¹ VS. The lack of enhanced methane production in the BW–FW co-digestion system (UASB_{FW+BW}) may be

attributable to the unknown FW:BW mixing ratios, which could have adversely affected anaerobic digestion (AD) performance.

Although the BW UASB system showed effective performance in terms of COD removal, methane production, and nutrient retention, and the full-scale UASB of BW is a suitable solution for circular sanitation solutions, future research should investigate the microbial communities responsible for hydrolysis to better understand sludge washout. In addition, removing sludge from mid-height of the UASB reactor could be tested to evaluate its impact on sludge washout. Finally, energy balances over longer operating periods could be conducted to further assess the energy balance of the full-scale system.

In FJPWW system (Chapter 5), the full-scale two-stage configuration was designed to separate hydrolysis/acidogenesis (the first stage) from acetogenesis/methanogenesis (the second stage). In the first stage (homogenization tank) generated a VFA-rich intermediate wastewater, with a VFA/sCOD of 38–41% and DoA values of approximately 21–25% across temperature groupings of 16–26°C. Temperature was reported as not significantly affecting COD removal or DoA during pseudo-steady state periods. Stage 2 (R2S reactors) delivered high removal performance (COD_{tot} dropped in the influent from 2902 to 349 mg COD L⁻¹ in the effluent; COD removal 88.1%), accompanied by strong VFA conversion to methane (in the influent from 1082 to 69.2 mg HAc L⁻¹ in the effluent) and stable operation, as indicated by a VFA/Alk of 0.07. Nevertheless, the effluent still required additional treatment before discharge, and TSS removal in the second stage (acetogenesis/methanogenesis) was low (6.2%), which was attributed to potential wash-out of solids. Similar to the BW UASB, the methanogenesis stage could perform well biologically while a downstream polishing requirement remained, driven by solids and/or residual COD highlighting the challenges of treating wastewater in full-scale applications. Although the two-stage AD system showed stable and effective performance in terms of DoA, COD removal, and methane production, the nutrient and alkalinity imbalance of the incoming FJPWW needs to be managed, especially in the first stage (hydrolysis/acidogenesis). One option is co-digesting with a substrate with high alkalinity and nutrient rich such as PS or animal manure. In addition, other parameters such as F/M in the first stage could be monitored to better understand the performance of this stage, given that temperature was the most dominant variable parameter and did not have a significant impact on DoA and COD removal in this stage. Moreover, the low TSS removal in the methanogenic reactors suggests the need for parameters other than SMA to assess granular biomass quality, such as settleability testing (e.g., cone test for 5 minutes), and for CFD studies to better understand the hydrodynamics of the methanogenic reactors.

The full-scale two-stage FJPWW system (Chapter 5) identifies nutrient and alkalinity management as an operational priority and suggests co-substrates such as PS as a potential solution to improve the nutrient and alkalinity imbalances. The batch hydrolysis/acidogenesis and BMP tests (Chapter 6) examined this hypothesis using defined vol./vol. mixing ratios (FJPWW alone, 97:3, and 90:10 with PS). The first stage (hydrolysis/acidogenesis) was conducted for 100 hours, and the resulting supernatant was used as the substrate for the second stage (acetogenesis/methanogenesis) BMP test (120 hours). Under these controlled conditions, adding PS did not increase VFA production or methane yield. Peak DoA occurred within ~24 hours (33% for FJPWW; 31% for 97:3 FJPWW+ PS; 15% for 90:10 FJPWW+ PS), and peak VFA yields

followed the same pattern (0.89, 0.86, and 0.45 g nTVFA-COD gVS⁻¹, for FJPWW, 97:3 FJPWW+PS, and 90:10 FJPWW+PS, respectively). Methane yields were essentially unchanged at 97:3 FJPWW+PS relative to FJPWW alone (504.5 vs 504.3 NmLCH₄ gVS⁻¹) but decreased for 90:10 FJPWW+PS (483.7 NmL CH₄ g VS⁻¹). The slightly lower methane yield at higher PS fractions was interpreted as potentially reflecting the introduction of less readily biodegradable fibrous/particulate material. In addition, because FJPWW is already highly biodegradable, the potential for further increasing methane production through PS addition in batch tests may have been limited given the low amount of added PS. Nevertheless, although PS had a limited effect on methane yield under the tested conditions, it may still be relevant in continuous systems, where its contribution to nutrient supply, buffering capacity, and overall process stability could become more important over longer operating periods. Gas production during the acidogenic phase was not measured; therefore, the fermentation pathways could not be confirmed. Overall, the BMP tests showed that adding PS did not improve VFA production or methane yield under the tested conditions. However, the results helped define when PS addition may or may not be beneficial for FJPWW co-digestion.

Together, the AD of BW and FJPWW show that reactor configuration, operational conditions and constraints should be interpreted in relation to substrate characteristics. In the full-scale BW UASB (35°C) (Chapter 4), methane conversion remained high, but overall treatment performance became increasingly dependent on solids retention, as reflected by the reduction in COD removal at higher OLR attributed to sludge washout. In the FJPWW system (Chapter 5), the two-stage AD system promoted acidification in the first stage (hydrolysis/acidogenesis), producing a VFA-rich intermediate, while the second stage (methanogenesis stage) converted VFAs effectively to methane under stable conditions. However, this performance relied on external nutrient and alkalinity management, and the system still required post-treatment for COD removal and showed limited TSS removal in the second stage. Collectively, these full-scale studies support a practical conclusion: stable methane conversion can coexist with residual effluent COD and/or solids, meaning that “good digestion” does not necessarily yield discharge-ready effluent without attention to solids management and polishing requirements. Moreover, it highlights the challenges in treating such substrates in full-scale applications.

A key role of the multi-scale approach adopted in this thesis is that it connects “what can be isolated and tested” with “what happens in practice”. The full-scale results show system performance and operational constraints (e.g., washout at higher OLR in a UASB; nutrient/alkalinity management and solids behavior in a two-stage industrial wastewater system). The batch and BMP experiments allowed the effect of PS addition to be tested under controlled mixing ratios and retention times. In this study, adding PS at 97:3 and 90:10 improved the COD:N ratio but did not increase VFA or methane yields. This may be because FJPWW is already highly biodegradable, and the amount of PS added was relatively low. However, PS addition should not be ruled out, as it may have a stronger role in continuous systems by supporting nutrient supply, buffering capacity, and long-term process stability.

In combination, these findings support evidence-based decisions on which optimization routes are likely to be beneficial, and which may introduce additional trade-offs.

7.2. Scientific Contributions of the Thesis

Chapter 4 provides long-term, full-scale operational evidence for AD of BW in a novel full-scale UASB system, including performance evaluation (COD removal and methane yield and production) under two OLR regimes and COD, N, and P mass- and energy-balance outcomes. The findings show that, in the full-scale BW UASB, methane production can remain high even when COD removal declines at higher OLR due to sludge washout. Nitrogen and phosphorus were maintained predominantly in soluble forms ($\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$), indicating that the system can support methane production and resource recovery. The chapter also provides operational recommendations for sludge removal at different heights (e.g., from the bottom and mid-height). In addition, it proposes that future research should examine microbial communities to better understand substrate hydrolysis.

Second, Chapter 5 presents a long-term performance assessment of a full-scale two-stage AD system treating FJPWW. Pseudo-steady-state periods were selected to link first-stage (hydrolysis/acidogenesis) performance (DoA, COD removal, VFA production) with second stage (acetogenesis/methanogenesis) performance (COD removal, VFA conversion, VFA/ALK, methane yield, and production). The work provides operationally relevant evidence that high COD removal during the second stage (acetogenesis/methanogenesis) may occur alongside limited solids removal and a continued requirement for post-treatment. The findings also highlight nutrient and alkalinity management as essential for stable operation with this type of wastewater. In addition, the chapter proposes plant operation procedures, including measuring the F/M ratio in the first stage (hydrolysis/acidogenesis) to better understand the main factors affecting performance. Further recommendations include measuring the settleability of the granular biomass and conducting CFD studies to better understand low TSS removal, sludge washout, and the hydrodynamics of the methanogenic reactors.

Third, Chapter 6 evaluates a co-digestion strategy (PS addition) using a staged experimental design (acidogenic fermentation followed by BMP) that explicitly links acidification metrics (DoA, VFA yield and composition) to methane potential outcomes. Notably, the results indicate that, at the tested volumetric ratios and experimental conditions, PS addition did not increase VFA production or methane yield, and that higher PS fractions reduced both acidification metrics and methane yield. These findings directly inform the feasibility of PS co-feeding as a performance enhancement strategy under comparable conditions.

Collectively, the thesis links full-scale performance constraints (washout, nutrient/alkalinity management, and effluent quality requirements) with targeted experimental testing of an operational hypothesis, yielding an integrated evidence base that spans real-world system behavior and controlled mechanistic evaluation.



Chapter 8

Overall Conclusions

Chapter 8. Conclusions and recommendations for future research

This chapter consists of two parts. The first part (Section 8.1) presents the overall conclusions drawn from **Chapters 4, 5, and 6**, while the second part (Section 8.2) provides recommendations for future research.

8.1. Overall Conclusions

This thesis examined anaerobic digestion performance through three complementary studies conducted across multiple bioreactors scales and configurations. Chapter 4 evaluated a full-scale UASB treating source-separated blackwater using long-term operational monitoring over 130 weeks at 35°C, under two operating phases targeting two different organic loading rates. The chapter also highlighted the performance of a 41 m³ full-scale UASB reactor co-digesting source-separated blackwater with food waste at two operating temperatures, 22.5 and 35 °C. The evaluation included COD removal, methane production, COD, nitrogen, and phosphorus mass balances, energy balance, and an acetotrophic SMA test. Chapter 5 evaluated a full-scale two-stage anaerobic digestion system treating fruit juice processing wastewater using long-term plant operational datasets to quantify the performance of each step in two-stage AD (hydrolysis/acidogenesis and acetogenesis/methanogenesis), including COD removal, methane production, VFA production, degree of acidification, and microbial activity evaluation by SMA testing. Chapter 6 evaluated whether co-fermenting and co-digesting fruit juice processing wastewater with primary sludge at different volume to volume ratios improves VFA and methane production, using controlled 100 hours batch acidification/fermentation (without gas measurements) followed by BMP tests at 35°C (ended after 120 hours) using supernatant from the acidification tests. This thesis examined AD performance through three complementary studies conducted across multiple scales.

1. Conclusions from the Full-scale BW UASB performance evaluation:
 - a. The system achieved its best performance in the first phase (lower OLR) at an average OLR of 0.95 kg COD m⁻³ d⁻¹, reaching 88% COD removal and a methanisation of 0.77 kg COD-CH₄ kg⁻¹ COD_{in}.
 - b. When the loading was increased to an average OLR of 1.5 kg COD m⁻³ d⁻¹, COD removal decreased to 72% as a result of sludge washout, whereas methanization remained high at 0.71 kg COD-CH₄ kg⁻¹ COD_{in}.
 - c. The COD balance indicated that 69% was converted to methane, 28% was discharged in the effluent, and 3% was associated with the removed sludge.
 - d. For nutrients, the decanted effluent contained 92% of N and 82% of P in the soluble form of NH₄⁺-N and PO₄³⁻-P, while the removed sludge accounted for 2% of N and 18% of P.
2. Conclusions from the Full-scale two-stage AD FJPWW performance evaluation:
 - a. The first stage (hydrolysis/acidogenesis) generated a VFA-rich intermediate, with VFA/sCOD of 38–41% and a DoA of 21–25%. Operating this stage at different temperatures (16–26°C) showed no significant effect on COD removal or DoA during the analyzed periods.

- b. In the second stage (acetogenesis/methanogenesis). AD performance was high and efficient, with COD_{total} decreasing from 2902 to 349 mg COD L⁻¹ (COD removal 88.1%) alongside substantial VFA removal (VFA as HAc from 1082 to 69.2 mg HAc L⁻¹). Process stability was maintained, as indicated by a VFA/Alk of 0.07.
 - i. The methane yield was 0.277 Nm³CH₄ kg⁻¹ COD_{removed}, with an average methane content of 80%.
 - ii. Although COD removal was efficient, TSS removal was low (6.2%) possibly attributed to sludge washout.
 3. Conclusions from the potential of co-digesting fruit juice processing wastewater with primary sludge:
 - a. Peak acidification was reached within ~24 hours. The maximum DoA values were 33% (FJPWW), 31% (97:3 FJPWW+PS), and 15% (90:10 FJPWW+PS), while the corresponding peak VFA yields were 0.89, 0.86, and 0.45 g nTVFA-COD g VS⁻¹, respectively.
 - b. Methane yields were comparable for FJPWW alone and FJPWW+PS (97:3) (504 N mL CH₄ g VS⁻¹) but declined at 90:10 (483.7 N mL CH₄ g VS⁻¹).
 - c. Overall, within the tested conditions and ratios, adding PS did not improve either VFA production or methane yield.

8.2. Recommendations for Future Research

- For the BW UASB in Chapter 4, strategies to limit sludge washout and improve effluent quality during operation at higher OLR should be assessed by (i) removing sludge from different heights (i.e., from the bottom and mid-height), (ii) investigating the microbial communities responsible for hydrolysis, and (iii) further characterizing the composition and biodegradability of the residual soluble COD in the effluent.
- To improve and extend energy balance assessments for the BW UASB (Chapter 4) beyond the studied period.
- For the full-scale two-stage FJPWW system in Chapter 5, to strengthen interpretation of the first stage (hydrolysis/acidogenesis) performance by monitoring biomass (e.g., VS or other proxies) and operational ratios (e.g., F/M).
- To further examine solids behavior and the reasons for low TSS removal in the R2S reactors (Chapter 5), as solids washout was identified.
- To evaluate nutrient and alkalinity management options for the first-stage hydrolysis/acidogenesis process in Chapter 5 beyond chemical dosing, including co-substrate-based approaches.
- To apply computational fluid dynamics (CFD) to optimize internal hydrodynamics in the methanogenic reactor, and conduct in-depth investigations of the microbial communities in both stages to further refine operating conditions for these microorganisms.
- Building on the co-digestion experiments, the experimental matrix should be expanded by testing primary sludge from WWTPs with different levels of industrial wastewater contribution, applying different mixing ratios, widening the S:I ratios under controlled pH, increasing the working volumes in the BMP tests, and operating a continuous pilot-

scale plant to evaluate the impact of co-digesting fruit juice processing wastewater with a substrate rich in alkalinity and nutrients. In future acidogenic fermentation experiments, to measure gas production ($H_2/CO_2/CH_4$) during the acidogenic phase to clarify pathways and close carbon balances that were not measurable in the current batch design.



References

References

- Abbasi, T., & Abbasi, S. A. (2012). Formation and impact of granules in fostering clean energy production and wastewater treatment in upflow anaerobic sludge blanket (UASB) reactors. *Renewable and Sustainable Energy Reviews*, 16(3), 1696-1708.
- Abdel-Shafy, H. I., El-Khateeb, M. A., Regelsberger, M., El-Sheikh, R., & Shehata, M., 2009. Integrated system for the treatment of blackwater and greywater via UASB and constructed wetland in Egypt. *Desalination and Water Treatment*, 8(1-3), 272-278.
- Abuzir, S., Gren, L., Domini, M., Bertanza, G., Davidsson, Å., & Kjerstadius, H. (2025). Performance of a full-scale anaerobic UASB digester treating blackwater from an urban city district in Helsingborg, Sweden. *Cleaner Water*, 4, 100082.
- Ağdağ, O. N., & Sponza, D. T. (2007). Co-digestion of mixed industrial sludge with municipal solid wastes in anaerobic simulated landfilling bioreactors. *Journal of hazardous materials*, 140(1-2), 75-85.
- Agrawal, A., Chaudhari, P. K., & Ghosh, P. (2023). Anaerobic digestion of fruit and vegetable waste: a critical review of associated challenges. *Environmental Science and Pollution Research*, 30(10), 24987-25012.
- Ait Hsine, E., Benhammou, A., & Pons, M. N. (2005). Water resources management in soft drink industry-water use and wastewater generation. *Environmental technology*, 26(12), 1309-1316.
- Akunna, J. C. (2018). *Anaerobic waste-wastewater treatment and biogas plants: a practical handbook*. CRC Press.
- Al bkoor Alrawashdeh, K., Al-Sameraie, L. A., Al Bsoul, A., Khasawneh, A., & Al-Tabbal, J. (2024). Correlation between kinetic parameters, reactor performance, and biogas and methane potential of co-digestion and mono-digestion of active sludge and olive mill wastewater. *International Journal of Low-Carbon Technologies*, 19, 1501-1515.
- Alexiou, I. E., Anderson, G. K., & Evison, L. M. (1994). Design of pre-acidification reactors for the anaerobic treatment of industrial wastewaters. *Water Science and Technology*, 29(9), 199-204.
- Algapani, D. E., Qiao, W., Su, M., di Pumpo, F., Wandera, S. M., Adani, F., & Dong, R. (2016). Bio-hydrolysis and bio-hydrogen production from food waste by thermophilic and hyperthermophilic anaerobic process. *Bioresource Technology*, 216, 768-777.
- Alkaya, E., & Demirer, G. N. (2015). Water recycling and reuse in soft drink/beverage industry: A case study for sustainable industrial water management in Turkey. *Resources, conservation and Recycling*, 104, 172-180.
- Amuda, O. S., & Amoo, I. A. (2007). Coagulation/flocculation process and sludge conditioning in beverage industrial wastewater treatment. *Journal of Hazardous Materials*, 141(3), 778-783.
- Angelidaki, I., & Sanders, W. (2004). Assessment of the anaerobic biodegradability of macropollutants. *Re/Views in Environmental Science & Bio/Technology*, 3(2), 117-129.
- Anukam, A., Mohammadi, A., Naqvi, M., & Granström, K. (2019). A review of the chemistry of anaerobic digestion: Methods of accelerating and optimizing process efficiency. *Processes*, 7(8), 504.

- APHA, AWWA, & WEF. (2005). Standard methods for the examination of water and wastewater (21st ed.). American Public Health Association.
- APHA. (1998). Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, American Water Works Association and Water Environmental.
- Appels, L., Baeyens, J., Degreè, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in energy and combustion science*, 34(6), 755-781.
- Aragón-Barroso, A. J., González-Martínez, A., González-López, J., & Osorio, F. (2026). Impact of operational conditions and microbial dynamics on expanded granular sludge bed reactor performance: A comprehensive review. *Bioresource Technology*, 134118.
- Arantes, M. K., Sequinel, R., Alves, H. J., Machado, B., Fiorini, A., & da Silva, E. A. (2020). Improvement of biohydrogen production from brewery wastewater: evaluation of inocula, support and reactor. *international journal of hydrogen energy*, 45(8), 5216-5226.
- Arif, S., Liaquat, R., & Adil, M. (2018). Applications of materials as additives in anaerobic digestion technology. *Renewable and Sustainable Energy Reviews*, 97, 354-366.
- 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.
- Austermann-Haun, U., Seyfried, C. F., & Rosenwinkel, K. H. (1997). UASB-reactor in the fruit juice industry. *Water Science and Technology*, 36(6-7), 407-414.
- Azarmanesh, R., Qaretepeh, M. Z., Zonoozi, M. H., Ghiasinejad, H., & Zhang, Y. (2023). Anaerobic co-digestion of sewage sludge with other organic wastes: a comprehensive review focusing on selection criteria, operational conditions, and microbiology. *Chemical Engineering Journal Advances*, 14, 100453.
- Azarmanesh, R., Zonoozi, M. H., & Ghiasinejad, H. (2020). Characterization of food waste and sewage sludge mesophilic anaerobic co-digestion under different mixing ratios of primary sludge, secondary sludge and food waste. *Biomass and Bioenergy*, 139, 105610.
- Azevedo, A., Lapa, N., Moldão, M., & Duarte, E. (2023). Opportunities and challenges in the anaerobic co-digestion of municipal sewage sludge and fruit and vegetable wastes: A review. *Energy Nexus*, 10, 100202.
- Baldi, F., Pecorini, I., & Iannelli, R. (2019). Comparison of single-stage and two-stage anaerobic co-digestion of food waste and activated sludge for hydrogen and methane production. *Renewable Energy*, 143, 1755-1765.
- Banu, J. R., Kavitha, S., Gunasekaran, M., & Kumar, G. (2020). Microalgae based biorefinery promoting circular bioeconomy-techno economic and life-cycle analysis. *Bioresource technology*, 302, 122822.
- Barber, W. P., & Stuckey, D. C. (1999). The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. *Water research*, 33(7), 1559-1578.
- Basinas, P., Rusín, J., & Chamrádová, K. (2021). Assessment of high-solid mesophilic and thermophilic anaerobic digestion of mechanically-separated municipal solid waste. *Environmental research*, 192, 110202.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., ... & Vavilin, V. A. (2002). The IWA anaerobic digestion model no 1 (ADM1). *Water Science and technology*, 45(10), 65-73.

Bergland, W., Haugen, F., & Bakke, R. (2010). A cost effective two stage biogas process for organic waste treatment. *Linnaeus Eco-Tech*, 540-548.

Bertanza, G., Boiocchi, R., & Pedrazzani, R. (2022). Improving the quality of wastewater treatment plant monitoring by adopting proper sampling strategies and data processing criteria. *Science of The Total Environment*, 806, 150724.

Bisschops, I., Kjerstadius, H., Meulman, B., & Van Eekert, M. (2019). Integrated nutrient recovery from source-separated domestic wastewaters for application as fertilisers. *Current Opinion in Environmental Sustainability*, 40, 7-13.

Bolzonella, D., Battistoni, P., Susini, C., & Cecchi, F. (2006). Anaerobic codigestion of waste activated sludge and OFMSW: the experiences of Viareggio and Treviso plants (Italy). *Water Science and Technology*, 53(8), 203-211.

Bong, C. P. C., Lim, L. Y., Lee, C. T., Klemeš, J. J., Ho, C. S., & Ho, W. S. (2018). The characterisation and treatment of food waste for improvement of biogas production during anaerobic digestion—A review. *Journal of cleaner production*, 172, 1545-1558.

Bonk, F., Popp, D., Weinrich, S., Sträuber, H., Kleinstüber, S., Harms, H., & Centler, F. (2018). Intermit-tent fasting for microbes: how discontinuous feeding increases functional stability in anaerobic digestion. *Biotechnology for biofuels*, 11(1), 274.

Bouallagui, H., Marouani, L., & Hamdi, M. (2010). Performances comparison between laboratory and full-scale anaerobic digesters treating a mixture of primary and waste activated sludge. *Resources, Conservation and Recycling*, 55(1), 29-33.

Braun, R., Brachtel, E., & Grasmug, M. (2003). Codigestion of proteinaceous industrial waste. *Applied bio-chemistry and biotechnology*, 109(1), 139-153.

Carvalho, M., Cassidy, J., Ribeiro, J. M., Oliveira, B. A., Freitas, E. B., Roca, C., ... & Reis, M. A. (2018). Performance of a two-stage anaerobic digestion system treating fruit pulp waste: The impact of substrate shift and operational conditions. *Waste Management*, 78, 434-445.

Catenacci, A., Boniardi, G., Mainardis, M., Gievers, F., Farru, G., Asunis, F., ... & Canziani, R. (2022). Processes, applications and legislative framework for carbonized anaerobic digestate: Opportunities and bottlenecks. A critical review. *Energy Conversion and Management*, 263, 115691.

Chen, G. H., van Loosdrecht, M. C., Ekama, G. A., & Brdjanovic, D. (Eds.). (2020). *Biological wastewater treatment: principles, modeling and design*. IWA publishing.

Chen, H., Zhang, H., Tian, J., Shi, J., Linhardt, R. J., Ye, T. D. X., & Chen, S. (2019). Recovery of high value-added nutrients from fruit and vegetable industrial wastewater. *Comprehensive Reviews in Food Science and Food Safety*, 18(5), 1388-1402.

Chen, S., He, J., Wang, H., Dong, B., Li, N., & Dai, X. (2018). Microbial responses and metabolic pathways reveal the recovery mechanism of an anaerobic digestion system subjected to progressive inhibition by ammonia. *Chemical Engineering Journal*, 350, 312-323.

- Chen, Y., Xiao, K., Jiang, X., Shen, N., Zeng, R. J., & Zhou, Y. (2018). Long solid retention time (SRT) has minor role in promoting methane production in a 65 C single-stage anaerobic sludge digester. *Biore-source Technology*, 247, 724-729.
- Chen, Y., Zhu, R., Jiang, Q., Sun, T., Li, M., Shi, J., ... & He, Q. (2019). Effects of green waste participation on the co-digestion of residual sludge and kitchen waste: A preliminary study. *Science of the Total Environment*, 671, 838-849.
- Chong, S., Sen, T. K., Kayaalp, A., & Ang, H. M. (2012). The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment—a state-of-the-art review. *Water research*, 46(11), 3434-3470.
- Choudhury, S. P., Panda, S., Haq, I., & Kalamdhad, A. S. (2022). Enhanced methane production and hydrocarbon removal from petroleum refinery sludge after *Pseudomonas putida* pretreatment and process scale-up. *Bioresource technology*, 343, 126127.
- Chow, W. L., Chong, S., Lim, J. W., Chan, Y. J., Chong, M. F., Tiong, T. J., ... & Pan, G. T. (2020). Anaerobic co-digestion of wastewater sludge: A review of potential co-substrates and operating factors for improved methane yield. *Processes*, 8(1), 39.
- Chozhavendhan, S., Karthigadevi, G., Bharathiraja, B., Kumar, R. P., Abo, L. D., Prabhu, S. V., ... & Jayakumar, M. (2023). Current and prognostic overview on the strategic exploitation of anaerobic digestion and digestate: A review. *Environmental research*, 216, 114526.
- Chun-Te Lin, J., Liu, Y. S., & Wang, W. K. (2020). A full-scale study of high-rate anaerobic bioreactors for whiskey distillery wastewater treatment with size fractionation and metagenomic analysis of granular sludge. *Bioresource Technology*, 306, 123032.
- Collins, G., Woods, A., McHugh, S., Carton, M. W., & O'Flaherty, V. (2003). Microbial community structure and methanogenic activity during start-up of psychrophilic anaerobic digesters treating synthetic industrial wastewaters. *FEMS microbiology ecology*, 46(2), 159-170.
- Conklin, A. S., Chapman, T., Zahller, J. D., Stensel, H. D., & Ferguson, J. F. (2008). Monitoring the role of acetoclasts in anaerobic digestion: activity and capacity. *Water research*, 42(20), 4895-4904.
- Corbari, S. D., Andreani, C. L., Torres, D. G., Eng, F., & Gomes, S. D. (2019). Strategies to improve the biohydrogen production from cassava wastewater in fixed-bed reactors. *International Journal of Hydrogen Energy*, 44(32), 17214-17223.
- Cremonez, P. A., Teleken, J. G., Meier, T. R. W., & Alves, H. J. (2021). Two-Stage anaerobic digestion in agroindustrial waste treatment: A review. *Journal of Environmental Management*, 281, 111854.
- Cruz-Salomón, A., Meza-Gordillo, R., Rosales-Quintero, A., Ventura-Canseco, C., Lagunas-Rivera, S., & Carrasco-Cervantes, J. (2017). Biogas production from a native beverage vinasse using a modified UASB bioreactor. *Fuel*, 198, 170-174.
- Cruz-Salomón, A., Ríos-Valdovinos, E., Pola-Albores, F., Lagunas-Rivera, S., Meza-Gordillo, R., Ruíz-Valdiviezo, V. M., & Cruz-Salomón, K. C. (2019). Expanded granular sludge bed bioreactor in wastewater treatment. *Global Journal of Environmental Science and Management*, 5(1), 119-138.
- Cuff, G., Turcios, A. E., Mohammad-Pajooh, E., Kujawski, O., Weichgrebe, D., & Rosenwinkel, K. H. (2018). High-rate anaerobic treatment of wastewater from soft drink industry: methods, performance and experiences. *Journal of environmental management*, 220, 8-15.

- da Silva, A. N., Macêdo, W. V., Sakamoto, I. K., Pereyra, D. D. L. A. D., Mendes, C. O., Maintinguer, S. I., ... & de Amorim, E. L. C. (2019). Biohydrogen production from dairy industry wastewater in an anaerobic fluidized-bed reactor. *Biomass and Bioenergy*, 120, 257-264.
- Damasceno, L. H., Rodrigues, J. A., Ratusznei, S. M., Zaiat, M., & Foresti, E. (2007). Effects of feeding time and organic loading in an anaerobic sequencing batch biofilm reactor (ASBBR) treating diluted whey. *Journal of Environmental Management*, 85(4), 927-935.
- De Graaff, M. S., Temmink, H., Zeeman, G., & Buisman, C. J. (2010). Anaerobic treatment of concentrated black water in a UASB reactor at a short HRT. *Water*, 2(1), 101-119.
- de Lemos Chernicharo, C. A. (2007). *Anaerobic reactors*. IWA publishing.
- De Prá, M. C., Anschau, A., Busso, C., Gabiatti, N., & Bortoli, M. (2019). Effect of short-chain fatty acid production on biogas generation. In *Improving Biogas Production: Technological Challenges, Alternative Sources, Future Developments* (pp. 199-216). Cham: Springer International Publishing.
- Delzeit, R., & Kellner, U. (2013). The impact of plant size and location on profitability of biogas plants in Germany under consideration of processing digestates. *Biomass and Bioenergy*, 52, 43-53.
- Demirel, B., & Yenigün, O. (2002). Two-phase anaerobic digestion processes: a review.
- Desmidt, E., Ghyselbrecht, K., Zhang, Y., Pinoy, L., Van der Bruggen, B., Verstraete, W., ... & Meesschaert, B. (2015). Global phosphorus scarcity and full-scale P-recovery techniques: a review. *Critical Reviews in Environmental Science and Technology*, 45(4), 336-384.
- Dhaked, R. K., Singh, P., & Singh, L. (2010). Biomethanation under psychrophilic conditions. *Waste Management*, 30(12), 2490-2496.
- Di Maria, F., Sordi, A., Cirulli, G., Gigliotti, G., Massaccesi, L., & Cucina, M. (2014). Co-treatment of fruit and vegetable waste in sludge digesters. An analysis of the relationship among bio-methane generation, process stability and digestate phytotoxicity. *Waste management*, 34(9), 1603-1608.
- Divya, D., Gopinath, L. R., & Christy, P. M. (2015). A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. *Renewable and sustainable energy reviews*, 42, 690-699.
- Dold P L, Ekama G A & Marais G V R, A General model for the activated sludge process, *Wat Technol*, 12 (1980) 47-77.
- Dold, P. L., & Ekama, G. A. (1981). A general model for the activated sludge process. In *Water pollution research and development* (pp. 47-77). Pergamon.
- Dold, P. L., Sam-Soon, A., Palmer, I. H., & Marais, G. V. R. (1987). Anaerobic treatment of an apple processing wastewater. *Water Science and Technology*, 19(1-2), 237-247.
- Donoso-Bravo, A., Retamal, C., Carballa, M., Ruiz-Filippi, G., & Chamy, R. (2009). Influence of temperature on the hydrolysis, acidogenesis and methanogenesis in mesophilic anaerobic digestion: parameter identification and modeling application. *Water science and technology*, 60(1), 9-17.
- Ebrahimi, S. R., Rönnqvist, M., Ouhimmou, M., & Stuart, P. (2025). A systematic literature review of the logistics planning for sustainable bioenergy based on Forestry, Agricultural, and municipal solid waste value chains. *Energy Conversion and Management: X*, 27, 101105.

- El-Kamah, H., Tawfik, A., Mahmoud, M., & Abdel-Halim, H. (2010). Treatment of high strength wastewater from fruit juice industry using integrated anaerobic/aerobic system. *Desalination*, 253(1-3), 158-163.
- El-Khateeb, M., Hassan, G. K., El-Liethy, M. A., El-Khatib, K. M., Abdel-Shafy, H. I., Hu, A., & Gad, M. (2023). Sustainable municipal wastewater treatment using an innovative integrated compact unit: Microbial communities, parasite removal, and techno-economic analysis. *Annals of Microbiology*, 73(1), 35.
- Elsayed, A., Kakar, F. L., Abdelrahman, A. M., Ahmed, N., Alsayed, A., Zaghloul, M. S., ... & Elbeshbishy, E. (2024). Enhancing anaerobic digestion Efficiency: A comprehensive review on innovative intensification technologies. *Energy Conversion and Management*, 320, 118979.
- Elsayed, M. A., Hassany, R., & Soliman, M. F. (2020). Methane production from anaerobic co-digestion of primary sludge and crop residues under mesophilic condition. *Aswan University Journal of Environmental Studies*, 1(1), 97-105.
- Elsayed, M., Andres, Y., Blel, W., Gad, A., & Ahmed, A. (2016). Effect of VS organic loads and buckwheat husk on methane production by anaerobic co-digestion of primary sludge and wheat straw. *Energy Conversion and Management*, 117, 538-547.
- Elsayed, M., Andres, Y., Blel, W., Hassan, R., & Ahmed, A. (2019). Effect of inoculum VS, organic loads and I/S on the biochemical methane potential of sludge, buckwheat husk and straw. *Desalination and Water Treatment*, 157, 69-78.
- Elsayed, M., Diab, A., & Soliman, M. (2021). Methane production from anaerobic co-digestion of sludge with fruit and vegetable wastes: Effect of mixing ratio and inoculum type. *Biomass Conversion and Biorefinery*, 11(3), 989-998.
- Enitan, A. M., Kumari, S., Odiyo, J. O., Bux, F., & Swalaha, F. M. (2018). Principal component analysis and characterization of methane community in a full-scale bioenergy producing UASB reactor treating brewery wastewater. *Physics and Chemistry of the Earth, Parts A/B/C*, 108, 1-8.
- Esfandiar, R., Najafpour-Darzi, G., & Mohammadi, M. (2024). Enhanced volatile fatty acids production from solid-state acidogenic fermentation of food waste by optimization of substrate to inoculum ratio. *Biomass Conversion and Biorefinery*, 1-16.
- Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., & Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, 11(4), 325-341.
- Fang, Q., Ji, S., Huang, D., Huang, Z., Huang, Z., Zeng, Y., & Liu, Y. (2019). Impact of alkaline pretreatment to enhance volatile fatty acids (VFAs) production from rice husk. *Biochemistry research international*, 2019(1), 8489747.
- Feiz, R., & Kanda, W. (2024). Nordic biogas model in international contexts: Early-stage decision support for adaptation. *Waste Management & Research*, 42(10), 882-888.
- Feng, K., Wang, Q., Li, H., Zhang, Y., Deng, Z., Liu, J., & Du, X. (2020). Effect of fermentation type regulation using alkaline addition on two-phase anaerobic digestion of food waste at different organic load rates. *Renewable Energy*, 154, 385-393.
- Ferrera, E., Ruigómez, I., & Vera, L. (2022). Preliminary study of Up-Flow Anaerobic Sludge Blanket (UASB) technology for energy recovery from domestic wastewater. *Renew Energy Power Qual J*, 20, 542-547.

- Filer, J., Ding, H. H., & Chang, S. (2019). Biochemical methane potential (BMP) assay method for anaerobic digestion research. *Water*, 11(5), 921.
- Florentino, A. P., Sharaf, A., Zhang, L., & Liu, Y. (2019). Overcoming ammonia inhibition in anaerobic blackwater treatment with granular activated carbon: the role of electroactive microorganisms. *Environmental Science: Water Research & Technology*, 5(2), 383-396.
- Fuentes, K. L., Torres-Lozada, P., & Chaparro, T. R. (2021). Beverage wastewater treatment by anaerobic digestion in two-stages for organic matter removal and energy production. *Biomass and Bioenergy*, 154, 106260.
- Fuess, L. T., Eng, F., Bovio-Winkler, P., Etchebehere, C., Zaiat, M., & Nascimento, C. A. O. D. (2024). Methanogenic consortia from thermophilic molasses-fed structured-bed reactors: microbial characterization and responses to varying food-to-microorganism ratios. *Brazilian Journal of Chemical Engineering*, 41(3), 777-797.
- Fukuhara, S. (2011). A basis for the space of modular forms. *Acta Arithmetica*, 151, 421-427.
- Gandhi, B. P., Otite, S. V., Fofie, E. A., Lag-Brotons, A. J., Ezemonye, L. I., Semple, K. T., & Martin, A. D. (2022). Kinetic investigations into the effect of inoculum to substrate ratio on batch anaerobic digestion of simulated food waste. *Renewable Energy*, 195, 311-321.
- Gao, M., Guo, B., Li, L., & Liu, Y. (2021). Role of syntrophic acetate oxidation and hydrogenotrophic methanogenesis in co-digestion of blackwater with food waste. *Journal of cleaner production*, 283, 125393.
- Gao, M., Guo, B., Zhang, L., Zhang, Y., & Liu, Y. (2019c). Microbial community dynamics in anaerobic digesters treating conventional and vacuum toilet flushed blackwater. *Water Research*, 160, 249-258.
- Gao, M., Zhang, L., & Liu, Y. (2020). High-loading food waste and blackwater anaerobic co-digestion: Maximizing bioenergy recovery. *Chemical Engineering Journal*, 394, 124911.
- Gao, M., Zhang, L., Florentino, A. P., & Liu, Y. (2019b). Performance of anaerobic treatment of blackwater collected from different toilet flushing systems: Can we achieve both energy recovery and water conservation?. *Journal of hazardous materials*, 365, 44-52.
- Gao, M., Zhang, L., Guo, B., Zhang, Y., & Liu, Y. (2019). Enhancing biomethane recovery from source-diverted blackwater through hydrogenotrophic methanogenesis dominant pathway. *Chemical Engineering Journal*, 378, 122258.
- Gao, T., Zhang, H., Xu, X., & Teng, J. (2021). Integrating microbial electrolysis cell based on electrochemical carbon dioxide reduction into anaerobic osmosis membrane reactor for biogas upgrading. *Water research*, 190, 116679.
- Garcia-Aguirre, J., Aymerich, E., de Goñi, J. G. M., & Esteban-Gutiérrez, M. (2017). Selective VFA production potential from organic waste streams: assessing temperature and pH influence. *Bioresource technology*, 244, 1081-1088.
- García-Depraet, O., Diaz-Cruces, V. F., & León-Becerril, E. (2020). Upgrading of anaerobic digestion of tequila vinasse by using an innovative two-stage system with dominant lactate-type fermentation in acidogenesis. *Fuel*, 280, 118606.
- Ghanavati, H. (2018). Biogas production systems: Operation, process control, and troubleshooting. In *Biogas: Fundamentals, Process, and Operation* (pp. 199-219). Cham: Springer International Publishing.

- Gianico, A., Braguglia, C. M., Gallipoli, A., & Mininni, G. (2015). Innovative two-stage mesophilic/thermophilic anaerobic degradation of sonicated sludge: performances and energy balance. *Environmental Science and Pollution Research*, 22(10), 7248-7256.
- Gómez, E., Martin, J., & Michel, F. C. (2011). Effects of organic loading rate on reactor performance and archaeal community structure in mesophilic anaerobic digesters treating municipal sewage sludge. *Waste management & research*, 29(11), 1117-1123.
- Gong, H., Liu, M., Li, K., Li, C., Xu, G., & Wang, K. (2020). Optimizing dry anaerobic digestion at pilot scale for start-up strategy and long-term operation: Organic loading rate, temperature and co-digestion. *Bioresource Technology*, 316, 123828.
- Gonzalez-Gil, G., Kleerebezem, R., & Lettinga, G. (2002). Assessment of metabolic properties and kinetic parameters of methanogenic sludge by on-line methane production rate measurements. *Applied microbiology and biotechnology*, 58(2), 248-254.
- Gopinath, A., Divyapriya, G., Srivastava, V., Laiju, A. R., Nidheesh, P. V., & Kumar, M. S. (2021). Conversion of sewage sludge into biochar: A potential resource in water and wastewater treatment. *Environmental Research*, 194, 110656.
- Granatto, C. F., Grosseli, G. M., Sakamoto, I. K., Fadini, P. S., & Varesche, M. B. A. (2021). Influence of metabolic cosubstrates on methanogenic potential and degradation of triclosan and propranolol in sanitary sewage. *Environmental research*, 199, 111220.
- Gren, L. (2024). The mass balance of compounds in source-separated blackwater treatment at RecoLab, Helsingborg [Master's thesis, Luleå tekniska universitet].
- Greses, S., Tomás-Pejó, E., & González-Fernández, C. (2021). Short-chain fatty acids and hydrogen production in one single anaerobic fermentation stage using carbohydrate-rich food waste. *Journal of Cleaner Production*, 284, 124727.
- Güneş Durak, S., Acarer, S., & Türkoğlu Demirkol, G. (2023). Treatment of citrus juice process wastewater with UASB and biogas production. *Environmental Research and Technology*, 6(1), 68-77.
- Guo, W., Wu, Q., Yang, S., Luo, H., Peng, S., & Ren, N. (2014). Optimization of ultrasonic pretreatment and substrate/inoculum ratio to enhance hydrolysis and volatile fatty acid production from food waste. *Rsc Advances*, 4(95), 53321-53326.
- Hagos, K., Zong, J., Li, D., Liu, C., & Lu, X. (2017). Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renewable and sustainable energy reviews*, 76, 1485-1496.
- Hartmann, H., & Ahring, B. K. (2006). Strategies for the anaerobic digestion of the organic fraction of municipal solid waste: an overview. *Water science and technology*, 53(8), 7-22.
- He, K., Li, W., Tang, L., Li, W., Lv, S., & Xing, D. (2022). Suppressing methane production to boost high-purity hydrogen production in microbial electrolysis cells. *Environmental science & technology*, 56(17), 11931-11951.
- Hernandez Leal, L., Zeeman, G., Temmink, H., & Buisman, C. (2007). Characterisation and biological treatment of greywater. *Water Science and Technology*, 56(5), 193-200.
- Hobson, P. N., & Wheatley, A. D. (1993). *Anaerobic digestion: modern theory and practice*.

- Holl, E., Steinbrenner, J., Merkle, W., Krümpel, J., Lansing, S., Baier, U., ... & Lemmer, A. (2022). Two-stage anaerobic digestion: State of technology and perspective roles in future energy systems. *Bioresource technology*, 360, 127633.
- Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., ... & Wierinck, I. (2016). Towards a standardization of biomethane potential tests. *Water Science and Technology*, 74(11), 2515-2522.
- Huang, L., Chen, Z., Xiong, D., Wen, Q., & Ji, Y. (2018). Oriented acidification of wasted activated sludge (WAS) focused on odd-carbon volatile fatty acid (VFA): regulation strategy and microbial community dynamics. *Water Research*, 142, 256-266.
- Huang, Q., Liu, Y., & Dhar, B. R. (2023). Boosting resilience of microbial electrolysis cell-assisted anaerobic digestion of blackwater with granular activated carbon amendment. *Bioresource Technology*, 381, 129136.
- Huang, Q., Zakaria, B. S., Zhang, Y., Zhang, L., Liu, Y., & Dhar, B. R. (2021). A high-rate anaerobic bio-film reactor for biomethane recovery from source-separated blackwater at ambient temperature. *Water Environment Research*, 93(1), 61-74.
- Hussain, A., & Dubey, S. K. (2014). Specific methanogenic activity test for anaerobic treatment of phenolic wastewater. *Desalination and Water Treatment*, 52(37-39), 7015-7025.
- Hussain, A., & Dubey, S. K. (2017). Specific methanogenic activity test for anaerobic degradation of influents. *Applied water science*, 7(2), 535-542.
- Ibáñez-López, M. E., Frison, N., Bolzonella, D., & García-Morales, J. L. (2023). Enhancing anaerobic digestion with an UASB reactor of the winery wastewater for producing volatile fatty acid effluent enriched in caproic acid. *Fermentation*, 9(11), 958.
- Ilo, O. P., Simatele, M. D., Nkomo, S. P. L., Mkhize, N. M., & Prabhu, N. G. (2021). Methodological approaches to optimising anaerobic digestion of water hyacinth for energy efficiency in south africa. *Sustainability*, 13(12), 6746.
- Imoobe, T. O. T., & Koye, P. I. O. (2011). Assessment of the impact of effluent from a soft drink processing factory on the physico-chemical parameters of Eruvbi stream Benin City, Nigeria. *Bayero Journal of Pure and Applied Sciences*, 4(1), 126-134.
- Induchoodan, T. G., Haq, I., & Kalamdhad, A. S. (2022). Factors affecting anaerobic digestion for biogas production: A review. *Advanced organic waste management*, 223-233.
- Isla, M. A., Comelli, R. N., & Seluy, L. G. (2013). Wastewater from the soft drinks industry as a source for bioethanol production. *Bioresource technology*, 136, 140-147.
- Jain, S., Jain, S., Wolf, I. T., Lee, J., & Tong, Y. W. (2015). A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. *Renewable and Sustainable Energy Reviews*, 52, 142-154.
- Jawed, M., & Tare, V. (1999). Microbial composition assessment of anaerobic biomass through methanogenic activity tests. *water SA*, 25(3), 345-350.
- Jiang, J., Zhang, Y., Li, K., Wang, Q., Gong, C., & Li, M. (2013). Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate. *Bioresource technology*, 143, 525-530.

- Jijai, S., Srisuwan, G., Sompong, O., Ismail, N., & Siripatana, C. (2015). Effect of granule sizes on the performance of upflow anaerobic sludge blanket (UASB) reactors for cassava wastewater treatment. *Energy Procedia*, 79, 90-97.
- Jiraprasertwong, A., Maitriwong, K., & Chavadej, S. (2019). Production of biogas from cassava wastewater using a three-stage upflow anaerobic sludge blanket (UASB) reactor. *Renewable Energy*, 130, 191-205.
- Jo, Y., Rhee, C., Choi, H., Shin, J., Shin, S. G., & Lee, C. (2021). Long-term effectiveness of bioaugmentation with rumen culture in continuous anaerobic digestion of food and vegetable wastes under feed composition fluctuations. *Bioresource technology*, 338, 125500.
- Jönsson, H., Baky, A., Jeppsson, U., Hellström, D., & Kärrman, E. (2005). Composition of urine, faeces, greywater and biowaste for utilisation in the URWARE model. *Urban water report*.
- Joshi, P., & Visvanathan, C. (2019). Sustainable management practices of food waste in Asia: Technological and policy drivers. *Journal of environmental management*, 247, 538-550.
- Kainthola, J., Kalamdhad, A. S., & Goud, V. V. (2019). A review on enhanced biogas production from anaerobic digestion of lignocellulosic biomass by different enhancement techniques. *Process Biochemistry*, 84, 81-90.
- Kamravamanesh, D., & Kokko, M. (2024). Source separation and anaerobic co-digestion of blackwater and food waste for biogas production and nutrient recovery. *Water Science & Technology*, 90(3), 1082-1098.
- Kanchanapiya, P., Jantharadej, K., & Chavanparit, O. (2017). Performance of precast anaerobic digestion tank combined with a food waste disposer system. A case study of household food waste in Thailand. *Environment Protection Engineering*, 43(2), 93-108.
- Karki, R., Chuenchart, W., Surendra, K. C., Shrestha, S., Raskin, L., Sung, S., ... & Khanal, S. K. (2021). Anaerobic co-digestion: Current status and perspectives. *Bioresource Technology*, 330, 125001.
- Karthikeyan, O. P., & Visvanathan, C. (2013). Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: a review. *Reviews in Environmental Science and Bio/Technology*, 12(3), 257-284.
- Kaykhaii, S., Kjerstadius, H., Hedström, A., Kvarnström, E., Eshetu-Moges, M., & Herrmann, I. (2025). Treatment of digested blackwater using a submerged microfiltration membrane system or a drum filter. *Separation and Purification Technology*, 359, 130884.
- Kazimierowicz, J., Dzienis, L., Dębowski, M., & Zieliński, M. (2021). Optimisation of methane fermentation as a valorisation method for food waste products. *Biomass and Bioenergy*, 144, 105913.
- Ketheesan, B., & Stuckey, D. C. (2015). Effects of hydraulic/organic shock/transient loads in anaerobic wastewater treatment: a review. *Critical Reviews in Environmental Science and Technology*, 45(24), 2693-2727.
- Kettunen, R. H., & Rintala, J. A. (1997). The effect of low temperature (5–29 C) and adaptation on the methanogenic activity of biomass. *Applied microbiology and biotechnology*, 48(4), 570-576.
- Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste management*, 31(8), 1737-1744.

- Khan, M. S., Raza, W., Gul, H., Hussain, M., Malik, B., Azam, M., & Winter, F. (2021). A study on the reaction kinetics of anaerobic microbes using batch anaerobic sludge technique for beverage industrial wastewater. *Separations*, 8(4), 43.
- Khanal, S. K. (2008). *Anaerobic biotechnology for bioenergy production. Principles and Application* Wiley and Blackwell, 161-86.
- Khatami, K., Atasoy, M., Ludtke, M., Baresel, C., Eyice, Ö., & Cetecioglu, Z. (2021). Bioconversion of food waste to volatile fatty acids: Impact of microbial community, pH and retention time. *Chemosphere*, 275, 129981.
- Kjerstadius, H., De Vrieze, J., la Cour Jansen, J., & Davidsson, Å. (2016). Detection of acidification limit in anaerobic membrane bioreactors at ambient temperature. *Water research*, 106, 429-438.
- Kjerstadius, H., Haghighatafshar, S., & Davidsson, Å. (2015). Potential for nutrient recovery and biogas production from blackwater, food waste and greywater in urban source control systems. *Environmental technology*, 36(13), 1707-1720.
- Kjerstadius, H., Saraiva, A. B., Spångberg, J., & Davidsson, Å. (2017). Carbon footprint of urban source separation for nutrient recovery. *Journal of environmental management*, 197, 250-257.
- Kongjan, P., O-Thong, S., & Angelidaki, I. (2013). Hydrogen and methane production from desugared molasses using a two-stage thermophilic anaerobic process. *Engineering in Life Sciences*, 13(2), 118-125.
- Kujawa-Roeleveld, K., Elmitwalli, T., & Zeeman, G. (2006). Enhanced primary treatment of concentrated black water and kitchen residues within DESAR concept using two types of anaerobic digesters. *Water Science and Technology*, 53(9), 159-168.
- Kujawa-Roeleveld, K., Fernandes, T., Wiryawan, Y., Tawfik, A., Visser, M., & Zeeman, G. (2005). Performance of UASB septic tank for treatment of concentrated black water within DESAR concept. *Water Science and Technology*, 52(1-2), 307-313.
- Kujawa-Roeleveld, K., Weijma, J., & Nanninga, T. (2012). *Nieuwe Sanitatie op Wijkniveau: Resultaten en Ervaringen Demo-site Sneek en Perspectieven voor Opschaling. LeAF (Lettinga Associates Foundation): Wageningen, the Netherlands.*
- Kumar, A., & Samadder, S. R. (2020). Performance evaluation of anaerobic digestion technology for energy recovery from organic fraction of municipal solid waste: A review. *Energy*, 197, 117253.
- Laiq Ur Rehman, M., Iqbal, A., Chang, C. C., Li, W., & Ju, M. (2019). Anaerobic digestion. *Water environment research*, 91(10), 1253-1271.
- Larsson, M., Truong, X. B., Björn, A., Ejlerthsson, J., Bastviken, D., Svensson, B. H., & Karlsson, A. (2015). Anaerobic digestion of alkaline bleaching wastewater from a kraft pulp and paper mill using UASB technique. *Environmental technology*, 36(12), 1489-1498.
- Latif, M. A., Ghufuran, R., Wahid, Z. A., & Ahmad, A. (2011). Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. *Water research*, 45(16), 4683-4699.
- Lauzurique, Y., Feroso, F. G., Sánchez, N., Castillo, A., Salazar, R., García, V., & Huiliñir, C. (2022). Biogas production from winery wastewater: Effect of the substrate-inoculum ratio on fly ash addition and iron availability. *Journal of Water Process Engineering*, 47, 102826.

- Lee, E., Min, K. J., & Park, K. Y. (2025). Changes in anaerobic digestion performance and microbial community by increasing SRT through sludge recycling in food waste leachate treatment. *Scientific Reports*, 15(1), 19845.
- Lee, E., Min, K. J., Choi, H., & Park, K. Y. (2024). Impact of dewatering inorganic coagulants on anaerobic digestion treating food waste leachate. *Bioresource Technology*, 393, 130136.
- Lettinga, G., & Hulshoff Pol, L. W. (1991). UASB-process design for various types of wastewaters. *Water science and technology*, 24(8), 87-107.
- Lettinga, G., Rebac, S., & Zeeman, G. (2001). Challenge of psychrophilic anaerobic wastewater treatment. *TRENDS in Biotechnology*, 19(9), 363-370.
- Li, L., Peng, X., Wang, X., & Wu, D. (2018). Anaerobic digestion of food waste: A review focusing on process stability. *Bioresource technology*, 248, 20-28.
- Li, Y., Chen, Y., & Wu, J. (2019). Enhancement of methane production in anaerobic digestion process: A review. *Applied energy*, 240, 120-137.
- Li, Y., Chen, Z., Peng, Y., Huang, W., Liu, J., Mironov, V., & Zhang, S. (2022). Deeper insights into the effects of substrate to inoculum ratio selection on the relationship of kinetic parameters, microbial communities, and key metabolic pathways during the anaerobic digestion of food waste. *Water Research*, 217, 118440.
- Li, Y., Zhang, R., Chen, C., Liu, G., He, Y., & Liu, X. (2013). Biogas production from co-digestion of corn stover and chicken manure under anaerobic wet, hemi-solid, and solid state conditions. *Bioresource technology*, 149, 406-412.
- Lim, S. J., & Kim, T. H. (2014). Applicability and trends of anaerobic granular sludge treatment processes. *Biomass and bioenergy*, 60, 189-202.
- Lin, Y. C., Ni, C. H., Wu, C. Y., & Lin, J. C. T. (2019). A full-scale study of external circulation sludge bed (ECSB) system for anaerobic wastewater treatment in a whiskey distillery. *Environmental Science and Pollution Research*, 26(33), 34261-34276.
- Lindner, J., Zielonka, S., Oechsner, H., & Lemmer, A. (2015). Effect of different pH-values on process parameters in two-phase anaerobic digestion of high-solid substrates. *Environmental technology*, 36(2), 198-207.
- Liu, L., Xiong, R., Li, Y., Chen, L., & Han, R. (2022). Anaerobic digestion characteristics and key microorganisms associated with low-temperature rapeseed cake and sheep manure fermentation. *Archives of Microbiology*, 204(3), 188.
- Liu, T., Shen, Z., Zhang, C., Song, Y., Li, J., Yang, Z., ... & Zhou, Y. (2019). Effect of influent pH on hydrolytic acidification performance and bacterial community structure in EGSB for pretreating crotonaldehyde manufacture wastewater after ozonation. *Water Science and Technology*, 79(6), 1174-1183.
- Lu, J. Y., Wang, X. M., Liu, H. Q., Yu, H. Q., & Li, W. W. (2019). Optimizing operation of municipal wastewater treatment plants in China: The remaining barriers and future implications. *Environment International*, 129, 273-278.
- Ma, H., Guo, Y., Qin, Y., & Li, Y. Y. (2018). Nutrient recovery technologies integrated with energy recovery by waste biomass anaerobic digestion. *Bioresource technology*, 269, 520-531.

- Ma, Y., Gu, J., & Liu, Y. (2018). Evaluation of anaerobic digestion of food waste and waste activated sludge: Soluble COD versus its chemical composition. *Science of the total environment*, 643, 21-27.
- Mahat, S. B., Omar, R., Man, H. C., Idris, A. M., Kamal, S. M., Idris, A., ... & Abdullah, L. C. (2021). Performance of dynamic anaerobic membrane bioreactor (DAnMBR) with phase separation in treating high strength food processing wastewater. *Journal of Environmental Chemical Engineering*, 9(3), 105245.
- Mahmood, Z., Cheng, H., & Tian, M. (2022). A critical review on advanced anaerobic membrane bioreactors (AnMBRs) for wastewater treatment: advanced membrane materials and energy demand. *Environmental Science: Water Research & Technology*, 8(10), 2126-2144.
- Mainardis, M., & Goi, D. (2019). Pilot-UASB reactor tests for anaerobic valorisation of high-loaded liquid substrates in friulian mountain area. *Journal of Environmental Chemical Engineering*, 7(5), 103348.
- Mainardis, M., Buttazzoni, M., & Goi, D. (2020). Up-flow anaerobic sludge blanket (UASB) technology for energy recovery: a review on state-of-the-art and recent technological advances. *Bioengineering*, 7(2), 43.
- Mainardis, M., Flaibani, S., Mazzolini, F., Peressotti, A., & Goi, D. (2019). Techno-economic analysis of anaerobic digestion implementation in small Italian breweries and evaluation of biochar and granular activated carbon addition effect on methane yield. *Journal of Environmental Chemical Engineering*, 7(3), 103184.
- Manchala, K. R., Sun, Y., Zhang, D., & Wang, Z. W. (2017). Anaerobic digestion modelling. In *Advances in Bioenergy* (Vol. 2, pp. 69-141). Elsevier.
- Mao, C., Feng, Y., Wang, X., & Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and sustainable energy reviews*, 45, 540-555.
- Mari, A. G., Andreani, C. L., Tonello, T. U., Leite, L. C., Fernandes, J. R., Lopes, D. D., ... & Gomes, S. D. (2020). Biohydrogen and biomethane production from cassava wastewater in a two-stage anaerobic sequencing batch biofilm reactor. *International Journal of Hydrogen Energy*, 45(8), 5165-5174.
- Mariraj Mohan, S., & Swathi, T. (2022). A review on upflow anaerobic sludge blanket reactor: Factors affecting performance, modification of configuration and its derivatives. *Water environment research*, 94(1), e1665.
- 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.
- Mateescu, C., Băran, G., & Băbuțanu, C. A. (2008). Opportunities and barriers for development of biogas technologies in Romania. *Environmental engineering and Management journal*, 7(5), 603-607.
- Mateus, S., Carvalho, M., Cassidy, J., Freitas, E., Oehmen, A., & Reis, M. A. (2020). Two-stage anaerobic digestion system treating different seasonal fruit pulp wastes: Impact on biogas and hydrogen production and total energy recovery potential. *Biomass and Bioenergy*, 141, 105694.
- Mehariya, S., Patel, A. K., Obulisamy, P. K., Punniyakotti, E., & Wong, J. W. (2018). Co-digestion of food waste and sewage sludge for methane production: Current status and perspective. *Bioresource technology*, 265, 519-531.
- Metcalf & Eddy, Inc. (2003). *Wastewater engineering: Treatment and reuse* (4th ed.). McGraw-Hill.

- Min, K. S., Khan, A. R., Kwon, M. K., Jung, Y. J., Yun, Z., & Kiso, Y. (2005). Acidogenic fermentation of blended food-waste in combination with primary sludge for the production of volatile fatty acids. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 80(8), 909-915.
- Mishra, P., Balachandar, G., & Das, D. (2017). Improvement in biohythane production using organic solid waste and distillery effluent. *Waste Management*, 66, 70-78.
- Moestedt, J., Nordell, E., Hallin, S., & Schnürer, A. (2016). Two-stage anaerobic digestion for reduced hydrogen sulphide production. *Journal of Chemical Technology & Biotechnology*, 91(4), 1055-1062.
- Moges, M. E., Heistad, A., & Heidorn, T. (2020). Nutrient recovery from anaerobically treated blackwater and improving its effluent quality through microalgae biomass production. *Water*, 12(2), 592.
- Morgan-Sagastume, F., Niero, L., Delatolla, R., & Lagerkvist, A. (2024). Anaerobic moving-bed biofilm reactors for the treatment of wastewater: a review of applicability. *Environmental Technology Reviews*, 13(1), 421-440.
- Mu, L., Zhang, L., Zhu, K., Ma, J., Ifran, M., & Li, A. (2020). Anaerobic co-digestion of sewage sludge, food waste and yard waste: Synergistic enhancement on process stability and biogas production. *Science of the Total Environment*, 704, 135429.
- Nayono, S. E. (2010). *Anaerobic digestion of organic solid waste for energy production* (Vol. 46). KIT scientific Publishing.
- Nghiem, L. D., Koch, K., Bolzonella, D., & Drewes, J. E. (2017). Full scale co-digestion of wastewater sludge and food waste: Bottlenecks and possibilities. *Renewable and Sustainable Energy Reviews*, 72, 354-362.
- Nguyen, D., Wu, Z., Shrestha, S., Lee, P. H., Raskin, L., & Khanal, S. K. (2019). Intermittent micro-aeration: New strategy to control volatile fatty acid accumulation in high organic loading anaerobic digestion. *Water Research*, 166, 115080.
- Nguyen, V. K., Chaudhary, D. K., Dahal, R. H., Trinh, N. H., Kim, J., Chang, S. W., ... & Nguyen, D. D. (2021). Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. *Fuel*, 285, 119105.
- Odedina, M. J., Charnnok, B., Saritpongteeraka, K., & Chaiprapat, S. (2017). Effects of size and thermophilic pre-hydrolysis of banana peel during anaerobic digestion, and biomethanation potential of key tropical fruit wastes. *Waste management*, 68, 128-138.
- Oktaý, S., Iskender, G., Babuna, F. G., Kutluay, G., & Orhon, D. (2007). Improving the wastewater management for a beverage industry with in-plant control. *Desalination*, 211(1-3), 138-143.
- Ossiansson, E., Persson, F., Bengtsson, S., Cimbritz, M., & Gustavsson, D. J. (2023). Seasonal variations in acidogenic fermentation of filter primary sludge. *Water Research*, 242, 120181.
- Oteng-Peprah, M., Acheampong, M. A., & DeVries, N. K. (2018). Greywater characteristics, treatment systems, reuse strategies and user perception—a review. *Water, Air, & Soil Pollution*, 229(8), 255.
- Otterpohl, R., Albold, A., & Oldenburg, M. (1999). Source control in urban sanitation and waste management: ten systems with reuse of resources. *Water Science and Technology*, 39(5), 153-160.

Otterpohl, R., Braun, U., & Oldenburg, M. (2004). Innovative technologies for decentralised water-, wastewater and biowaste management in urban and peri-urban areas. *Water science and technology*, 48(11-12), 23-32.

Ouyang, E., Lu, Y., Ouyang, J., Wang, L., & Wang, X. (2019). Performance and dynamic characteristics of microbial communities in multi-stage anaerobic reactors treating gibberellin wastewater. *Journal of bio-science and bioengineering*, 127(3), 318-325.

Owusu-Agyeman, I., Plaza, E., & Cetecioglu, Z. (2020). Production of volatile fatty acids through co-digestion of sewage sludge and external organic waste: Effect of substrate proportions and long-term operation. *Waste Management*, 112, 30-39.

Ozbas, E. E., Tufekci, N., Yilmaz, G., & Ovez, S. (2006). Aerobic and anaerobic treatment of fruit juice industry effluents.

Pan, H., fan Hu, Y., hong Pu, W., fen Dan, J., & kuan Yang, J. (2017). CFD optimization of the baffle angle of an expanded granular sludge bed reactor. *Journal of Environmental Chemical Engineering*, 5(5), 4531–4538.

Pan, H., Feng, Q., Zhao, Y., Li, X., & Zi, H. (2023). Influence of organic loading rate on methane production from brewery wastewater in bioelectrochemical anaerobic digestion. *Fermentation*, 9(11), 932.

Pan, X., Zhao, L., Li, C., Angelidaki, I., Lv, N., Ning, J., ... & Zhu, G. (2021). Deep insights into the network of acetate metabolism in anaerobic digestion: focusing on syntrophic acetate oxidation and homoacetogenesis. *Water Research*, 190, 116774.

Papa, M., Foladori, P., Guglielmi, L., & Bertanza, G. (2017). How far are we from closing the loop of sewage resource recovery? A real picture of municipal wastewater treatment plants in Italy. *Journal of Environmental Management*, 198, 9-15.

Paranjpe, A., Saxena, S., & Jain, P. (2023). A review on performance improvement of anaerobic digestion using co-digestion of food waste and sewage sludge. *Journal of Environmental Management*, 338, 117733.

Paranjpe, A., Saxena, S., & Jain, P. (2023). Biogas yield using single and two stage anaerobic digestion: An experimental approach. *Energy for Sustainable Development*, 74, 6-19.

Parra-Orobio, B. A., Cruz-Bournazou, M. N., & Torres-Lozada, P. (2021). Single-stage and two-stage anaerobic digestion of food waste: effect of the organic loading rate on the methane production and volatile fatty acids. *Water, Air, & Soil Pollution*, 232(3), 105.

Paudel, S., Kang, Y., Yoo, Y. S., & Seo, G. T. (2017). Effect of volumetric organic loading rate (OLR) on H₂ and CH₄ production by two-stage anaerobic co-digestion of food waste and brown water. *Waste Management*, 61, 484-493.

Pearse, L. F., Hettiaratchi, J. P., & Kumar, S. (2018). Towards developing a representative biochemical methane potential (BMP) assay for landfilled municipal solid waste—A review. *Bioresource technology*, 254, 312-324.

Pellegrin, M. L., Aguinaldo, J., Arabi, S., Sadler, M. E., Min, K., Liu, M., ... & Padhye, L. P. (2013). Membrane processes. *Water Environment Research*, 85(10), 1092-1175.

- Perera, K. R. J., Ketheesan, B., Arudchelvam, Y., & Nirmalakhandan, N. (2012). Fermentative biohydrogen production II: Net energy gain from organic wastes. *international journal of hydrogen energy*, 37(1), 167-178.
- Pererva, Y., Miller, C. D., & Sims, R. C. (2020). Approaches in design of laboratory-scale UASB reactors. *Processes* 8: 734.
- Perez-Esteban, N., Vinardell, S., Vidal-Antich, C., Peña-Picola, S., Chimenos, J. M., Peces, M., ... & Astals, S. (2022). Potential of anaerobic co-fermentation in wastewater treatments plants: A review. *Science of the Total Environment*, 813, 152498.
- Pitk, P., Kaparaju, P., Palatsi, J., Affes, R., & Vilu, R. (2013). Co-digestion of sewage sludge and sterilized solid slaughterhouse waste: methane production efficiency and process limitations. *Bioresource technology*, 134, 227-232.
- Pramanik, S. K., Suja, F. B., Zain, S. M., & Pramanik, B. K. (2019). The anaerobic digestion process of biogas production from food waste: Prospects and constraints. *Bioresource Technology Reports*, 8, 100310.
- Rabii, A., Aldin, S., Dahman, Y., & Elbeshbishy, E. (2019). A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. *Energies*, 12(6), 1106.
- Rajendran, K., Mahapatra, D., Venkatraman, A. V., Muthuswamy, S., & Pugazhendhi, A. (2020). Advancing anaerobic digestion through two-stage processes: Current developments and future trends. *Renewable and Sustainable Energy Reviews*, 123, 109746.
- Ramos-Suarez, M., Zhang, Y., & Outram, V. (2021). Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste. *Reviews in Environmental Science and Bio/Technology*, 20(2), 439-478.
- Rao, P. V., & Baral, S. S. (2011). Experimental design of mixture for the anaerobic co-digestion of sewage sludge. *Chemical Engineering Journal*, 172(2-3), 977-986.
- Rasapoor, M., Young, B., Brar, R., Sarmah, A., Zhuang, W. Q., & Baroutian, S. (2020). Recognizing the challenges of anaerobic digestion: Critical steps toward improving biogas generation. *Fuel*, 261, 116497.
- Remigi, E. U., & Buckley, C. A. (2006). Co-digestion of high-strength/toxic organic effluents in anaerobic digesters and wastewater treatment works. Pretoria, South Africa: Water Research Commission.
- Rocamora, I., Wagland, S. T., Villa, R., Simpson, E. W., Fernández, O., & Bajón-Fernández, Y. (2020). Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance. *Bioresource technology*, 299, 122681.
- Rocha, D. H. D., Sakamoto, I. K., & Varesche, M. B. A. (2023). Evaluation of significant factors in hydrogen production and volatile fatty acids in co-fermentation of citrus peel waste and processing wastewater. *Fuel*, 354, 129306.
- Rocha, D. H. D., Sakamoto, I. K., & Varesche, M. B. A. (2024). Alkali-buffering effect to increase hydrogen production on co-fermentation of citrus agroindustrial waste. *International Journal of Hydrogen Energy*, 53, 364-382.

- Rodrigues, C. V., de Oliveira Filho, L. M., Camargo, F. P., de Souza Dornelles, H., Sakamoto, I. K., Maintinguer, S. I., ... & Varesche, M. B. A. (2024). New approach to agro-industrial solid and liquid waste management: Performance of an EGSB reactor at different hydraulic retention times for methane production. *Renewable Energy*, 237, 121822.
- Ruffino, B., Cerutti, A., Campo, G., Scibilia, G., Lorenzi, E., & Zanetti, M. (2020). Thermophilic vs. mesophilic anaerobic digestion of waste activated sludge: Modelling and energy balance for its applicability at a full scale WWTP. *Renewable Energy*, 156, 235-248.
- Sanchez-Ledesma, L. M., Rodríguez-Victoria, J. A., & Ramírez-Malule, H. (2024). Acidogenic Fermentation of Cassava Wastewater: Effect of the Substrate-to-Microorganism Ratio and Temperature on Volatile Fatty Acids Production. *Water*, 16(23), 3344.
- Santos, F. S., Ricci, B. C., Neta, L. S. F., & Amaral, M. C. (2017). Sugarcane vinasse treatment by two-stage anaerobic membrane bioreactor: Effect of hydraulic retention time on changes in efficiency, biogas production and membrane fouling. *Bioresource technology*, 245, 342-350.
- Saratale, R. G., Kumar, G., Banu, R., Xia, A., Periyasamy, S., & Saratale, G. D. (2018). A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. *Bioresource technology*, 262, 319-332.
- Senadheera, U. E., Abeykoon, A. M. W. D. C. B., Sewmini, P. M. N., Weerasekara, W. M. R. B., Darshani, N. P., Jayasanka, J., ... & Jayawardena, T. U. (2025). Up-Flow Anaerobic Sludge Bed Reactors for Sustainable Wastewater Management: Challenges, Innovations, and Future Directions. *Water* (20734441), 17(4).
- Seneesrisakul, K., Sutabutr, T., & Chavadej, S. (2018). The effect of temperature on the methanogenic activity in relation to micronutrient availability. *Energies*, 11(5), 1057.
- Shah, S. V., Lamba, B. Y., Tiwari, A. K., & Chen, W. H. (2024). Sustainable biogas production via anaerobic digestion with focus on CSTR technology: A review. *Journal of the Taiwan Institute of Chemical Engineers*, 162, 105575.
- Sheldon, M. S., & Erdogan, I. G. (2016). Multi-stage EGSB/MBR treatment of soft drink industry wastewater. *Chemical Engineering Journal*, 285, 368-377.
- Shelton, D. R., & Tiedje, J. M. (1984). General method for determining anaerobic biodegradation potential. *Applied and environmental microbiology*, 47(4), 850-857.
- Siddique, M. N. I., & Wahid, Z. A. (2018). Achievements and perspectives of anaerobic co-digestion: A review. *Journal of cleaner production*, 194, 359-371.
- Silva, F. C., Serafim, L. S., Nadais, H., Arroja, L., & Capela, I. (2013). Acidogenic fermentation towards valorisation of organic waste streams into volatile fatty acids. *Chemical and Biochemical Engineering Quarterly*, 27(4), 467-476.
- Silvestre, G., Rodríguez-Abalde, A., Fernández, B., Flotats, X., & Bonmatí, A. (2011). Biomass adaptation over anaerobic co-digestion of sewage sludge and trapped grease waste. *Bioresource technology*, 102(13), 6830-6836.
- Simeonov, I., Chorukova, E., & Kabaivanova, L. (2025). Two-stage anaerobic digestion for green energy production: A review. *Processes*, 13(2), 294.

- Singh, B., Szamosi, Z., & Siménfalvi, Z. (2019). State of the art on mixing in an anaerobic digester: A review. *Renewable Energy*, 141, 922-936.
- Skambraks, A. K., Kjerstadius, H., Meier, M., Davidsson, Å., Wuttke, M., & Giese, T. (2017). Source separation sewage systems as a trend in urban wastewater management: Drivers for the implementation of pilot areas in Northern Europe. *Sustainable Cities and Society*, 28, 287-296.
- Sosnowski, P., Klepacz-Smolka, A., Kaczorek, K., & Ledakowicz, S. (2008). Kinetic investigations of methane co-fermentation of sewage sludge and organic fraction of municipal solid wastes. *Bioresource technology*, 99(13), 5731-5737.
- Souto, T. F., Aquino, S. F., Silva, S. Q., & Chernicharo, C. A. (2010). Influence of incubation conditions on the specific methanogenic activity test. *Biodegradation*, 21(3), 411-424.
- Srisowmeya, G., Chakravarthy, M., & Devi, G. N. (2020). Critical considerations in two-stage anaerobic digestion of food waste—A review. *Renewable and Sustainable Energy Reviews*, 119, 109587.
- Strömberg, S., Nistor, M., & Liu, J. (2014). Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. *Waste management*, 34(11), 1939-1948.
- Suksong, W., Kongjan, P., & Sompong, O. (2015). Biohythane production from co-digestion of palm oil mill effluent with solid residues by two-stage solid state anaerobic digestion process. *Energy Procedia*, 79, 943-949.
- Świątek, M., Lewicki, A., Szymanowska, D., & Kubiak, P. (2019). The effect of introduction of chicken manure on the biodiversity and performance of an anaerobic digester. *Electronic Journal of Biotechnology*, 37, 25-33.
- Tassew, F. A., Bergland, W. H., Dinamarca, C., Kommedal, R., & Bakke, R. (2019). Granular sludge bed processes in anaerobic digestion of particle-rich substrates. *Energies*, 12(15), 2940.
- Tauseef, S. M., Abbasi, T., & Abbasi, S. A. (2013). Energy recovery from wastewaters with high-rate anaerobic digesters. *Renewable and Sustainable Energy Reviews*, 19, 704-741.
- Tawfik, A., & El-Kamah, H. (2012). Treatment of fruit-juice industry wastewater in a two-stage anaerobic hybrid (AH) reactor system followed by a sequencing batch reactor (SBR). *Environmental technology*, 33(4), 429-436.
- Tho, B. L., & Thanh, L. T. (2025). A critical review on factors affecting the two-stage anaerobic digestion of biodegradable solid waste. *Vietnam Journal of Science and Technology*, 63(2), 226-248.
- Tian, L., Pan, L., & Wang, L. (2024). Effect of inoculum pretreatment and substrate/inoculum ratio on acidogenic fermentation of chemically enhanced primary treatment sludge. *Sustainability*, 16(8), 3347.
- Tyagi, V. K., Liu, J., Poh, L. S., & Ng, W. J. (2019). Anaerobic–aerobic system for beverage effluent treatment: performance evaluation and microbial community dynamics. *Bioresource Technology Reports*, 7, 100309.
- Uddin, M. M., & Wright, M. M. (2023). Anaerobic digestion fundamentals, challenges, and technological advances. *Physical Sciences Reviews*, 8(9), 2819-2837.
- Ueno, Y., & Fujii, M. (2001). Three years experience of operating and selling recovered struvite from full-scale plant. *Environmental Technology*, 22(11), 1373-1381.

- Valta, K., Kosanovic, T., Malamis, D., Moustakas, K., & Loizidou, M. (2015). Overview of water usage and wastewater management in the food and beverage industry. *Desalination and Water Treatment*, 53(12), 3335-3347.
- van Lier, J. B., & Huibers, F. P. (2010). From unplanned to planned agricultural use: Making an asset out of wastewater. *Irrigation and drainage systems*, 24(1), 143-152.
- van Niel, E. W., Claassen, P. A., & Stams, A. J. (2003). Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnology and bioengineering*, 81(3), 255-262.
- Van, D. P., Fujiwara, T., Tho, B. L., Toan, P. P. S., & Minh, G. H. (2020). A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. *Environmental Engineering Research*, 25(1), 1-17.
- Vang, T. T. T., Nguyen, D. P., Le, T. L., Le, L. N. T., Pham, A. T. N., Do Le, T. Q., ... & Pham, T. T. B. (2023). Enrichment of anammox sludge by using an Internal Circulation Reactor inoculated with anaerobic granular sludge and old landfill leachate. *Science & Technology Development Journal: Science of the Earth & Environment*, 7(1), 683-690.
- Vanwonterghem, I., Jensen, P. D., Rabaey, K., & Tyson, G. W. (2015). Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. *Scientific reports*, 5(1), 8496.
- Vassalle, L., Díez-Montero, R., Machado, A. T. R., Moreira, C., Ferrer, I., Mota, C. R., & Passos, F. (2020). Upflow anaerobic sludge blanket in microalgae-based sewage treatment: co-digestion for improving biogas production. *Bioresource Technology*, 300, 122677.
- Venkiteshwaran, K., Bocher, B., Maki, J., & Zitomer, D. (2015). Relating anaerobic digestion microbial community and process function: supplementary issue: water microbiology. *Microbiology insights*, 8, MBI-S33593.
- Vergine, P., Sousa, F., Lopes, M., Silva, F., Gameiro, T., Nadais, H., & Capela, I. (2015). Synthetic soft drink wastewater suitability for the production of volatile fatty acids. *Process Biochemistry*, 50(8), 1308-1312.
- Wang, H., Li, Z., Zhou, X., Wang, X., & Zuo, S. (2020). Anaerobic co-digestion of kitchen waste and blackwater for different practical application scenarios in decentralized scale: from wastes to energy recovery. *Water*, 12(9), 2556.
- Wang, Q. Y., Tian, J., Kato, M. T., Rong, Y. J., He, Y. L., & Ji, F. (2018, September). Anaerobic co-digestion of wastes from fruit processing and activated sludge reactor in juice production industry. In *IOP Conference Series: Earth and Environmental Science* (Vol. 186, No. 3, p. 012042). IOP Publishing.
- Wang, Q., Liang, Y., Zhao, P., Li, Q. X., Guo, S., & Chen, C. (2016). Potential and optimization of two-phase anaerobic digestion of oil refinery waste activated sludge and microbial community study. *Scientific Reports*, 6(1), 38245.
- Wang, S., An, Z., & Wang, Z. W. (2020). Bioconversion of methane to chemicals and fuels by methane-oxidizing bacteria. In *Advances in bioenergy* (Vol. 5, pp. 169-247). Elsevier.

- Wang, T., Huang, Z., Ruan, W., Zhao, M., Shao, Y., & Miao, H. (2018). Insights into sludge granulation during anaerobic treatment of high-strength leachate via a full-scale IC reactor with external circulation system. *Journal of environmental sciences*, 64, 227-234.
- Wang, X., Chen, J., Li, Z., Cheng, S., Mang, H. P., Zheng, L., ... & Harada, H. (2023). Nutrient recovery technologies for management of blackwater: A review. *Frontiers in Environmental Science*, 10, 1080536.
- Wang, Z., Yin, Q., & Wu, G. (2024). Ammonia-induced inhibition and its alleviation strategies in anaerobic digestion ecosystems. In *Anaerobic Digestion: Fundamentals, Modelling, and Applications* (pp. 97-116). Cham: Springer Nature Switzerland.
- Weiland, P. (2006). State of the art of solid-state digestion—recent developments. *Solid-State Digestion—State of the Art and Further R&D Requirements*, Gulzower Fachgespräche, 24, 22-38.
- Wen, C., Dai, Z., Cheng, F., Cheng, H., Yang, Z., Cai, Q., ... & Lu, X. (2024). Review on research achievements of blackwater anaerobic digestion for enhanced resource recovery: C. Wen et al. *Environment, Development and Sustainability*, 26(1), 1-31.
- Wendland, C., Deegener, S., Behrendt, J., Toshev, P., & Otterpohl, R. (2007). Anaerobic digestion of blackwater from vacuum toilets and kitchen refuse in a continuous stirred tank reactor (CSTR). *Water science and technology*, 55(7), 187-194.
- Wickham, R., Xie, S., Galway, B., Bustamante, H., & Nghiem, L. D. (2019). Pilot-scale operation experience of anaerobic Co-digestion for possible full scale implementation. *International Biodeterioration & Biodegradation*, 142, 137-142.
- Wu, J., Jiang, B., Feng, B., Li, L., Moideen, S. N. F., Chen, H., ... & Li, Y. Y. (2020). Pre-acidification greatly improved granules physicochemical properties and operational stability of Upflow anaerobic sludge Blanket (UASB) reactor treating low-strength starch wastewater. *Bioresource Technology*, 302, 122810.
- Xie, S., Higgins, M. J., Bustamante, H., Galway, B., & Nghiem, L. D. (2018). Current status and perspectives on anaerobic co-digestion and associated downstream processes. *Environmental Science: Water Research & Technology*, 4(11), 1759-1770.
- Xing, X., Wang, X. T., Zhao, L., Wang, W., Xing, D., Ren, N., ... & Chen, C. (2024). Anaerobic treatment of fruit and vegetable wastewater using EGSB: From strategies for regulating over-acidification to microbial community. *Renewable Energy*, 230, 120882.
- Xu, J., Yang, L., & Zhou, X. (2023). A systematical review of blackwater treatment and resource recovery: Advance in technologies and applications. *Resources, Conservation and Recycling*, 197, 107066.
- Xu, S. Y., Karthikeyan, O. P., Selvam, A., & Wong, J. W. (2012). Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor. *Bioresource technology*, 126, 425-430.
- Yan, H. H., Han, L., Yin, Q., Guo, X. Y., & Nian, Y. G. (2021, May). Corn starch processing wastewater treated by a full-scale expanded granular sludge bed reactor and comprehensive analysis of microbial community at low and high organic loading rate. In *IOP Conference Series: Earth and Environmental Science* (Vol. 776, No. 1, p. 012003). IOP Publishing.
- Yu, P., Tu, W., Wu, M., Zhang, Z., & Wang, H. (2021). Pilot-scale fermentation of urban food waste for volatile fatty acids production: The importance of pH. *Bioresource Technology*, 332, 125116.

Zahan, Z., Georgiou, S., Muster, T. H., & Othman, M. Z. (2018). Semi-continuous anaerobic co-digestion of chicken litter with agricultural and food wastes: a case study on the effect of carbon/nitrogen ratio, substrates mixing ratio and organic loading. *Bioresource technology*, 270, 245-254.

Zaher, U., Cheong, D. Y., Wu, B., & Chen, S. (2007). Producing energy and fertilizer from organic municipal solid waste. Department of Biological Systems Engineering, Washington State University.

Zamri, M. F. M. A., Hasmady, S., Akhiar, A., Ideris, F., Shamsuddin, A. H., Mofijur, M., ... & Mahlia, T. M. I. (2021). A comprehensive review on anaerobic digestion of organic fraction of municipal solid waste. *Renewable and Sustainable Energy Reviews*, 137, 110637.

Zapata Martínez, O., Serrano, A., Aguilera Flores, M. M., & Villa-Gómez, D. K. (2019). Rabbit manure as a potential inoculum for anaerobic digestion. *Journal of Environmental Science and Health, Part A*, 54(9), 943-950.

Zerrouki, S., Rihani, R., Bentahar, F., & Belkacemi, K. (2015). Anaerobic digestion of wastewater from the fruit juice industry: experiments and modeling. *Water Science and Technology*, 72(1), 123-134.

Zerrouki, S., Rihani, R., Lekikot, K., & Ramdhane, I. (2021). Enhanced biogas production from anaerobic digestion of wastewater from the fruit juice industry by sonolysis: experiments and modelling. *Water Science and Technology*, 84(3), 644-655.

Zhang, L., Guo, B., Mou, A., Li, R., & Liu, Y. (2020). Blackwater biomethane recovery using a thermophilic upflow anaerobic sludge blanket reactor: Impacts of effluent recirculation on reactor performance. *Journal of environmental management*, 274, 111157.

Zhang, L., Guo, B., Zhang, Q., Florentino, A., Xu, R., Zhang, Y., & Liu, Y. (2019). Co-digestion of blackwater with kitchen organic waste: Effects of mixing ratios and insights into microbial community. *Journal of cleaner production*, 236, 117703.

Zhang, L., Peng, B., Wang, L., & Wang, Q. (2022). Potential of anaerobic co-digestion of acidic fruit processing waste and waste-activated sludge for biogas production. *Green Processing and Synthesis*, 11(1), 1013-1025.

Zhang, L., Zhang, Y., Yuan, Y., Mou, A., Park, S., & Liu, Y. (2022). Impacts of granular activated carbon addition on anaerobic granulation in blackwater treatment. *Environmental research*, 206, 112406.

Zhang, P., Chen, Y., & Zhou, Q. (2009). Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH. *Water research*, 43(15), 3735-3742.

Zhang, Q., Zeng, L., Fu, X., Pan, F., Shi, X., & Wang, T. (2021). Comparison of anaerobic co-digestion of pig manure and sludge at different mixing ratios at thermophilic and mesophilic temperatures. *Bioresource technology*, 337, 125425.

Zheng, X., & Li, R. (2024). Critical review on two-stage anaerobic digestion with H₂ and CH₄ production from various wastes. *Water*, 16(11), 1608.

Zhou, H., Jiang, J., Zhao, Q., Li, L., Wang, K., & Wei, L. (2022). Effects of organic loading rates on high-solids anaerobic digestion of food waste in horizontal flow reactor: Methane production, stability and mechanism. *Chemosphere*, 293, 133650.

Zhu, X., Campanaro, S., Treu, L., Seshadri, R., Ivanova, N., Kougias, P. G., ... & Angelidaki, I. (2020). Metabolic dependencies govern microbial syntrophies during methanogenesis in an anaerobic digestion ecosystem. *Microbiome*, 8(1), 22.

Zieliński, M., Kazimierowicz, J., & Dębowski, M. (2022). Advantages and limitations of anaerobic wastewater treatment—Technological basics, development directions, and technological innovations. *Energies*, 16(1), 83.



Supplementary Information

Supplementary Information

Appendix A. Supplementary material for chapter 4

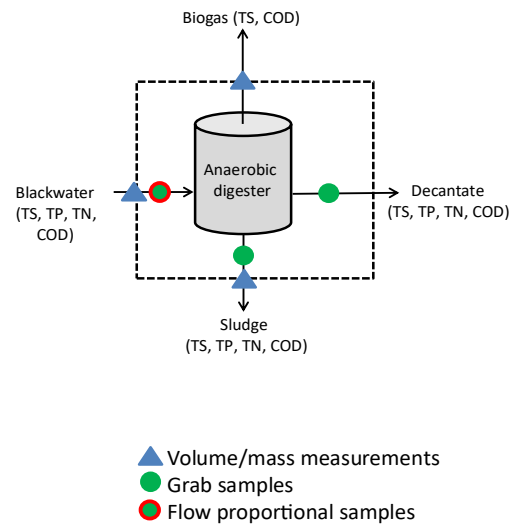


Fig. A.1. Blackwater System overview.

Table A1. Population Equivalent (PE) Estimation for RecoLab Based on Influent Loads and Flow (Using Jönsson et al., 2005; Adapted from Gren, 2024).

Parameter	Default values (Jönsson et al. 2005) [g/pe/day]	RecoLab [kg/week]	PE
COD	71	429	860
BOD7	38	247	919
TS	71	260	522
TP	1.4	7.8	802
TN	12	83	957
FLOW	5	63.5	1815
Estimated pe	-	-	885
Number of pe used for mass balance in present work	-	-	1088

Table A2. Comparison of Default Per-Capita Loads and Present Work Values for Estimating Population Equivalent (PE) and Flow per PE (Jönsson et al., 2005; Adapted from Gren, 2024)

Parameter	Default values (Jönsson et al. 2005) [g/pe/day]	Present work [g/pe/day]	PE	Flow per pe [L pe ⁻¹ d ⁻¹]
COD	72.6	552.8	1088	
BOD7				
TS				
TP	1.4	7.8	801	
TN	12.5	82.3	944	
Flow	6	63.3	1508	
Flow per pe COD				8.3
Flow per pe P				11.3
Flow per pe N				9.6

Table A3. Weekly Mass Balance of Wet Mass, COD, Nitrogen, and Phosphorus: Average Values of the Main Parameters (Standard Deviations); n = Number of Data Points Used to Calculate the Average and Standard Deviation (Adapted from Gren, 2024).

Parameter	Inflow	Biogas	Sludge	Decantate	Sum out-flows	% outflow/in-flow
Wet mass [kg week ⁻¹]	63.3 (n=50) (8.4)	-	1023 (n=26) (153)	62.3 (8.4)	53.3	100
COD [kg week ⁻¹]	553 (n=47) (97)	406 (n=40) (95)	20 (n=10) (8)	166 (n=36) (80)	592	109
Nitrogen [kg week ⁻¹]	82.6 (n=90) (12.0)	-	1.7 (n=10) (0.6)	87.0 (n=32) (20)	88.7	108
Phosphorus [kg week ⁻¹]	7.85 (n=47) (1.61)	-	1.47 (n=10) (0.48)	6.80 (n=33) (1.23)	8.27	108

Table A4. Weekly Mass Balance Normalized to per-COD (72.6 gCOD pe⁻¹ d⁻¹): average values of the main parameters (Adapted from Gren, 2024).

Parameter	Inflow	Biogas	Sludge	Decantate	Sum out-flows	% outflow/in-flow
Wet mass [kg pe ⁻¹ d ⁻¹]	8.32	-	0	8	8	100
COD [g pe ⁻¹ d ⁻¹]	72.6	53	3	22	78	109
Nitrogen [g pe ⁻¹ d ⁻¹]	10.8	-	0.2	11.4	11.7	108
Phosphorus [g pe ⁻¹ d ⁻¹]	1.03	-	0.19	0.89	1.09	108

Table A5. Influent Characteristics Normalized to per-COD (72.6 g COD pe⁻¹ d⁻¹): average values of the main parameters (Adapted from Gren, 2024).

Parameter	Present work	Values in Jönsson et al. 2005
Wet mass [kg pe ⁻¹ d ⁻¹]	8.32	5
COD [g pe ⁻¹ d ⁻¹]	72.6	73
Nitrogen [g pe ⁻¹ d ⁻¹]	10.8	12.5
Phosphorus [g pe ⁻¹ d ⁻¹]	1.03	1.40

Table A6. BW UASB energy calculations: average values of the main parameters (standard deviations); n = number of data points used to calculate the average and standard deviation.

Parameter	Value
Temperature lift winter time - Feb/March [K]	19.1 (n=30) (0.7)
District heating Energy usage winter time [kWh m ⁻³]	48.6 (n=30) (6)
Temperature lift summer time - May [K]	14.2 (n=30) (1.8)
District heating Energy usage summer time [kWh m ⁻³]	30.6 (n=30) (2.0)
Biogas production [m ³ biogas m ⁻³ blackwater]	3
Energy Biomethane kWh (65% methane)	18.9
Energy ratio Biomethane kWh/district heating kWh – winter [KWh_methane kWh ⁻¹ _heat]	0.39
Energy ratio Biomethane kWh/district heating kWh – summer [KWh_methane kWh ⁻¹ _heat]	0.62
Primary energy factor district heating [-]	0.07
Primary energy factor biomethane [-]	1
Primary Energy ratio Biomethane kWh/district heating kWh – winter [KWh_methane kWh ⁻¹ _heat]	5.56
Primary Energy ratio Biomethane kWh/district heating kWh – summer [KWh_methane kWh ⁻¹ _heat]	8.84

Table A7. Operational parameters applied to UASB systems treating BW: average values of the main parameters (standard deviations).

Parameter	Unit	Full-scale UASB _{BW} (this study)		Full-scale UASB _{BW+FW} (this study)		Lab- scale UASB ^a	Lab-scale UASB septic tank ^b		Lab- scale UASB ^c	Lab-scale UASB ^d
		Phase1 (OLR<1)	Phase2 (OLR=1.5)	22.5°C	35°C		UASB _{st1}	UASB _{st2}	Phase IV	Phase 3
Operating weeks	[-]	56	74	49	33	136	52	52	~7	~4
Flow	[m ³ d ⁻¹]	5.36 (0.8)	8.9 (0.9)	3.54 (0.25)	3.54 (0.25)	0.006*	0.007*	0.007*	-	-
Reactor volume	[L]	50,000	50,000	41,000	41,000	50	200	200	5	5
Temperature	[°C]	35 (0.03)	35 (0.03)	22.5 (0.4)	34.7 (0.5)	25	15	25	35	35
HRT	[d]	9.5 (1.4)	5.7 (1.0)	11.55 (0.68)	11.55 (0.68)	8.7 (1.0)	27-29	27-29	6.28	12
SRT	[d]	75 (15)	56 (26)	-	-	254	>365	>365	-	-
OLR	[kgCOD m ⁻³ d ⁻¹]	0.95 (0.2)	1.5 (0.45)	0.8 (0.2)	0.8 (0.2)	1.0	0.3-0.4	0.3-0.4	1.64	0.8 (0.07)

Appendix B. Supplementary material for Chapter 5

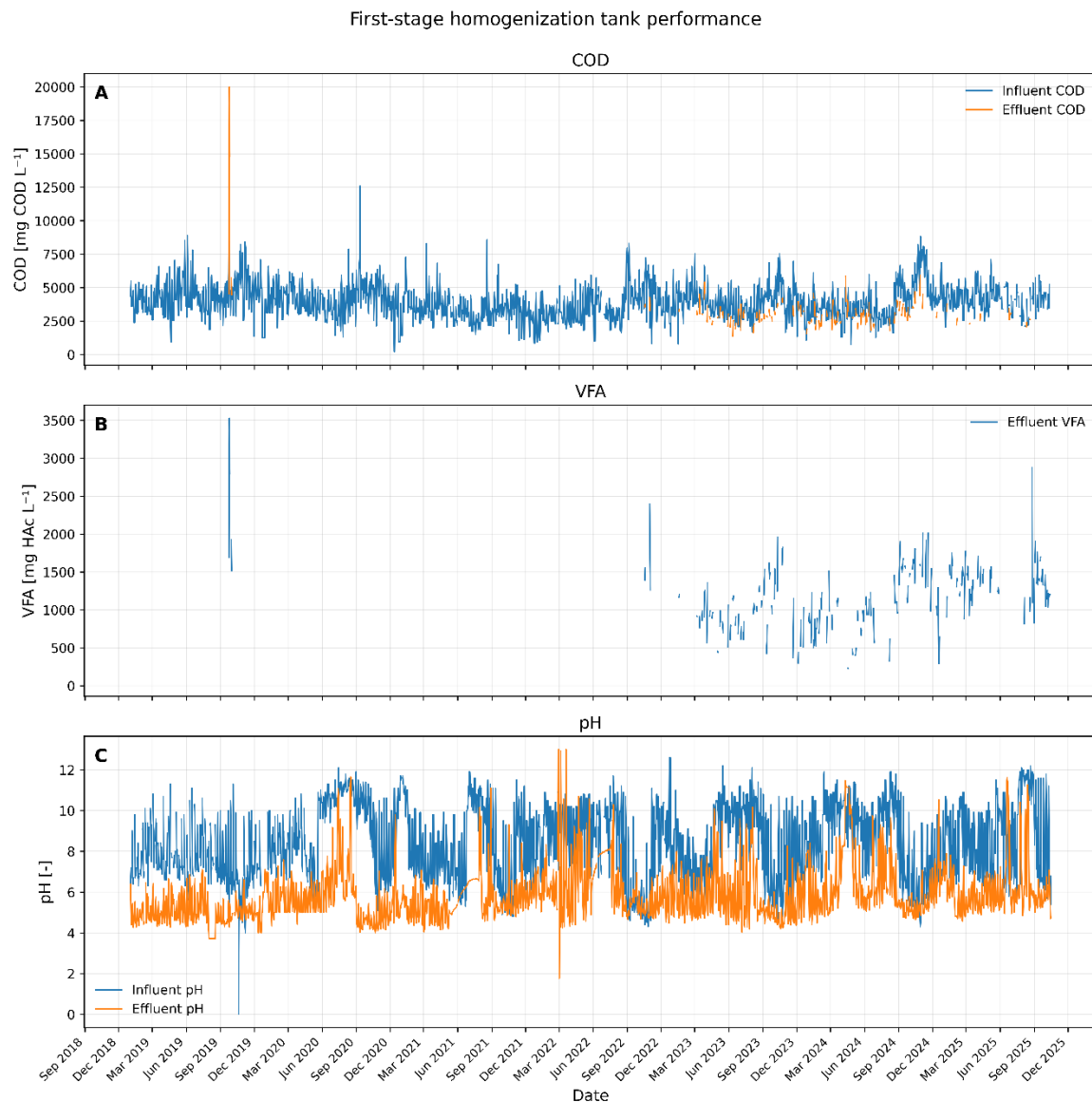


Fig. B.1. Temporal dynamics of COD, VFA, and pH in the first-stage homogenization tank of the two-stage anaerobic digestion plant. A) Influent and effluent COD concentrations. B) Effluent VFA concentrations expressed as HAC. C) Influent and effluent pH.

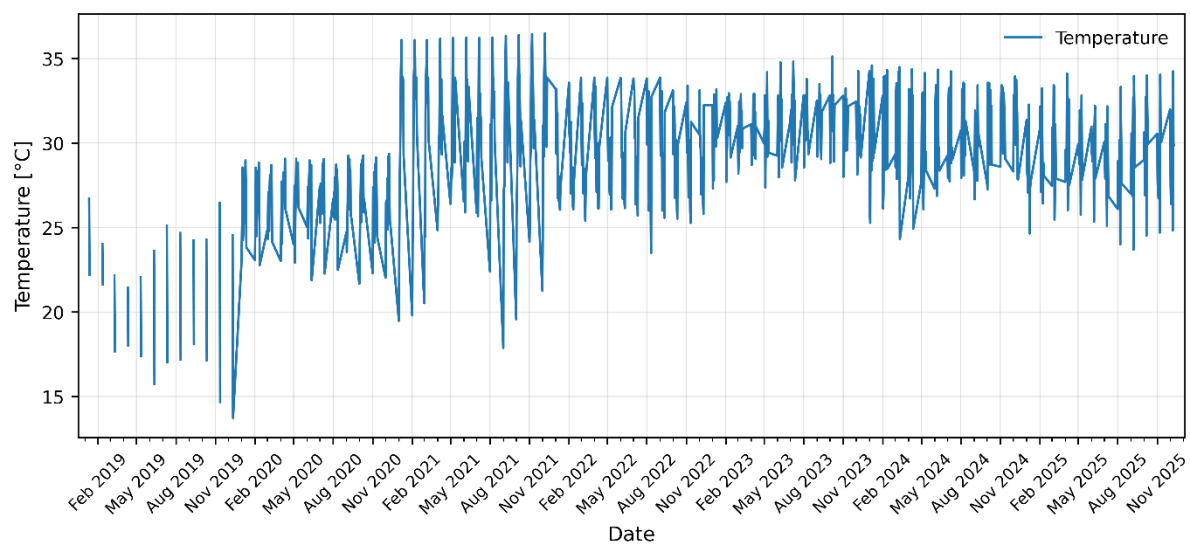


Fig. B.2. Temporal variation in the temperature of the R2S reactors.

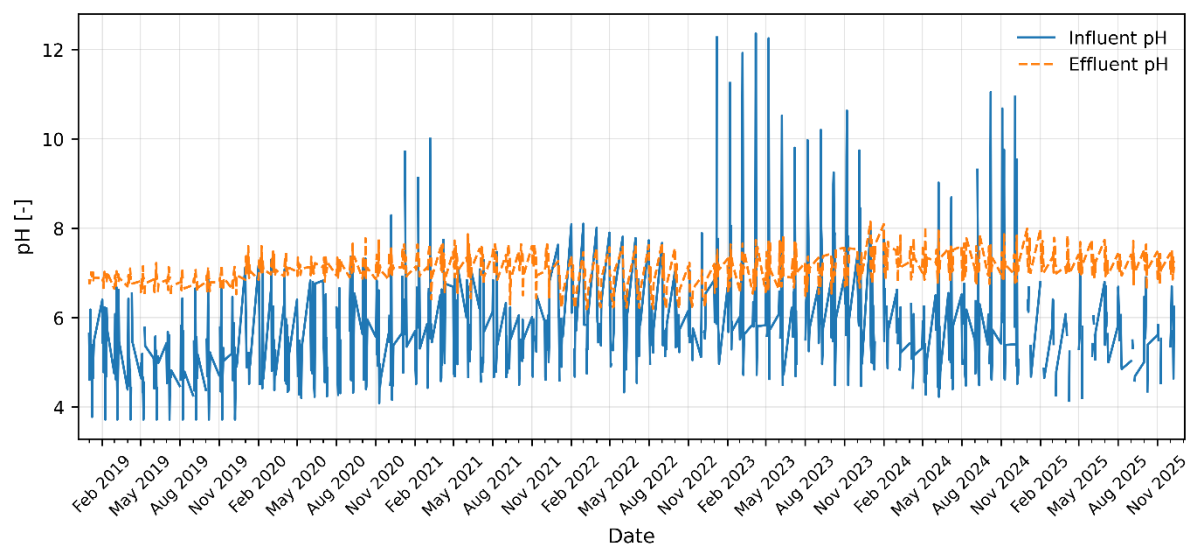


Fig. B.3. Temporal variation in influent and effluent pH in the R2S reactors.

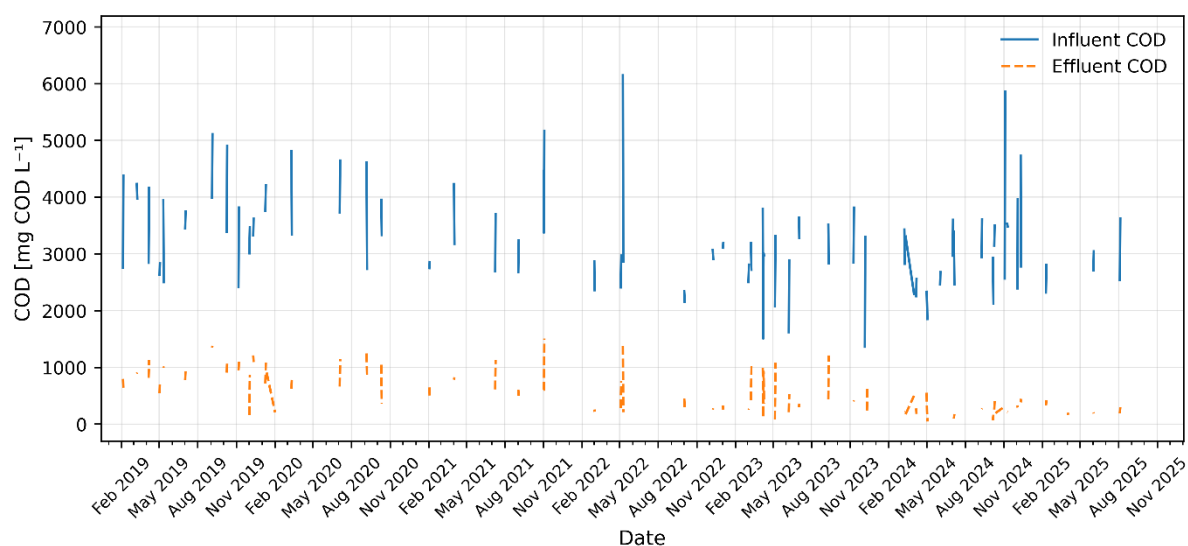


Fig. B.4. Temporal variation in influent and effluent COD concentrations in the R2S reactors.

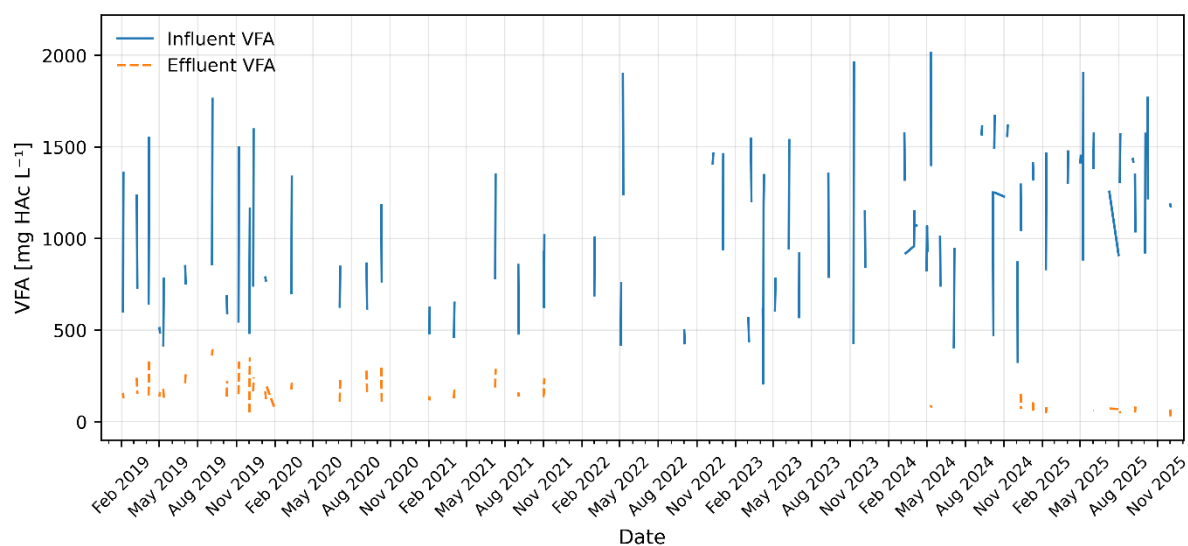


Fig. B.5. Temporal variation in influent and effluent COD concentrations in the R2S reactors.

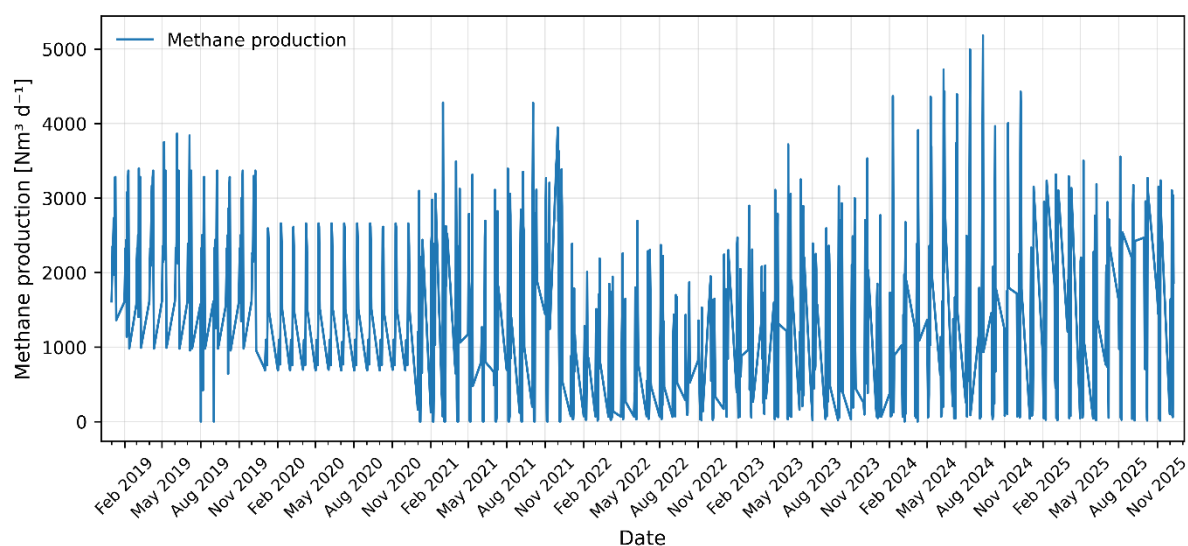


Fig. B.6. Temporal variation in daily methane production from the R2S reactors.

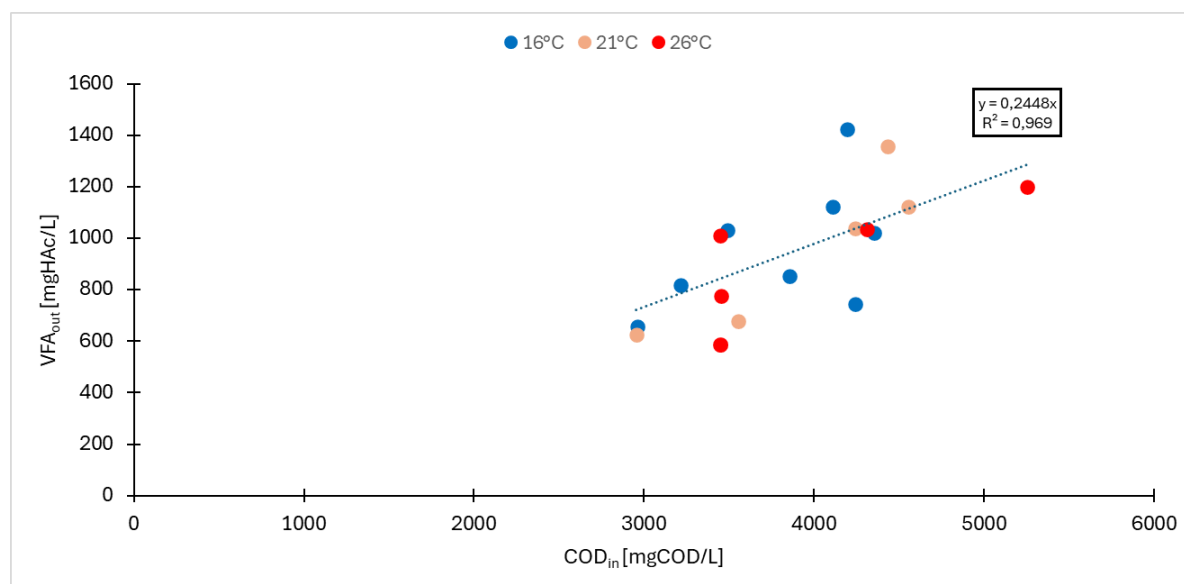


Fig. B.7. Relationship between influent total COD and VFA production in the homogenization tank under different operating temperatures.

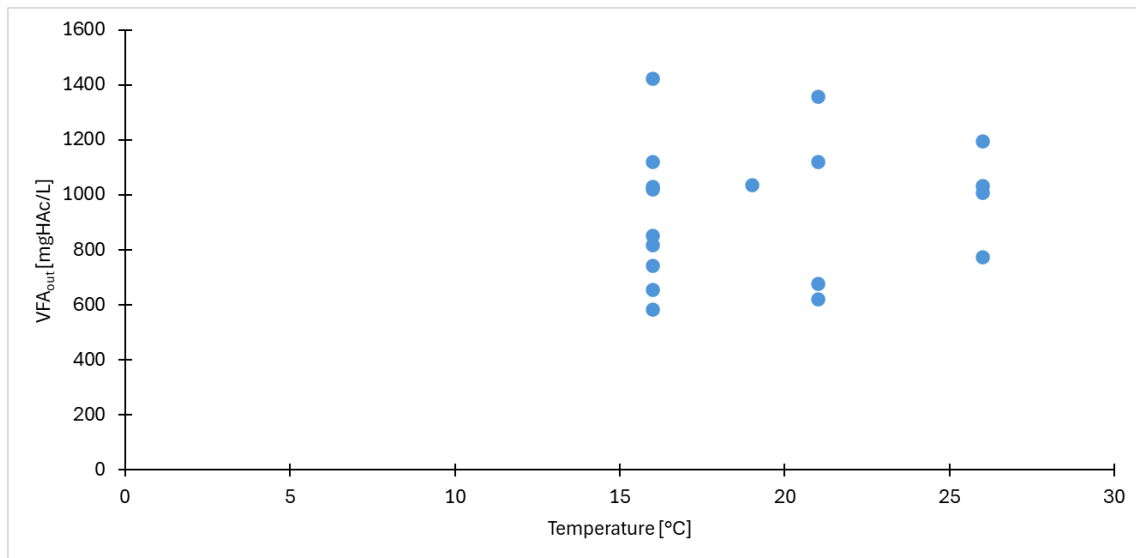


Fig. B.8. Relationship between temperature and VFA production in the homogenization tank.

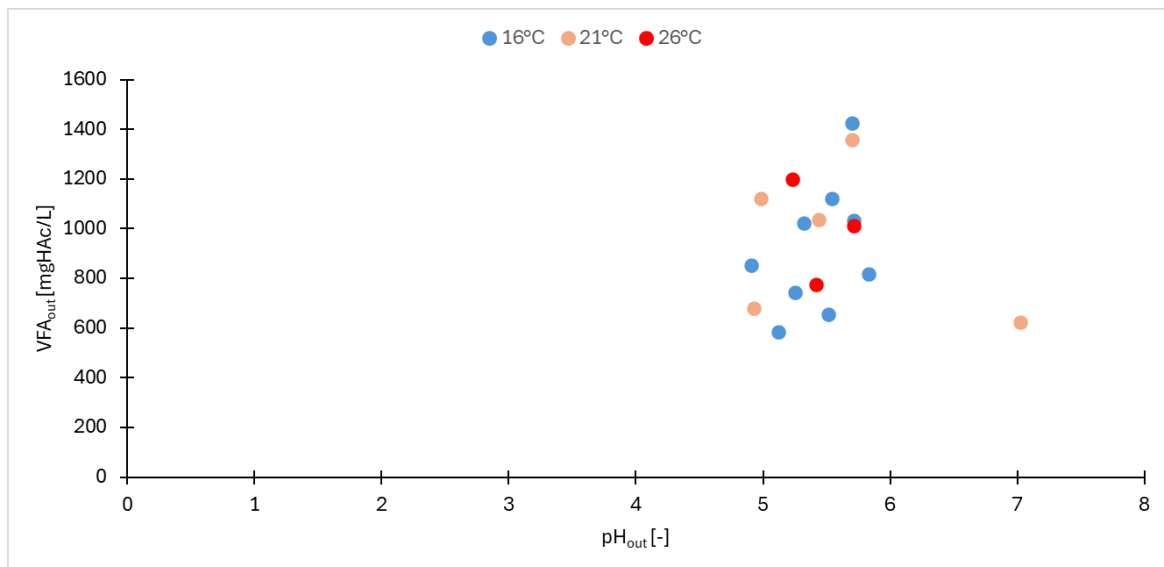


Fig. B.9. Relationship between pH and VFA production in the homogenization tank under different operating temperatures.

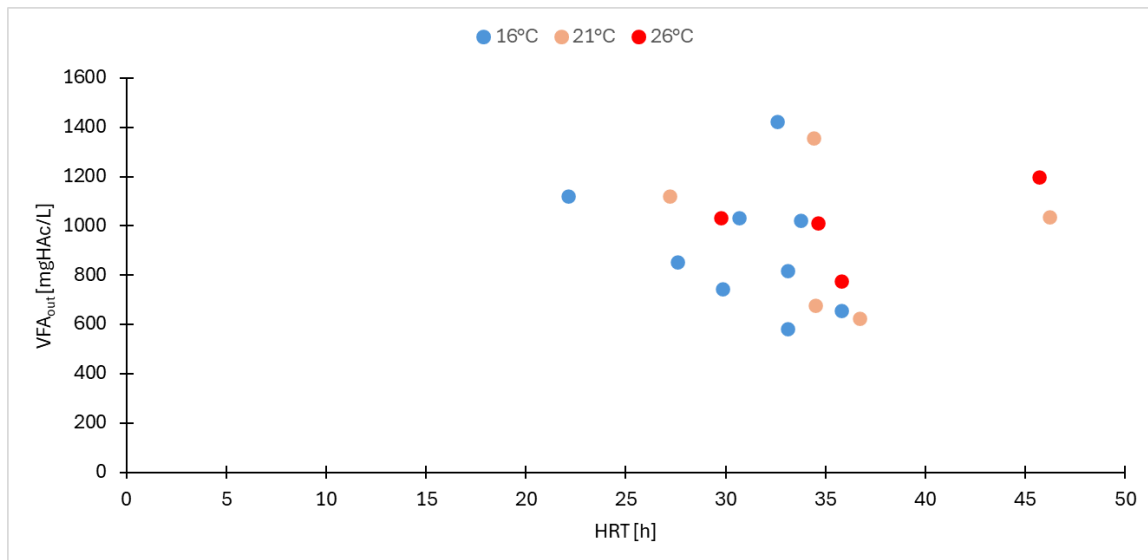


Fig. B.10. Relationship between hydraulic retention time (HRT) and VFA production in the homogenization tank under different operating temperatures.

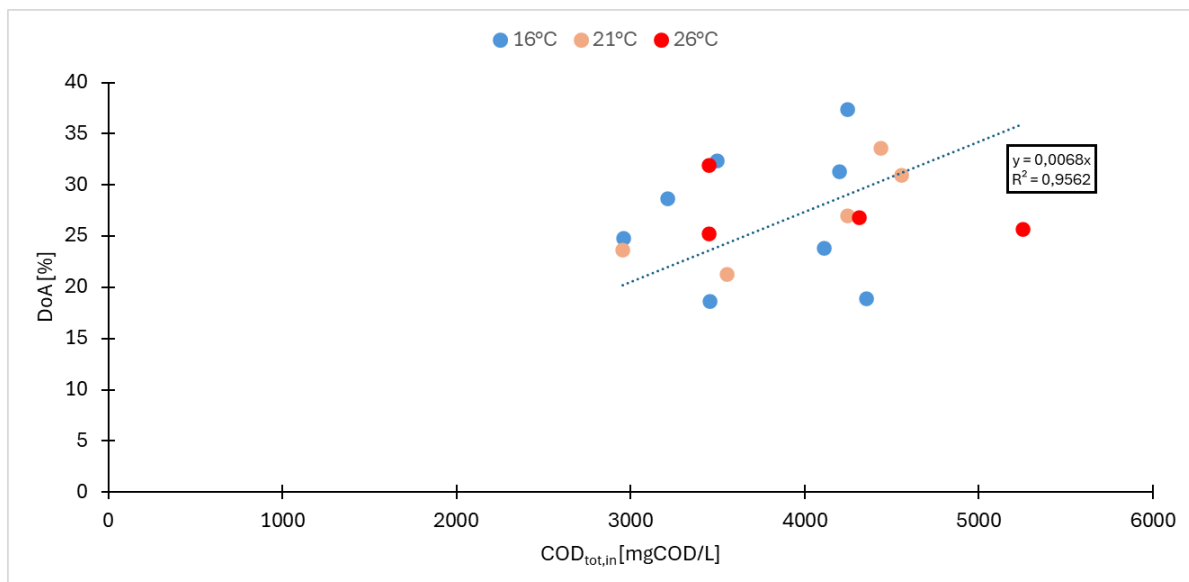


Fig. B.11. Relationship between influent total COD and degree of acidification (DoA) in the homogenization tank under different operating temperatures.

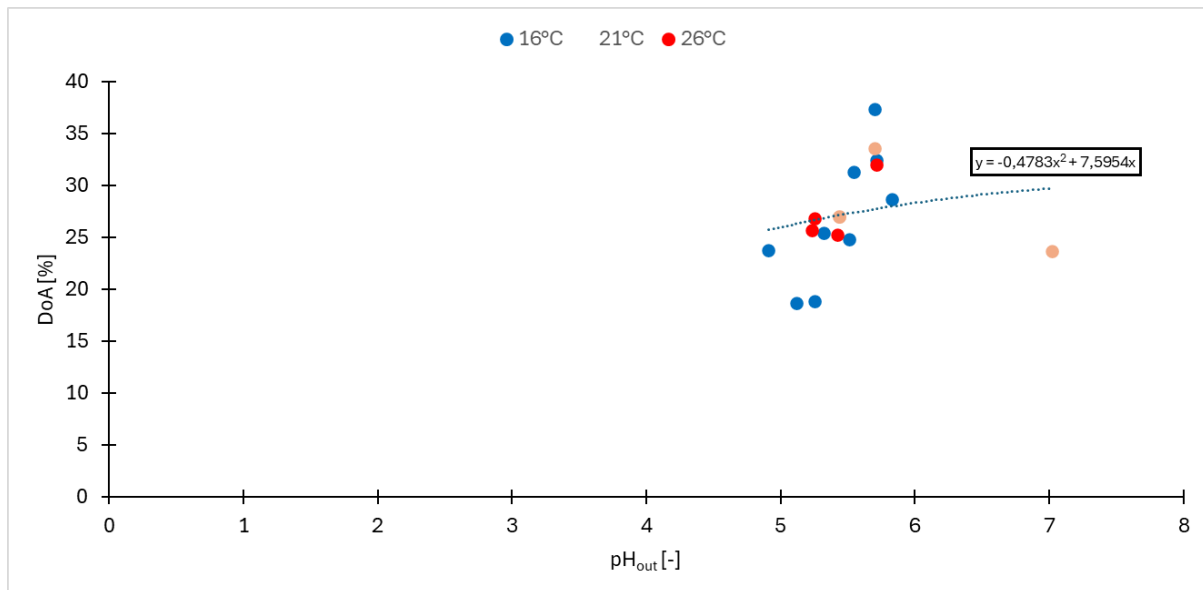


Fig. B.12. Relationship between pH and degree of acidification (DoA) in the homogenization tank under different operating temperatures.

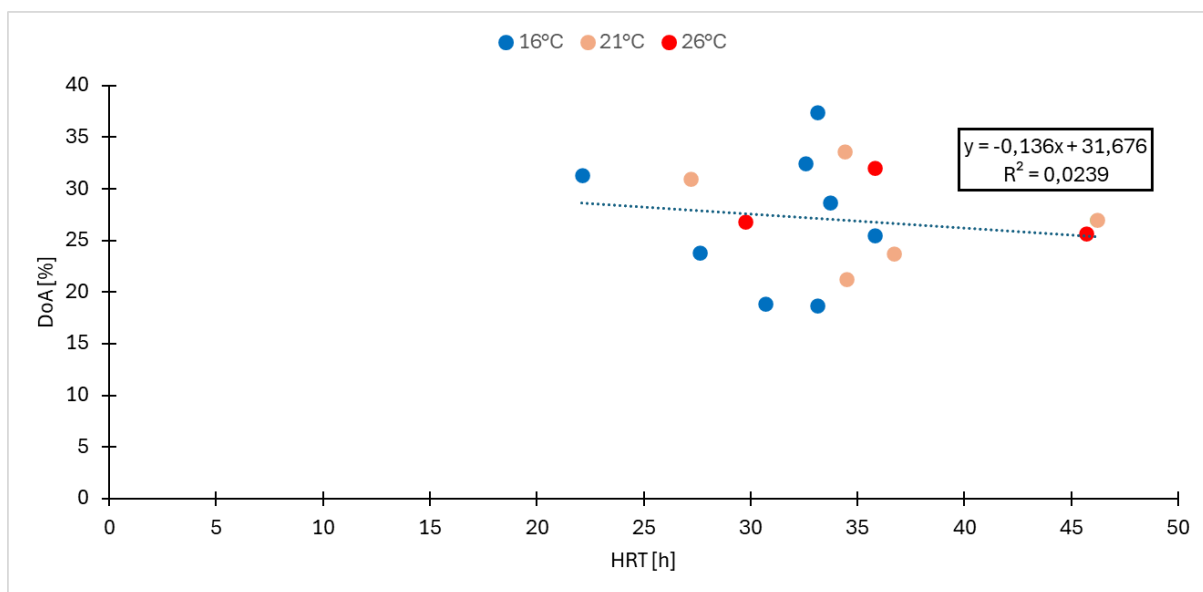


Fig. B.13. Relationship between HRT and degree of acidification (DoA) in the homogenization tank under different operating temperatures.

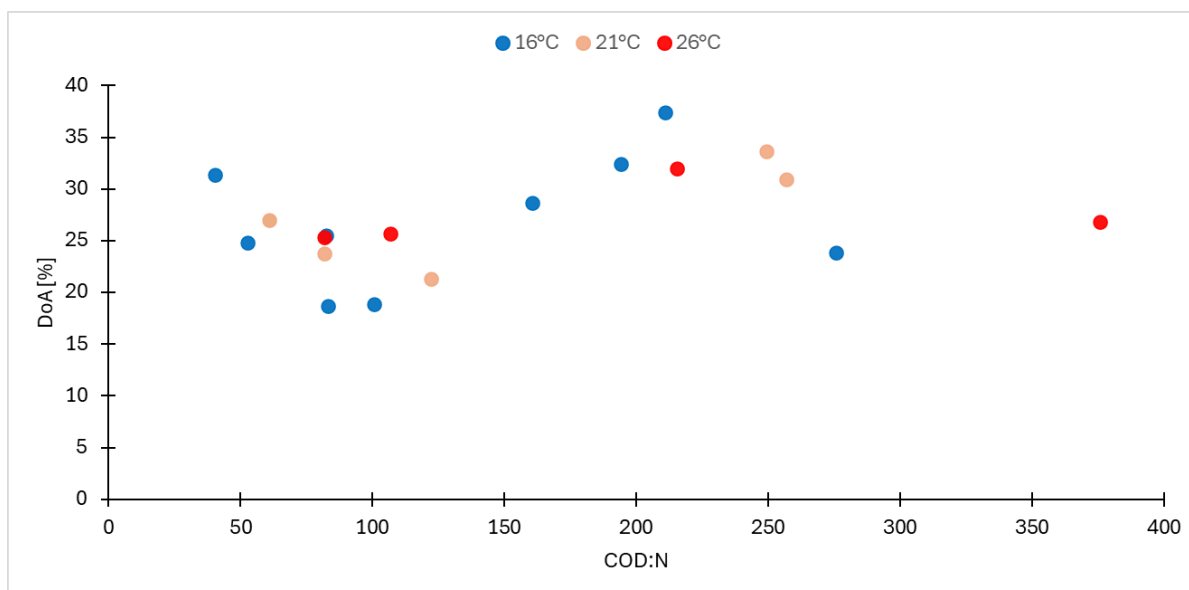


Fig. B.14. Relationship between COD:N ratio and degree of acidification (DoA) in the homogenization tank under different operating temperatures.

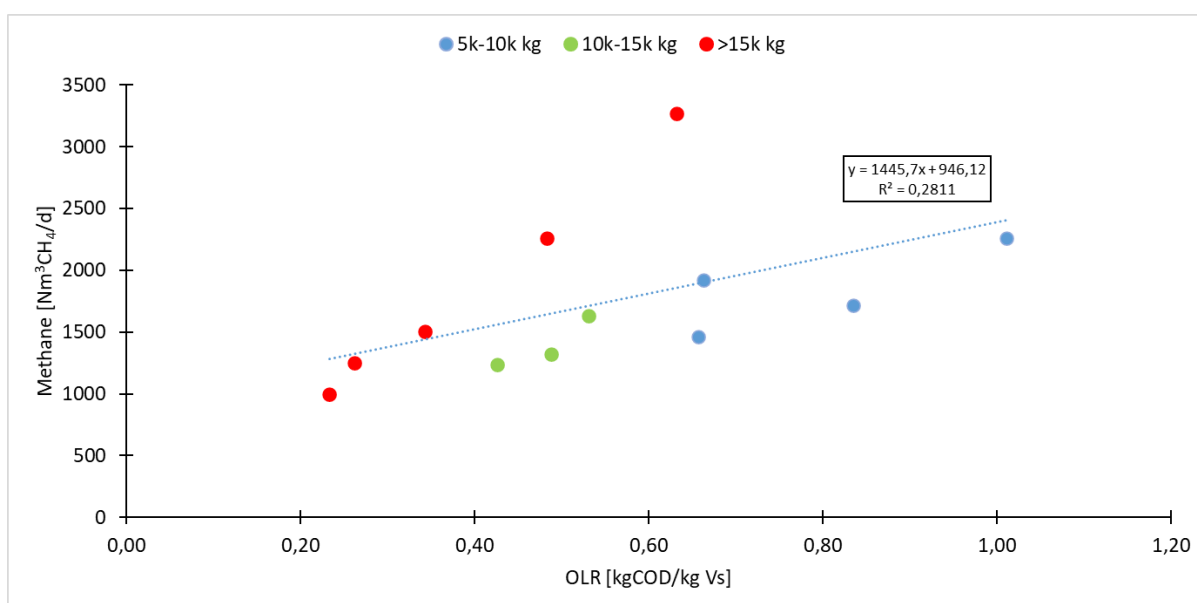


Fig. B.15. Relationship between organic loading rate (OLR) and daily methane production in the methanogenic reactors at different biomass masses.

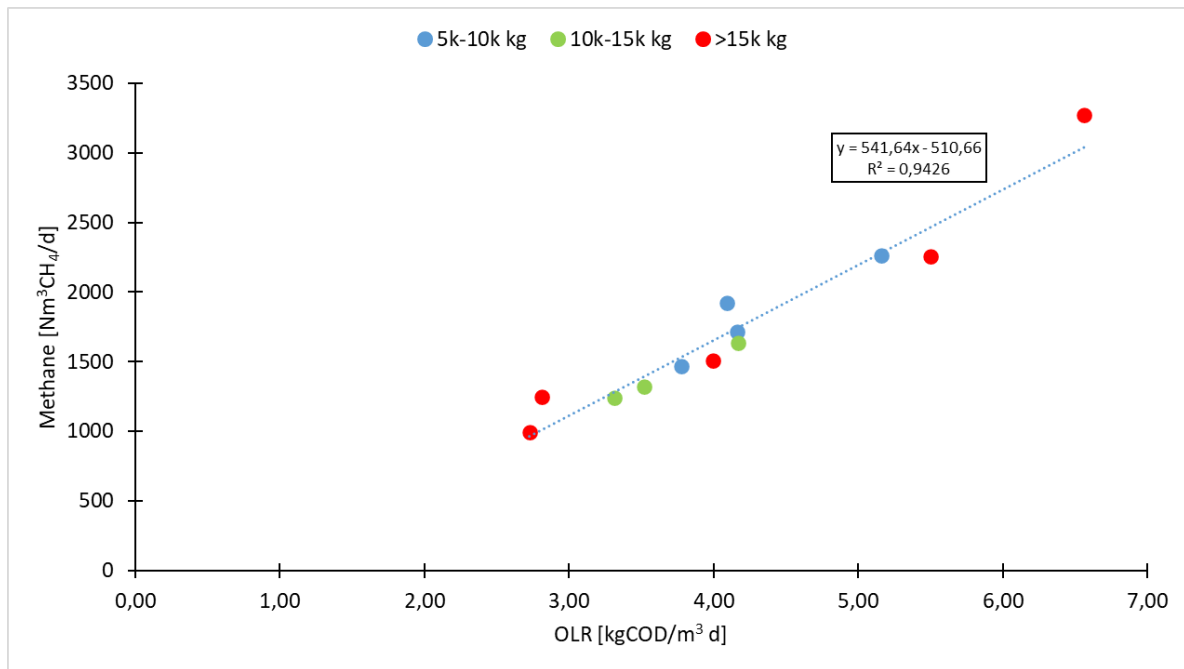


Fig. B.16. Relationship between volumetric organic loading rate (OLR) and daily methane production in the methanogenic reactors at different biomass masses.

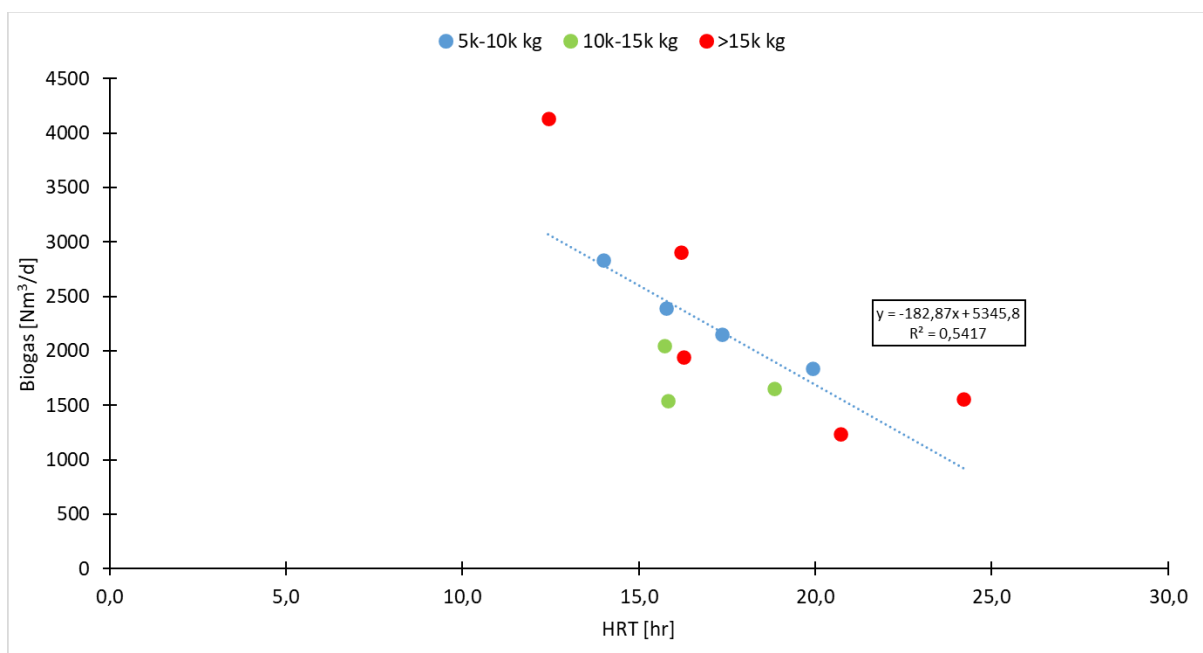


Fig. B.17. Relationship between hydraulic retention time (HRT) and daily biogas production in the methanogenic reactors at different biomass masses.

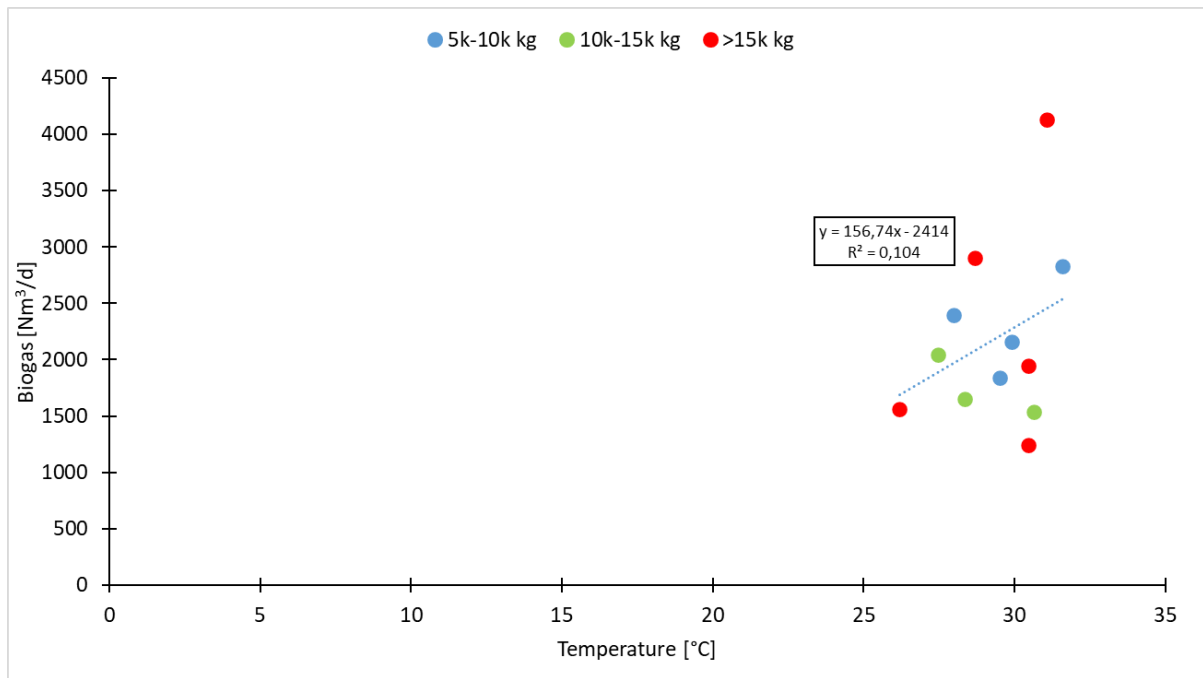


Fig. B.18. Relationship between temperature and daily biogas production in the methanogenic reactors at different biomass masses.

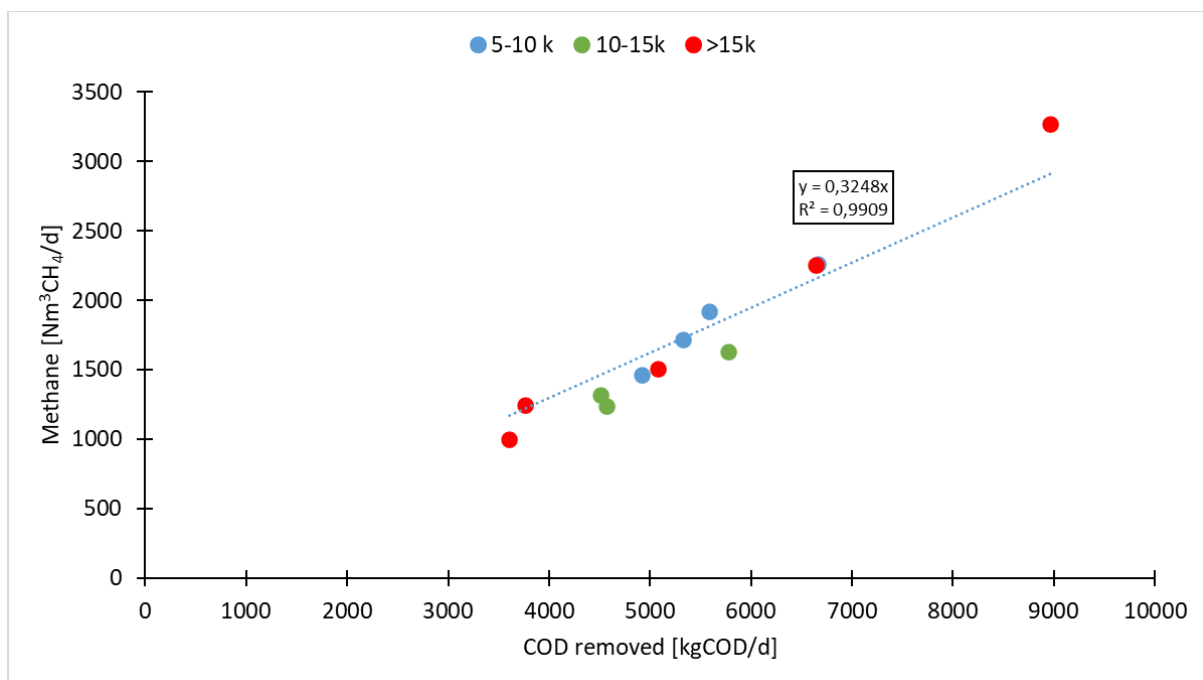


Fig. B.19. Relationship between daily removed COD mass and daily methane production in the methanogenic reactors at different biomass masses.

Table B1. Operating conditions of the homogenization tank during 2019-2016 (average values and standard deviations).

Operating temperature	Inflow [m ³ d ⁻¹]	HRT [hr]	Outflow [m ³ d ⁻¹]	OLR [kgCOD m ⁻³ d ⁻¹]
16°C	2904 (604) (n=261)	29.1 (5.87) (n=260)	2128 (945) (n=353)	3.35 (1.14) (n=260)
21°C	2627 (680) (n=159)	32.6 (8.25) (n=161)	2267 (730) (n=214)	3.18 (1.22) (n=158)
26°C	2632 (660) (n=115)	33.3 (8.28) (n=114)	2167 (777) (n=148)	3.06 (1.19) (n=113)

Table B2. Operating conditions of the EGSB/R2S reactors during 2019-2026 (average values and standard deviations).

	HRT [hr]	OLR [kg COD m ⁻³ d ⁻¹]	Inflow and out-flow [m ³ d ⁻¹]	Temperature [°C]
Value	17.0 (5.62) (n=547)	4.77 (1.43) (n=214)	2313 (603) (n=547)	29.4 (2.15) (n=593)

Appendix C. Supplementary material for Chapter 6

Table C1. Characteristics of Raw Substrates Used in the Fermentation and Co-fermentation Experiment. (average values and standard deviations).

Parameter	Unit	FJPWW	PS	FJPWW+PS (97:3)	FJPWW+PS (90:10)	Inoculum
COD _{total}	[g COD L ⁻¹]	3.5 (0.0)	13.7 (0.2)	3.8 (0.06)	4.2 (0.1)	46.4 (0.5)
sCOD	[g COD L ⁻¹]	3.0 (0.0)	2.25 (0.01)	2.9 (0.0)	2.84 (0.02)	3.0 (0.13)
TS	[g TS L ⁻¹]	2.85 (0.17)	9.8 (0.0)	3.1 (0.05)	3.5 (0.06)	28.2 (3.4)
VS	[g VS L ⁻¹]	2.34 (0.0)	7.1 (0.02)	2.5 (0.06)	2.7 (0.01)	22.2 (2.75)
VS/TS	[%]	82.2	78.1	81.5	78.7	78.6
pH	[-]	5.3 (0.1)	5.52 (0.3)	5.34 (0.1)	5.37 (0.1)	5.77 (0.5)
VFA	[g COD L ⁻¹]	0.27 (0.0)	0.98 (0.0)	0.3 (0.0)	0.36 (0.0)	-
Alkalinity	[g CaCO ₃ L ⁻¹]	0.25 (0.0)	0.41 (0.0)	0.26 (0.0)	0.28 (0.0)	0.76 (0.0)
TN	[g N L ⁻¹]	0.01 (0.0)	0.3 (0.0)	0.02 (0.0)	0.03 (0.0)	-
NH ₄ ⁺ -N	[g N L ⁻¹]	0.003 (0.0)	0.04 (0.0)	0.004 (0.0)	0.004 (0.0)	-
TP	[g P L ⁻¹]	0.004 (0.0)	0.1 (0.0)	0.006 (0.0)	0.012 (0.0)	-
PO ₄ ⁻³ -P	[g P L ⁻¹]	0.0003 (0.0)	0.03 (0.0)	0.0004 (0.0)	0.0006 (0.0)	-

Appendix D. Operation of a pilot two-stage anaerobic digestion system

This appendix details the operation of a pilot-scale, two-stage anaerobic digestion system and is structured into three sections. The first section defines the objectives of operating the pilot system, the second describes the materials and methods applied, and the final section summarizes the operational challenges and provides operational insights associated with running this type of system.

D.1. The Aim

The aim of operating the pilot two-stage anaerobic digestion system is to evaluate the impact of co-digesting fruit juice processing wastewater with primary sludge on VFA and methane production.

D.2. Methods and Materials

D.2.1. Substrate

Fruit juice processing wastewater was collected at the influent of the Bronzolo wastewater treatment plant (North Italy), which receives this stream from a nearby fruit juice industry. Samples were taken as grab (instantaneous) samples, and their characteristics are reported in Table D1.

Table D1. Fruit juice processing wastewater influent characteristics.

Parameter	Phase 1
sCOD [mg COD L ⁻¹]	2000-9700
COD _{tot} [mg COD L ⁻¹]	2200-10,000
pH [-]	4.5-12
VFA [mg HAc L ⁻¹]	10-160
Alkalinity [mg CaCO ₃ L ⁻¹]	10-200
TSS [mg TSS L ⁻¹]	300-600
N _{tot} [mg N L ⁻¹]	4-11
P _{tot} [mg P L ⁻¹]	1-6

D.2.2. Operation of the pilot-scale two-stage anaerobic digestion system

The two-stage anaerobic digestion pilot system comprised a storage tank (55.7 L) (Figures D1 and D2) in which fruit juice processing wastewater was introduced daily and mixed with urea and phosphoric acid for nutrient supplementation (nitrogen and phosphorus). The wastewater was then pumped (flow rate of 4-8 L h⁻¹) to a homogenization tank (no temperature control) consisting of two compartments with a total volume of 20 L (Figures D1 and D2). The first compartment was cylindrical (5.8 L) (Figures D1 and D2), continuously mixed, and contained the microorganisms responsible for hydrolysis and acidogenesis. The second compartment was trapezoidal (14.2 L) (Figures D1 and D2) and functioned primarily as a settler, allowing solids to accumulate at the bottom. This compartment was equipped with a

level sensor that, upon reaching a defined level, triggered the pump to transfer wastewater from the storage tank to the homogenization tank.

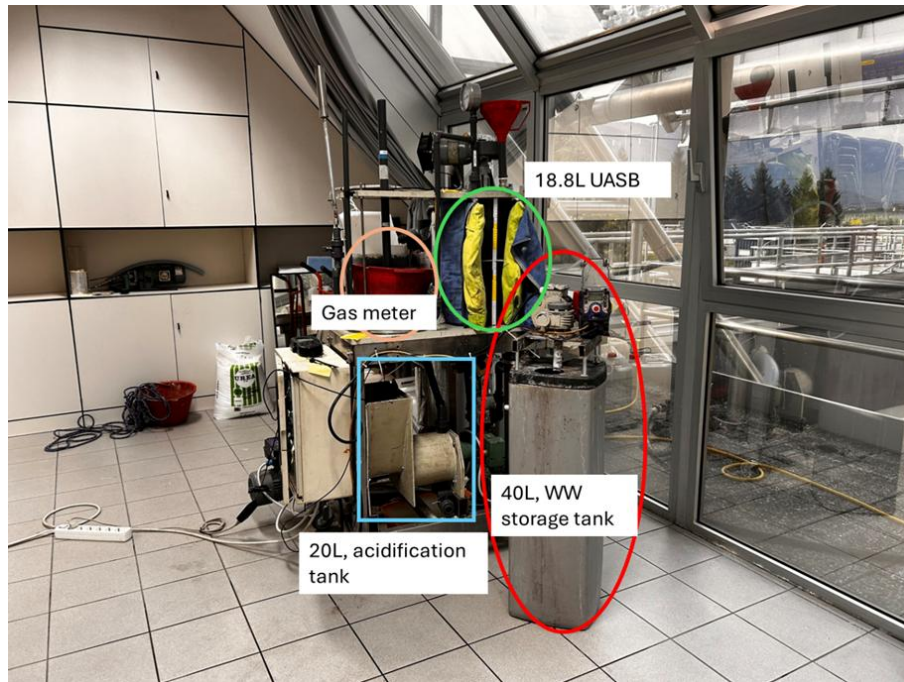


Fig. D.1. Pilot-scale two-stage AD system.

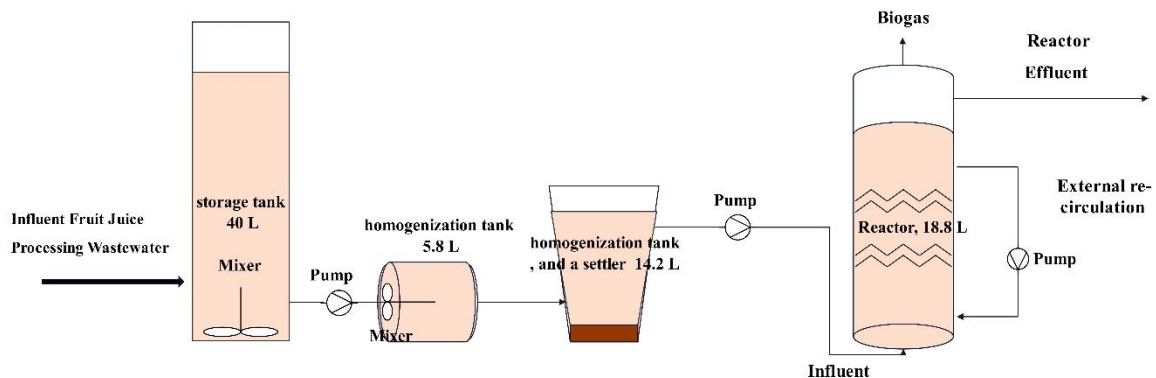


Fig. D.2. Schematic of the pilot-scale two-stage AD system.

The homogenization tank supernatant was subsequently pumped (flow rate of 3.4 L h^{-1}) to the UASB reactor (18.8 L), which was operated with external re-circulation ($50\text{--}100 \text{ L h}^{-1}$) of the biogas reactor effluent to ensure effective mixing. The reactor was heated to maintain 35°C . Biogas volume was measured using a balloon (17 L) connected to a meter, as shown in Figure D1. Part of the effluent was discharged via a tube.

The two-stage anaerobic digestion pilot system was operated in 4 phases, each lasting 2-3 weeks. During the first three phases, the OLR ($\text{kg COD kg}^{-1}\text{VS}$) was progressively increased for the UASB reactor, whereas it remained relatively comparable for the homogenization tank. In the fourth phase, the pilot-scale two-stage system was converted into a pilot-scale single-stage configuration by cancelling the homogenization tank. The operating conditions are summarized in Tables D2 and D3.

Table D2. Operating conditions of the homogenization tank.

Parameter	Phase 1	Phase 2	Phase 3	Phase 4
OLR [kg COD Kg ⁻¹ VS]	0.5-2	0.5-2	0.5-2	-
pH [-]	4.5-5.5	4.5-5.5	4.5-5.5	-
HRT [h]	16-24	16-24	16-24	-

Table D3. Operating conditions for the biogas production reactor.

Parameter	Phase 1	Phase 2	Phase 3	Phase 4
OLR [kgCOD Kg ⁻¹ VS]	0.1	0.25	0.5	0.25
pH [-]	6.8-7.2	6.8-7.2	6.8-7.2	6.8-7.2
HRT [h]	4.5-9	4.5-9	4.5-9	4.5-9

The biomass in the UASB reactor was granular biomass obtained from a full-scale EGSB reactor treating fruit juice processing wastewater at the treatment plant of Bronozlo. The VS of the granular biomass varied between 80-90 gVS L⁻¹. At the start of each phase, the reactor content was completely emptied and replaced with new biomass from the full-scale EGSB reactor at the Bronzolo plant.

D.3. Operational challenges and insights from running the pilot-scale two-stage anaerobic digestion system

The pilot-scale two-stage anaerobic digestion system showed effective performance in VFA production in the homogenization tank (VFAs concentrations 500-1100 mg HAc L⁻¹). However, biogas was rarely measured due to undiscovered leakages in the UASB reactor. Biogas was recorded only on the day of inoculation and the following day (volumes of 10-24 L of biogas), after which no further biogas measurements were obtained. These measurements on the inoculation day and the next day could potentially be attributed to biogas produced by the granules and from substrates attached to them.

Leakages in the UASB reactor were assumed because bubble formation was evident, yet no gas was collected in the gas meter ballon. In addition, COD removal was between 75-90% and effluent VFAs were low (20-100 mg HAc L⁻¹), which indicated effective performance.

During the third phase (when OLR was increased to 0.5kg COD kg⁻¹ VS), only a minimal amount of biogas was measured (< 2L). This indicated that at higher OLR there is higher biogas production; however, effluent VFAs increased to higher than 400 mg HAc L⁻¹ and pH values went below 6.5.

During the last phase, the homogenization tank was removed because, in the previous phases, flocculant biomass from the homogenization tank was often observed to be pumped into the UASB, coinciding with a 30-40% decrease in VFA production in the homogeizantian tank. In this phase, the UASB was observed to operate effectively at an OLR of 0.3 kg COD kg⁻¹ VS, achieving COD removals of 80-90%, although biogas production was still not measured. When

the OLR was increased to 1 kgCOD kg⁻¹VS, biogas production was measured (volumes of 5-8 L), but pH was lower than 6.5 and VFAs were higher than 400 mg HAc L⁻¹.

Appendix E. Experimental photos



Fig. E.1. Sampling from the full-scale BW UASB.

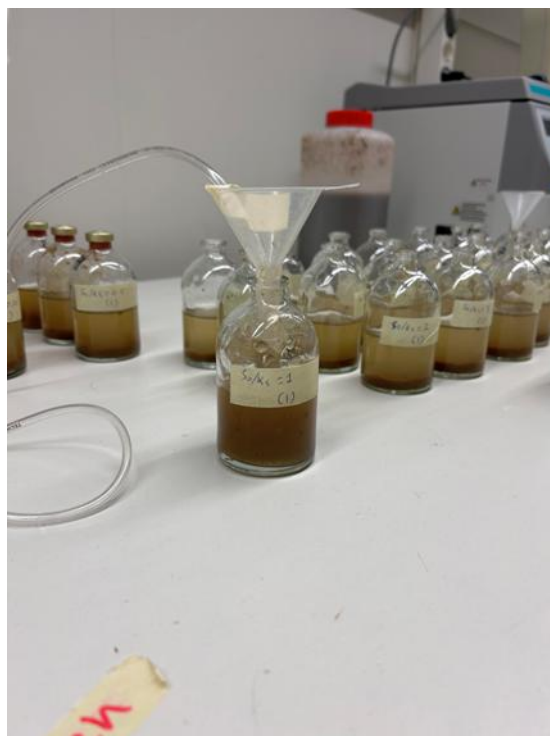


Fig. E.2. SMA test for BW samples.

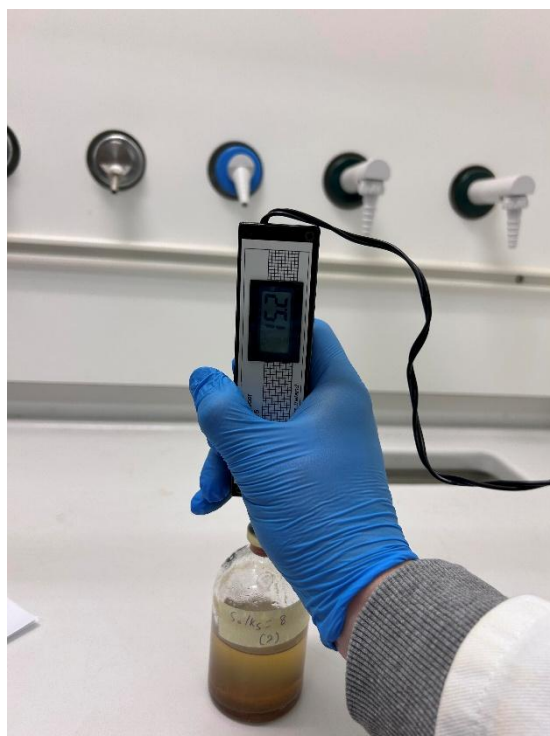


Fig. E.3. Pressure measurement during the SMA test on BW samples.



Fig. E.4. HPLC.



Fig. E.5. FJPWW and PS co-fermentation test preparation.



Fig. E.6. SMA of granules from R2S reactors treating FJPWW using the AMPTS II® system.

About the author

Saleh was born in Palestine. He completed his bachelor's degree in civil engineering, with a focus on structural engineering in Palestine. During his undergraduate studies, he took a course on wastewater treatment, which he initially found challenging. Two years later, he received a scholarship to pursue a master's degree in Civil and Environmental Engineering at the University of Brescia, Italy. There, he decided to challenge himself by taking a course in wastewater and waste management. This proved to be a turning point: he discovered his passion for the field and was inspired by Prof. Giorgio Bertanza's story of transitioning from mechanical engineering to wastewater engineering.

Along the way, Saleh was further motivated by researchers and professionals he met during his master's internship at AnoxKaldnes–Veolia Water Technologies, as well as during his PhD visit to Lund University and NSVA. This growing interest led him to undertake and write this thesis.

After completing his PhD, he aims to contribute to a greener, brighter future by continuing to develop his expertise in wastewater treatment and energy production.

