DOCTORAL THESIS

UNIVERSIDAD DEL VALLE
FACULTAD DE INGENIERÍA

ACIDOGENIC FERMENTATION FOR THE PRODUCTION OF VOLATILE FATTY ACIDS FROM CASSAVA SOUR STARCH EXTRACTION PROCESS WASTEWATER

ACIDOGENIC FERMENTATION FOR THE PRODUCTION OF VOLATILE FATTY ACIDS FROM CASSAVA SOUR STARCH EXTRACTION PROCESS WASTEWATER

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A cumulative dissertation for the degree of Doctor of Engineering with an emphasis in Sanitary and Environmental Engineering

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Posgrado en Ingeniería Sanitaria y Ambiental
Facultad de Ingeniería
Escuela de Ingeniería de Recursos Naturales y del Ambiente
Universidad del Valle
Santiago de Cali
2025

To God and to my parents.

ACKNOWLEDGMENTS

I am deeply grateful to God, the source of inspiration and guidance throughout this arduous journey. His light has illuminated moments of uncertainty and given me the strength to persevere.

To my parents, Alba Nory and Pioquinto, whose love and sacrifices have taught me the value of effort and dedication. Their unwavering support has been fundamental to my personal and academic growth, helping me achieve my goals.

To my sisters, Lady Julieth and Natalia, for their steadfast support, companionship, and for being my accomplices in life.

To my nephew, Nicolás, whose joy and curiosity remind me of the importance of dreaming big and never giving up.

To my advisors, Howard Diego Ramírez Malule and Jenny Alexandra Rodríguez Victoria, whose wisdom and guidance have been invaluable throughout this journey. I deeply appreciate their patience, advice, respect, and unwavering encouragement.

To Professor Patricia Torres Lozada for her generous collaboration and willingness to guide me whenever needed.

To Engineers Mario León Moncaleano and Germán Andrés Oleas from the CAVASA Wastewater Treatment Plant for facilitating the collection of the inoculum used in this research.

To the Postgraduate Program in Sanitary and Environmental Engineering (PISA) at Universidad del Valle and all the professors who were part of the program, for their invaluable teachings during my doctoral studies.

To Dany Mercedes Acevedo from the Environmental Microbiology Laboratory at Universidad del Valle for her valuable collaboration in the characterization of volatile fatty acids.

To Nhora Suaterna Ortiz and Gloria Delfina Lasso from the Teaching and Research Laboratory of the School of Chemical Engineering for their willingness and collaboration in providing essential equipment for sample analysis.

To the School of Natural Resources and Environmental Engineering (EIDENAR) and the School of Chemical Engineering at Universidad del Valle for their support in the formation process.

To the research group Estudio y Control de la Contaminación Ambiental (ECCA) for endorsing my doctoral project.

To Universidad del Valle for the opportunity to serve as a teaching assistant during various semesters of my doctoral studies.

To Universidad del Valle for funding this research through the Internal Call Project CI 21237.

To the Ministerio de Ciencia, Tecnología e Innovación (Minciencias) for awarding the doctoral scholarship through Call No. 933 of 2023.

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the scholarship through the Move La América Program for my doctoral internship.

To Universidade Federal de Santa Catarina (UFSC), as well as Professors Paulo Belli Filho and Nelson Libardi, for enabling the development of my doctoral internship.

Finally, my heartfelt thanks to all the friends, family, and colleagues who may not be mentioned by name but have been part of this journey, always ready to support me whenever I needed it.

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List of Abbreviations

AD	Anaerobic Digestion
AF	Acidogenic Fermentation
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
$CaCO_3$	Calcium Carbonate
CH_4	Methane
COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
CRD	Completely Randomized Design
CSTR	Continuous Stirred Tank Reactor
CWW	Cassava Wastewater
FT	Fermentation Time
HAc	Acetic acid
HCl	Hydrochloric acid
HCN	Hydrocyanic acid
HRT	Hydraulic Retention Time
H_2	Hydrogen
NaOH	Sodium Hydroxide
NH_3	Ammonia
NH_4^+	Ammonium
OLR	Organic Loading Rate
PHA	Polyhydroxyalkanoates
pH_2	Partial Pressure of Hydrogen
RCCD	Rotatable Central Composite Design
RMSE	Root Mean Square Error
R^2	Coefficient of Determination
SCOD	Soluble Chemical Oxygen Demand
SRT	Solids Retention Time
S/M	Substrate-to-Microorganism
T	Temperature
TCOD	Total Chemical Oxygen Demand
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

Abstract

Cassava and its processed products, such as sour starch, form the economic backbone of various communities in Colombia. However, their production generates effluents with high organic loads, cyanide concentrations, and acidic pH levels, leading to significant deterioration of receiving water sources. If these discharges are not properly treated beforehand, bodies of water may become unsuitable for human consumption, fishing, or recreation.

Previous research indicates that this type of wastewater can be treated through anaerobic digestion, yielding methane as the primary product. However, studies also suggest that the volatile fatty acids produced during the acidogenic phase of anaerobic digestion, known as acidogenic fermentation, have valuable applications in various chemical and biological processes.

This doctoral thesis begins with an introduction to the environmental issues caused by effluents from sour starch extraction from cassava, along with an analysis of previous research and emerging trends in its treatment. It also outlines the challenges that motivated this study and led to the establishment of three specific objectives (Chapter 1). Next, acidogenic fermentation and volatile fatty acid production are examined as alternatives for wastewater treatment through a bibliometric analysis of existing scientific literature (Chapter 2).

This analysis identified key operational variables, which were evaluated at the laboratory scale for the substrate of interest. The individual and interactive effects of fermentation time and pH (Chapter 3), as well as the substrate-microorganism relationship and temperature (Chapter 4), were examined. Additionally, recognizing the importance of modeling in complementing experimental trials and enhancing the understanding of biological processes, a kinetic study was conducted on the acidogenic fermentation of wastewater from cassava processing. This study identified the models that best fit soluble organic matter consumption and volatile fatty acid production (Chapter 5).

Finally, this thesis synthesizes the results and outlines perspectives for future research in the field (Chapter 6).

Keywords: acidogenic fermentation, wastewater, cassava, volatile fatty acids, kinetic modeling, fermentation time, pH, substrate-microorganism ratio, temperature.

Resumen

La yuca y los productos derivados de su procesamiento, como el almidón agrio, se han convertido en la base de la economía de diversas poblaciones en Colombia. Sin embargo, su producción genera efluentes potencialmente contaminantes debido a la elevada carga orgánica, las concentraciones de cianuro y el pH ácido, lo que provoca un deterioro significativo de las fuentes hídricas receptoras. Si estos vertimientos no reciben un tratamiento previo, los cuerpos de agua pueden quedar inhabilitados para otros usos, como el consumo humano, la pesca o la recreación.

Investigaciones previas han demostrado que este tipo de agua residual puede tratarse mediante digestión anaerobia, obteniéndose metano como principal producto. No obstante, otros estudios han evidenciado que los ácidos grasos volátiles generados en la fase acidogénica de la digestión anaerobia, conocida como fermentación acidogénica, pueden tener aplicaciones valiosas en diversos procesos químicos y biológicos.

Esta tesis doctoral inicia con una introducción a la problemática ambiental generada por los efluentes de la extracción de almidón agrio a partir de la yuca, así como un análisis de antecedentes y nuevas tendencias en su tratamiento. Además, se presentan los desafíos que justificaron el desarrollo de este trabajo y permitieron el planteamiento de tres objetivos específicos (Capítulo 1). Posteriormente, se explora la fermentación acidogénica y la producción de ácidos grasos volátiles como alternativa para el tratamiento del agua residual, a través de un análisis bibliométrico de la literatura científica existente (Capítulo 2).

Como resultado de este análisis, se identificaron variables operacionales clave del proceso, las cuales fueron evaluadas a escala de laboratorio para el sustrato de interés. En este contexto, se analizaron los efectos individuales e interactivos del tiempo de fermentación y el pH (Capítulo 3), así como la relación sustrato-microorganismo y la temperatura (Capítulo 4). Adicionalmente, considerando la importancia de la modelación para complementar los ensayos experimentales y mejorar la comprensión de los procesos biológicos, se realizó un estudio cinético de la fermentación acidogénica del agua residual generada en el procesamiento de la yuca. En este estudio, se identificaron los modelos que mejor se ajustan al consumo de materia orgánica soluble y a la producción de ácidos grasos volátiles (Capítulo 5).

Finalmente, esta tesis presenta una síntesis de los resultados obtenidos y plantea algunas perspectivas para futuras investigaciones en este campo (Capítulo 6).

Palabras clave: fermentación acidogénica, agua residual, yuca, ácidos grasos volátiles, modelación cinética, tiempo de fermentación, pH, relación sustrato – microorganismo, temperatura.

1 Introduction

1.1 Background and justification

According to the United Nations World Water Development Report (2017), there is growing momentum for a paradigm shift in wastewater treatment as part of a circular economy. Trends in wastewater management indicate a focus on greater water reuse and resource recovery. Additionally, there is a need for alternative production chains utilizing renewable resources to reduce dependence on oil and minimize environmental impacts [1,2].

The generation of wastewater is a significant environmental aspect in most production processes, including agroindustry. Improper disposal of wastewater negatively impacts water sources, affecting their economic, social, and ecological uses. This is due to decreased dissolved oxygen, harm to aquatic species, the growth of microbial species, and eutrophication, which diminishes water mirror [3].

Colombia is a predominantly agricultural country, with cassava (*Manihot esculenta Crantz*) ranking sixth in production, after sugarcane, panela sugarcane, banana, potato, and rice. Also known as mandioca, tapioca, or manioc in other parts of the world, cassava is a valuable source of food, employment, and income for many farming communities in developing regions, particularly in Africa, Asia, and Latin America [4]. In 2024, Colombia produced 2.6 million tons of cassava from a cultivated area of 237,118 hectares, reflecting a 16% increase in production from 2021 to 2024. While cassava is cultivated in all 32 departments of Colombia, it is most prevalent in the Atlantic Coast, Llanos, and Cauca regions [5].

In Colombia, commercial cassava production primarily consists of 93% sweet cassava, used mainly for direct human consumption, and 7% sour cassava, which is processed for industrial use to produce both sweet and sour starches [5]. Sour starch is produced in small and medium-scale processing plants, known as *rallanderías* (spanish term), through a fermentation process. It is primarily used in the production of baked goods, such as pandebono, cassava bread, fritters, and doughnuts [6]. In contrast, sweet or native starch is produced by large industries, without undergoing fermentation. It lacks odor and flavor and is primarily used as a binder in the national food industry (e.g., biscuits, meat products, pasta, and dressing sauces). Additionally, it has industrial applications in the paper and cardboard industry, adhesives, and textiles [7].

Industrial cassava production is registered in 11 of the 32 departments of Colombia, with Cauca, Córdoba, and Sucre contributing the most to national production, at 30, 27, and 40%, respectively [5]. In the department of Cauca, the processing of sour cassava starch is the most commercially important product and forms the foundation of the local economy [8].

Sour starch production generates significant amounts of solid and liquid waste, with the type and composition varying depending on the processing method and technological sophistication used [9]. Solid by-products, such as bran and stain, can be used as inputs in the production of animal feed concentrates. Additionally, approximately 85% of the water required to produce one ton of starch is discharged as wastewater, with the remainder lost to evaporation [10].

The water that comes into contact with cassava dissolves a considerable amount of materials, producing an effluent with high pollutant potential. This is primarily due to the substantial organic load from soluble carbohydrates and proteins (COD of 3400 to 5400 mg/L), cyanide concentrations (~0.71 mg/L), and acidic characteristics (pH between 4.1 and 4.4). These acidic conditions result from the high concentrations of lactic and acetic acids, as well as smaller amounts of formic, propionic, and butyric acids [11–13]. Consequently, the effluent causes significant degradation of nearby surface water sources, making them unsuitable for uses such as human consumption, fishing, and recreation [8].

All cassava varieties contain cyanogenic glycosides, which, when subjected to acid or enzymatic hydrolysis, release hydrocyanic acid (HCN). Between 40 and 70% of this acid ends up in the water used for washing the starch. If the cyanide concentration exceeds 0.3 mg/L, it can have detrimental effects on local agriculture, as well as on fish and crustacean populations in nearby water bodies [10].

Moreover, depending on the pollutant load of the wastewater, eutrophication can occur in lentic water systems, worsening the situation during drought periods [9]. Eutrophication leads to increased microorganism and algal growth, which alters water quality and disrupts aquatic ecosystems. This process negatively affects plants, animals, and human health. Effluent from the cassava sour starch extraction process can therefore have significant environmental impacts on lakes and reservoirs, ultimately affecting local communities [10].

Laboratory and pilot-scale studies have demonstrated that this type of wastewater is suitable for treatment using Anaerobic Digestion (AD) technologies [8,11,13,14] due to the abundance of readily biodegradable sugars. While methane is traditionally considered the main product of AD, research indicates that intermediate products, such as volatile fatty acids (VFA) and hydrogen, offer greater economic value [2,15]. These intermediates are produced during the acidogenic stage of AD, also referred to as acidogenic fermentation (AF).

VFA have significant potential as a renewable carbon source and are widely utilized across various applications. They play a key role in biological nutrient removal processes and are extensively used in the pharmaceutical, food, and chemical industries. Additionally, VFA serve as feedstock for producing biogas, biodiesel, bioplastics, biohydrogen, biofertilizers, and biosurfactants [16,17]. From an economic perspective, VFA (2 to 5 carbon atoms) have a higher gross added value than methane and hydrogen but a lower value than medium-chain carboxylic acids (6 to 12 carbon atoms) [18,19].

Economically, it may be more advantageous to produce acids biologically through AF rather than generating methane via conventional AD [2]. This shift could contribute to both the economic and environmental sustainability of wastewater treatment systems. Studies have also shown that the theoretical energy yield of VFA is comparable to or exceeds that of biogas. Furthermore, their production cost is competitive with acids derived from chemical markets, making VFA a viable option for mass production [15].

In this context, AF emerges as a promising alternative process. It not only stabilizes organic matter and reduces the environmental impact of effluents from the cassava sour starch extraction process but also produces valuable chemical compounds, such as VFA. These compounds, which are typically generated by the less sustainable petrochemical industry, highlight the potential of AF as a greener and more sustainable alternative [1,19].

In recent years, the production of VFA from wastewater has gained significant attention in research [16]. Laboratory and pilot-scale studies have investigated VFA production using various substrates, focusing on the influence of environmental and operational factors [16,19,20]. Both continuous [21,22] and batch reactors [23] have been employed to evaluate process performance, with researchers also developing kinetic and mathematical models to better understand and simulate process dynamics [23–25].

Despite these advancements, significant challenges remain in utilizing wastewater as an alternative industrial substrate, particularly for cassava processing. One major challenge is the persistence of methanogenic microorganisms during VFA production, which limits the achievement of high VFA concentrations needed for effective separation and application. Additionally, the valorization of VFA requires further exploration to identify new markets and applications [16,18,26,27].

Other obstacles include the lack of optimization studies for batch and continuous reactors systems [28], the development of integrated systems to streamline production, and improvements in statistical models of optimized properties for large-scale production. Advancing knowledge of microbial gene expression and enzyme regulation is also crucial for increasing product specificity and yield [29].

Furthermore, there is limited control over metabolic activities and an incomplete understanding of the factors driving changes in the product spectrum during metabolic processes. These variations are influenced by operational variables such as hydraulic retention time, pH, temperature, substrate type and concentration, nutrient availability, and the partial pressure of gases [1,19,30].

Studying the operational factors and metabolic reactions involved in AF is inherently complex in experimental settings. However, modeling and simulation tools can enhance interpretation and provide valuable insights. According to Lorenzo-Llanes et al. [31], utilizing process simulation tools enables a more comprehensive understanding of the acidogenic stage of AF. These tools allow for faster and more cost-effective predictions and optimizations compared to laboratory or full-scale experiments.

The wastewater from cassava sour starch extraction has proven to be a suitable substrate for VFA production through AF. This approach not only addresses the environmental issue of water pollution caused by cassava processing but also generates valuable compounds that can be used as industrial raw materials. To further understand the AF process, this doctoral thesis focused on experimental research and advances in kinetic modeling of VFA production from cassava wastewater (CWW), aiming to maximize yield.

1.2 Objectives

1.2.1 General Objective

To assess the production of VFA from the acidogenic fermentation of wastewater generated during the cassava sour starch extraction process using mathematical model fitting.

1.2.2 Specific Objectives

- To identify the effects of pH, temperature, substrate-to-microorganism ratio (S/M), and fermentation time on the production of VFA during the acidogenic fermentation of wastewater from cassava sour starch extraction.

- To examine the kinetics of VFA production from cassava sour starch wastewater under defined operating conditions, aiming to optimize acids production.
- To adjust a model for the fermentative production of VFA from cassava sour starch wastewater, based on models reported in the literature.

1.3 Organization of the Thesis

This doctoral thesis follows an article-based format, incorporating published and submitted articles in indexed scientific journals derived from the research. It presents a bibliometric analysis of scientific literature on VFA production through AF of wastewater. Additionally, it examines the influence of key operational variables on VFA production from wastewater generated by the cassava sour starch extraction process and explores the models that best describe the kinetics of soluble organic matter consumption and VFA production.

Chapter 2 establishes the reference framework for the thesis. It included a bibliometric analysis of the scientific literature on VFA production from wastewater fermentation published between 1981 and June 21, 2021. Additionally, it examined key process variables that can be optimized and modeled to enhance VFA yield as a value-added product. The chapter also reviewed studies focused on modeling of VFA production via AF of wastewater.

Chapter 3 evaluates the individual and interactive effects of fermentation time and pH on VFA production and composition during the acidogenic fermentation of cassava wastewater. This analysis employed a completely randomized design and a second-order response surface methodology (rotating central composite design). The chapter also described pH and pressure variations within the bioreactors and concluded with a section on future perspectives.

Chapter 4 investigates the effects of the substrate-to-microorganism ratio and temperature on VFA production and composition during the fermentation of wastewater from cassava starch extraction. It also highlighted the challenges of VFA production using mixed cultures in continuous reactors at laboratory and pilot scales, as well as during the transition to industrial-scale implementation.

Chapter 5 analyzes the kinetics of VFA production in batch reactors using cassava wastewater as a substrate under conditions established in Chapter 3 and 4. It also identified the best-fit models for estimating kinetic parameters related to soluble organic matter consumption and VFA production.

Finally, **Chapter 6** provides an overview of the thesis, presents key conclusions, and offers recommendations for future research in this field.

2 Volatile Fatty Acids Production by Acidogenic Fermentation of Wastewater: A Bibliometric Analysis

Published as: Sanchez-Ledesma, L. M., Ramírez-Malule, H., & Rodríguez-Victoria, J. A. (2023). Volatile Fatty Acids Production by Acidogenic Fermentation of Wastewater: A Bibliometric Analysis. Sustainability, 15(3), 2370. https://doi.org/10.3390/su15032370

Note: Item 2.3.4.4 on acidogenic fermentation modeling has been added. Additionally, the conclusions (Item 2.4) have been updated accordingly.

Abstract

This study presents a bibliometric analysis of the scientific literature on volatile fatty acids (VFA) production from wastewater fermentation published from 1981 to 21 June 2021. A total of 618 papers obtained from the Scopus database were analyzed using VOSviewer 1.6.16 software. According to the results, this topic has been capturing the attention of researchers over the years, but with different research approaches, including optimization of anaerobic digestion in two-stage reactors, biological removal of nutrients from wastewater, energy production in bioelectrochemical systems, and recovery of VFA as value-added intermediate products to be used as inputs in a variety of industries. In addition, the bibliometric networks obtained from the authors' keyword frequency showed that wastewater treatment by using fermentation to obtain VFA as a value-added by-product is an emerging topic that undoubtedly requires further research and collaboration between scientific institutions. In this regard, different types of wastewater have been used as a substrate for acidogenic fermentation; however, and based on the results, the production of VFA from cassava processing wastewater is seen as one of the emerging issues of this field. Finally, evaluating the effect of operating conditions on the fermentation process, such as pH, hydraulic retention time, organic loading rate, temperature, inoculum and substrate concentration, independent of the final application of the VFA produced, is a relevant aspect for bioprocess optimization and implementation on a large scale.

Keywords: acidogenic fermentation; volatile fatty acids; wastewater; bibliometric analysis

2.1 Introduction

Recently, wastewater management has become increasingly one of the most critical problems faced by society. Its inadequate disposal generates a negative environmental impact on the water resource. Those disposals affect the different economic, social and ecological uses of water due to the decrease in dissolved oxygen and its impact on the species of the aquatic environment, the growth of microbial species and eutrophication that reduces the water mirror [3].

Wastewater can be treated through physicochemical and biological processes. Considering technical and economic aspects, physicochemical processes are preferably applied in the treatment of water with inorganic contaminants, non-biodegradable organic matter or compounds toxic to microorganisms, while biological processes are used when the main contaminants are biodegradable organic compounds and inorganic anions are present, such as nitrates, nitrites, sulfates, sulfides and phosphates [32]. To carry out biological treatment there are a variety of aerobic and anaerobic processes, depending on the presence or absence of oxygen as an electron acceptor, respectively.

Anaerobic digestion (AD), a process that involves a series of reactions (including hydrolysis, acidogenesis, acetogenesis and methanogenesis stages), was initially established to sludge stabilization, and later found an area of interest in wastewater treatment, especially in wastewaters with high organic matter content. However, today AD is considered, in addition to a technology for wastewater and waste treatment, a technology for resource recovery [33].

According to the United Nations World Water Development Report (2017), there is growing impetus for a paradigm shift in wastewater treatment as a component of a circular economy. In wastewater management, trends point to greater water reuse and resource recovery. In addition, alternative production chains involving the use of renewable resources are needed to reduce dependence on oil and associated environmental impacts [1,2].

Although methane is considered the main product of AD, studies confirm that some intermediate products have a greater added value and variety of applications in chemical and biological processes, as is the case with hydrogen and carboxylic acids [2] generated in the acidogenic stage of AD (also known as Acidogenic Fermentation—AF).

In AF, simple organic compounds (monosaccharides, long-chain fatty acids, glycerol and amino acids) generated by the hydrolysis of complex particulate material are metabolized inside the cells through fermentative metabolism, producing organic acids, mainly short-chain fatty acids (C1 to C5), commonly called Volatile Fatty Acids or VFA, such as acetic, lactic, butyric, propionic and formic acids. In addition, alcohols (ethanol, butanol, methanol, glycerol, isopropanol, propanol), ketones (acetone), carbon dioxide (CO_2), hydrogen (H_2), ammonia (NH_3, NH_4^+), hydrogen sulfide and new bacterial cells are produced [34,35]. AF is carried out by a large and diverse group of acidogenic bacteria through a series of oxidation-reduction reactions, whose activity depends on changes in environmental and operational conditions [19].

To give an example of their applications, VFA have high potential as renewable carbon sources and are widely used for biological nutrient removal, in the pharmaceutical, food and chemical industries, and as feedstock for products such as biogas, biodiesel, bioplastic and biohydrogen [16].

The production of VFA from AF of wastewater has been studied for several decades with different research approaches, which is reflected in a considerable volume of scientific publications. Approximately 3% of the indexed records are reviews on the subject, and most refer to a specific focus (biodegradable polymers and hydrogen production are two examples) [36–38]. Furthermore, and to the best of our knowledge, the available reviews do not make use of bibliometric analysis to analyze and follow up scientific publications on this topic, a tool that has been widely used for mapping a large volume of scientific studies.

Therefore, this work aims to identify and analyze the studies of the production of VFA from AF of wastewater by a bibliometric analysis of the world scientific literature published from 1981 to 21 June 2021. The work also includes the identifying, description and analysis of critical process variables that are susceptible to optimization and/or modeling to obtain a higher yield of VFA as a value-added product.

2.2 Materials and Methods

Data for the bibliometric analysis was obtained from the Scopus database on 21 June 2021. To address all articles related to VFA and wastewater fermentation, the following search equation with its respective Boolean operators was designed: “volatile fatty acid” AND wastewater AND fermentation. This search equation was used in the “article title, abstract and keywords” field (see Ramírez-Malule et al. [39] and Ramírez-Malule et al. [40] for further details of bibliometric analysis approach/methodology), and was adopted after performing the search with three preliminary equations, observing a greater number of records and verifying that key documents on the topic of interest previously reviewed were found within the results.

The information retrieved from the Scopus database was not limited in terms of type of document and corresponded to citation information, bibliographic information, abstract, keywords and funding details. Additionally, during the search the data was further refined (i.e., timespan) before errors such as duplicates and erroneous entries were eliminated. All this information was used for the analysis of bibliometric indicators, and for the elaboration and visualization of bibliometric networks based on co-authorship between countries and the author’s keyword co-occurrence by the VOSviewer 1.6.16 software [41]. One and nine were established as the minimum number of documents from a country and the minimum number of occurrences of a keyword for each type of analysis, respectively. The keywords were exported to Microsoft Excel® to adjust the thesaurus.

In addition, the titles and abstracts of each of the records were reviewed, and many of them (articles) were read in full.

Figure 2.1 shows a scheme of the methodology used.

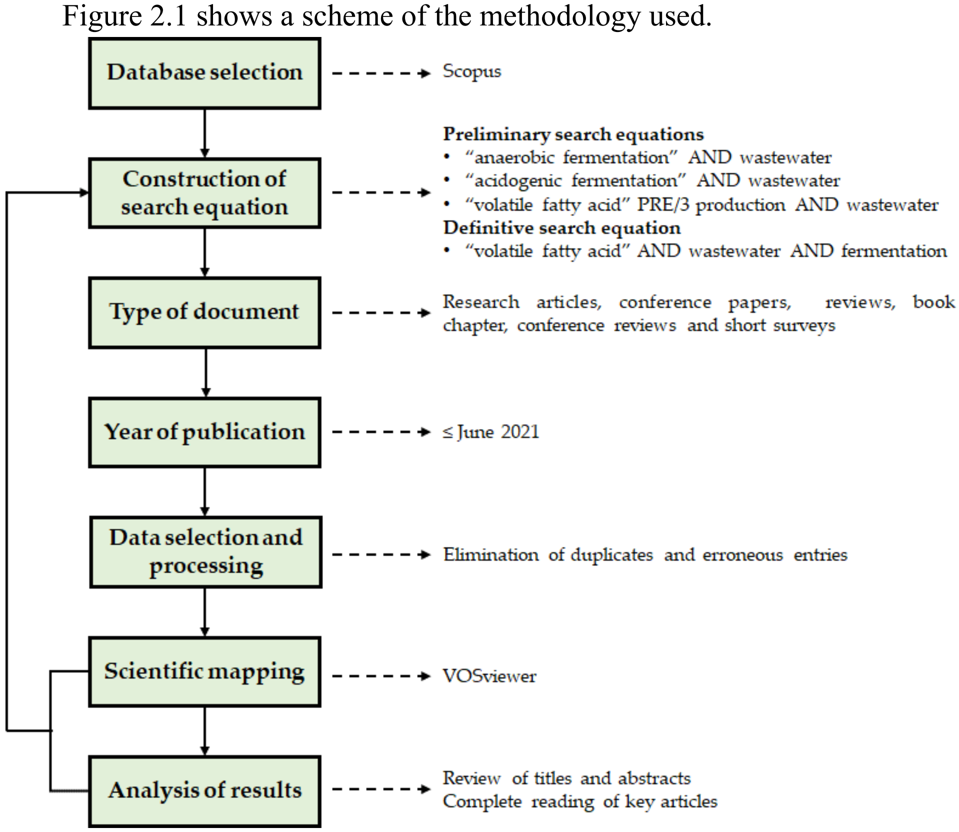


Figure 2.1 Methodological scheme for bibliometric analysis.

2.3 Results and Discussion

2.3.1 Worldwide Scientific Production Related to VFA and Wastewater Fermentation

The search process yielded 618 publications, of which 85.6%, 9.7% and 2.9% were research articles, conference papers and reviews, respectively. The remaining 1.8% represented book chapters, conference reviews and short surveys.

Figure 2.2 shows the evolution in the number of papers related to VFA and wastewater fermentation published annually from 1981 to 21 June 2021, the journals that published them, and the countries that conducted the research.

Prior to 1997, the number of publications on the topic of interest in journals indexed in Scopus was low. Annually, the number of documents did not exceed four and the number of publications represented only 4.0% of the total worldwide scientific production identified for the period evaluated.

From 1997 onward, the overall production of scientific publications on this topic reflects a slight growth compared to the period 1981–1996; however, this has not been continuous, as short periods with marked decreases (e.g., 2001–2004, 2011–2013, 2014–2018) indicate.

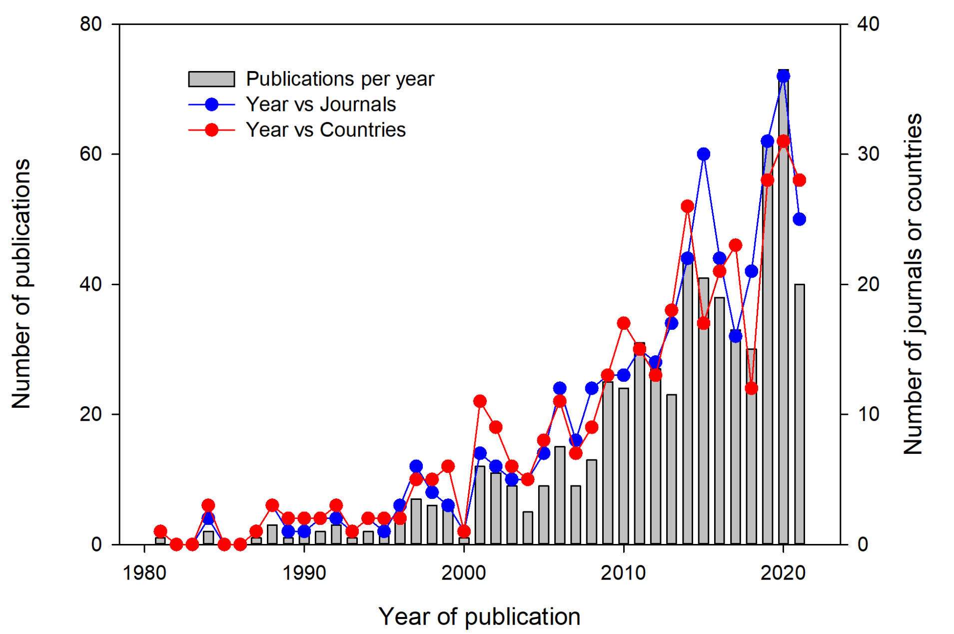


Figure 2.2 Annual consolidation of publications on VFA and wastewater fermentation, and the numbers of countries and journals that issued those studies from 1981 to 21 June 2021. Source: Scopus database.

Publications in scientific journals related to the topic of interest became more relevant in the scientific community as of 2001, increasing from 7 journals and 11 countries to 36 and 31, respectively, in 2020.

The number of publications increased in the last two decades due to various reasons. There has been a shift in awareness regarding the removal of nutrients from wastewater as an important measure to preserve the quality of water bodies receiving effluent from treatment systems [42,43]. In addition, the term "green biorefinery" was introduced in 1997 and major research and development contributions in the field of biorefinery systems were notable in Europe [44].

Likewise, resource maintenance and management were key policy areas within the sustainable development approved in Agenda 21 as a program of action for the 21st century. This required a search for novel solutions to reduce the rapid consumption of non-renewable fossil resources, demanding new research and development approaches that would redirect energy and material production towards biological raw materials as the main source [44]. For Ahmed et al. [45], the production of fuels and chemicals by biomass processing has become a worldwide practice to expand energy resources and reduce global warming, because, due to its characteristics, biomass is considered a renewable and carbon-neutral natural source.

Regarding the most cited articles on VFA and wastewater fermentation, the top 10 is available as Supplementary Material Table 2S1. This file shows the title of the article, the authors, the journal, the year of publication, the number of citations, the number of citations per year and some comments related to the research.

2.3.2 Most Productive Countries and Research Institutions Worldwide in the Scientific Literature on VFA and Wastewater Fermentation

Studies on VFA production and wastewater fermentation have been carried out in Asia (395 papers), Europe (245 papers), North America (98 papers), Oceania (37 papers), Africa (20 papers) and South America (15 papers). China is the leader with 202 papers, equivalent to 32.7% of the world's scientific production for the period evaluated (Figure 2.3), followed by the United States (9.9%), India (7.1%) and Australia (5.3%).

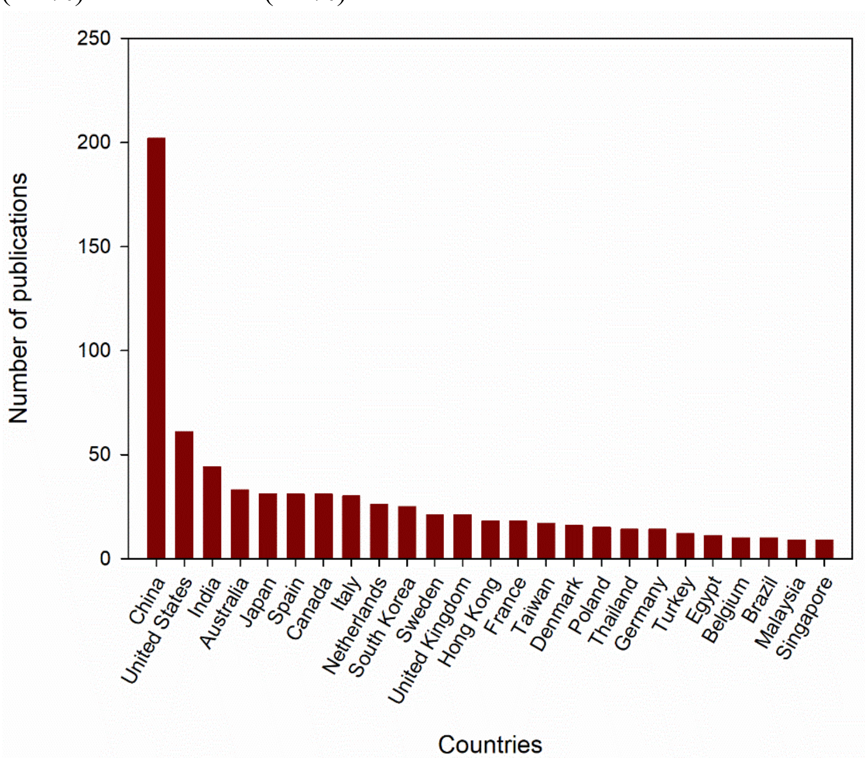


Figure 2.3 Top 25 countries with the most publications on VFA and wastewater fermentation. Source: Scopus database.

Of the 52 countries reporting publications, the majority are European. Major research and development contributions in biorefinery systems were initially notable in Europe, with a high degree of contribution from Germany [46].

For its part, the U.S. President and Congress pushed for significant industrial developments for the first time in 2000 [46]. In the United States, in 2002, the Biomass Technical Advisory Committee published the work plan "Biomass technology in the United States", in which research, development and construction of demonstration biorefineries were established as necessary, as reviewed by Kamm and Kamm [44]. China, which had the largest number of records, began publishing studies on this subject in 2001.

In the top 25 countries, Brazil is the only South American country publishing in this area of knowledge (ranking 22nd with 10 publications). Prior to 1997, publications were related to the optimization of the primary sludge fermentation process to maximize VFA production, essential for biological phosphorus removal. The most recent publications (after 2008) have focused on process optimization to produce value-added compounds. Brazil is no stranger to the global research interest in wastewater treatment, which in recent decades has been marked by the convergence of two areas: treatment and recycling, and reuse as a renewable source of energy and value-added products [47].

For the period studied, other Latin American countries such as Colombia and Chile are only in 34th and 43rd position with 4 and 1 publications, respectively. This shows the need to allocate more funding sources and establish effective policies that promote research and development to solve problems that can be addressed with technologies that have a more sustainable approach.

Regarding the topic of co-authorship, Figure 2.4 shows a collaboration network among different countries for scientific production related to VFA and wastewater fermentation. A total of 52 countries complied with the threshold of having at least one publication on the topic studied, but only 50 are linked in the network map. In this Figure, each node represents a country, the size of the circle represents the amount of publications and the width of the line linking them is proportional to the strength of the relationship between them [48,49]. China, in addition to being the most productive country, represented the place with the closest collaboration with other countries. This may be related to the country's research capabilities and the availability of high-level research institutes, which becomes an attraction for foreign researchers to form partnerships.

Australia and the United States are also countries with a robust collaboration network.

If the analysis focuses on scientific production by research institutions, the predominance of Chinese institutions in the top 10 becomes evident, the exceptions being the Indian Institute of Chemical Technology (India) and the University of Queensland (Australia), which occupy the fourth and sixth positions, respectively (Table 2.1). It is worth noting that, although the United States has the second highest number of publications worldwide, none of its institutions are among the top 10, which is occupied by highly productive institutions.

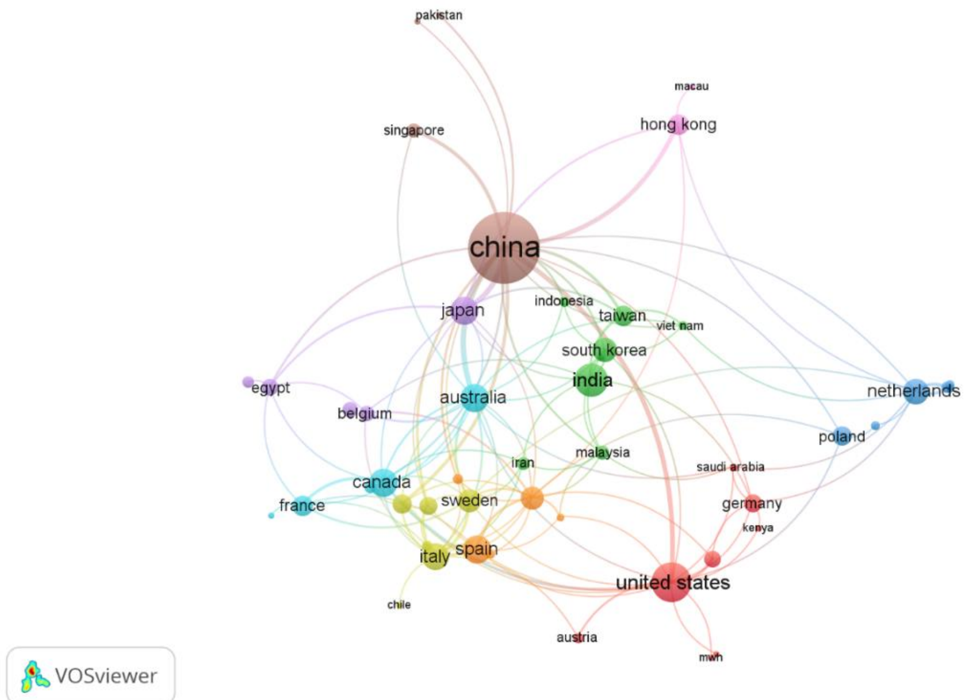


Figure 2.4 Collaboration network between authors from different countries: minimum threshold for publication of 1 document. Source: Scopus database.

Table 2.1 Top 10 most productive research institutions worldwide on the topic of VFA production from wastewater fermentation.

Position	Research Institution	Number of Publications
1	Tongji University	31
2	Harbin Institute of Technology	26
3	Ministry of Education China	25
4	Indian Institute of Chemical Technology	24
5	Chinese Academy of Sciences	19
6	The University of Queensland	18
7	Beijing University of Technology	16
8	The University of Hong Kong	14
9	Tsinghua University	14
10	University of Science and Technology of China	13
11	Jiangnan University	13

2.3.3 Main Journals Publishing on VFA and Wastewater Fermentation

According to the documents reviewed, most of the publications have addressed the topic of interest from an environmental and technological perspective. Therefore, the journals with the highest number of publications are of environmental interest.

Figure 2.5a shows the top 10 scientific journals with the highest number of publications on VFA and wastewater fermentation, in which Bioresource Technology, Water Research, Water Science and Technology, International Journal of Hydrogen Energy and Water Environment Research stand out.

These journals account for 40.3% of all the papers retrieved for the period evaluated (1981–21 June 2021). Figure 2.5b shows the evolution of the number of articles published per year in the five most important journals. This is consistent with the growth that these journals have experienced over the years due to increases in the frequency of publication.

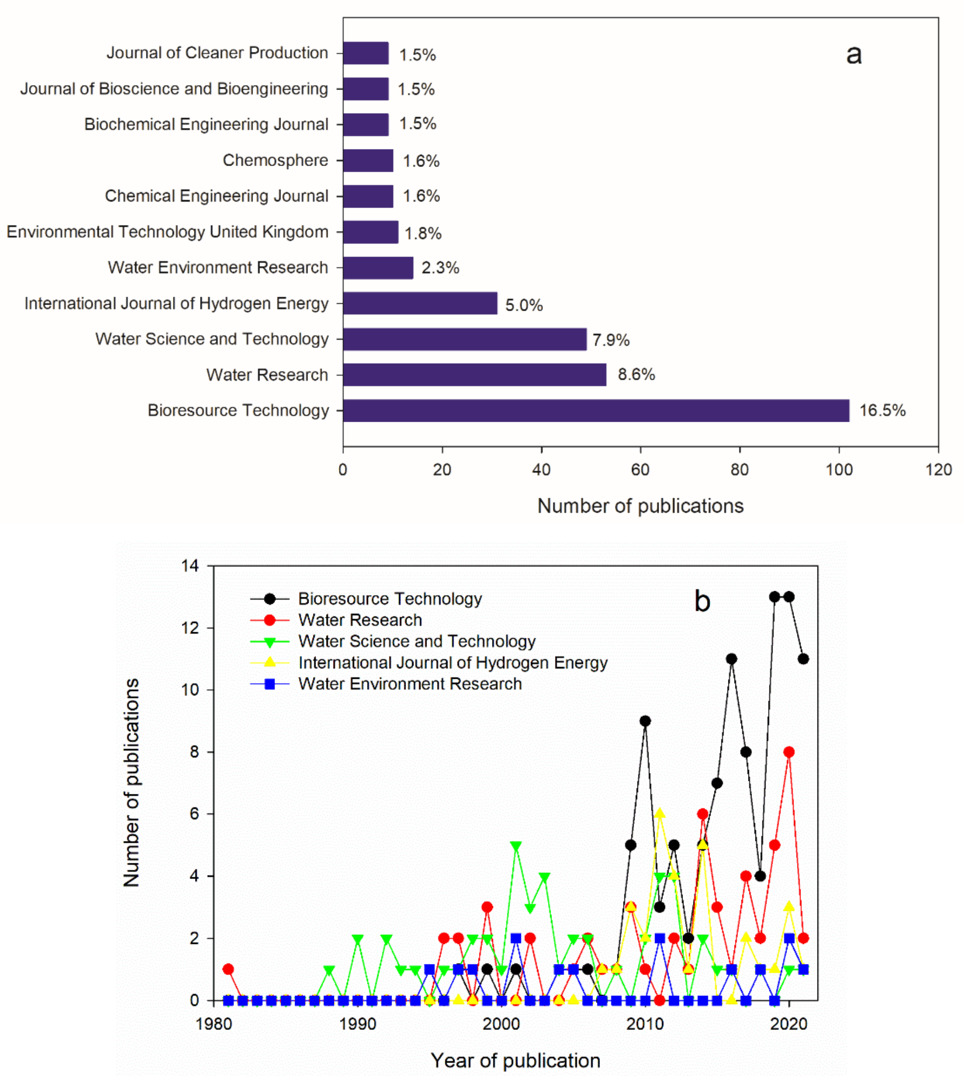


Figure 2.5 Main journals publishing on VFA and wastewater fermentation. **(a)** Top 10 scientific journals with the highest number of publications. **(b)** Annual evolution in the number of publications for the top 5 scientific journals with the highest number of publications. Source: Scopus database.

The journal Bioresource Technology ranks first, with 102 publications since 1997. It is an international journal currently publishing on topics related to biomass, biowaste treatment, bioenergy, biotransformation and bioresource systems analysis, as well as technologies associated with conversion or production. The journals that occupy the second, third and fifth positions publish mainly on topics related to water cycle science and technology, water quality and management, wastewater and stormwater. The International Journal of Hydrogen Energy, which ranks fourth with publications reported since 2007, prioritizes aspects of hydrogen energy, such as production, storage, transmission, utilization, supporting technologies, environmental impact and economic and international aspects of hydrogen.

2.3.4 Main Research Approaches on VFA Production

2.3.4.1 Subject Areas of VFA Production

The studies were mainly related to the area of Environmental Sciences followed by the areas of Chemical Engineering, Energy, Biochemistry, Genetics and Molecular Biology, Engineering, and Immunology and Microbiology, where the number of publications corresponded to 71.0, 35.3, 30.7, 15.5, 11.8 and 11.2%, respectively (Figure 2.6).

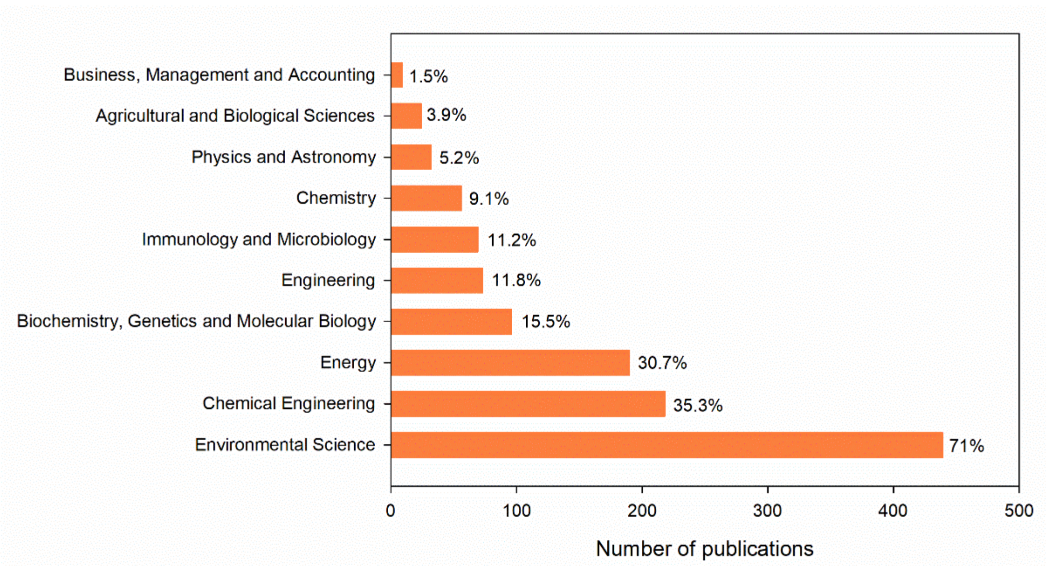


Figure 2.6 Publications on VFA and wastewater fermentation grouped by subject area (Top 10). Note: Percentages add up to more than 100 because a paper may be indexed in more than one area. Other areas contributed 6.3%. Source: Scopus database.

It was to be expected that environmental sciences would take the first place because since 1981 the fermentation of wastewater for VFA production has had various research approaches in this area. The production of VFA to optimize AD for biogas production in two-stage reactors—for the biological removal of nutrients in wastewater—to be transformed into energy in bio-electrochemical systems and to be recovered as value-added intermediate products are some examples of those approaches.

However, most of the publications indexed in environmental sciences were also indexed in the areas of chemical engineering and energy. There is a strong connection between these areas, as the different environmental research approaches identified involve different chemical and biological transformations. The connection is mainly due to those investigations where the production of hydrogen, biogas, methane, PHA and the use of microbial fuel cells were part of the main objective.

2.3.4.2 Bibliometric Networks of VFA and Wastewater Fermentation Studies

The bibliometric network generated with the information exported from Scopus is shown in Figure 2.7. As a result, 1557 keywords were identified, of which 37 had a minimum number of nine occurrences; that is, each of these terms appears at least nine times (frequency). Five clusters were formed, consisting of 37 nodes.

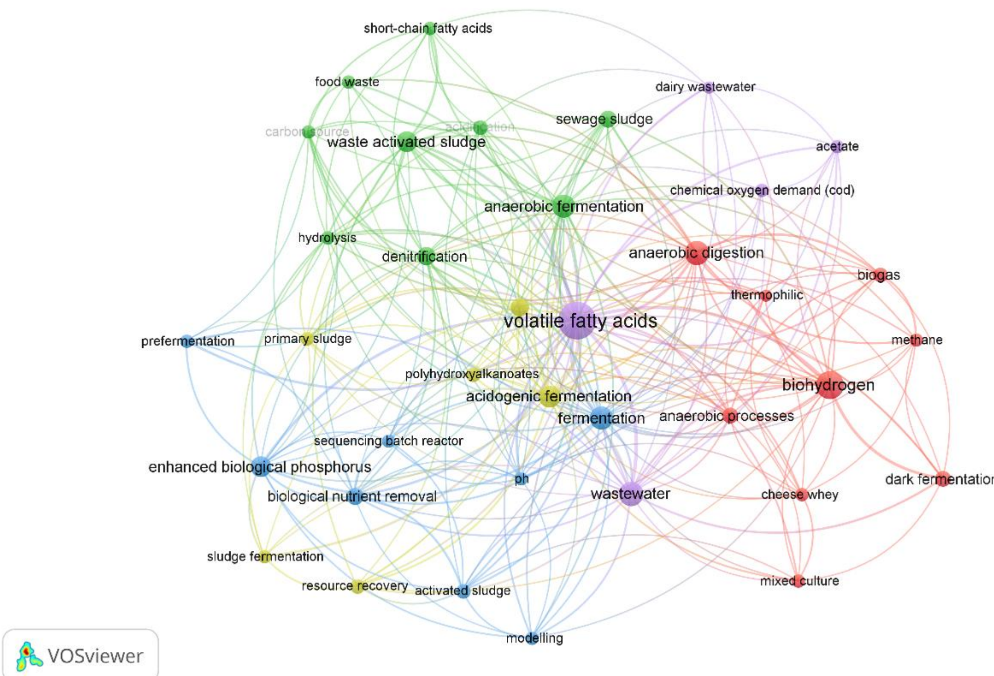


Figure 2.7 Bibliometric network for studies on VFA and wastewater fermentation.

Figure 2.8 Word cloud of the abstracts. Words repeated more than 100 times.

The network analysis allows us to identify different thematic groups related to the field of knowledge of interest. In addition to the keywords used in the search, the terms with the highest frequency were biohydrogen, anaerobic digestion, anaerobic fermentation, acidogenic fermentation, waste activated sludge and enhanced biological phosphorus removal, representing the largest nodes in the network (Figure 2.7). Moreover, Figure 2.8 exhibits a word cloud of the abstracts of the articles used in this study, which supports the findings presented in Figure 2.7.

Cluster No. 1 (red color) is formed of the biohydrogen, anaerobic digestion, dark fermentation, anaerobic process, biogas, methane, mixed culture, cheese whey and thermophilic nodes (Figure 2.7). This cluster represents research oriented towards AD and dark fermentation processes for the transformation of organic matter into by-products such as biogas/methane and hydrogen, respectively.

AD is a process that was applied almost exclusively to the stabilization of sludge from treatment plants; however, in the 1980s AD found an important area of interest in the treatment of wastewater, especially wastewaters with high carbonaceous organic matter content such as those generated in agri-food industries [33]. This is consistent with the dates of the oldest publications indexed in Scopus used in this bibliometric analysis.

The aim of those investigations were mainly to decrease the pollutant load of the wastewater to optimize biogas production and/or to evaluate the efficiency of different reactor configurations [50,51]. Athanasopoulos et al. [50] evaluated at pilot scale the applicability of an upflow fixed-bed anaerobic reactor for the treatment of sulfite-rich wastewater from the raisin finishing process, obtaining chemical oxygen demand (COD) removals between 90 and 95%. The authors observed that, with small organic loads, VFA concentrations in the digester were less than 0.5 g/L and the biogas had 63% *v*/*v* CH_4.

Additionally, hydrogen production from petroleum compounds or fossil fuels involves the release of greenhouse gases (e.g., CO_2, CH_4) into the environment, which has made it necessary to investigate a renewable and environmentally friendly route for its production. Dark fermentation is considered one of the most appropriate methods for hydrogen production [52], which involves anaerobic microorganisms that grow in the dark and use carbohydrate-rich substrates (wastewater, solid waste, biomass).

Wu and Lin [53] evaluated the hydrogen production potential of molasses wastewater, with waste activated sludge from a municipal wastewater treatment plant (WWTP) as inoculum and a fermentation time of 104 h, to optimize the substrate concentration (10 to 160 g COD/L) and pH (4 to 8). The authors found that hydrogen production depended on the two parameters evaluated (substrate concentration and pH). In this study, hydrogen contents in the digestate gas of 49–58% and 20–27% (*v*/*v*) were observed for concentrations of 10–60 and 80–160 g COD/L, respectively, indicating some inhibition at higher substrate concentrations. In addition, hydrogen production was accompanied by VFA and solvents, among which butyric acid was present in the highest proportions, and the fraction (butyric acid) increased gradually from 7.8 to 17 g COD/L (41–62.5%) when the substrate concentration increased from 10 to 160 g COD/L. A substrate concentration of 40 g COD/L and a pH close to 6.0–7.0 were considered optimal for molasses wastewater fermentation.

Cluster No. 2 (green color) consists of the nodes of anaerobic fermentation, activated sludge withdrawal, denitrification, treatment plant sludge, acidification, hydrolysis, carbon source, food waste and short-chain fatty acids. This cluster represents research focused on the first stages of AD (hydrolysis and acidogenesis) to improve the production of VFA as a carbon source for the optimization of methanogenesis, the increase of AD stability [51] and the removal of nitrogen from wastewater or sludge fermentation liquid from a WWTP.

According to Dinopoulou et al. [51], the separation of the acidogenic and methanogenic phases represented advantages over conventional AD:

Increased reactor stability by avoiding methane overload because of adequate control of the acidogenic phase.

Maintenance of appropriate densities of acid and methane forming bacteria in separate reactors to maximize acidification and methanogenesis rates under optimal operating conditions.

The acidification reactor acts as a buffer system with wastewater of varying composition and helps to remove toxic compounds from methanogenic archaea.

The acidification reactor provides a constant substrate for methanogenic archaea, which adapt slowly to varying substrate content and composition.

Undoubtedly, the two-stage AD process is favorable because the conditions in the two reactors can be optimized separately for acidogenic and methanogenic microorganisms. However, the need for an additional large reactor for wastewater acidification can make the two-stage process more costly than the single-stage process [54].

Two-stage AD systems have been widely used for solid waste treatment [55–57], mainly with food waste as substrate [56,57]. Wang et al. [57] investigated a hybrid two-stage batch mode system for food waste digestion. The authors used two reactors for the acidification stage (inoculated with sludge from a WWTP anaerobic digester) and a methanogenic reactor (UASB type) (inoculated with sludge adapted to synthetic wastewater containing VFA). With each acidification reactor, one cycle (A and B) was carried out at 35 °C and times of 16 and 10 days, respectively. The results showed that the two-phase hybrid system is effective and efficient for the conversion of biodegradable food waste into biogas. The authors reported treatment efficiencies of 77–79% total organic carbon removal, 57–60% volatile solids removal and 79–80% total of COD reduction. Approximately, 99% of the total biogas yield came from the methanogenic phase with a high CH_4 content (68–70%).

On the other hand, WWTPs face the problem of the scarcity of carbon sources for denitrification, using as a carbon source the VFA obtained from the AF of the sludge (primary sludge and waste activated sludge). However, the AF of activated sludge withdrawal can present drawbacks related to the separation of VFA from nitrogen and phosphate, and low production efficiency, for which pretreatment and alkaline fermentation are effective alternative solutions[58].

Cluster No. 3 (blue color), formed by the nodes of fermentation, enhanced biological phosphorus removal, biological nutrient removal, activated sludge, pH, pre-fermentation, modeling and sequential batch reactor, includes research focused on the improvement of conventional treatment processes (e.g., activated sludge, UASB) to optimize the biological removal of nutrients (mainly phosphorus).

Enhanced biological phosphorus removal in the activated sludge process is a mechanism where the organisms present in the activated process remove a greater mass of phosphorus from the wastewater than they need for their basic metabolic purposes. For this removal to take place in the activated sludge process, the mixture must be subjected to anaerobic conditions (free of dissolved oxygen and nitrate/nitrite) and the influent from the anaerobic zone must have a certain concentration of simple carbonaceous substrates, with VFA such as acetate and propionate being the preferred substrates [59].

The production of VFA can take place in a separate fermenter (pre-fermentation) or directly in the anaerobic reactor of the treatment process [60]. Comeau et al. [61] demonstrated at the laboratory scale its feasibility and determined the maximum phosphorus removal potential of wastewater from a phosphorus-rich cheese industry (60 to 80 mg P/L), which was being treated in a plant composed of an anaerobic equalization tank, followed by a UASB reactor and aerated lagoons. In the laboratory, they used the effluent from the equalization tank and the UASB reactor in a sequential batch reactor. The authors observed that nutrient removal in the UASB reactor effluent was difficult due to the low VFA concentration, while it improved with the equalization tank effluent (higher VFA concentration), generating an effluent with a concentration of 5 to 10 mg P/L.

Several types of research have been directed to the kinetic and mathematical modeling of pre-fermenters or of the acidogenic stage [60,62,63] with the final aim of favoring the activity of phosphate accumulating organisms.

Cluster No. 4 (yellow color) consists of the nodes of acidogenic fermentation, microbial community, polyhydroxyalkanoates, resource recovery, sludge fermentation and primary sludge. This cluster represents the research where AF emerges as an interesting process in which, in addition to treating organic matter, favoring the biological removal of nutrients and mitigating the environmental impact, it produces chemical compounds that are currently generated by the petrochemical industry and have diverse applications in chemical and biological processes [1,19].

In recent years, there has been increasing research interest in the recovery of VFA from solid waste [64] and wastewater [21,65]. Research has aimed to evaluate and optimize operational parameters such as pH, temperature, organic loading rate, substrate concentration, hydraulic retention time and inoculum [14,21,66,67], through laboratory-scale experiments in continuous [21,22] and batch [24] reactors. VFA production presents additional challenges because the products are soluble and there is no automatic phase separation [68]. Therefore, the production of medium-chain acids, such as caproic acid, perhaps represents greater advantages than VFA because, being less soluble, their separation can be easier [69].

Other work has focused on the production of polyhydroxyalkanoates (PHA), which are bio-based and biodegradable polymers that are usually produced from expensive sugars or VFA; these biopolymers are considered an alternative for the replacement of traditional plastics (petroleum polymers) [70,71]. Elain et al. [70] evaluated two wastewaters rich in vegetable protein (legume processing water) and reduced sugars (fruit processing water) as a growth medium for PHA synthesis with a single marine bacterial species (*Halomonas* i4786). The authors demonstrated that agro-industry by-products offer an effective alternative to an expensive conventional culture medium (marine broth) to produce PHA. In that study, biomass was produced from the media studied to obtain PHA productivities of 1.6 and 1.8 g/L in 72 h for legume and fruit processing water, respectively. The results showed that these alternative media produce relative PHA contents (in relation to the dry mass of the cells) above 50% and reach almost 80% in the case of fruit processing water. For their part, Karaca et al. [71] investigated the optimization of PHA production from VFA produced in the fermentation of dairy wastewater and food waste, using activated sludge acclimated with domestic wastewater and VFA supplements under different conditions (feed–starvation regime and anaerobic–aerobic–anoxic operation). However, the authors demonstrated that the PHA accumulation potential of acclimatized activated sludge was mainly dependent on the VFA components available for polymer storage, rather than on the acclimatization conditions. In this case, the researchers obtained maximum PHA contents of 67.3 and 4.5% using VFA obtained from the acidification of solid food waste and dairy wastewater, respectively.

Finally, cluster No. 5 (purple color) includes as nodes two of the key terms used to obtain the information (volatile fatty acids and wastewater) and three keywords directly related to them (acetate, chemical oxygen demand and dairy industry wastewater). However, this cluster is small and does not clearly define a research approach related to the topic under study.

Figure 2.9 shows the areas with the most and least development, as well as the thematic areas where future scientific projects are most needed. The recovery of resources such as VFA (see the yellow nodes in Figure 2.9) from various substrates is an unconsolidated/emergent topic which requires further laboratory- and pilot-scale research to provide inputs for projecting this sustainable technology on a larger scale. It is perceived that the production of compounds from other substrates also rich in biodegradable organic matter (see item 2.3.4.3), to be used as a carbon source in subsequent processes and the evaluation of the microbial community involved in the AF process, are also emerging topics.

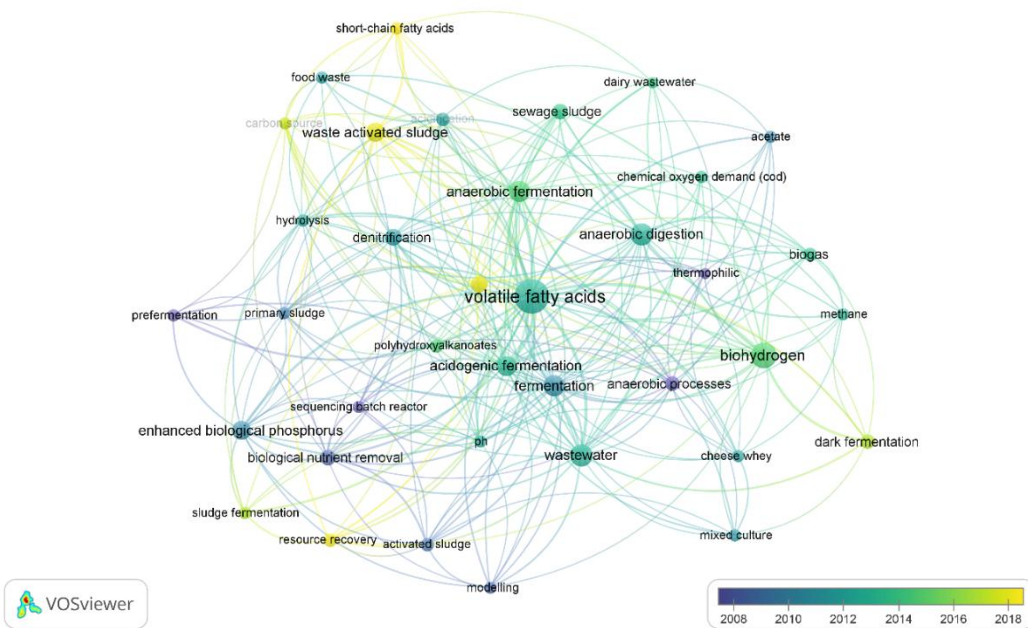


Figure 2.9 Bibliometric network for studies related to VFA and wastewater fermentation. The colors indicate the year in which the topic was most researched.

Therefore, this bibliometric analysis delves into the review of scientific literature related to the influence of the main operational parameters on the production of VFA from the AF of wastewater, since it is an indispensable aspect for understanding the phenomena involved in the process.

2.3.4.3 Operational Parameters of VFA Production by Wastewater Fermentation

Wastewater (real and synthetic) derived from agricultural or food sources, or more complex effluents such as municipal or industrial wastewater, have been used for VFA production through AF in laboratory and pilot scale experiments. Some examples include dairy wastewater [72–75], cheese whey [21,76], slaughterhouse wastewater [64], paper mill wastewater [21,64], winery wastewater [64], olive oil mill wastewater [77], saline cooked mussel processing wastewater [78], synthetic soft drink wastewater [79], wood mill wastewater [80], swine wastewater [54,81,82], sucrose-rich wastewater [83,84], municipal wastewater [85] and cassava processing wastewater [14,65].

The concentration, composition and efficiency of VFA production from the AF process depends on wastewater characteristics, reactor configuration and operational parameters such as pH, hydraulic retention time, temperature, organic loading rate and inoculum, among others [14,51,86,87]. Operational conditions largely determine which thermodynamically viable reactions and microorganisms can grow most efficiently [64,88], allowing researchers to exert some control over the product spectrum. This section describes the operating parameters that are commonly evaluated in studies related to the production of VFA through wastewater fermentation.

- Effect of pH on VFA production by AF

The pH in the reactor is a key factor in producing carboxylic acids as most hydrolytic and fermentative bacteria cannot survive in extremely acidic or alkaline environments [87]. pH value affects the growth of organisms, which influences the product spectrum, mainly of acetic, propionic and butyric acids, in this process [14,89].

The optimum pH to produce VFA can range from 5.25 to 11.0; however, the specific value will depend mainly on the type and characteristics of the substrate [87] and the species and enzymes involved in the process [68]. Gallert and Winter [89] reported that hydrolysis of proteins and lipids requires a neutral or slightly alkaline pH, while for carbohydrates a slightly acidic pH is desirable. An increase in the degree of hydrolysis is reflected in a higher concentration of total VFA. Niz et al. [65], who used wastewater from cassava processing, concluded that neither sludge adaptation nor heat treatment to eliminate methanogenic archaea activity was as relevant as pH in improving VFA production.

Table 2.2 lists several works showing the optimum pH for the production of carboxylic acids from different types of wastewater.

Table 2.2 Optimal pH for obtaining carboxylic acids from different types of wastewater.

Type of Wastewater	Substrate Concentration (mgCOD /L)	Optimum pH	Total Acids (mg/L)	Acid Composition	Degree of Acidification	Reference
Meat extract	2920	7.0	NA	Acetic acid (39%) Propionic acid (35%)	40%	[51]
Cheese whey	4590	5.25–5.5	2270	Acetic acid (43%) Butyric acid (42%) Propionic acid (15%)	83%	[21]
Pulp and paper	7740	5.5–6.0	3960	Butyric acid (78%) Acetic acid (9%) Propionic acid (6%) Others (7%)	76%	[21]
Cassava processing	6040 to 7600	5.50–6.09	5080 to 5760	Butyric acid (87%)	84.10%	[65]
Cassava processing	8865	5.9	4000	Butyric acid (42%) Acetic acid (35%) Propionic acid (20%)	63%	[14]

Table 2.2 Optimal pH for obtaining carboxylic acids from different types of wastewater (continuation).

Cassava processing	50,000 (glucose)	6.5	40,000 (lactic acid)	Lactic acid Acetic acid Propionic acid	NA Note: the paper was oriented only to the production of lactic acid.	[67]
Dairy wastewater	111,450 ± 1937	8	20,585	Acetic acid (17.5%) Butyric acid (61.1%) Propionic acid (9.3%) Valeric acid (12.0%)	NA	[90]
Cheese processing	20,000	5.0	3688	Propionic acid (24%) Acetic acid (21%) Valeric acid (20%) Butyric acid (17%)	93%	[91]
Cassava Processing	1000–25,440	5.5–6.0	200 to 4520	Lactic Acid (82%) Butyric Acid (6%) Acetic Acid (5%) Small concentrations of tartaric, succinic, propionic, iso-butyric and iso-valeric acids were detected.	NA	[92]
Cassava vinasse	36,000	8.0	24,000	Butyric acid (40%) Acetic acid (36.5%) Propionic acid (20%)	71%	[93]
Starch	19,400	8.0	8600	Acetic acid (68.3%) Propionic acid (15.5%) Butyric acid (13.8%)	68%	[93]
Agro-industrial (oil mill, cheese whey and cow manure slurry—55:40:5 *v*/*v*/*v*)	95,000	6.5	13,430	Butyric acid Propionic acid Acetic acid Ethanol Lactic acid	NA	[94]
Synthetic wastewater (with glucose) DQO:N:P—300:5:1	4000	6.0	2139.11 as mgHAc/L	Acetic acid (49.88%) Butyric acid Propionic acid	56.64%	[95]

NA: Information not available.

From the studies reviewed, it is perceived that, when wastewater is used as substrate, the optimum pH can vary between 5 and 8 (see Table 2.2). However, its specific value must be determined for each case since it depends on the substrate characteristics (e.g., substrate concentration) and possibly on other operational parameters (e.g., hydraulic retention time). The optimum pH range is generally very tight. Bengtsson et al. [21] identified an optimum pH for cheese whey wastewater of 5.25–5.5 and for pulp and paper industry effluent of 5.5–6.0, with acidification degrees of 0.83 and 0.76, respectively. For wastewater from cassava processing, VFA production generally happens under acidic conditions, with optimum values ranging from 5.0 to 6.5.

A considerable amount of research has been conducted on the optimal pH range to produce carboxylic acids from fermentation processes. The results are often diverse due to differences in the type of substrate, inoculum and other operating conditions adopted. In addition, the aim of the studies must be taken into account because, while a significant number of works are oriented towards identifying the pH necessary to optimize the total production of carboxylic acids, others seek the optimization of a specific acid. Da Silva et al. [67] used wastewater from cassava processing to optimize lactic acid production, while the work of Dareioti et al. [94], despite having evaluated VFA production, took optimize hydrogen production as its main objective.

The pH value influences the composition of the carboxylic acids. Bengtsson et al. [21] observed—for cheese whey wastewater—that the acid composition was stable between pH 3.5 and 5.25. When the pH value increased from 5.25 to 6, the amount of acetate and butyrate decreased and the amount of propionate was favored (51:19:24 to 31:41:10 acetic:propionic:butyric). In the case of pulp and paper industry effluent, butyrate and propionate increased with pH in the range of 4.9–6.0, varying the VFA composition from 42:13:18 to 22:23:33 in % acetic:propionic:butyric. Dareioti et al. [94] observed an increase in propionic acid concentration from 0.09 to 4.02 g/L when the pH varied from 4.5 to 7.5. Wu and Lin [53], who evaluated hydrogen production from molasses wastewater, observed that a pH between 4 and 5 favored acetic acid production (50.6–55.4%), while a pH between 6 and 8 increased butyric acid production (47.2–58.7%). According to Guo et al. [96] metabolic pathways involving the production of acetic and butyric acid are favored at pH ranging from 4.5 to 6.0, while a neutral or higher pH favors ethanol and propionic acid production.

- Effect of Hydraulic Retention Time on VFA production by AF

The hydraulic retention time (HRT) represents the average time that the substrate remains in the reactor. It can vary between 0.5 to 10 days depending mainly on the characteristics and type of substrate as this considerably influences the rate of hydrolysis of particulate organic matter [87]. The HRT should be sufficient to allow the solubilization of complex organic matter and favor subsequent AF. A very long HRT, on the other hand, will reduce the amount of substrate available per day, making it easier for methanogenic archaea to develop at an appropriate pH [97].

Dinopoulou et al. [51] observed a tendency for the degree of acidification to vary proportionally with HRT. However, the authors state that there is a critical time that may vary slightly with substrate concentration and under which the degree of acidification drops sharply. Of the parameters evaluated (HRT, substrate concentration and organic loading rate), the authors identified HRT as the most statistically important variable for predicting the degree of acidification and indicated the need for long HRT, even for less concentrated substrates. Fang and Yu [75] used dairy wastewater and observed that VFA production nearly doubled when HRT increased from 4 to 12 h, but when it varied from 16 to 24 h the production only increased by 4 to 5%. For the authors, this behavior may be due to the complexity of the wastewater (composition and concentration) since more than 70% of the glucose, starch or other easily biodegradable carbohydrates could be acidified in less than 12 h.

Ben et al. [98] evaluated the possibility of producing bioplastics from wood mill wastewater. The AF tests were performed in a continuous stirred tank reactor (CSTR) at pH 5.5, 30 °C and HRT of 1 and 1.5 d, observing that the concentration of VFA in the effluent was similar for the two HRT tested, with acidification degrees of 37 and 42%, respectively. However, the VFA composition was different, with an increase in the concentration of long-chain VFA at higher HRT evaluated.

Khan et al. [22] investigated VFA production from low-strength wastewater at different HRT and organic loading rates in a continuous anaerobic membrane bioreactor using glucose as carbon source, no inhibition of methanogenesis, pH of 7 ± 0.1, constant influent COD of 550 mg/L and organic loading rate of 68.75 mg COD/(L.h). The authors evaluated HRT of 48, 24, 18, 12, 8 and 6 h. The results showed that the overall VFA yield in the reactor (mg VFA/mg COD in the feed) changed from 48 to 24 h, registering a rapid increase in the yield (13.39 ± 1.21 to 32.88 ± 2.56%). The influent carbon content increased at the shortest HRT and VFA yield increased to 48.20 ± 1.21% at 8 h. However, a further decrease in HRT (6 h) caused a drop in the overall VFA yield to 42.32 ± 2.32%, indicating that this was an insufficient contact time between the microorganisms and the substrate.

Likewise, Lv et al. [95] varied the HRT between 12 and 2 h for VFA production from synthetic wastewater with glucose as sole carbon source at pH 6, 35 ± 1 °C and 4000 mg COD/L in a CSTR. The results showed that, when the HRT gradually decreased from 12 to 6 h, the VFA concentration progressively increased and peaked at HRT of 6 h with value about 2540 mg HAc/L and an acidification degree of 68.28%. Consistently, when HRT was reduced to 4 h, the VFA concentration decreased abruptly and achieved the minimum of 1892.90 mg HAc/L.

Most studies on AF of wastewater were conducted in batch bioreactors [14,65,66]. Therefore, there is limited information in the scientific literature evaluating the effect of HRT on the production of carboxylic acids, mainly if wastewater from cassava processing is treated. However, some studies that did not assess the impact of HRT reported the fermentation time in which the maximum concentration of organic acids was observed. Da Silva et al. [67] aimed to evaluate the effect of initial pH on the lactic acid production from cassava processing wastewater in 48 h experiments. The authors indicated a rapid decrease in pH to 4.5 within 6 h after starting fermentation, but after 24 h pH remained stable between 4.0 and 4.5 until the end of the process. In this case, the pH value can be considered an indirect indicator of the amount of organic acids present in the medium.

Controlling HRT can favor specific microbial populations. Because other operating parameters (for example, substrate concentration, pH and temperature) also affect mixed cultures, the HRT will affect not only the total concentration of organic acids but also their composition, especially when using pure cultures (Arslan et al., 2016a).

Demirel and Yenigun [74] evaluated the production and distribution of VFA from dairy wastewater at the laboratory scale in a continuous-flow, complete-mix acidogenic reactor coupled to a conventional gravity settling tank and a continuous recycling system. The authors varied HRT between 24 and 12 h, and up to an organic loading rate of 9.3 kg COD/(m^3.d), without pH control and at 35 ± 1 °C. The results showed a gradual increase in VFA production, proportional to the organic loading rate, with decreasing HRT. The highest degree of acidification and VFA production rate was 56% and 3.1 g/(dm^3.d), respectively, at 12 h of HRT. The VFA produced were acetic, propionic, butyric and valeric, with a higher production of propionic acid for the optimum HRT of the evaluated range. However, in the experiments performed by Lv et al. [95], the VFA were mainly composed of acetate, butyrate and propionate. In addition, HRT reduction favored butyrate production and formate retainment.

- Effect of Organic Loading Rate on VFA production by AF

Organic loading rate (OLR) refers to the amount of substrate fed to the bioreactor per day per unit volume in terms of total solids (TS), volatile solids (VS) or COD [97]. The ORL combines the effect of substrate concentration and HRT, and determines the feed–microorganism ratio [68].

Theoretically, increasing the ORL can generate an increase in the concentration of carboxylic acids; however, studies show that this is not always the case and that the effect of this variable on acid production also depends on the type and characteristics of the substrate [51]. Dinopoulou et al. [51] conducted experiments with meat extract wastewater and constant HRT. They observed a tendency for the degree of acidification to vary inversely with OLR and initial substrate concentration. However, the authors reported that changes in the degree of acidification were not significant in most of the experiments as it remained almost constant for COD loads of 9.4 and 72 g/(L.d).

Azbar et al. [99], in experiments aimed at hydrogen production from the fermentation of cheese whey wastewater under thermophilic conditions (55 °C) and pH 5.5 ± 0.05, evaluated the effect of different ORL (47, 35 and 21 g COD/(L.d)) in a CSTR and constant HRT of 1 d. The authors observed in the effluent a concentration of VFA ranging from 118 to 27,012 mg/L and mainly composed of acetic acid, iso butyric acid, butyric acid, propionic acid, formate and lactate. The most abundant VFA in all OLR tested was lactate, as lactose was converted to lactic acid by mixed culture instead of acetic acid being formed. The second most abundant VFA in the experiments was formate.

Other studies mention the influence of ORL on VFA composition and point out that low values (5 g ST/(L.d)) favor the production of propionic and butyric acids, while high values (16 g ST/(L.d)) promote the production of acetic and valeric acids [100]. Additionally, the VFA composition may depend on how the ORL is adjusted. According to Arslan et al. [68], in the range of 5 to 240 g COD/(L.d) when a high ORL is achieved by increasing the substrate concentration, the carboxylate concentration increases and the product type tends to be reduced. In contrast, when a high ORL is achieved by decreasing the HRT, hydrolysis may be less effective, resulting in a lower concentration of total VFA and/or shifts towards more acetate, because only fast-growing bacteria such as acetate or lactate-producing bacteria are retained in the reactor.

Operating a reactor with a high ORL (11 to 16 g ST/(L.d)) must consider the presence of inhibitory substances in the substrate as it can affect not only methanogenic archaea but also hydrolytic and fermentative bacteria [97].

Khan et al. [22] evaluated ORL of 43.75, 68.75 and 89.38 mg COD/(L.h) adjusting the input COD (350, 550 and 715 mg COD fed, respectively) in wastewater with glucose as carbon source, HRT of 8 h, pH of 7 ± 0.1 and temperature of 22 ± 1 °C. The maximum acetic and propanoic acid concentrations were obtained (1.1845 ± 0.0165 and 0.5160 ± 0.0141 mmol/L, respectively) at 550 mg COD, the concentration at which the highest VFA production yield was also observed. The isobutyric acid concentration was highest (0.3580 ± 0.0407 mmol/L) at 715 mg COD fed, indicating butyric-type fermentation at a higher ORL.

- Effect of Temperature on VFA production by AF

Temperature influences microbial growth and metabolism, and most fermentative bacteria cannot survive in extreme temperatures. Each type of microorganism has an optimal temperature range; therefore, a change in bioreactor temperature can alter the structure of the microbial consortium involved in AF [97].

Reviewing the scientific literature reveals a wide range of outcomes. Dinopoulou et al. [51] evaluated the effect of temperature on the percentage of acidification using meat extract wastewater as a substrate and observed an increase with increasing temperature in the range studied (25–40 °C). For their part, Hasan et al. [14] evaluated the effect of alkalinity and temperature in the same range on VFA production from wastewater of cassava processing and observed a negative effect of temperature on VFA production, indicating higher yields at a lower temperature (30 °C).

Infantes et al. [101] concluded that pH, temperature and concentration of non-dissociated acids (formic, acetic, propionic, lactic, butyric acid) influence the fermentation process (oriented to hydrogen production), as they had a significant effect on cell growth and substrate consumption. The authors observed less biomass growth as temperature increased. At pH of 6, the biomass increased up to 1.4 and 0.9 g/L when the temperature was 26 and 40 °C, respectively, showing a similar behavior at pH 4 and 5, although at these pH values the increase in biomass growth was negligible. Regarding substrate consumption, the effect was more important at pH 4 and temperature values above 33 °C, where glucose was not completely consumed, and biomass growth decreased due to the undissociated acid concentrations reached (50 to 70 mM).

In addition, the concentrations of undissociated acids affected fermentation exponentially, mainly at high temperatures (40 °C), because the higher the temperature the higher the permeability of the cell membrane, and the undissociated acids can pass through it more easily.

According to the review by Lee et al. [87], the effect of temperature on VFA composition appears minor when compared to pH, challenging the widespread idea that microbial composition changes with temperature. The authors raise the concern of whether similar microbial species are present at different temperatures or whether there are different microbial species that produce similar types of VFA, being relevant to examine the microbial community involved in AF at different temperatures. Khan et al. [102] also mentioned that the type of VFA produced does not alter much when the temperature is changed during VFA production.

Kim et al. [54] investigated the influence of HRT and temperature on VFA production in thermophilic acidogenesis of swine wastewater. The authors verified through ANOVA of the response surface methodology that VFA produced by acidogenic bacteria were more affected by HRT than by temperature. However, from the qualitative analysis of the microbial consortium, they observed that the changes were due more to temperature variation than to HRT. The authors deduced that VFA production was affected by the physicochemical properties of the acidogenic microorganisms (specific growth rate or contact time with the substrate), rather than by the change in microbial diversity.

Additionally, Yang et al. [82] conducted experiments to optimize VFA production in a laboratory-scale CSTR from swine wastewater under mesophilic conditions. Initially, the authors evaluated different concentrations of ammonia nitrogen, finding that a concentration lower than 1.2 g/L did not significantly affect the biochemical acidogenic potential of the swine wastewater. Additionally, the authors used a response surface as a statistical methodology, simultaneously varying HRT (1 to 3 d) and temperature (25 to 35 °C), and keeping the substrate concentration at 80,000 mg COD/L for all trials. The results showed optimal physiological conditions for acetic and butyric acid production of 2.4 d of HRT at 34 °C and 2.1 d of HRT at 35 °C, respectively.

- Effect of Inoculum on VFA production by AF

The biomass of acidogenic reactors and anaerobic digesters is rich in acid-producing organisms and methane-producing organisms, requiring pretreatment to prevent methanogenic archaeal activity if this control is not done by managing some operational parameter, e.g., HRT [68]. The most common pretreatment methods are thermal [65,66,103], acid/alkali conditioning and chemical inhibitors [24,104,105]. Pretreatments may not be as cost-effective or technically feasible at the industrial level, but they do allow for increased production of organic acids from a given substrate, if there is a risk that these are consumed for methane production [68].

Amorim et al. [66] evaluated the effect of methanogenesis inhibition on the production of carboxylic acids and hydrogen from cassava processing wastewater and indicated that chemical (with acetylene) and thermal treatment effectively inhibited methanogenesis, avoiding methane production. However, the maximum hydrogen production potential (~563 mL) upon application of the heat treatment was more than twice that of the acetylene treatment (~257 mL). Moreover, butyrate was the main carboxylic acid produced (~3 g/L). Furthermore, Niz et al. [65] investigated, in batch reactors, the effect of inoculum adapted and not adapted to cassava wastewater, collected from the upflow anaerobic reactor of a beverage industry, with and without methanogenic archaea inhibition techniques. The study aimed to improve the production of VFA by fermentation of cassava processing wastewater. The authors observed that in non-adapted sludge an acidification of 84.1 and 66.1% was obtained for thermally inhibited and non-inhibited conditions, respectively, 10% and 45% higher than the acidification degrees obtained in the same conditions with adapted sludge.

Amorim et al. [66] evaluated three types of inoculums obtained from anaerobic sludge: municipal WWTP sludge, bovine rumen and WWTP sludge from the textile industry. The authors observed that the best inoculum was bovine rumen, which produced the highest amount of butyric acid at the lowest substrate concentrations (10 and 20 g O_2/L); however, they observed a decrease after 20 days of operation, as acetic acid became available. This behavior was like that observed by Hasan et al. [14], but after 32 h of operation and using sludge from an anaerobic digester treating swine manure as inoculum. The consumption of hydrogen and subsequent decrease in the partial pressure thermodynamically favored the conversion of butyric acid to acetic acid [66].

- Effect of Substrate Concentration on VFA production by AF

Although the product concentration increases as the substrate concentration in the reactor rises, the increase in VFA concentration is limited when the feed level exceeds 40 g COD/L due to overload or inhibition [68]. However, some studies show that inhibition can occur at lower concentrations. Dinopoulou et al. [51] conducted experiments with influent COD concentrations of 3.15 and 12 g/L and similar HRT and showed that the degree of acidification achieved during the first series (3.15 g/L) was significantly higher than that obtained during the second (12 g/L), evidencing a negative effect at higher initial substrate concentrations.

Da Silva et al. [67] evaluated the effect of initial pH and five glucose concentrations (10, 15.8, 30, 44.2 and 50 g/L) on lactic acid production from cassava processing wastewater. In the experiments with higher initial glucose concentrations, the authors observed a stabilization of fermentation 24 h after the start of the process. Nevertheless, in the experiments with lower glucose concentration, there was a rapid decrease in the first 12 h and it remained stable until the end of the experiment. Statistical analysis showed that only substrate concentration had a significant effect on lactic acid production at the 95% confidence level. High substrate concentrations (50 g/L) favor lactic acid production.

Wu and Lin [53] evaluated hydrogen production from molasses wastewater with concentrations ranging from 10 to 160 g COD/L, at 35 °C, pH 6 and 104 h of fermentation, but during the experiments

VFA production was monitored. The results showed butyric acid, acetic acid and ethanol as the dominant liquid fermentation products. The butyric acid fraction gradually increased from 7831 to 17,000 mg COD/L (41.0–62.5%) when substrate concentrations increased from 10 to 160 g COD/L.

The initial substrate concentration can also influence the composition of VFA in the reactor. The number of electrons available per cell and the $NADH/NAD^+$ ratio in the reactor increase as the substrate concentration rises, and an excess of electrons and NADH favors reactions in which NADH can be consumed, leading to the formation of more reduced compounds such as ethanol, n-butyrate or n-caproate instead of acetate and propionate [68].

Yu and Fang [106] performed experiments to evaluate the acidogenesis of synthetic dairy wastewater in batch reactors at pH 5.5 and 55 °C, with feed solutions containing 2, 4, 8, 12, 20 and 30 g COD/L. For all concentrations tested, the authors identified acetic, propionic and butyric acids as the main VFA. The concentrations of propionic acid and its time to peak increased with increasing substrate concentration. However, for acetic and butyric acid concentrations a different behavior was perceived. The concentrations of the acids increased rapidly at the beginning, peaked at some point during the experiment, and then decreased in reactors with initial concentrations equal to or greater than 8 g COD/L. A decrease in the degree of acidification from 49.6% at 2 g COD/L to 30.4% at 30 g COD/L was observed in this study, allowing the authors to conclude the significant influence of substrate concentration on AF.

Despite the large amount of research that has been oriented towards evaluating the effect of operational parameters on the production of VFA from wastewater, there is still much diversity among the results. This confirms the need for further research and improved understanding of the phenomena involved in the process, before its application at pilot and real scale, mainly when less explored substrates such as wastewater from cassava processing are used. In addition, it is worth noting that a complete physicochemical characterization, mainly of the organic content, can alleviate efforts to transform wastewater into value-added by-products.

Finally, since several recent studies of VFA production by AF of wastewater from cassava processing were found during the analysis of the information retrieved in this bibliometric analysis, it may be considered an emerging topic in this field.

2.3.4.4 Modeling of Acidogenic Fermentation

Modeling is a hypothetical approach used to explain biochemical processes. Models can be classified based on various criteria, including their characteristics (kinetic, parametric, non-ideal reactor), prior knowledge of the process (mechanistic, empirical, hybrid), and the level of detail (structured, unstructured, segregated, non-segregated) [107–109].

The understanding of AF is enhanced through models that facilitate the estimation of kinetic and stoichiometric parameters [25,66,105]. In the context of wastewater resource recovery, modeling provides valuable insights for developing new bioreactors, predicting reactor performance, supporting pilot-scale studies, and optimizing fermentation process [25,110].

Several studies have modeled VFA production from wastewater fermentation. The scientific literature includes research that has developed new models or modified existing ones. Münch et al. [63] developed a model to describe VFA production from the anaerobic fermentation of wastewater, aiming to optimize pre-fermenter design and operation while improving predictions of biological phosphorus removal. The model represented real processes in pre-fermenters, accounting for the influence of methanogenic archaea but excluding sulfate-reducing bacteria. It consisted of nine differential equations and accurately described the effects of HRT, solids retention time (SRT), and influent characteristics on VFA concentration, soluble COD, and ammonia nitrogen in the effluent.

Martin Ruel et al. [60] developed a model to describe AF under varying conditions, including the presence or absence of sulfate-reducing bacteria and the exclusion of methanogenic archaea. They adjusted the model using experimental data from Biochemical Acidogenic Potential tests, with the same objective as Münch et al. [63]: optimizing biological phosphorus removal. The model incorporated 11 state variables, 18 parameters, and 9 processes, primarily representing anaerobic hydrolysis of particulate organic matter, fermentation of soluble substrates, sulfate reduction from butyric and propionic acids, acetic acid mineralization to CO_2, and microbial decomposition.

Other studies have assessed pre-existing models—including first-order, first-order with residual, second-order, logistic, Monod with growth, Fitzhugh, Monomolecular, Modified Gompertz, Transfer, and Richards—to determine which best fits experimental data. To this purpose, statistical indicators such as the coefficient of determination, root mean square error, and Akaike information criterion have been applied [24,25,101,105].

Coelho et al. [24] conducted batch reactor experiments using wastewater from the dairy industry, an inoculum from a brewery's UASB reactor, and inhibited methanogenesis with 0.05% (v/v) chloroform. The first-order kinetic model with residual provided the best fit for particulate organic matter hydrolysis and substrate consumption curves. Meanwhile, kinetic models describing an exponential phase, such as first-order and Fitzhugh, effectively represented VFA production. In that study, the hydrolysis rate constant (K_H), soluble substrate degradation rate constant (K_B), and VFA production rate constant (K_{AC}) were 0.47 ± 0.50 d^{-1}, 0.86 ± 0.31 d^{-1}, and 0.60 ± 0.06 d^{-1}, respectively. The process yield was 0.66 $g_{AC}/gCOD_A$, corresponding to 0.83 $gCOD_{AC}/gCOD_A$.

Morais et al. [25] found that the first-order kinetic model with residual best fit both the hydrolysis curve of particulate organic matter and the substrate consumption curve when bovine slaughterhouse wastewater was used. According to the authors, mathematical models describing exponential growth, such as the first-order exponential model, the cone model, and the Fitzhugh model, were the most suitable for representing VFA production kinetics. In these experiments, the K_H, K_B, and K_{AC} constants were 0.15 ± 0.04 d^{-1}, 0.26 ± 0.07 d^{-1}, and 0.30 ± 0.07 d^{-1}, respectively. The process yield was 0.55 $g_{AC}/gCOD_A$, corresponding to 0.76 $gCOD/gCOD_A$.

Additionally, various models and approaches have been applied to fermentation, including the Monod model [107,111,112], ADM1 [113,114], AM2 [115,116], Gompertz [66], Michaelis-Menten [21], and artificial neural networks [117,118]. Hybrid models [119] and bioenergetic approaches [27,120] have also been evaluated. While these studies provide valuable tools for AF modeling, many focus on biogas or hydrogen production, as well as nutrient removal optimization. Few have specifically addressed fermentation and VFA production using wastewater, highlighting a gap in AF modeling, particularly for wastewater derived from cassava processing.

Finally, the true value of a model lies in its ability to predict, evaluate, and explore various scenarios related to the system and its environment. A model should be viewed primarily as a tool for answering questions and as a complement or alternative to laboratory experiments [121].

2.3.5 Future Perspectives and Challenges

In the 21st century, providing clean, accessible and reliable sources of energy and chemicals has become a priority from both socioeconomic and environmental perspectives [45]. To manage this problem, it has been shown that low-cost renewable resources such as solid waste and wastewater from various sources (e.g., industrial, agricultural, etc.) can be an ideal source of raw material for the production of valuable compounds such as VFA. These are produced synthetically from petrochemical derivatives, a production method supplying about 90% of this market [2,122].

Undoubtedly, technologies normally responsible for eliminating pollutants from wastewater (e.g., cheese whey, swine, dairy, cassava processing, etc.), which have a high biodegradable organic load, should be oriented towards the generation of products to be incorporated into a circular economy. A massive change in current practices is not expected in the short term, but more research focused on improving these technologies with a focus on recovery rather than compound removal is a priority.

It is in this context that AF should be considered an alternative and advanced strategy which consists of stopping methanogenesis in the conventional AD process to favor the production of VFA. These are direct precursors of energy or higher value-added products when subjected to downstream processes, playing an important role in the future market of sustainable energy and green chemistry technologies, and representing an important process if the resource–waste–resource cycle is to be completed [17].

However, major obstacles have been identified in this issue, such as lack of control of metabolic activities, the persistence of methanogenic microorganisms in the biological production of VFA, maximizing the production of VFA in appropriate proportions to facilitate their subsequent use, limitations for the extraction of produced acids, developing integrated systems, to detect the genetic expression of microorganisms and regulation of key enzymes in order to increase the specificity and yield of specific products [16,27,29].

Limited understanding is perceived of the mechanisms involved during metabolic changes, i.e., changes in the product range, which mainly depend on operational parameters such as HRT, pH, temperature, nature and concentration of the substrate, nutrient composition and availability, and partial pressure [1,19,30]. Optimizing the biological process—that is, finding the perfect combination of operational parameters to maximize the product—can be achieved by combining experimental studies with modeling tools. According to Lorenzo-Llanes et al. [31], an understanding and description of the acidogenic stage of AD would improve with the use of process simulation tools, allowing predictions and optimization in less time and at lower cost, compared to laboratory- or real-scale work.

Concerning the extraction of the produced acids, it is interesting to evaluate the biological process of chain elongation to favor the transformation of short- to medium-chain carboxylic acids, since their chemical properties would make them easier to separate from the fermentation broth. Furthermore, according to de Sousa e Silva et al. [2] the added value of short-chain carboxylic acids (400–2500 US$/ton) is lower than the market price of medium-chain carboxylic acids (2000–2500 US$/ton).

Although the optimal set of operational parameters to produce VFA and their separation from the fermentation broth remains a challenge, their high energy potential and competitive cost make them a strong contender for AD technology [15].

Few pilot-scale studies have been conducted using wastewater as a substrate for VFA production. The pilot scale is an intermediate stage in which the operating conditions (e.g., type of regime, agitation speeds, etc.) of the processes are as stable as possible in order to establish the most optimal operating parameters, as well as to determine the economic feasibility of the project. In addition, it is necessary to evaluate the reactor configuration (batch, continuous, semi-continuous, etc.) that best suits the desired application.

Finally, bioaugmentation strategies should be considered to identify bacterial strains that participate in a specific metabolic pathway and produce an acid of interest, isolate them, promote their growth and introduce them into the acidogenic reactor. This in order to favor the predominance of a particular microorganism within the reactor.

Therefore, due to the economic and environmental advantages of this field of knowledge, an accelerated development of research in this area is expected in the near future.

2.4 Conclusions

The bibliometric networks showed five clusters, which identified thematic groups where VFA production through wastewater fermentation is a key aspect. Methane production through AD, hydrogen production in dark fermentation, AD in two-phase reactors, biological nutrient removal, generation of value-added chemicals and energy production in bio-electrochemical systems were the main research focuses identified.

Additionally, in the context of resource recovery from wastewater, VFA production is an emerging issue and requires further research, mainly when less explored substrates such as cassava processing wastewater are used.

The technological challenges in this field of knowledge should be mainly oriented towards always trying to maximize VFA concentration, avoid product inhibition, modeling VFA production, and direct the fermentation process towards the production of a specific VFA depending on the desired application; all this in order to reduce costs associated with the concentration and purification of VFA.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table 2S1: The top-10 cited publications in the field of VFA and wastewater fermentation.

Author Contributions: Conceptualization, J.A.R.-V. and H.R.-M.; methodology, L.M.S.-L. and H.R.-M.; software, L.M.S.-L.; validation, L.M.S.-L.; formal analysis, L.M.S.-L.; investigation, L.M.S.-L.; resources, L.M.S.-L.; data curation, L.M.S.-L.; writing—original draft preparation, L.M.S.-L.; writing—review and editing, J.A.R.-V. and H.R.-M.; visualization, L.M.S.-L.; supervision, J.A.R.-V. and H.R.-M.; project administration, H.R.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its supplementary information file.

Conflicts of Interest: The authors declare no conflict of interest.

3 Effect of Fermentation Time, pH, and Their Interaction on the Production of Volatile Fatty Acids from Cassava Wastewater

Published as: Sanchez-Ledesma, L. M., Rodríguez-Victoria, J. A., & Ramírez-Malule, H. (2024). Effect of Fermentation Time, pH, and Their Interaction on the Production of Volatile Fatty Acids from Cassava Wastewater. Water, 16(11), 1514. https://doi.org/10.3390/w16111514

Abstract

Acidogenic fermentation is a technology that involves halting methanogenesis in the conventional anaerobic digestion process to produce mainly volatile fatty acids (VFAs). VFAs serve as direct precursors to energy-rich or higher value-added products upon undergoing additional processing. In this study, batch reactors were utilized to assess the individual and interaction effects of fer-mentation time and pH variables on VFA production from acidogenic fermentation of cassava wastewater through the establishment of a completely randomized design and a second-order response surface (rotatable central composite design), respectively. The maximum VFA production observed was 3444.04 mg of acetic acid (HAc)/L (0.58 $gCOD_{VFA}$/gCOD) in a fermentation time of 6 days, with acetic (48.5%), propionic (28.3%), and butyric (13.6%) acids identified as the main metabolites. Additionally, in the assessment of the effect of pH, the maximum VFA production reached 2547.72 mgHAc/L (0.34 $gCOD_{VFA}$/gCOD) at pH 5.9, and acetic acid was identified as the predominant organic acid. Statistically, the fermentation time and pH variables individually affect VFA production from cassava wastewater; however, the interaction between them generated a non-significant effect.

Keywords: acidogenic fermentation; volatile fatty acids; fermentation time; pH; cassava wastewater

3.1 Introduction

For several decades, cassava (*Manihot esculenta Crantz*) has been considered a valuable source of food, employment, and income for many farming communities in developing countries [4]. Worldwide, the largest cassava production is in Africa (63% of the global volume), with more than 208 million tons in 2022, followed by Asia (29%) and Latin America (7%) [123].

In Colombia, cassava and the products derived from its processing, such as starch, have become the basis of the economy for different populations. Nevertheless, the production process generates potentially polluting effluents due to the high organic load, cyanide concentrations, and pH. These effluents can cause a significant deterioration in the receiving water sources if discharges arrive without prior treatment. Consequently, the water bodies become unfit for other uses such as human consumption, fishing, and recreation [8,124].

Proper management of wastewater from cassava processing can reduce contamination of the ecological environment and promote the development of a circular economy. Previous studies have highlighted anaerobic digestion (AD) as a viable treatment method [125,126]. However, in recent years, there has been growing investigation interest in treating it by means of acidogenic fermentation (AF) [14,65,67], a stage within conventional AD.

AF not only reduces organic matter and mitigates environmental impact but also generates value-added chemical compounds, such as volatile fatty acids (VFAs). VFAs have diverse applications in chemical and biological processes (e.g., biological nutrient removal, bioplastic production, feedstock in the pharmaceutical, chemical, and food industries, etc.) [16,28] and are usually refined from petroleum. Petroleum-based production methods supply about 90% of the demand of this market [2]. These methods involve the use of high temperatures, pressures, and metals as catalysts [127], as well as greater contribution to environmental pollution, climate change, and scarcity of raw materials [128].

Therefore, biological routes of VFA production have gained considerable attention, as they can use renewable carbon sources (e.g., solid waste and wastewater) as a substrate. Consequently, these processes are more environmentally friendly and become an interesting alternative to replace petrochemical refining [129,130].

Studies in this field typically explore the influence of operational and environmental factors on the process, such as pH, hydraulic retention time (HRT), solids retention time (SRT), temperature (T), organic loading rate (OLR), substrate to microorganism (S/M) ratio, headspace partial pressure, and inoculum [91,95,131,132]. Adequate control of these operational variables is important to ensure high yield and composition of VFA production.

AF of cassava wastewater (CWW) aimed at direct VFA production has been poorly studied [14,65,67]. Hasan et al. [14] evaluated the effect of temperature (25 to 40 °C) and alkalinity (1 to 3 g/L of sodium bicarbonate) on VFA production, while Niz et al. [65] investigated the effect of CWW adapted and non-adapted inoculum with and without methanogenic archaeal inhibition techniques in order to improve VFA production. Additionally, the study by da Silva et al. [67] aimed to evaluate the impact of pH (4.5 to 7.0) and initial substrate concentration (10 to 50 g/L in terms of glucose) on lactic acid production yield particularly. Therefore, this work highlights the significance of understanding the influence of fermentation time (FT) and pH value in batch reactors, given the scarcity of studies on these variables in the scientific literature for CWW as a substrate. Undoubtedly, FT and pH are critical factors that affect the performance and composition of VFAs and can be directly modified during the operation of the fermentation system [76,95]. Regarding FT, it is desired that it be as short as possible (maximizing the production of VFAs), because if it is reduced, productivity is increased. The FT is notably influenced by substrate characteristics and type, significantly impacting the hydrolysis rate of particulate organic matter [97]. Furthermore, the pH has a significant influence on fermentative bacteria as it affects the activity of certain essential enzymes and metabolic pathways [101]. Operating at lower pH levels can reduce the need of basic chemicals for pH control, especially in wastewater with relatively low alkalinity, thus enhancing the economic feasibility of large-scale technology application [133].

Therefore, the aim of this study was to assess the individual and interactive effects of FT and pH on the production and composition of VFAs through AF of CWW using a microbiome with thermal inhibition of methanogenesis. This was achieved by the establishment of a completely randomized design and a second-order response surface (rotatable central composite design), respectively. These findings may provide a theoretical basis for controlling and optimizing VFA production from CWW.

3.2 Materials and Methods

3.2.1 Substrate

Wastewater from the cassava starch extraction process was used as a substrate for the AF process. The substrate was physicochemical characterized and stored at 4 °C for less than 24 h to preserve its properties.

3.2.2 Inoculum

Sludge from an Upflow Anaerobic Sludge Blanket (UASB) reactor at a wastewater treatment plant from a pig slaughterhouse was used.

The inoculum was progressively adapted to the substrate, aiming to obtain an enriched inoculum of acidogenic microorganisms capable of transforming the carbohydrates present in the CWW into VFAs. After adaptation, the inoculum was subjected to a heat pretreatment consisting of a temperature of 85 °C for 30 min [134] before starting the experiments.

3.2.3 Acidogenic Fermentation Experiments

Batch flow amber glass flasks with 400 mL total volume (200 mL reaction volume and 200 mL of headspace) were used as acidogenic reactors. Three experiments (E_1, E_2, and E_3) were performed to evaluate the individual effect of FT, pH, and the effect of their interaction, respectively. In addition, for each experiment, a blank reproducing the test with distilled water instead of the substrate was established.

The substrate and inoculum were placed in each reactor at an S/M ratio of 4 gCOD/g volatile solids (VS). The initial pH of the mixture was adjusted using drops of sodium phosphate solution (Na_2HPO_4, Thermo Fisher Scientific Inc., Waltham, MA, USA) or hydrochloric acid (HCl, Merck KGaA, Darmstadt, Germany). The pH values were selected based on the results of previous studies [134,135]. A magnetic bar was inserted into each reactor to promote complete mixing, and a cap with 2 NaOH (Merck KGaA, Darmstadt, Germany) pellets for CO_2 absorption was placed. Then, the reactors were sealed with rubber septa and aluminum seals. Finally, the reactors were incubated at a temperature of 34 ± 1 °C during the established FT. Figure 3.1 illustrates the experimental unit used in all experiments.

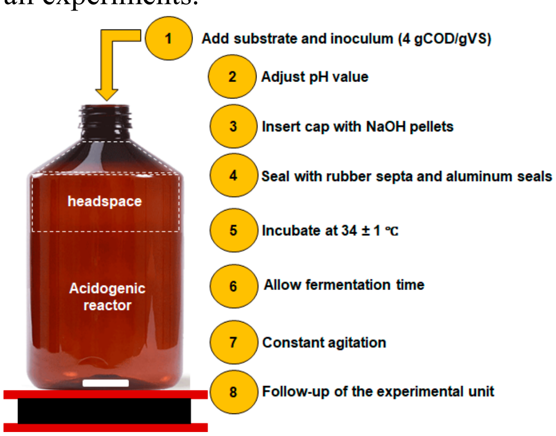


Figure 3.1 Experimental unit used in the AF of CWW.

The internal pressure of each reactor was regularly monitored. In addition, at the end of the experiment, a sample of the liquid phase was collected and characterized for pH, total solids (TS), VS, soluble chemical oxygen demand (SCOD), total VFAs, total alkalinity, bicarbonate alkalinity, total carbohydrates, and the composition of VFAs.

The yield for each evaluated experimental condition was calculated by using Equation (3.1) [136]:

$$\text{yield} = \frac{\text{COD}_{\text{VFA(final)}} - \text{COD}_{\text{VFA(initial)}}}{\text{TCOD}_{\text{initial}} - \text{COD}_{\text{VFA(initial)}}} \tag{3.1}$$

where $\text{COD}_{\text{VFA(final)}}$ represents the COD of VFAs produced in the reactors, and $\text{TCOD}_{\text{initial}}$ and $\text{COD}_{\text{VFA(initial)}}$ are the total COD and the COD of the VFAs present in the feed, respectively.

3.2.4 Experimental Design and Statistical Analyses

A completely randomized design (CRD) was utilized as the statistical methodology to assess the individual effect of the variables FT and pH. Eight FTs and six pH values were evaluated in the first (E_1) and second experiment (E_2), respectively. Each experimental condition was performed in triplicate. Table 3.1 displays the conditions of E_1 and E_2.

Table 3.1 Characteristics of the experiments conducted to evaluate the influence of FT and pH on the production of VFAs by AF of CWW.

Experiment	E_1	E_2
Variable evaluated	FT	pH
Evaluated conditions	1, 2, 3, 4, 5, 6, 7 and 8 d	5.0, 5.2, 5.4, 5.5, 5.7 and 5.9
Adjusted conditions	S/M: 4 gCOD/gVS pH: 5.4 T: 34 ± 1 °C	S/M: 4 gCOD/gVS T: 34 ± 1 °C FT: 6 d
Replicates	3	3

A one-way analysis of variance (ANOVA) with a 95% confidence level and 5% probability ($p < 0.05$) was applied to investigate the existence of significant differences among the FT and pH conditions evaluated. When experimental results were considered different by ANOVA, a multiple comparison test was employed. All ANOVAs were run using R Studio software version 2023.06.1+524.

Finally, the third experiment (E_3) utilized a second-order response surface, specifically a rotatable central composite design (RCCD), to assess the effect of the interaction of the variables FT and pH on VFA production. For the development of response surface, the levels of the FT and pH variables were coded as low (−1), middle (0), and high (1), as presented in Table 3.2.

Table 3.2 Coding of the factors used in the RCCD.

Treatment	Factors		Codified Factors		Replicates
	pH	FT	pH	FT	
1	5.40	4.00	−1	−1	2
2	6.00	4.00	1	−1	2
3	5.40	8.00	−1	1	2
4	6.00	8.00	1	1	2
5	5.70	6.00	0	0	4
6	5.70	8.83	0	1.414	2
7	5.70	3.17	0	−1.414	2
8	6.12	6.00	1.414	0	2
9	5.28	6.00	−1.414	0	2

For the regression model shown in Equation (3.2), which corresponds to a second-order response surface (the model included linear, quadratic, and factor interaction terms), the fitted variable was as follows:

$$\mu_y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon, \quad (3.2)$$

where μ_y is the variable predicted from the response surface (dependent), X_1 and X_2 are the independent variables, β_0 corresponds to the term to be countered, β_1 and β_2 are the linear coefficients, β_{11} and β_{22} are the quadratic coefficients, β_{12} is the interaction of the coefficients, and ε is the residual error.

3.2.5 Analytical Methods

The pH, total and bicarbonate alkalinity, TS, and VS were determined following standard methods [137]. An iris HI801 spectrophotometer (Hanna Instruments, Woonsocket, RI, USA) was used for the determination of TCOD, SCOD, ammonia nitrogen, and orthophosphates. Total carbohydrates were analyzed following the method described by Dubois et al. [138]. Total VFAs were analyzed by adapting the method described by DiLallo and Albertson [139]. A Perkin Elmer gas chromatograph, model Clarus 590, was used for the analysis of VFA composition.

The internal pressure of the reactors was monitored with OxiTop® system heads (WTW, Giessen, Germany) in differential pressure mode (negative or positive difference with respect to the initial pressure in the reactor).

3.3 Results and Discussion

3.3.1 Physicochemical Characterization of CWW and Inoculum Solids Concentration

The physicochemical characteristics of the substrate and the solids concentration of the inoculum used to evaluate the effect of FT, pH, and their interaction on VFA production are shown in Tables 3.3 and 3.4, respectively. Those values were similar to studies using either the same or a similar substrate [134].

Table 3.3 Physicochemical characterization of wastewater from the cassava starch extraction process in the set of experiments.

Parameter	Units	E_1	E_2	E_3
pH		4.29	4.44	4.47
TCOD	mg/L	5000.00	4775.00	5000.00
SCOD	mg/L	4580.00	4580.00	4700.00
VFAs	mgHAc/L	1749.58	1553.31	1265.57
Carbohydrates	mg/L	546.34	1658.54	1776.42
Total alkalinity	$mgCaCO_3/L$	0	162.72	100.12
Bicarbonate alkalinity	$mgCaCO_3/L$	0	0	0
Total acidity	$mgCaCO_3/L$	1173.55	822.60	853.67
TS	mg/L	6310.00	5520.00	5230.00
VS	mg/L	4480.00	4010.00	3715.00
Ammonia nitrogen	$mgNH_4^+/L$	60.05	57.85	76.10
Orthophosphates	$mgPO_4^{3-}/L$	0.62	1.07	0.80

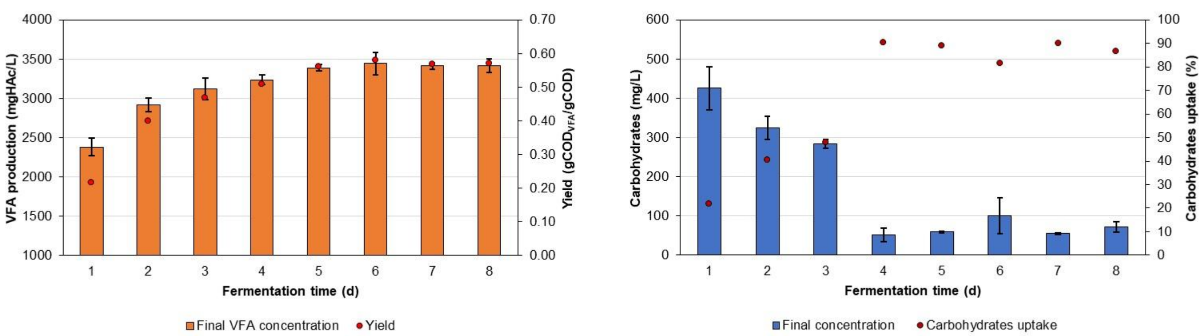
Table 3.4 Concentration of total and volatile solids in the inoculum utilized in the set of experiments.

Parameter	Units	E_1	E_2	E_3
TS	g/L	57.63	105.73	63.93
VS	g/L	41.31	52.56	53.52

3.3.2 Effect of Fermentation Time on VFA Production

3.3.2.1 VFA Production, Yields, and Substrate Uptake

Figure 3.2 shows the VFA production, yields, and carbohydrate uptake for each FT evaluated.



(**a**) (**b**)

Figure 3.2 Effect of FT in the AF of CWW. **(a)** Production and yield of VFAs and **(b)** carbohydrate concentrations and uptake.

An increase in total VFA production was observed as the FT increased from 1 to 6 d (Figure 3.2a). Later, a slight decrease was observed, with a tendency to stabilize. The maximum VFA production was 3444.04 mgHAc/L for 6 d of fermentation, corresponding to a yield of 0.58 $gCOD_{VFA}/gCOD$. Conversely, the minimum VFA production was 2382.99 mgHAc/L for an FT of 1 d, with a yield of 0.22 $gCOD_{VFA}/gCOD$. However, the VFA production for the FT of 1 d represented an increase of 36.2% with respect to the initial VFA concentration of the substrate utilized.

Few studies in the scientific literature investigate the influence of FT on the AF of wastewater in batch reactors, especially if the substrate is CWW. Nonetheless, the trend of increasing VFA production rate with FT is consistent with the observations of Jankowska et al. [140]. The authors evaluated a range of 5 to 15 d for a mixture of primary sludge and waste activated sludge, justifying the increase in VFAs with the presence of more soluble proteins, available carbohydrates, and disintegration of waste activated sludge by prolonging the FT.

The production of VFAs from glucose-rich substrates requires an FT of a few hours [141]. Nevertheless, for more complex and less soluble substrates, a longer duration (5 to 15 d) is required [76], which aligns with our findings.

Bengtsson et al. [21] concluded that variations in FT and pH in reactors significantly affected the quantity and composition of VFAs produced from industrial wastewaters, including cheese whey permeate and effluents from three pulp and paper mills. The authors verified that increasing FT occasioned higher degrees of acidification up to a maximum of 0.93 and 0.75 $gCOD_{VFA}/gCOD$ at 95 and 24 h of FT for the whey and the paper mill effluent, respectively.

Despite the similarity in trend with the previously cited works, the results of our study differ from those observed by other authors for CWW. Hasan et al. [14] identified the maximum VFA production from CWW at 45 h; however, different operating conditions (pH 5.9, 30 °C, and 3 g/L sodium bicarbonate) were used in their experiments. The authors obtained 4000 mg/L of total VFAs with an initial concentration of 8865 mgCOD/L in the influent wastewater, achieving a yield of 0.45 gTVFA/gCOD. Conversely, Niz et al. [65] observed for the same substrate with an adapted inoculum a maximum degree of acidification of 75.8% for day 4 of the 20 monitored. According to Jankowska et al. [76], different types of substrates involve varied kinetics and require different FTs.

Statistically, there were significant differences ($p < 0.05$) in VFA production between the FTs tested in our study. Table 3.5 presents the summary of the ANOVA for the results of the FT variable.

Table 3.5 Summary of one-way ANOVA for the FT variable.

Source	df	Sum of Squares	Mean Square	F-Value	ρ-Value
Treatment	7	1,869,472	267,067	28.087	5.064×10^{-5}
Residuals	8	76,068	9508		

Note: df = degree of freedom.

Fisher’s test was applied to identify the treatments that generated the difference, clustering the results into four groups: group a (FT of 1 d), group b (FTs of 2 and 3 d), group c (FTs of 3 and 4 d), and group d (FTs of 4, 5, 6, 7, and 8 d).

Considering the results obtained in this study, an FT of 6 d is deemed appropriate for VFA production from the substrate studied.

Additionally, in this experiment, carbohydrates were assumed as the predominant carbon source in the acidogenic reactors to produce VFAs. However, according to Colin et al. [142], CWW can contain carbohydrates, acetic acid, and lactic acid. In our experiment, in addition to carbohydrates, concentrations of total VFAs (approximately 1749 mgHAc/L) were quantified in the substrate, mainly composed of acetic and valeric acids.

Figure 3.2b shows the carbohydrate concentration in the supernatant of each reactor at the end of the AF test. A progressive decreasing trend as FT increases is discernible. An average carbohydrate concentration of 67.76 mg/L was measured for FTs between 4 and 8 d, which represents a consumption of 87.6% of the initially existing carbohydrates.

High carbohydrate uptakes have been reported in similar studies [143,144], demonstrating a high affinity of the microorganisms for the substrate. Alibardi and Cossu [143] noted a correlation between VFAs and biogas production with carbohydrate consumption, while protein and lipid content did not display a significant effect. The authors observed that substrates with higher carbohydrate content yielded a concentration of butyric acid close to that of acetic acid, resulting in a butyric acid/acetic acid ratio above 0.8. In contrast, for substrates where proteins and lipids predominated, the ratio was only 0.36.

3.3.2.2 VFA Distribution for Different Fermentation Times

The distribution of VFAs at the end of the trial for the different FTs is illustrated in Figure 3.3. A greater diversity of produced VFAs is observed as FT increases. Acetic, butyric, and propionic acids were predominant in trials ranging from 2 to 8 d. However, lower concentrations of isovaleric, valeric, isobutyric, and hexanoic acids were also detected for FTs longer than 5 d.

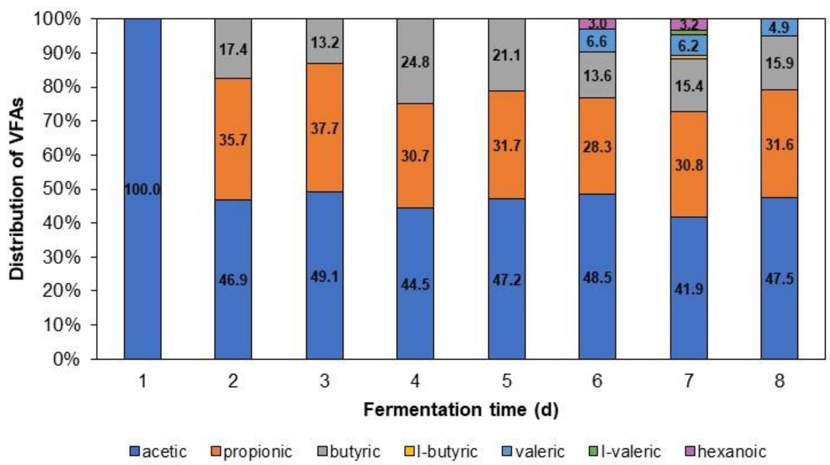


Figure 3.3 VFA distribution for the FTs evaluated in AF of CWW.

The results align with those reported by Hasan et al. [14], who, in experiments evaluating the influence of temperature and alkalinity on the AF of CWW using a response surface, confirmed a predominance of acetic, butyric, and propionic acids (63, 22, and 12%, respectively).

Two common types of fermentation exist: the butyrate type, which yields butyrate, acetate, CO_2, and H_2, and the propionate type, which generates propionate, acetate, and some valerate, with minimal H_2 production [145]. Therefore, in the tests performed for the FT variable, a predominance of propionate-type fermentation can be assumed. Consistent with the above, the pressure differential monitored in the reactors was lower compared to those measured for other variables, where butyric acid instead of propionic acid was predominant.

Acetic acid is the primary and preferred product of acidogenesis because of its higher generation of reducing equivalents during ATP synthesis [129]. Nevertheless, under elevated H_2 partial pressure

(pH_2), acetic acid is formed via the butyrate oxidation intermediate by syntrophic bacteria to avoid inhibition of accumulated products [146].

Acetic acid production is carried out by the acetogens (e.g., *Acetobacter*, *Gluconobacter*, and *Clostridium* genera), which are characterized by a diverse group of microorganisms that include bacteria of many different phyla, allowing them to metabolize a wide spectrum of substrates. In most cases, the substrate is oxidized to acetyl-CoA; this central intermediate is then converted to acetate as the end product, coupled to substrate-level phosphorylation and thus providing ATP [147].

Propionate can be synthesized from pyruvate through two metabolic pathways. In one pathway, lactate and propionate are directly formed by the reduction of pyruvate, utilizing NADH and releasing NAD^+ [148]. The other pathway does not use lactate as an intermediate but involves other compounds such as oxaloacetic, malic, fumaric, and succinic acids, as well as succinyl-CoA, methylmalonyl-CoA, and propionyl-CoA for the synthesis of propionic acid [149]. Propionic acid is mainly produced by propionic acid bacteria like *Corynebacteria*, *Propionibacterium*, and *Bifidobacterium* [129].

The predominance of the propionic acid metabolic pathway in this experiment may be due to the presence of proteins in the substrate [91], which were not monitored in this study. This hypothesis gains strength, particularly since this experiment was characterized by the production of high concentrations of total VFAs (approximately 3444 mgHAc/L) and the use of a substrate with a low carbohydrate concentration (546.34 mg/L), in contrast to the concentrations present in the substrate utilized in our previous experiments. As reported by de Oliveira Schmidt et al. [150], the composition of CWW includes glucose, cyanide, carbohydrates, proteins, lipids, and minerals, which vary depending on environmental factors like soil, climate, and variety of cassava. According to Yu and Fang [151], acetate, propionate, butyrate, and i-butyrate can directly result from the fermentation of carbohydrates, proteins, and lipids. However, higher-molecular-weight VFAs, such as valerate, i-valerate, and caproate, are primarily linked to the fermentation of protein-rich substrates, as observed by Jankowska et al. [76] in the AF of microalgae biomass and whey. These metabolites were also detected in this experiment (Figure 3.3). The AF of non-protein substrates produced few or none of these three previously mentioned VFAs [111,144].

In addition, the production of hexanoic acid (also called caproate) reported in this study aligns with findings from Jankowska et al. [76], who concluded that prolonging FT favored chain elongation and the buildup of medium-chain fatty acids. The authors observed the presence of caproate and caprylate at an FT of 15 d, which was the maximum time tested by the researchers.

Despite the above, the VFA distribution trend in this study differs from that found by Amorim et al. [66], who observed predominance of butyric acid in experiments that assessed the effect of the type of methanogenesis inhibition (chemical or thermal) on the production of H_2 and carboxylic acids from CWW.

There are indications that the presence of H_2 and CO_2 in the headspace may influence certain fermentation pathways [129,152,153]. The pH_2 can affect fermentation performance and the composition of the final products [143]. High pH_2 can alter metabolic pathways, resulting in the production of more reduced intermediates, such as propionate, butyrate, and lactate [154], while low pH_2 reduces the butyrate/acetate ratio and increases H_2 yields [143,155]. Furthermore, according to Zhou et al. [156], pH_2 has an important influence on the richness and diversity of microbial populations in AF, and a reduction in pH_2 potentially leads to increased bacterial diversity.

3.3.2.3 Variation in pH and Internal Pressure in Acidogenic Reactors

In contrast to studies correlating higher VFA production with a more pronounced decrease in pH value [157], in our study, a light increase in pH values (ranged from 5.92 to 6.06) was observed at the end of the trials for all FTs evaluated, being more evident for the longer FTs (7 and 8 d).

The increase in pH value may be due to the use of NaOH pellets. According to Pabón Pereira et al. [154], NaOH pellets for CO_2 capture from the gas phase can force the dissociation and disappearance of bicarbonate and carbonic acid from the liquid phase, causing the increase in pH. However, other studies (specifically related to biochemical methane potential tests) do not describe this effect and, on the contrary, promote the use of NaOH pellets [158]. In some cases, subsequent to the pH decrease generated by VFA production, the buffering capacity may be related to the reaction of water-soluble CO_2 with hydroxide ions to form bicarbonate ions (HCO_3^-), which tends to restore the pH of the process towards neutrality [159]. Also, Dinamarca et al. [160] reported the possibility of maintaining a stable pH by producing volatile buffers in the anaerobic system through the buffering effect of macro-molecules (e.g., proteins and other compounds) present in the residues.

All of the above could be possible explanations for the increase in pH in the experiments, despite the production of VFAs.

In thermodynamically stable dark fermentative processes, H_2 is always produced with VFAs or alcohols [129]. In our study, the biogas could be mainly composed of H_2, as the inoculum was thermally pretreated for the inhibition of methanogenic archaea, and absorption of most of the CO_2 by NaOH pellets is expected.

The biogas produced in the acidogenic reactors for the FTs evaluated was analyzed by measuring the internal pressures shown in Figure 3.4.

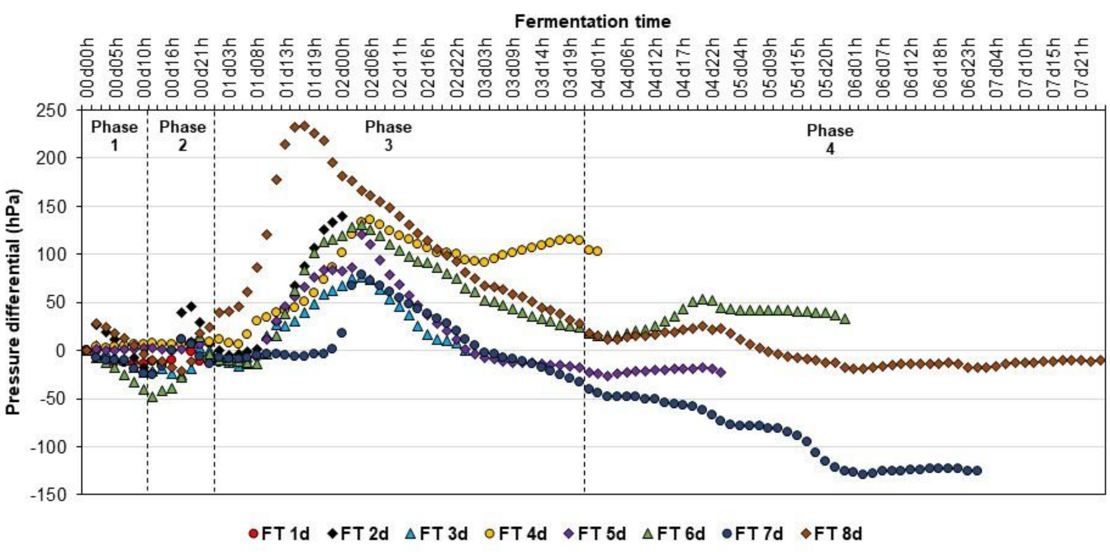


Figure 3.4 Pressure differentials measured in the acidogenic reactors for each FT evaluated.

In general, the curves exhibit four phases. A first phase of gas consumption between 10 and 18 h of fermentation is visualized as a decrease in pressure over time. In this phase, the rate of consumption of gaseous compounds was higher than the production rate. The decrease in the internal pressure of the reactor may be mainly due to the absorption of CO_2 (present in the air or produced by biodegradation of the substrate during the preparatory stage of the reactors) by NaOH pellets since gas purging at the beginning of the experiment was not performed. Additionally, the inoculum is characterized by the presence of a variety of microorganisms, which could have contributed to the decrease in internal pressure due to the consumption of oxygen present in the headspace by facultative microorganisms.

Subsequently, a second phase lasting 10 to 20 h shows a small peak (progressive increase in the reactor's internal pressure, followed by a decrease). A high CO_2 production in the initial hours of the test may be reflected in a relatively lower gas absorption rate compared to production, showing an initial pressure peak that tends to decrease as the production/absorption rates balance out. Additionally, this phase could also signify the onset of a substrate assimilation stage with the consequent production of biogas (mainly H_2) and acetic acid as the primary VFA (Figure 3.3).

The third phase was characterized by the formation of a noticeably larger peak than the previous one. For FTs less than or equal to 3 d, the peak was inconclusive due to the interruption of the assay as described in material and methods (Table 3.1). However, for FTs greater than or equal to 4 d, this phase ranged from 1.5 to 5 d.

On one hand, the exponential growth of pressure in this phase could indicate that microorganisms exhibit a high level of activity, as they have recognized the substrate as a significant food source for their metabolic needs [161], producing mainly acetic, propionic, and butyric acids (Figure 3.3). On the other hand, the decrease in pressure may be due to the possible assimilation of H_2 by hydrogenotrophic methanogenic archaea that may not have been fully inhibited by the thermal pretreatment [162] or by consumption in other metabolic pathways leading to the production of VFAs [163].

Among the factors that likely contributed to the formation of CH_4 by hydrogenotrophic methanogenic archaea is the final pH value of the medium, which was close to 6.0 in all acidogenic reactors. According to Torres Lozada et al. [164], methanogenic archaea can maintain stability for methane formation within the pH range of 6.0 to 8.0.

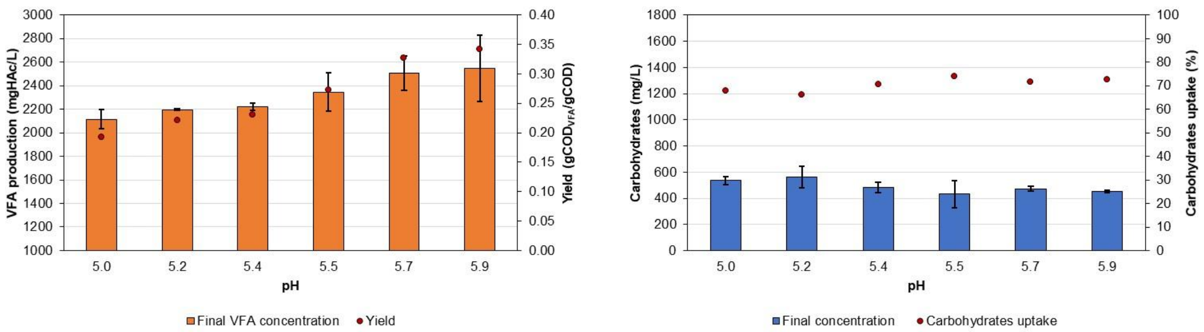
Finally, a fourth phase is observed in which the headspace pressure tends to stabilize. In this phase, there are no significant increases or decreases in the internal pressure in the reactor, which could be influenced by substrate depletion evidenced from the fourth day of fermentation (Figure 3.2b).

The maintenance of an adequate and optimized headspace level is necessary, as authors acknowledge that it contributes to increasing the production of VFAs and biogas [129]. According to Huang et al. [165], a lower pH_2 in the headspace thermodynamically favors the conversion of hydrolyzed products into VFAs.

3.3.3 Effect of Initial pH on VFA Production

3.3.3.1 VFA Production, Yields, and Substrate Uptake

Figure 3.5 shows the total VFA production, yields, and carbohydrate uptake at the end of the fermentation trial for each pH value evaluated. The FT was adjusted to 6 d considering the results of previous experiments performed and reported in this study.



(**a**) (**b**)

Figure 3.5 Effect of pH in the AF of CWW. **(a)** Production and yield of VFAs and **(b)** carbohydrate concentrations and uptake.

An increase in the total VFA concentration was observed as the initial pH increased (Figure 3.5a). The VFA concentration ranged from 2114.33 to 2547.72 mgHAc/L, corresponding to yields between 0.19 and 0.34 $gCOD_{VFA}/gCOD$ for initial pH values of 5.0 and 5.9, respectively. The VFA concentration obtained at an initial pH of 5.0 represented an increase of 36% compared to the VFA concentration present in the substrate used.

Statistically, there were significant differences ($p < 0.05$) in VFA production between the different pH values tested. Table 3.6 presents the summary of the ANOVA for the results of the pH variable.

Table 3.6 Summary of one-way ANOVA for the pH variable.

Source	df	Sum of Squares	Mean Square	F-Value	ρ-Value
Treatment	5	461,052	92,210	4.0958	0.0211
Residuals	12	270,162	22,513		

Note: df = degree of freedom.

Fisher’s test was applied to identify the treatments that generated the difference, grouping the results into two groups: group a (pH of 5.0, 5.2, 5.4, and 5.5) and group b (pH of 5.5, 5.7, and 5.9).

The production trend found for this experiment is consistent with that observed by Lv et al. [95] during glucose fermentation in a continuous stirred tank acidogenic reactor (CSTR). The authors found that the concentration of total VFAs showed significant variations at different pH levels (5.0, 5.5, and 6.0), being favored at pH 6.0 with a maximum production of 2139.11 mgHAc/L. A decrease in pH to 5.5 and 5.0 was reflected in the reduction in total VFA concentration by 21.78% and 14.45%, respectively. Also, Jiang et al. [100] evaluated the effect of pH (5.0, 6.0, 7.0, and uncontrolled at 35 °C) on VFA production from food waste and found that VFA concentration and yield were higher at pH 6.0. The authors achieved a maximum total VFA concentration of 39.46 g/L (yield of 0.316 g/gVS_{fed}) composed mainly of acetate and butyrate (77%), attributing the higher production to optimal hydrolytic enzyme activity at pH 6.0.

According to results reported by Infantes et al. [101], the concentration of undissociated acids increased as pH decreased (6, 5, and 4), causing inhibition. The undissociated forms of VFAs are capable of crossing the cell membrane and dissociating inside the cell due to the higher internal pH, releasing a proton inside the cell. The uptake of protons in this way uncouples the proton motive force, leading to increased energy requirements to maintain the intracellular pH close to neutrality, resulting in reduced biomass growth [101].

Previous studies have inquired about the pH value suitable for VFA production from CWW [14,65,92]. However, the scientific literature specifically aimed at evaluating the effect of pH for this substrate is still limited. Niz et al. [65] investigated AF with adapted and non-adapted biomass and with and without methanogenesis inhibition techniques in a batch process. The authors concluded that inoculum adaptation and thermal treatment were not as relevant in improving VFA production as was the pH of the fermentation broth. The optimum pH range observed by the authors oscillated from 5.5 to 6.0, with a total VFA production of 5080 to 5760 mg/L, respectively. Meanwhile, the study by Barana and Cereda [92], which investigated CWW treatment using a two-phase anaerobic digester, maintained a pH value between 5.5 and 6.0 during the AF stage and observed concentrations ranging from 200 to 4520 mgHAc/L.

Figure 3.5b shows the concentration in the supernatant at the end of the AF assay and the carbohydrate consumption for each evaluated pH condition.

The carbohydrate concentration in the supernatant ranged from 432.68 to 560.98 mg/L, representing a consumption of 66.2 to 73.9% for conversion into cell mass, organic acids, and gaseous products. The lowest and highest carbohydrate consumptions were measured at pH 5.2 and 5.5, respectively. It is evident that there was a significant consumption of carbohydrates from the substrate, but not as observed for the FT variable, which was around 87.60% from 4 to 8 d of fermentation (Figure 3.2b).

Substrate consumption can be influenced by different factors (e.g., temperature, inoculum, pH, substrate concentration, etc.). As reported by Infantes et al. [101], substrate consumption exhibited a pH- and temperature-dependent behavior. The authors observed that glucose was completely utilized at pH 5.0 and 6.0 at all temperatures evaluated (26, 33, and 40 °C), but this was not the case when the pH was 4.0, as glucose was only fully consumed at 26 °C. The amount of glucose consumed decreased to 42 and 23 mM when the temperature increased to 33 and 40 °C, respectively. These results reflect the difficulty that persists in comparing existing studies in the literature, as different factors can be determinants in the final outcome, including the nature of the inoculum.

3.3.3.2 VFA Distribution for Different pH Values

Figure 3.6 shows the distribution of VFAs at the end of the test for each pH value tested. The VFAs were mainly composed of acetic acid, except for the reactors operated at pH 5.7, whose composition was acetic, butyric, and propionic acids at 34.5, 61.5, and 4.0%, respectively. Undoubtedly, at this pH value, butyrate-type fermentation predominated, since the percentage of acetate and butyrate represented approximately 70 to 90% of the total VFAs [95].

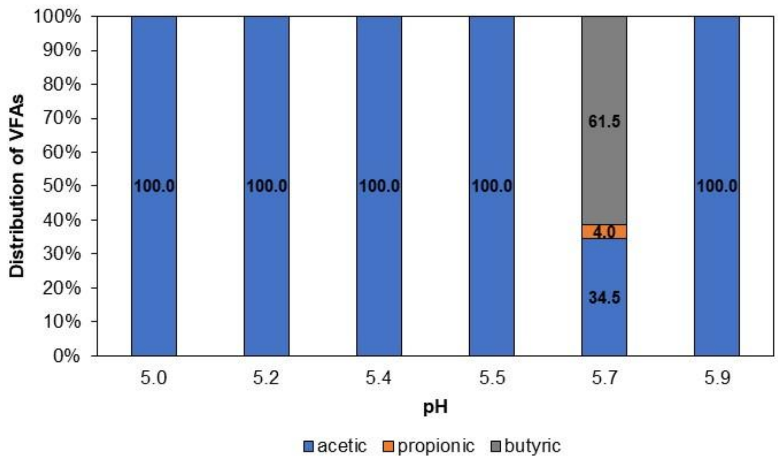


Figure 3.6 VFA distribution for different pH values evaluated in AF of CWW.

A variety of studies have examined the production and distribution of VFAs according to the operating pH, mostly for a glucose substrate and with mixed culture [76,95,166,167]. However, the results obtained in our work differ slightly from those found in previous studies. Jankowska et al. [76] evaluated acidic (5.0), neutral (7.0), and alkaline (11.0) initial pH conditions for four different substrates (maize silage, cheese whey, microalgae biomass, and glucose). The authors found that acetate and butyrate were the main acids under acidic (pH < 5.0) or partially neutral (pH > 5.5) conditions, whereas acetate and propionate were dominant under alkaline conditions. However, the authors concluded that the distribution of VFAs was only dependent on the type of substrate, and not on the pH value. Lv et al. [95] investigated the effect of pH (5.0–6.0) and HRT (2–12 h) in a CSTR, and their results showed that acetate and butyrate dominated during pH regulation. Also, Atasoy et al. [166] demonstrated that acetic acid (44 ± 7%) was predominant under neutral pH conditions (without pH control), while a mixture of acetic (35 ± 8%) and butyric (37 ± 4%) acids dominated under acidic conditions (pH 5.0), and butyric acid (60 ± 12%) under alkaline conditions (pH 8.0 and 10.0). Temudo et al. [167], when assessing low pH values (4.0 to 5.5), detected butyrate and acetate as the main dissolved organic products, while at higher pH values (6.25 to 8.50), butyrate production decreased, and ethanol and acetate became the primary products. Finally, Yu and Fang [151] in AF of dairy wastewater verified that acetate and butyrate were the dominant products at pH > 5.5, while propionate was dominant at pH < 5.5.

According to the scientific literature, an acidic pH favors the formation of acetic and butyric acids for glucose, which is consistent with the findings in our study for reactors operated at pH 5.7, where the proportions of acetic and butyric acids varied slightly (34.5 and 61.5%, respectively). However, for the other acid pH values evaluated, only acetic acid was produced.

On one hand, for CWW, Niz et al. [65] found the highest VFA production at pH 5.50 to 6.09, with butyric acid (87%) as the main metabolite. On the other hand, Hasan et al. [14] found butyric (42%), acetic (35%), and propionic (20%) acids (for pH 5.9), which is not consistent with what was observed for the same pH value in our study.

The above suggests that the pH value is not the only determinant of VFA composition, but the substrate type and the nature of the inoculum may also have a significant influence on VFA distribution.

According to Lv et al. [95], researchers have demonstrated that the regulation of operational parameters not only modifies the specific acid production but also influences the microbial community. According to the results of Atasoy and Cetecioglu [91], the adaptation of the bacterial community to different pH conditions can determine the acid profile. The authors observed the predominance of the phyla *Bacteroidetes* at pH 10, *Proteobacteria* at neutral pH, and *Firmicutes* at pH 5 during the AF of cheese wastewater. This is consistent with the findings of Horiuchi et al. [141], as the shift in the main products in their experiments was caused by the change in dominant microbial populations in the acidogenic reactor due to the pH change, shifting from bacteria producing butyric acid to bacteria producing propionic acid. Furthermore, Eng et al. [168] identified a strong link between experimental conditions (pH and temperature) and bacterial composition in sugarcane vinasse fermentation experiments for VFA production.

Considering the diversity of the acid profile obtained in the conducted research, it is believed that the spectrum of VFAs in AF with mixed cultures is not reproducible or independent of the inoculum. However, based on our results and previous studies for CWW, it is highly probable that a mixture of VFAs, primarily composed of acetic, propionic, and butyric acids, is formed. The generation of a VFA-rich effluent (mainly composed of acetic and butyric acids) through wastewater fermentation offers versatile opportunities for its utilization in various energy and chemical manufacturing processes. These processes include but are not limited to photofermentation and microbial electrolysis cells for H_2 production, the synthesis of biopolymers like polyhydroxyalkanoates (PHA), and microalgae cultivation for the generation of high-value products (e.g., biodiesel) [169].

In this experiment, a pH of 5.7 was considered the suitable value for the AF of CWW, based on the maximum production of total VFAs and diversity in the acids produced.

3.3.3.3 Variation in pH and Internal Pressure in Acidogenic Reactors

After the AF experiment ended, it was observed that the pH of the supernatant decreased in some conditions and increased in others with respect to the initial adjusted value. Overall, a decrease in pH was observed in the reactors adjusted to 5.0, 5.2, 5.4, and 5.9, while in the reactors adjusted to 5.5 and 5.7, the trend was upward. Other researchers have also observed decreasing and increasing trends [100,170] in pH value during fermentation.

The increase in pH value during fermentation, in addition to the reasons previously explained (Section 3.3.2.3), may be attributed to the rise in bicarbonate alkalinity due to the production of ammonia nitrogen (NH_4^+-N). In this experiment, the replicates that showed the greatest increases in pH value were those with bicarbonate alkalinity at the end of the trial. The values ranged from 107.62 to 424.78 mg$CaCO_3$/L. According to Xiao et al. [171], in fermentation processes (glucose and proteins) with low substrate concentration (e.g., glucose $\leq$ 1603.65 mg/L), the hydrolysis and acidification of the inoculum promote the release of ammonia, which is an alkaline substance with high buffering capacity [172]. According to Jiang et al. [100], a higher pH (6.0 and 7.0) leads to a higher release of ammonia.

The biogas produced in the acidogenic reactors for the different pH values was analyzed through the internal pressures shown in Figure 3.7.

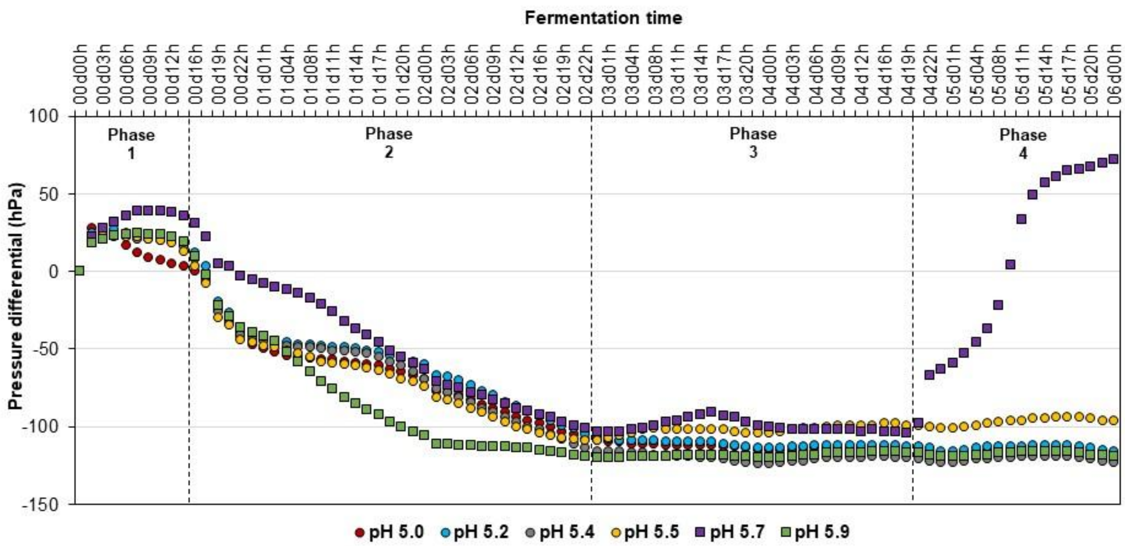


Figure 3.7 Pressure differentials measured in the acidogenic reactors for each pH evaluated.

Three phases were observed for all initial pH values evaluated, except for pH 5.7, where an additional phase was identified. In the first phase, an increase in the internal pressure of the reactor during the first hour of operation due to the increase in temperature (incubation at 34 ± 1 °C) was observed, followed by a trend of stable pressures that extended from 3 to 14 h. This phase could be related to the latency or adaptation stage of microorganisms to the established operating conditions (e.g., pH, S/M ratio, temperature, etc.). The first phase was quite short (3 h) in the acidogenic reactor adjusted to pH 5.0, probably because it was the condition where microorganisms had to undergo fewer changes compared to the conditions in which the inoculum was before the experimental setup.

Subsequently, a second phase characterized by a progressive decrease in the internal pressure in the reactor was observed, which lasted between 35 and 70 h. In the third phase, it was observed that the internal pressures varied very little among them, that is, a stable trend was observed. This phase extended from 40 to 95 h. Finally, a fourth phase was detected only for the pH 5.7 condition, in which there was a progressive and significant increase in the internal pressure of the reactor. The reasons for the perceived decrease or increase in internal pressure in acidogenic reactors were described in Section 3.3.2.3.

Undoubtedly, differences in the dynamics of the internal pressure of the reactors that evaluated the effect of FT and pH on VFA production are observed (Figures 3.4 and 3.7). This indicates that in the AF process, it was not only the parameters evaluated that were responsible for the behavior of the internal pressure of the reactors, since in both experiments, there was a condition at pH 5.4 and FT of 6 d, which behaved differently. This could be due to the heterogeneity of the inoculum used in the two experiments.

3.3.4 Effect of the Interaction among Fermentation Time and pH Variables on VFA Production

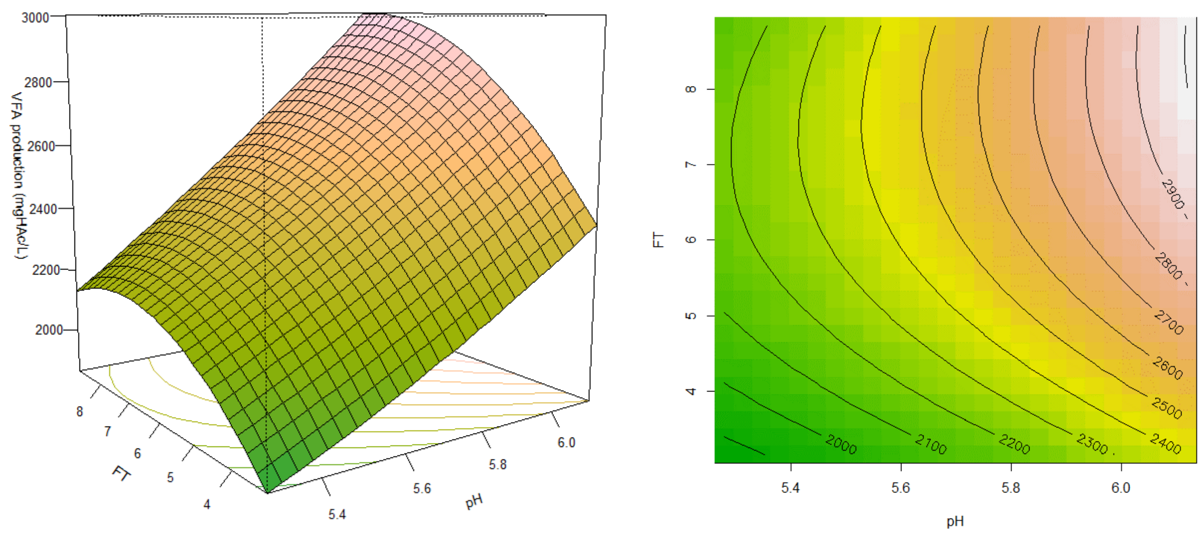
An RCCD was conducted to fit a nonlinear model. The aim was to develop and evaluate a statistical approach to better understand the relationships between the two variables under consideration. Hence, nine treatments (equivalent to 20 experimental runs) were conducted to evaluate the resulting effect of the interaction between the pH and FT variables on VFA production in AF of CWW. Those results are summarized in Table 3.7. The studied variables ranged from 5.28 to 6.12 for pH and from 3.17 to 8.83 d for FT. The central point was set at a pH of 5.7 and an FT of 6 d.

Table 3.7 Experimental results of the RCCD for the production of VFAs by AF of CWW.

Treatment	Factors		VFA Production	Yields
	pH	FT	(mgHAc/L)	$gCOD_{VFA}/gCOD$
1	5.40	4.00	1980.40	0.21
2	6.00	4.00	2428.92	0.34
3	5.40	8.00	2202.23	0.27
4	6.00	8.00	2820.41	0.46
5	5.70	6.00	2486.98	0.36
6	5.70	8.83	2601.37	0.39
7	5.70	3.17	2165.37	0.26
8	6.12	6.00	2913.59	0.48
9	5.28	6.00	2248.12	0.29

The variation in total VFA concentration as a function of the independent variables initial pH (X_1) and FT (X_2) is explained by the quadratic model presented in Equation (3.3), corresponding to the response surface shown in Figure 3.8. Additionally, the ANOVA of the RCCD is presented in Table 3.8.

$$\mathrm{VFAs} = 2490.075 + 252.318X_1 + 153.697X_2 + 14.064X_1^2 - 84.199X_2^2 + 42.415X_1X_2, \quad (3.3)$$



(a) (b)

Figure 3.8 Resulting effect of the interaction among pH and FT on the production of VFAs in AF of CWW. **(a)** Response surface and **(b)** contour plot.

Table 3.8 ANOVA for the quadratic model describing total VFA production as a function of the independent variables and interactions at a 95% confidence level for the RCCD.

Source	Sum of Squares	F-Value	ρ-Value
Model		6.855	0.001982
Linear terms	1,386,605	15.9045	0.000249
Interaction term	14,393	0.3302	0.574671
Quadratic terms	93,047	1.0673	0.370346
Lack of fit	65,337	0.4396	0.729221
Pure error	544,947		
R^2	0.71		
Adjusted R^2	0.61		

According to the previous results (Table 3.8), the model was significant as evidenced by the F-value equivalent to 6.855 (p-value of 0.001982) for total VFA concentration. The R^2 value obtained for the regression model describing the experimental variation of the response variable was 0.71, representing a moderate fit between experimental and predicted values. The value obtained for the adjusted coefficient of determination (adjusted R^2) was 0.61. The lack-of-fit test was not significant for total VFA concentration (p-value of 0.729221).

Additionally, it is observed that the two variables individually (linear terms) exert a significant influence ($p < 0.05$) on the response variable. The analysis showed a greater influence of initial pH than FT. However, the interaction term and the quadratic terms of the model were not significant (p-values of 0.5747 and 0.3703, respectively). This, coupled with the moderate curvature observed (Figure 3.8a), suggests that the optimal values of FT and pH are not within the adopted range. These results are not consistent with findings by Eng et al. [168] for VFA production from sugarcane vinasse, as the quadratic model was suitable for predicting the maximum VFA production.

The production of organic acids increased gradually with pH as with FT. The highest concentration and yield of VFAs were measured at pH 6.12 and FT 6 d, equivalent to 2913.59 mgHAc/L and 0.48 $gCOD_{VFA}$/gCOD, respectively (Table 3.7).

The region where VFA production tends to be maximized is at pH and FT values above 5.6 and 5 d, respectively (Figure 3.8b). It is feasible to perform experiments in a wider range to identify the optimal conditions. However, for AF processes where the main objective is the production of VFAs, pH values higher than 6.0 are not recommended, since they may favor the activity of methanogenic archaea and therefore cause the consumption of the VFAs produced. According to Eng et al. [168], experimental evidence indicates that methane production is favored in a pH range (6.6–7.5) close to neutral conditions. Pittmann and Steinmetz [173] assessed the effect of operational conditions (temperature, pH, retention time, and withdrawal) and reactor operation mode on VFA production as a first step to produce of polyhydroxyalkanoates. The authors observed that a pH of 7.0 gave the best result, but methane production proved to be a problem at this value. After 15 d, the acetate concentration decreased rapidly, and the total VFA concentration was less than 44% of the maximum after 18 d, concluding that to prevent methanogenic conditions, a pH level of 6 should be maintained.

Based on our results, it is considered that the second-order response surface may be suitable for determining the optimal region (in terms of FT and pH) for VFA production from CWW. However, it is recommended that experimental trials be conducted beforehand to determine the evaluation range, using the same inoculum lot that will be utilized for the response surface experiments.

The predominant metabolites in the treatments were acetic, propionic, and butyric acids, except for the conditions pH 5.7 and FT 3.17 d with acetic acid, and pH 5.4 and FTs 4 and 8 d with acetic and butyric acids produced mainly. It is confirmed that for CWW, butyrate and propionate-type fermentations prevail in the experiments. Furthermore, the nature of the inoculum (individual characteristics, microbial consortia, time of adaptation to the substrate, homogenization prior to the experiments, etc.) is a determining aspect of the metabolic pathway established in the fermentation process. According to Khatami et al. [170], the impact of microbial structure on VFA production remains a gray area, and more research is needed to increase the efficiency of VFA production from waste and commercialize bio-based VFA production.

3.3.5 Future Perspectives

Establishing suitable operational conditions is crucial for optimizing the AF of CWW for VFA production. However, scientific studies evaluating the effect of operational variables on AF for CWW are scarce.

Research suggests that the outcome of the fermentation process may be influenced by a combination of operational variables (such as pH, FT or HRT, temperature, S/M ratio, headspace pressure, etc.), as well as factors like inoculum characteristics, bacterial structure, and substrate composition. This complexity makes it challenging to compare different scientific studies regarding total VFA production, yields, and the effects of variables on the process. Also, standardizing the method for calculating the yield of the wastewater AF process is necessary.

Additionally, characterizing the microbial community is essential for enhancing the understanding of fermentation processes and their association with the produced metabolites. In our experiments investigating the effect of FT and pH on VFA production, the inoculum was collected from the same UASB reactor at different times. It underwent identical adaptation and pretreatment protocols and ensured the same inoculum mass according to the established S/M ratio. Nevertheless, we did not control or ensure the homogeneity of specific characteristics of the added mass, such as granule size and individual microbiological aspects, which could influence its response to certain biological processes [174]. These variations could explain the observed variability in substrate consumption, VFA composition, and internal pressure behavior of the reactor under certain evaluated conditions.

Hence, future research should encompass an analysis of the microbial community utilized as inoculum in AF processes, the assessment of the individual and interactive effects of other variables (such as S/M ratio, temperature, and headspace pressure, among others) specific to CWW, and the configuration and design of reactors. Addressing these aspects is crucial to render this process economically viable and enable VFA production on a large scale.

3.4 Conclusions

The production of VFAs from AF of CWW is feasible and can be enhanced by controlling the FT and pH variables. A pH of 5.7 and an FT of 6 d are considered adequate to produce VFAs from CWW using a thermally pretreated inoculum. Under these conditions, yields of approximately 0.36 $gCOD_{VFA}/gCOD$ were achieved, with variability observed in the VFAs produced, primarily consisting of acetic, propionic, and butyric acids. The mixture of organic acids produced could serve as a raw material in subsequent processes to obtain biological products (biofuels, bioplastics, biofertilizers, etc.). While individual variables FT and pH significantly influenced VFA production from CWW, their interaction was found to be not significant for the range evaluated.

Author Contributions: Conceptualization, L.M.S.-L., J.A.R.-V. and H.R.-M.; methodology, L.M.S.-L., J.A.R.-V. and H.R.-M.; validation, L.M.S.-L.; formal analysis, L.M.S.-L.; investigation, L.M.S.-L.; resources, L.M.S.-L.; data curation, L.M.S.-L.; writing—original draft preparation, L.M.S.-L.; writing—review and editing, J.A.R.-V. and H.R.-M.; visualization, L.M.S.-L.; supervision, J.A.R.-V. and H.R.-M.; project administration, H.R.-M.; funding acquisition, H.R.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Universidad del Valle, Project CI-21237.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflicts of interest.

4 Acidogenic Fermentation of Cassava Wastewater: Effect of the Substrate-to-Microorganism Ratio and Temperature on Volatile Fatty Acids Production

Published as: 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. https://doi.org/10.3390/w16233344

Abstract

The production of volatile fatty acids (VFAs) through the acidogenic fermentation of wastewater is an emerging technology that requires further research to optimize operational variables for specific substrates. Cassava wastewater, which is a byproduct of the cassava sour starch extraction process, has been minimally studied regarding its potential for VFA production through acidogenic fermentation. Batch reactors were used to evaluate the effects of the substrate-to-microorganism (S/M) ratio and temperature on VFA production from cassava wastewater. The results showed no statistically significant differences between the evaluated S/M ratios. The maximum total VFA concentration observed was 2214.64 mg of acetic acid (HAc)/L (0.32 $gCOD_{VFA}$/gCOD), which was achieved at a S/M ratio of 4 gCOD/gVS. This concentration was predominantly composed of acetic acid (42.7%), followed by butyric acid (30.1%) and propionic acid (24.6%), with a minor quantity of isovaleric acid (2.6%). The statistical analysis for the temperature variable showed significant differences between the evaluated conditions. The maximum concentration of total VFAs was 2650.19 mgHAc/L (0.45 $gCOD_{VFA}$/gCOD) at 34 ± 1 °C, with acetic (40.9%), butyric (29.8%), and propionic (29.3%) acids as primary metabolites. Cassava wastewater shows promise as a potential substrate for VFA production, warranting evaluation in continuous reactors.

Keywords: volatile fatty acids; acidogenic fermentation; substrate to microorganism ratio; temperature; cassava wastewater

4.1 Introduction

Volatile fatty acids (VFAs) are organic acids that are primarily derived from petroleum refining, meeting approximately 90% of market demand [2]. However, these acids are also intermediates in natural microbial metabolic pathways and have the potential to be produced through the acidogenic fermentation (AF) of organic biomass by mixed microbial cultures [129,135]. AF is a stage derived from the initial phase of conventional anaerobic digestion, in which fermentative bacteria metabolize simple organic compounds—such as monosaccharides, long-chain fatty acids, glycerol, and amino acids—generated through the hydrolysis of complex particulate material to produce VFAs, alcohols, ketones, carbon dioxide, hydrogen, ammonia, sulfur, and new bacterial cells [34,35]. VFAs have wide applications in biological nutrient removal, pharmaceuticals, and the food and chemical industries. Moreover, they serve as feedstock for various products, including biogas, biodiesel, bioplastic, biohydrogen, biofertilizer, and biosurfactant [16].

In recent years, there has been increasing interest in researching the production of VFAs from wastewater [24,25,105,135,175]. Laboratory and pilot-scale studies, employing both continuous and batch reactors, have explored VFA production from various substrates. These investigations aimed to assess the impact of operational factors, such as hydraulic retention time (HRT), pH, temperature (T), the substrate-to-microorganism (S/M) ratio, substrate characteristics and concentrations, and nutrient composition and availability, as well as headspace partial pressure on the process. Operating parameters play a significant role in determining thermodynamically viable reactions and the growth of the most efficient microorganisms [64].

Cassava wastewater (CWW) is a byproduct of the cassava sour starch extraction process. By 2020, global starch production ranged from 88.1 to 97.7 million tons, with 14% of it derived from cassava [176]. In Colombia, sour starch is produced in small- and medium-sized processing plants through a fermentation process and is primarily used in baked goods [6]. Sweet or native starch is produced by large industries, is not fermented, and has no odor or flavor. It is primarily used as a binder in the food industry—such as in cookies, meat products, pasta, and seasoning sauces—but also finds applications in other sectors including paper and cardboard, adhesives, and textiles [7].

Treating CWW through AF not only produces VFAs but also helps mitigate environmental impacts on receiving water sources. These impacts are mainly due to the addition of substances (such as organic matter, cyanide, and acids), heat, and microorganisms to the water, which cause temperature fluctuations, increased populations of microorganisms, and the eutrophication of the receiving body [10].

AF holds promise as a technology; however, there is limited information on yields and an insufficient understanding of the mechanisms involved in metabolic changes (changes in the type of products), especially concerning CWW. These metabolic changes are primarily influenced by operational factors. For CWW, previous studies evaluated the effect of fermentation time, pH, alkalinity, biomass adaptation, and methanogenesis inhibition techniques on VFA production [14,65,177]. Nevertheless, research on the impact of S/M ratio and temperature on AF of CWW is lacking. To our knowledge, the scientific literature reveals no studies evaluating the effect of the S/M ratio on VFA production from the substrate of interest, and temperature analysis has been conducted in only one study [14].

On the one hand, the S/M ratio can influence the oxidative and reductive pathways involved in VFA production [73]. Proper adjustment of the amount of inoculum is also crucial in fermentation processes. According to Arevalo Ortiz and Arias Arroyo [178], large inoculum concentrations can lead to substrate loss due to intense competition among microorganisms, resulting in cell death and excess heat. Conversely, low inoculum concentrations increase the risk of contamination by undesirable microorganisms and prolong fermentation time, consequently reducing productivity. De Sousa e Silva et al. [73] evaluated the impact of the S/M ratio (0.8 to 1.9 gCOD/gVSS) on the profile and kinetics of VFA production from the AF of dairy wastewater. The authors concluded that although increasing the S/M ratio did not affect the percentage conversion of dairy wastewater to carboxylic acids (42 to 44%), it positively influenced productivity. Pérez-Morales et al. [112] analyzed the effects of pH (5.04 to

7.41) and the S/M ratio (25.86 to 71.99 gCOD/gVSS) on VFA production from raw cheese whey. They found that decreasing the S/M ratio at a neutral pH enhanced VFA production, achieving the highest degree of acidification (45.37%) at an S/M ratio of 32.63 gCOD/gVSS and a pH of 7.0. Vergine et al. [79] tested three S/M ratios (1.6, 4.0, and 6.4 gCOD/gVSS) and different initial alkalinity values (1.0 to 2.5 $gCaCO_3/L$) for synthetic soft drink wastewater. The authors achieved the maximum degree of acidification (70.3 ± 0.4%) at an S/M ratio of 4.0 gCOD/gVSS and an initial alkalinity of 2.0 $gCaCO_3/L$. Similarly, Silva et al. [179] maximized VFA production (up to 0.63 gVFA/gCOD) from cheese whey with an initial alkalinity of 5 to 7 $gCaCO_3/L$ and an S/M ratio of 2 to 4 gCOD/gVSS. Finally, in a study focused on hydrogen production from CWW, Mañunga [134] evaluated the effect of the S/M ratio and observed that VFA concentration increased as the S/M ratio rose from 2 to 6 gCOD/gVS.

On the other hand, temperature plays a crucial role in microbial growth and metabolism, as most fermentative bacteria are unable to survive in extreme temperature conditions. Increasing temperature reduces the activation energy of enzymes [19]. Furthermore, each type of microorganism has an optimal temperature range, and variations in bioreactor temperature can modify the structure of the microbial consortium involved in AF [19,97].

Hasan et al. [14] investigated the effect of temperature (30 to 50 °C) and alkalinity (2 to 4 g/L sodium bicarbonate) on VFA production from CWW using a central composite design and found the highest yield (3400 mgTVFA/L) at 30 °C and 3 g/L sodium bicarbonate. The authors recommended a mesophilic temperature due to its lower energy demand and the more stable operation of the fermentation process. Furthermore, in their study on hydrogen production from glucose in batch reactors, Wang and Wan [180] analyzed VFA production across a temperature range of 20 to 55 °C. They observed that the production of ethanol and acetic acid—accounting for more than 86% of the total soluble metabolites—increased as the temperature rose from 20 to 35 °C but began to decline when the temperature was raised further from 35 to 55 °C. In a separate study, Yu and Fang [181] investigated the influence of pH (4.0 to 7.0) and temperature (20 to 55 °C) on the acidification of gelatin-rich wastewater using an up-flow anaerobic reactor. The authors observed that both the degree of acidification and the rate of VFA and alcohol formation increased slightly with rising temperature. Most studies evaluating the impact of temperature on VFA production utilized biological waste or food waste as a substrate [64,182,183].

Since the exploration of CWW for VFA production is still in its early stages, this study aimed to assess the individual effects of the S/M ratio and temperature on VFA production and composition through AF, employing a completely randomized design. Understanding the acidogenic potential of an organic waste stream, which refers to the quantity of VFAs produced through the fermentation of organic constituents and knowledge of their profiles, is crucial for developing locally based biorefinery concepts aimed at producing value-added compounds [179]. This study stands out as one of the few to assess the potential for recovering soluble metabolites from CWW, offering valuable insights to orient future research on continuous acidogenic reactors.

4.2 Materials and Methods

4.2.1 Substrate and Inoculum

CWW was used as a substrate for the AF process. The substrate underwent physicochemical characterization and was stored at 4 °C for less than 24 h to preserve its properties.

Sludge from an up-flow anaerobic sludge blanket (UASB) reactor at a pig slaughterhouse wastewater treatment plant served as the inoculum. The inoculum was gradually adapted to the substrate to enrich it with acidogenic microorganisms capable of converting the carbohydrates present in the CWW into VFAs. After adaptation, the inoculum was heat-pretreated at 85 °C for 30 min [134] to inhibit methanogenic archaea activity and promote the accumulation of VFAs, thereby preventing their conversion to methane.

4.2.2 Acidogenic Fermentation Assays

Two experiments (E_1 and E_2) evaluated the individual effects of the S/M ratio and temperature on VFA production from CWW by AF. Batch flow amber glass flasks with a total volume of 400 mL (200 mL for reaction and 200 mL for headspace) served as acidogenic reactors. Each reactor was loaded with the substrate and inoculum at the specified S/M ratio. The initial pH of the mixture was adjusted to 5.4 using Na_2HPO_4 (Thermo Fisher Scientific Inc., Waltham, MA, USA) or HCl (Merck KGaA, Darmstadt, Germany) solutions. The pH value was chosen based on the results of previous studies [134,135]. A magnetic bar to promote complete mixing and a cap with two NaOH (Merck KGaA, Darmstadt, Germany) pellets for CO_2 absorption were inserted into each reactor. The reactors were then sealed with rubber septa and aluminum seals and incubated at the designated temperature for 6 d [177]. A control was established for each experiment.

At the end of the experiment, a sample of the liquid phase was collected and analyzed for pH, total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), total VFAs, total alkalinity, bicarbonate alkalinity, total carbohydrates, and VFA composition. Figure 4.1 illustrates the unit and procedure adopted in experiments E_1 and E_2.

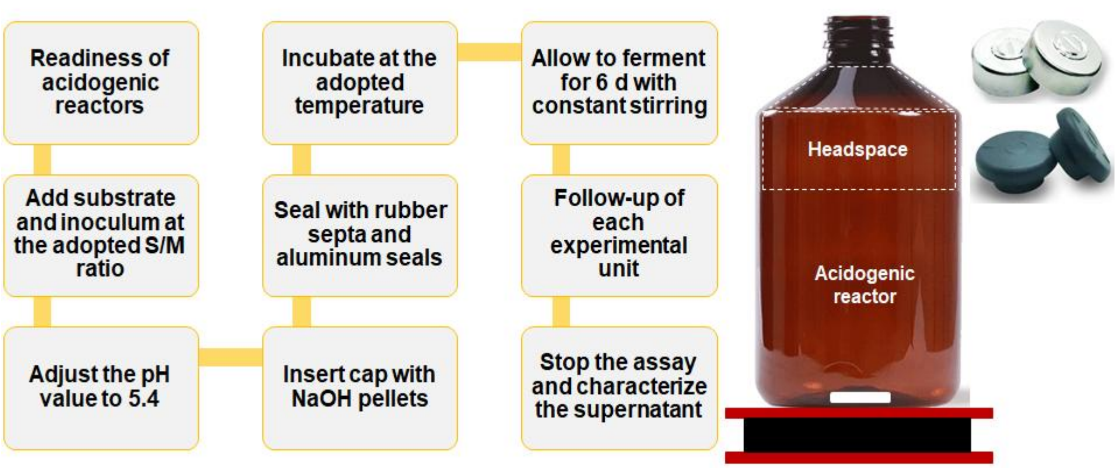


Figure 4.1 Scheme of the unit and experimental procedure adopted in the AF of CWW.

The yield for each evaluated experimental condition was calculated using Equation (4.1), as follows [136]:

$$\text{yield} = \frac{\text{COD}_{\text{VFA(final)}} - \text{COD}_{\text{VFA(initial)}}}{\text{TCOD}_{\text{initial}} - \text{COD}_{\text{VFA(initial)}}}, \tag{4.1}$$

where $\text{COD}_{\text{VFA(final)}}$ represents the COD of VFAs produced in the reactors, and $\text{TCOD}_{\text{initial}}$ and $\text{COD}_{\text{VFA(initial)}}$ are the total COD and the COD of the VFAs present in the feed, respectively.

4.2.3 Experimental Design and Statistical Analyses

A completely randomized design was used to evaluate the individual effects of the S/M ratio and temperature variables. Five S/M ratios and four temperatures were tested in experiments E_1 and E_2, respectively, with each condition performed in triplicate. The evaluated ranges were adopted based on results reported in the scientific literature. Table 4.1 displays the conditions of E_1 and E_2.

Table 4.1 Characteristics of the experiments assessing the effects of S/M ratio and temperature on VFA production through the AF of CWW.

Criteria	E_1	E_2
Variable evaluated	S/M ratio	Temperature
Evaluated conditions	2, 4, 6, 8, and 10 gCOD/gVS	20, 27, 34, and 42 ± 1 °C
Adjusted conditions	Fermentation time: 6 d pH: 5.4 T: 34 ± 1 °C	Fermentation time: 6 d pH: 5.4 S/M: 4 gCOD/gVS
Replicates	3	3

A one-way analysis of variance (ANOVA) with a 95% confidence level ($\rho < 0.05$) was used to investigate significant differences among the S/M ratios and temperatures evaluated. When ANOVA indicated significant differences, a multiple comparison test was employed. All analyses were performed using R Studio software version 2023.06.1+524.

4.2.4 Analytical Methods

The pH, total and bicarbonate alkalinity, TS, and VS were determined following standard methods [137]. An iris HI801 spectrophotometer (Hanna Instruments, Woonsocket, RI, USA) was used to measure TCOD, SCOD, ammonia nitrogen, and orthophosphates by adapting USEPA method 410.4, the Nessler method, and the ascorbic acid method, respectively. Total carbohydrates were analyzed using the phenol-sulfuric acid method described by Dubois et al. [138]. Total VFAs were analyzed by adapting the potentiometric titration method described by DiLallo and Albertson [139].

A Perkin Elmer gas chromatograph model Clarus 590 equipped with a flame ionization detector (FID) (T = 250 °C) was used to analyze VFA composition. Nitrogen served as the carrier gas, and filtered liquid samples were injected into an Elite-FFAP capillary column (30 m length, 0.25 mm ID, 0.25 µm DF) in split mode with a 20:1 split ratio. A mixture of VFAs (acetic, propionic, butyric, isobutyric, valeric, isovaleric, 4-methylvaleric, hexanoic, and heptanoic acids) at a concentration of 10 mM was used to prepare the standards for the calibration curve. From this concentration, six standards of 1, 2, 4, 6, 8, and 10 mM were prepared. The weight of the mixture and solvent (0.12 M HCl) was measured to convert the concentration to parts per million (ppm) and to correct the concentration readings from the gas chromatograph. Using the corrected weights of each standard and the areas provided by the gas chromatograph, calibration curves for each acid were constructed, along with calculations of the method's precision. Finally, the precision and accuracy of the method were evaluated by measuring a standard five times using the gas chromatograph and assessing the variability of the results. A 5 mM standard was prepared, and the coefficient of variation for each acid was calculated, with good precision defined as a value below 5%.

4.3 Results and Discussion

4.3.1 Physicochemical Characterization of CWW and Inoculum Solids Concentration

The physicochemical characteristics of the substrate and the solids concentration of the inoculum used to evaluate the effect of the S/M ratio and temperature on VFA production are shown in Tables 4.2 and 4.3, respectively. These values are consistent with those found in studies using the same or similar substrates [134,177].

Table 4.2 Physicochemical characterization of CWW in the set of experiments.

Parameter	Units	E_1	E_2
pH	---	4.48	4.71
Total COD	mg/L	4910.00	4480.00
Soluble COD	mg/L	4400.00	3870.00
Total VFAs	mgHAc/L	1116.01	1416.53
Carbohydrates	mg/L	2730.69	1107.22
Total alkalinity	$mgCaCO_3/L$	30.19	223.96
Bicarbonate alkalinity	$mgCaCO_3/L$	0	0
Total acidity	$mgCaCO_3/L$	669.25	842.11
TS	mg/L	5040.00	5760.00
VS	mg/L	3805.00	4035.00
Ammonia nitrogen	$mgNH_4^+/L$	45.00	42.90
Orthophosphates	$mgPO_4^{3-}/L$	1.01	0.74

Table 4.3 Concentration of total and volatile solids in the inoculum utilized in the set of experiments.

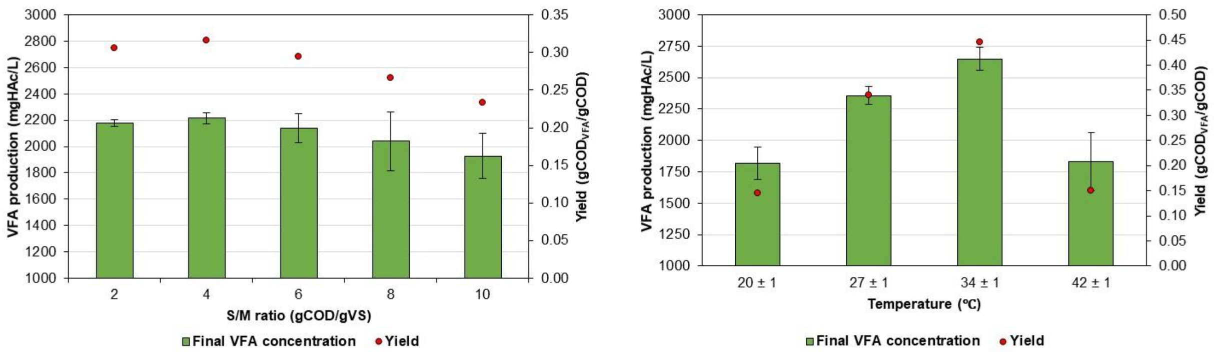
Parameter	Units	E_1	E_2
TS	g/L	76.33	115.15
VS	g/L	49.02	47.34

4.3.2 VFA Production, Yields, and Substrate Uptake from CWW

Figure 4.2 shows the production and yield of VFAs in the AF of CWW at different S/M ratios and temperatures. The results for each condition evaluated in experiments E_1 and E_2 are presented as the average of three replicates, which is a decision informed by a thorough cost and time analysis to ensure the feasibility and efficiency of this study.

Figure 4.2a shows an increase in VFA production when the S/M ratio changed from 2 to 4 gCOD/gVS. Nevertheless, S/M ratios greater than 4 gCOD/gVS resulted in decreased VFA production in the bioreactors. A maximum VFA concentration of 2214.64 mgHAc/L, representing an increase of

98.4% compared to the initial VFA concentration and a yield of 0.32 $gCOD_{VFA}/gCOD$, was measured at an S/M ratio of 4 gCOD/gVS. The lowest VFA concentration was measured at an S/M ratio of 10 gCOD/gVS, which was equivalent to 1928.33 mgHAc/L and a yield of 0.23 $gCOD_{VFA}/gCOD$.



(**a**) (**b**)

Figure 4.2 Production and yield of VFAs by AF of CWW. **(a)** Effect of the S/M ratio and **(b)** effect of the temperature.

A statistical analysis showed no significant differences (ρ = 0.1514) in VFA production between the S/M ratios tested. Table 4.4 summarizes the ANOVA results for the S/M ratio variable.

Table 4.4 Summary of one-way ANOVA results for the S/M ratio variable.

Source	df	Sum of Squares	Mean Square	F-Value	ρ-Value
Treatment	4	161,436	40,359	2.1308	0.1514
Residuals	10	189,408	18,941		

Note: df = degree of freedom.

Few studies in the scientific literature evaluate the impact of the S/M ratio on VFA production from wastewater [73,112,179]. However, the production trend observed in our study and the S/M ratio that favored VFA production are consistent with findings from previous studies. Silva et al. [179] evaluated the effect of S/M ratio (2, 4, 7, and 10 gCOD/gVSS) and alkalinity (1, 2, 5, and 7 $gCaCO_3/L$) on VFA production from cheese whey in 320 mL batch reactors, and the experiments were conducted in triplicate. Using response surface analysis, they confirmed that at lower alkalinity levels, raising the S/M ratio from 2 to 10 gCOD/gVSS significantly reduced the degree of acidification, from 50% to less than 29%. Moreover, they achieved maximum VFA production (up to 0.63 $gVFA/gCOD_{fed}$) with initial alkalinity levels between 5 and 7 $gCaCO_3/L$ and S/M ratios between 3 and 4 gCOD/gVSS. De Sousa e Silva et al. [73] investigated the influence of the S/M ratio (0.8, 1.2, 1.6, and 1.9 gCOD/gVSS) on the profile and kinetics of VFA production from the AF of dairy wastewater, using 300 mL batch reactors and conducting three replicates for each experimental condition. The authors concluded that increasing the S/M ratio did not affect the percentage conversion of dairy wastewater to VFAs (42–44%), but it did significantly enhance productivity (100–200%). Based on their kinetic parameter estimates, an S/M ratio of 1.6 was deemed most suitable for VFA production from the AF of dairy wastewater under the evaluated conditions. However, the study by Vergine et al. [79] found that an

S/M ratio of 1.6 gCOD/gVSS was unstable, as acidogenic conditions were not maintained until the end of the experiment. The authors evaluated the AF of synthetic soft drink wastewater in triplicate using 320 mL batch reactors with a mixed microbial culture. They varied the S/M ratio (1.6, 4.0, and 6.4 gCOD/gVSS) and the initial alkalinity (1.0 to 2.5 $gCaCO_3/L$). After 21 d of fermentation, the highest acidification rate achieved was 70.3 ± 0.4%, which was observed at an S/M ratio of 4.0 gCOD/gVSS and an initial alkalinity of 2.0 $gCaCO_3/L$. In this study, the S/M ratio proved to be the key factor in sustaining acidogenic conditions by suppressing methanogenic archaea and other microorganisms that consume VFAs. Also, Wang et al. [184] evaluated the influence of the S/M ratio (0.2 to 4.09 $gTS_{cassava}/gVSS_{sludge}$) on the AF of cassava powder using triplicate 1000 mL CSTR reactors operated at a pH of 6 and a temperature of 35 °C. During the first 22 h of fermentation, the authors observed that the VFA concentration increased with the S/M ratio, except at 4.09 $gTS_{cassava}/gVSS_{sludge}$, at which the VFA production rate was lower than at S/M ratios of 2.05 and 3.07 $gTS_{cassava}/gVSS_{sludge}$. The authors attributed this behavior to inhibition by high substrate concentrations and the need for a longer acclimatization period for the acidogenic microorganisms. However, after 22 h and by the end of the fermentation period, the S/M ratio of 4.09 $gTS_{cassava}/gVSS_{sludge}$ outperformed the others, achieving a VFA concentration of 6.79 gCOD/L (VFA/COD of 72%).

Nevertheless, other studies reported differing findings. Karaca et al. [71] investigated the optimization of polyhydroxyalkanoate (PHA) production using acidified dairy wastewater as a substrate. In batch reactors at 37 °C, they evaluated the initial S/M ratios of 1, 2.5, 5, and 10 gCOD/gVSS on VFA production. The authors noted that the acidification efficiency increased with the S/M ratio, achieving a maximum yield of 5.2 gCOD/gVSS (51.7%) at 10 gCOD/gVSS on the fourth day of fermentation. Also, Pérez-Morales et al. [112], evaluating the same substrate (cheese whey) used by Silva et al. [179], arrived at different conclusions. The authors evaluated the influence of pH (5.04 to 7.41), the S/M ratio (25.86 to 71.99 gCOD/gVSS), and their interaction in wide ranges within the limits of process feasibility. The authors concluded that both pH and the S/M ratio significantly affected VFA production, while their interaction was insignificant. Using response surface analysis, they observed that at a constant pH, a decrease in the S/M ratio favored the yield. The maximum yield (45.37%) was achieved with an S/M ratio of 32.63 gCOD/gVSS and a pH of 7.0. The significant impact of the S/M ratio in this study may be attributed to the broader range evaluated compared to the one tested in our study, as well as differences in the operational conditions used (e.g., pH, temperature, alkalinity, fermentation time). Consistent with our findings, Casero-Díaz et al. [185] concluded that the tested S/M ratios (2 and 4 gCOD/gVS) had no significant effect on VFA yield during the AF of three fish canning wastewaters using duplicate 500 mL batch reactors.

Based on the findings of our study and previous reports, an S/M ratio of 4 gCOD/gVS could be used to produce VFA from CWW.

Figure 4.2b shows that VFA production increases with temperature up to 34 ± 1 °C, after which it declines. At this temperature, a VFA concentration of 2650.19 mgHAc/L was measured, corresponding to a yield of 0.45 $gCOD_{VFA}/gCOD$. Increasing the temperature to 42 ± 1 °C resulted in a drop in yield to 0.15 $gCOD_{VFA}/gCOD$.

Statistical analysis revealed significant differences in VFA production between the temperatures tested ($\rho = 0.0002271$). Table 4.5 summarizes the ANOVA results for the temperature variable.

Table 4.5 Summary of one-way ANOVA results for the temperature variable.

Source	df	Sum of Squares	Mean Square	F-Value	ρ-Value
Treatment	3	1,510,583	503,528	24.261	0.0002271
Residuals	8	166,037	20,755		

Note: df = degree of freedom.

Fisher's test was applied to identify the treatments that generated the difference, clustering the results into three groups: Group A (temperatures of 20 and 42 ± 1 °C), Group B (temperature of 27 ± 1 °C), and Group C (temperature of 34 ± 1 °C).

Studies emphasized the significant influence of temperature on the AF process for VFA production. However, there is a scarcity of research examining the specific impact of temperature on VFA production from wastewater. Most studies have focused on biological sludge or food waste as substrates [64,131,183]. Furthermore, it is observed that the optimal temperature depends on the type of substrate used. Fernández-Domínguez et al. [183] analyzed the impact of temperature (20, 35, 45, 55, 70 ℃) on the production of VFAs in triplicate 250 mL batch reactors using biowaste collected in a mechanical–biological treatment plant. The highest VFA yields (0.49 to 0.59 $gCOD_{VFA}/gVS$) were observed at 35 °C. However, the differences in the tests performed at 20, 45, and 55 °C were minimal (maximum difference of 10%). Valentino et al. [182] evaluated the effect of temperatures ranging from 20 to 70 °C on the degree of acidification and production yields in batch reactors with thermally pretreated sewage sludge. They found that 55 °C was the optimal temperature for AF, achieving a degree of acidification of 0.81 ± 0.03 COD_{VFA}/COD_{SOL} and a yield of 0.29 ± 0.04 $gCOD_{VFA}/gVS_0$. In addition, Garcia-Aguirre et al. [64] investigated the impact of the pH level (5.5 and 10) and temperature (35 and 55 °C) on VFA production potential in duplicate 500 mL batch reactors using seven urban and agro-industrial waste streams. The authors found that VFA production kinetics were more favorable under acidic conditions and mesophilic temperatures, leading to a quicker achievement of maximum VFA concentration. Similarly, Song et al. [186] evaluated the effects of inoculum amount, initial pH, and temperature (25 to 55 °C) on VFA production from banana waste juice in triplicate, obtaining the highest yield (223.34 mgCOD/gVS) at 35 °C due to the higher activity of VFA-producing microorganisms at that temperature. Eng et al. [187] evaluated the impact of temperature (30, 40, 50, and 60 °C) and initial pH (6, 7, and 8) in triplicate 500 mL batch reactors to recover soluble metabolites using sugarcane vinasse as substrate. Maximum yields of 0.319 and 0.337 $gCOD/gCOD_{tinitial}$ were observed under slightly basic conditions (pH 8) for both mesophilic (40 °C) and thermophilic (60 °C) temperatures, respectively. For wastewater, Koottatep et al. [175] evaluated temperatures between 30 and 60 °C using mixed wastewater from toilets and a cafeteria (ratio of 90:10% *v*/*v*) and determined the highest production of total VFAs at 40 °C.

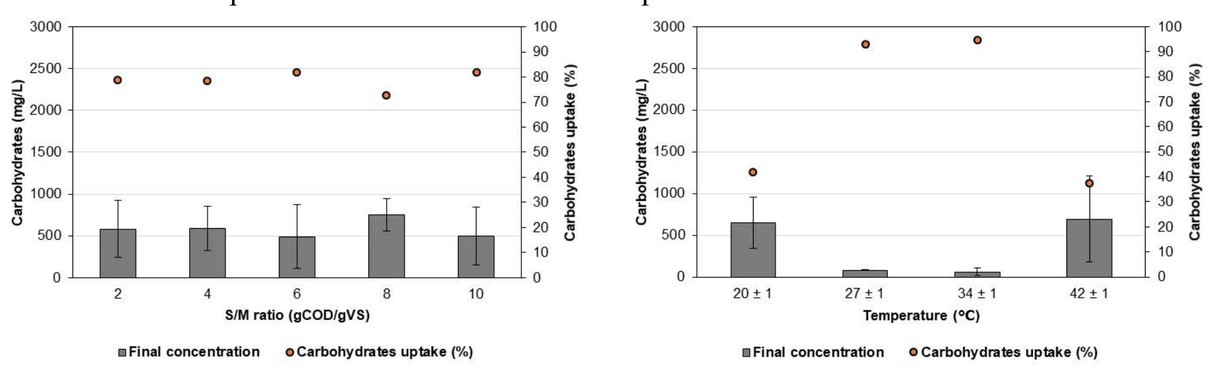
Additionally, in a mainly mesophilic temperature range, the VFA production trend observed in our study is consistent with that reported by other researchers. Pittmann and Steinmetz [173] evaluated the generation of VFAs as the initial stage of the polyhydroxyalkanoate production cycle using different sludges from a WWTP (primary sludge, excess sludge, a mixture of primary and digested sludge, and a mixture of excess and digested sludge). The sludges were tested under controlled pH conditions (pH 6) and without pH control at temperatures of approximately 20 and 30 °C. In six out of eight combinations, raising the temperature from 20 to 30 °C resulted in increased VFA production. Specifically, when using primary sludge as a substrate under pH-controlled conditions, the temperature change increased the degree of acidification from 14 to 31%. Eng et al. [168] assessed VFA production from sugarcane vinasse in triplicate 500 mL batch reactors using a response surface methodology, with temperatures ranging from 33 to 47 °C and the initial pH ranging from 7.1 to 9.9. The authors observed a gradual increase in soluble metabolite production with rising temperature and initial pH, reaching peak levels near optimal conditions (39.6 °C and initial pH of 8.8). For CWW, Hasan et al. [14] investigated the impact of alkalinity and temperature (between 30 and 50 °C) on VFA production and observed a negative influence for certain temperature values, measuring the highest production yield at 30 °C.

The particularities of the AF process, such as the source of inoculum and substrate complexity, undoubtedly influence fermentation performance to varying degrees. Therefore, selecting well-balanced microbial consortia helps minimize limitations associated with temperature and pH [187].

According to our results, a temperature of 34 ± 1 °C, using the selected inoculum source, is deemed suitable for VFA production from CWW. This temperature can facilitate the growth of acid-forming bacteria and promote acid-acetogenesis reactions within the system [175]. Perez-Esteban et al. [188] demonstrated higher microorganism growth (51%) at 35 °C through biomass mass balance analysis. However, they also observed acetic acid consumption at this temperature, suggesting an increased risk of such consumption likely due to the proliferation of methanogenic archaea introduced from the waste-activated sludge used as part of the substrate. In our study, the inoculum pretreatment aimed to decrease the activity of acid-consuming microorganisms.

According to Jung et al. [189], temperatures exceeding 40 °C may delay acidogenic activity due to decreased microbial growth rates, potentially resulting in reduced VFA production. Infantes et al. [101] conducted experiments with glucose at temperatures of 26, 33, and 40 °C and concluded that the energy required for cell maintenance increased at low pH and high temperatures. The authors noted that at a pH of 6, biomass increased to 1.4 and 0.9 g/L at 26 and 40 °C, respectively, confirming reduced biomass growth with increasing temperature. At elevated temperatures, the heightened permeability of the cell membrane would facilitate the passage of undissociated acids more readily, thereby increasing the energy requirements for cell maintenance.

Figure 4.3 shows the carbohydrate concentration in the reactor supernatant at the end of the AF trials and its consumption when the S/M ratio and temperature were evaluated.



(a) (b)

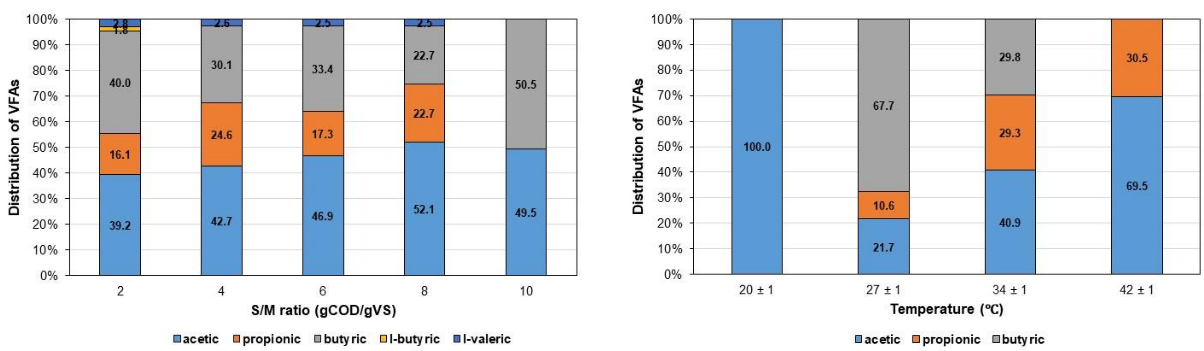
Figure 4.3 Final carbohydrate concentration and uptake in AF of CWW. **(a)** Effect of the S/M ratio and **(b)** effect of the temperature.

In all evaluated S/M ratios, carbohydrate concentrations in the supernatant remained below 750 mg/L, indicating consumptions exceeding 72% (Figure 4.3a). Our findings partly corroborate those of Pérez-Morales et al. [112], who reported carbohydrate consumption ranging from 50.34 to 97.24% during AF of cheese whey for VFA production. Nevertheless, they observed the highest consumption at a pH of 7.01 and an S/M ratio of 32.62 gCOD/gVSS. When evaluating the temperature effect (Figure 4.3b), the extracted supernatant from the reactors exhibited concentrations below 520 mg/L, indicating carbohydrate consumption exceeding 90% at temperatures of 27 and 34 ± 1 °C. Infantes et al. [101] demonstrated that both biomass growth and substrate consumption are influenced by pH and temperature. The authors observed complete glucose consumption at pH levels of 5 and 6 across all evaluated temperatures (26, 33, and 40 °C). However, at a pH of 4, complete glucose consumption occurred only at 26 °C, with consumption decreasing to 42 and 23 mM as the temperature increased to 33 and 40 °C, respectively. Similar to our results, Song et al. [186] observed a decreasing trend in soluble sugar concentration during the AF of banana waste juice at temperatures of 25, 35, 40, and 45 °C, with a rapid decrease from 19.47 to 0.96, 0.50, 0.71, and 1.37 g/L, respectively, by day 4. However, at 55 °C, the soluble sugar concentration decreased more slowly, reaching a minimum value of 6.68 g/L.

The high carbohydrate consumption observed in E_1 and E_2 confirms the high affinity of the microorganisms for the substrate, which was also reported in previous studies [143,144].

4.3.3 Distribution of VFAs Produced from CWW

Figure 4.4 shows the composition of VFAs produced at the end of the AF trials, which investigated the influence of the S/M ratio and temperature variables.



(**a**) (**b**)

Figure 4.4 Distribution of VFAs produced in AF of CWW. **(a)** Effect of the S/M ratio and **(b)** effect of the temperature.

At the beginning of the experiments, only acetic acid was detected in the CWW used as substrate. Figure 4.4a shows for the S/M ratio variable that at the end of the experiment, acetic and butyric acids were quantified in the greater proportion for all S/M ratios tested, followed by propionic and isovaleric acids for ratios of 2 to 8 gCOD/gVS. Also, a discrete formation of isobutyric acid was measured at 2 gCOD/gVS, suggesting a greater variety of VFAs at lower S/M ratios. Additionally, the proportion of acetic acid (ranging from 39.2 to 52.1%) tended to increase as the S/M ratio rose, except for the ratio of 10 gCOD/gVS. However, this trend contrasts with the findings of de Sousa e Silva et al. [73], who examined the impact of the S/M ratio within a range of 0.8 to 1.9 gCOD/gVSS for dairy wastewater and concluded that the percentage of COD as acetic acid decreased as the S/M ratio increased.

In our study, the percentages of butyric acid (ranging from 22.7 to 50.5%) and propionic acid (0 to 24.6%) exhibited an oscillatory behavior, resembling a zigzag pattern, as the S/M ratio increased. Interestingly, this behavior was the opposite for the two acids. Additionally, the proportion of isovaleric acid (ranging from 2.5 to 2.8%) showed minimal variation across the different S/M ratios where it was detected.

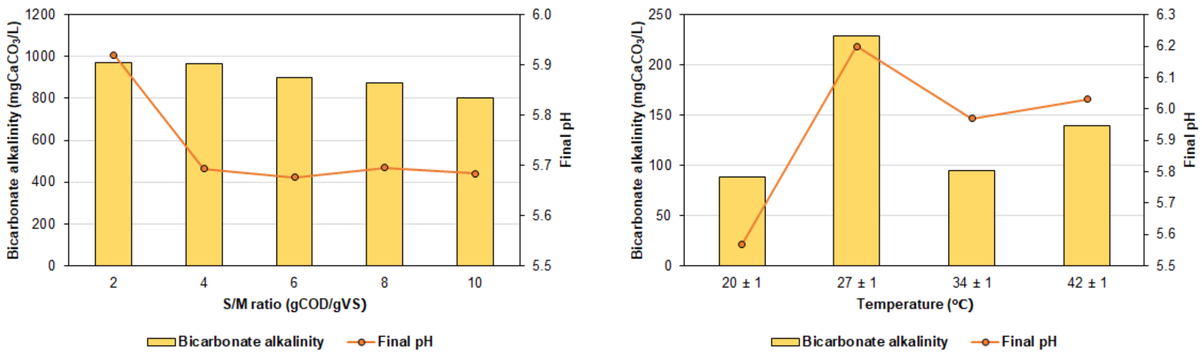
Our findings are consistent with those reported by Silva et al. [179] from AF experiments using cheese whey. The authors observed that acetic and n-butyric acids accounted for 70–90% of the total VFAs produced in all their trials. Additionally, they demonstrated that a low S/M ratio (ranging from 2 to 10 gCOD/gVSS) combined with a high alkalinity supply (ranging from 1 to 7 $gCaCO_3/L$) could modify the VFA profile by increasing the production of propionic and n-valeric acids (up to 500 mgCOD/L). For the same substrate (cheese whey), Pérez-Morales et al. [112] observed a similar predominance of acetic, butyric, and propionic acids. However, the authors noted that the proportion of propionic acid was higher than that of butyric acid. The maximum VFA production (7.91 gCOD/L) was achieved at a pH of 7 and an S/M ratio of 32.62 gCOD/gVSS, which was composed of 32.35, 41.58, 19.45, and 6.62% of acetic, propionic, butyric (normal and iso), and valeric (normal and iso) acids, respectively. Vergine et al. [79] observed that at pH levels near 7, synthetic soft drink wastewater produced butyric and acetic acids across all tested S/M ratios (1.6, 4.0, and 6.4 gCOD/gVSS) and alkalinity conditions (ranging from 1.0 to 2.5 $gCaCO_3/L$). Propionic acid was also produced, albeit at lower levels (approximately 10%). The study concluded that with constant initial alkalinity, increasing the S/M ratio resulted in higher percentages of butyric acid, rising from 5 to 44%. Wang et al. [184] observed a mixture of acetic, propionic, and butyric acids at the end of AF of cassava powder (55 h) for S/M ratios of 0.2 and 0.61 $gTS_{cassava}/gVSS_{sludge}$. However, for higher S/M ratios (1.02, 2.05, 3.07, and 4.09 $gTS_{cassava}/gVSS_{sludge}$), the mixture also included lactic acid. Acetic acid was the predominant metabolite in all S/M ratios in this study, accounting for 39 to 56% of total VFAs.

Figure 4.4b presents the composition of VFAs produced at the conclusion of the AF test for the four temperatures being evaluated. At 20 ± 1 °C, only acetic acid was produced in the bioreactors, whereas at 27 and 34 ± 1 °C, a mixture of acetic (21.7 and 40.9%), butyric (67.7 and 29.8%), and propionic (10.6 and 29.3%) acids was observed. At 42 ± 1 °C, butyric acid was absent from the bioreactor mixture. Moreover, there was an upward trend in the proportion of acetic and propionic acids with increasing temperature from 27 to 42 ± 1 °C, while conversely, a declining trend was noted for butyric acid. The findings at higher temperatures (34 and 42 ± 1 °C) are consistent with those reported by Jiang et al. [100] for food waste. The authors observed that acetic and propionic acids were the predominant VFAs generated at 35 and 45 °C, collectively constituting approximately 70% of the total VFAs. Additionally, Wang et al. [84] used sucrose-rich wastewater as a substrate and observed that AF was individually and interactively affected by pH, temperature, and substrate concentration, which were evaluated in ranges of 4.7 to 6.3, 25 to 45 °C, and 15 to 35 g/L, respectively. The optimal conditions for total VFA yield (1769 mmol/mol-sucrose) were determined to be a pH of 5.6, a temperature of 33.5 °C, and a sucrose concentration of 24.2 g/L. The major metabolites observed were acetate, propionate, and butyrate, along with minor quantities of isobutyrate, valerate, and caproate. Moreover, ethanol was the sole alcohol detected. Fernández-Domínguez et al. [183] analyzed the influence of temperature (20, 35, 45, 55, 70 °C) on VFA production from biowaste collected in a mechanical–biological treatment plant and concluded that the VFA profile, which was dominated by acetic, propionic, and butyric acids (75 to 86% COD_{VFA}), remained consistent regardless of fermentation temperature or seasonality. In contrast, Perez-Esteban et al. [188] found that temperature (25, 35, 45, and 55 °C) notably influenced the VFA profile in a continuous co-fermentation process of waste-activated sludge and food waste. At 25 and 35 °C, the VFA profiles were mainly composed of acetic (34 and 37%), butyric (32 and 31%), and propionic (18 and 17%) acids, aligning qualitatively with our results at 27 and 34 ± 1 °C. However, at 45 °C, the authors observed an accumulation of caproic acid, which was not observed in our study at any evaluated temperature. At 55 °C, acetic and butyric acids predominated (40% each) in Perez-Esteban et al.'s study.

In our study, acetic, butyric, and propionic acids were evidently the primary metabolites for CWW and the variables evaluated, which is consistent with previous findings in studies using the same substrate [14,177]. Acetic acid typically forms from pyruvate and via the branching of acetyl-CoA, accompanied by the simultaneous generation of H_2 and CO_2. Conversely, the butyrate metabolic pathway involves pyruvate and various intermediates like acetyl-CoA, acetoacetyl-CoA, and butyryl-CoA, resulting in the generation of CO_2 and ATP, alongside the utilization of NADH to reduce the intermediate [148,156]. Propionate synthesis can occur through the reduction of pyruvate, utilizing lactate as an intermediate and involving the conversion of NADH to NAD^+. Alternatively, it can proceed via a pathway that includes various intermediary compounds, including oxaloacetic, malic, fumaric, and succinic acids, along with succinyl-CoA, methylmalonyl-CoA, and propionyl-CoA [148,149].

The production of VFAs in this study did not result in a decrease in pH, contrary to observations reported in previous studies [157]. In all bioreactors that evaluated the S/M ratio and temperature, the final pH of the test was higher than that initially adjusted (pH of 5.40 ± 0.01). This behavior may result from the increased bicarbonate alkalinity and buffering capacity of the medium. Although the bicarbonate alkalinity of the CWW used in the experiments was 0 mg $CaCO_3$/L (Table 4.2), much of it was initially established in the system through the alkalinizer used to adjust the initial pH in the bioreactors, as Na_2HPO_4 can influence the acid-base equilibrium. According to Ceron et al. [190], substances with buffering capacity can prevent a drop in pH for a certain period. However, only when the alkalinity of the medium is insufficient to neutralize VFAs, a drop in pH may occur. Figure 4.5 illustrates the final pH for each condition evaluated in E_1 and E_2, along with the observed bicarbonate alkalinity at the end of the test.

When evaluating the S/M ratio, Figure 4.5a indicates that pH values ranged between 5.68 and 5.92. Although the final pH was higher than the initial in all bioreactors, the lowest values were observed for S/M ratios greater than or equal to 4 gCOD/gVS. In terms of bicarbonate alkalinity, the initial value in the bioreactors was 410.56 ± 16.84 mg$CaCO_3$/L, increasing to between 803.20 and 969.97 mg$CaCO_3$/L by the end of the experiment (6 d). The final bicarbonate alkalinity decreased as the S/M ratio increased. Additionally, in Figure 4.5b, the final pH ranged between 5.57 and 6.20 when the temperature was evaluated. The higher pH was recorded at 27 ± 1 °C. Furthermore, the initial bicarbonate alkalinity was 0 mg$CaCO_3$/L, increasing to a range of 88.69 to 229.37 mg$CaCO_3$/L by the end of the test. Alkalinity was notably higher at temperatures of 27 °C (229.37 $mgCaCO_3/L$) and 42 °C (139.71 $mgCaCO_3/L$). In both experiments (E_1 and E_2), the conditions with the highest alkalinity also exhibited the highest pH values at the conclusion of the AF test.



(**a**) (**b**)

Figure 4.5 Variation of pH and bicarbonate alkalinity in AF of CWW. **(a)** Effect of the S/M ratio and **(b)** effect of the temperature.

The increase in pH value can be attributed to several factors mentioned in our previous study [177]. These factors include the capture of CO_2 from the gas phase through NaOH pellets [154], the buffering capacity generated by the reaction of water-soluble CO_2 with hydroxide ions to form bicarbonate ions [159], and the increase in bicarbonate alkalinity due to the production of ammonia nitrogen. Additionally, the increase in system pH at the end could also be due to the breakdown of fatty acids by obligate proton-reducing bacteria, methanogenic archaea, or heterotrophic acetogens [191].

Bicarbonate alkalinity can influence VFA production and composition. Our results indicate a trend in VFA production (Figure 4.2) that mirrors the patterns observed for bicarbonate alkalinity (Figure 4.5). Dahiya et al. [191] evaluated the AF of food waste with biohydrogen cogeneration and concluded that the buffering capacity of the system correlated well with total VFA production. Additionally, Hasan et al. [14] reported that the alkalinity of the medium enhanced VFA production in experiments assessing the effects of temperature and alkalinity (2 to 4 g $NaHCO_3/L$) on CWW fermentation. Therefore, we recommend that future research evaluate the impact of alkalinity on VFA production and composition in CWW.

Finally, we would like to emphasize that significant challenges must be addressed in producing VFA from wastewater using mixed cultures in continuous reactors, both at the laboratory and pilot scale, as well as during the transition to the industrial scale.

Batch reactor studies are valuable for assessing the potential of AF for specific substrates. However, they provide limited insights into industrial processes, particularly regarding organic loading rate, recirculation, methanogen washing, inoculum adaptation, and product removal [28]. Therefore, it is essential to evaluate VFA production in continuous reactors. In continuous reactor operations, variability in wastewater composition and the inability to control optimized operational parameters can negatively affect fermentation efficiency, VFA production yield, and reproducibility. According to Ramos-Suarez et al. [28], lower productivity compared to batch fermentations is a significant disadvantage of continuous processes, making it challenging to optimize quickly and understand parameter interactions.

Optimized values of biological process operating parameters at small scales may change during scale-up, potentially leading to lower yields [192]. For the transition to a sustainable and economically viable industrial scale, AF faces several challenges, including insufficient control over the reactions occurring during fermentation (acidogenesis and acetogenesis) and the need to direct the formation of specific end products at an appropriate rate [68,193]; inhibition caused by the accumulation of fermentation products, which hinders further substrate conversion; the economic inhibition of methanogens [194]; and the separation and purification of produced VFAs, as a variety of products are generated at low concentrations and with similar physicochemical properties. This necessitates the evaluation and selection of cost-effective recovery methods to maximize VFA recovery [16].

The production of VFAs from wastewater is a promising technology, but it faces significant technical and economic challenges. Therefore, interdisciplinary collaboration, along with continuous research and innovation, is essential to overcome these obstacles, optimize processes, and ensure sustainability.

4.4 Conclusions

VFA production from the AF of CWW is feasible and can be enhanced by controlling the S/M ratio and temperature. While the S/M ratio had a non-significant influence, temperature significantly impacted VFA production. An S/M ratio of 4 gCOD/gVS and a temperature of 34 ± 1 °C are considered adequate to produce VFAs from CWW using a thermally pretreated inoculum. Under these conditions, yields of approximately 0.45 $gCOD_{VFA}$/gCOD were achieved, with variability observed in the VFAs produced, primarily consisting of acetic, butyric, and propionic acids. Overall, CWW is a potential substrate for VFA production. Future research should pay special attention to butyric acid production, given its relatively high market price and the natural predominance of butyric-type fermentation in acidogenic systems fed with CWW.

Author Contributions: Conceptualization, L.M.S.-L., J.A.R.-V., and H.R.-M.; methodology, L.M.S.-L., J.A.R.-V., and H.R.-M.; validation, L.M.S.-L.; formal analysis, L.M.S.-L.; investigation, L.M.S.-L.; resources, L.M.S.-L.; data curation, L.M.S.-L.; writing—original draft preparation, L.M.S.-L.; writing—review and editing, J.A.R.-V. and H.R.-M.; visualization, L.M.S.-L.; supervision, J.A.R.-V. and H.R.-M.; project administration, H.R.-M.; funding acquisition, H.R.-M. and L.M.S.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universidad del Valle (project CI-21237) and the Ministerio de Ciencia, Tecnología e Innovación de Colombia—MinCiencias (convocatoria No. 933—2023).

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflicts of interest.

5 Kinetic Modeling of Volatile Fatty Acids Production Using Cassava Wastewater as Low-Cost Substrate

Published as: Sanchez-Ledesma, L. M., Rodríguez-Victoria, J. A., & Ramírez-Malule, H. (2025). Volatile Fatty Acids Production Using Cassava Wastewater as Low-Cost Substrate. Water, 17(7), 991. https://doi.org/10.3390/w17070991

Abstract

The production of volatile fatty acids (VFAs) through the acidogenic fermentation of wastewater has garnered significant attention in recent years. This study examines the kinetics of VFA production in batch reactors using cassava wastewater as a substrate under previously identified conditions (initial pH of 5.7, S/M ratio of 4 gCOD/gVS, and temperature of 34 ± 1 °C). Additionally, this study identifies the best-fit models for estimating kinetic parameters related to the consumption of soluble organic matter and VFA production. VFA production yields ranged from 0.15 to 0.44 $gCOD_{VFA}$/gCOD over the 12-day fermentation period, with the highest yield observed on day 9. The acids produced consisted of 29.7% acetic acid, 43.3% propionic acid, and 27.0% butyric acid. The modified Gompertz and first-order with residual models effectively described the consumption of soluble organic matter, while the first-order and BPK models accurately represented the VFA production. These models showed the highest R^2 values and the lowest RMSE and AIC values. Cassava wastewater is a low-cost substrate with potential for VFA recovery. Its kinetic modeling provides valuable insights for the design, control, and scale-up of acidogenic reactors.

Keywords: volatile fatty acids, kinetic modeling, cassava wastewater, acidogenic fermentation

5.1 Introduction

Cassava wastewater (CWW) is a by-product of the process used to extract sour starch from cassava. Approximately 85% of the water required to produce one ton of starch is discharged as wastewater, while the remainder evaporates [10]. Water that comes into contact with cassava dissolves a significant amount of materials, resulting in a potentially polluting effluent. This effluent has a high organic load, including soluble carbohydrates and proteins, as well as elevated cyanide concentrations. It is also acidic, due to high levels of lactic and acetic acids, and smaller amounts of formic, propionic, and butyric acids [11–13]. If untreated, this effluent can significantly deteriorate surface water sources near starch production areas, making them unsuitable for other uses, such as human consumption, fishing, and recreation [8].

Laboratory and pilot-scale studies have demonstrated that CWW can be treated using anaerobic digestion technologies [8,11,13,14], owing to the high concentration of readily biodegradable sugars. While methane is typically considered the primary product of this process, research indicates that certain intermediate products can have higher value. For instance, volatile fatty acids (VFAs) generated during the acidogenic stage of anaerobic digestion, also known as acidogenic fermentation (AF), are of particular interest [2,15]. VFAs play a crucial role in biological nutrient removal and are widely used industries such as pharmaceuticals, food, and chemicals. Additionally, they serve as precursors for various bio-based products, including biogas, biodiesel, bioplastics, biohydrogen, biofertilizers, and biosurfactants [16,17], along with other promising real-world applications [195,196].

The process of AF for VFA production involves the hydrolysis of particulate matter, followed by acidification. Both stages are significantly influenced by environmental and operational variables [197]. Analyzing the impact of these variables, along with the metabolic reactions in AF, is complex in experimental studies. However, modeling and simulation tools can aid in interpreting these effects [25,66,105]. These tools also enable the extrapolation and prediction of microbial community dynamics and behavior under untested conditions, facilitating the rapid and efficient optimization of the AF process [198].

Several studies have evaluated and modeled VFA production from wastewater [23,24,73,105,199]. The choice of the model type depends on the research objectives and the available data to determine essential parameters [198]. Models can be classified as kinetic, parametric, or non-ideal reactor models based on their characteristics [107]. Kinetic models are used when bioreactor data include time series of concentrations for various components. They enable the estimation of kinetic parameters related to microbial growth, substrate utilization, and product formation under varying operational and environmental conditions [198,200]. Kinetic models can be either structured or unstructured. Structured models account for metabolic pathways and are typically more complex. In contrast, unstructured models are simpler and treat microorganisms as a component or reactant of the system [201]. Given that AF is a multi-product process, the development of unstructured models to describe it has become a viable alternative. Kinetic modeling provides valuable information for understanding and developing new bioreactors for AF, predicting reactor performance, supporting pilot-scale studies, and optimizing processes [25,110]. According to Ramos and Márquez [202], kinetic models are an effective tool during the design phase to enhance process productivity.

The scientific literature demonstrates that various kinetic models—such as first-order, first-order with residue, second-order, Logistic, Monod with growth, Fitzhugh, Monomolecular, modified Gompertz, Transfer, and Richards—have been applied to experimental data from the AF of wastewater [24,25,101,105]. The effectiveness of kinetic models in describing wastewater AF dynamics depends on their assumptions, complexity, and ability to accurately capture key process characteristics, such as substrate consumption, microbial growth, inhibition, lag phases, and system limitations. Coelho et al. [24] used dairy wastewater as a substrate in batch reactors, employing an inoculum collected from a brewery UASB reactor. They also chemically inhibited methanogenesis using chloroform at a concentration of 0.05% v/v. The authors determined that the first-order kinetic model with residual provided the best fit for the hydrolysis of particulate organic matter and substrate consumption, as it accounts for a fraction that remains unhydrolyzed or undegraded. Additionally, the first-order and Fitzhugh models, which describe an exponential phase, were suitable for representing VFA production. Morais et al. [105] used swine wastewater under conditions similar to those of Coelho et al. [24]. They found that the first-order kinetic model with residual was the best fit for the hydrolysis of particulate organic matter. However, none of the evaluated models accurately represented the soluble biodegradable organic matter consumption curve, as it did not account for the increase in soluble organic matter concentration due to hydrolysis. The models describing logistic functions, such as the Richards and Logistic models, were the most appropriate for representing VFA production from swine wastewater. In a subsequent study, Morais et al. [25] found that the first-order kinetic model with residual was the best fit for both the hydrolysis curve of particulate organic matter and substrate consumption when bovine slaughterhouse wastewater was used. They concluded that mathematical models describing exponential growth, such as the first-order, cone, and Fitzhugh models, were the most suitable for representing the kinetics of VFA production. Trisakti et al. [203] evaluated various kinetic models—Modified Gompertz, Logistic, and Monod—to describe microbial growth (based on volatile suspended solids values) and VFA production during the acidogenesis of palm oil mill effluent at an agitation speed of 250 rpm, a pH of 5.5, and a temperature of 55 °C. The authors concluded that the Logistic model most accurately represented both microbial growth and VFA production.

Based on the above, the best-fitting model for VFA production depends on the type and characteristics of the substrate used in AF. Kinetic modeling of VFA production from CWW remains limited, with only one study on acid production using cassava residues identified to date [204]. In this study, Undiandeye et al. [204] investigated the potential of ensiled mixtures of cassava peel and CWW as substrates for medium-chain carboxylate production. They also modeled the chain elongation process using first-order, modified Gompertz, and dual pool models. Therefore, this study aims to examine the kinetics of VFA production from CWW at a laboratory scale and to fit a model for the process, specifically for substrate utilization and VFA production. The results provide valuable insights for the design, operation, and scale-up of acidogenic reactors.

5.2 Materials and Methods

5.2.1 Substrate and Inoculum

CWW was used as a substrate for the AF process. The substrate underwent physicochemical characterization and was stored at 4 °C for less than 24 h to preserve its properties.

The acidogenic reactors used in the experimental setup were inoculated with sludge from an Upflow Anaerobic Sludge Blanket (UASB) reactor at a pig slaughterhouse wastewater treatment plant. To enrich the inoculum with acidogenic microorganisms, it was gradually adapted to the CWW. These microorganisms transform the carbohydrates in CWW into VFAs. After adaptation, the inoculum underwent heat pretreatment at 85 °C for 30 min [134] to inhibit methanogenic archaea activity and promote VFA accumulation, preventing their conversion to methane.

5.2.2 Experimental Setup

To examine the kinetics of VFA production, batch flow amber glass flasks with a total volume of 400 mL (200 mL for the reaction and 200 mL for the headspace) were used as acidogenic reactors. Each reactor was operated at a substrate to microorganism (S/M) ratio of 4 gCOD/gVS. The initial pH of the mixture was adjusted to 5.7 using Na_2HPO_4 (Thermo Fisher Scientific Inc., Waltham, MA, USA) or HCl (Merck KGaA, Darmstadt, Germany) solutions. Each reactor was equipped with a magnetic stir bar to ensure complete mixing and a cap containing two NaOH (Merck KGaA, Darmstadt, Germany) pellets for CO_2 absorption. The reactors were sealed with rubber septa and aluminum seals and incubated at 34 ± 1 °C for the established fermentation period. The S/M ratio, pH, and temperature were selected based on the results of our previous studies [177,205]. The kinetics of VFA production from CWW were evaluated over a 12-day period, with daily measurements. For each fermentation time, three acidogenic reactors were set up, resulting in a total of 36 reactors for the experimental setup. After the fermentation period, a sample of the liquid phase was collected from each reactor and analyzed for pH, total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), total VFAs, total alkalinity, bicarbonate alkalinity, total carbohydrates, and VFA composition.

The yield, VFA productivity, and available soluble organic matter (COD_A) were calculated using Equations (5.1) [136], (5.2), and (5.3) [25], respectively.

$$\text{Yield} = \frac{COD_{VFA\_f} - COD_{VFA\_i}}{TCOD_i - COD_{VFA\_i}} \quad (5.1)$$

$$\text{Productivity} = \frac{COD_{VFA\_t} - COD_{VFA\_i}}{t} \quad (5.2)$$

$$COD_A = SCOD_f - COD_{VFA\_f} \quad (5.3)$$

where COD_{VFA_f} refers to the fraction of soluble COD in the VFA form at the end of the trial, COD_{VFA_i} represents the fraction of soluble COD in the VFA form at the beginning of the trial (day zero), $TCOD_i$ indicates the total COD added in the batch reactor, COD_{VFA_t} represents the total VFA concentration expressed as COD on a specific day, t refers to the collection time, COD_A indicates the fraction of COD soluble available and not referring to VFAs, and $SCOD_f$ denotes the soluble COD measured at the end of the trial.

5.2.3 Analytical Methods

The pH, total and bicarbonate alkalinity, TS, and VS were determined following standard methods [137]. An iris HI801 spectrophotometer (Hanna Instruments, Woonsocket, RI, USA) was used to measure TCOD, SCOD, ammonia nitrogen, and orthophosphates. These measurements were performed by adapting the USEPA method 410.4, the Nessler method, and the ascorbic acid method, respectively. Total carbohydrates were analyzed using the phenol–sulfuric acid method described by Dubois et al. [138]. Total VFAs were analyzed by adapting the potentiometric titration method described by DiLallo and Albertson [139]. VFA composition was determined using a Perkin Elmer gas chromatograph model Clarus 590 equipped with a flame ionization detector (FID) (T = 250 °C). Nitrogen served as the gas carrier. Filtered liquid samples were injected into an Elite-FFAP capillary column (30 m length, 0.25 mm ID, 0.25 μm DF) in split mode with a 20:1 split ratio [205].

5.2.4 Kinetic Model Fitting

The kinetic models selected to describe the consumption of soluble organic matter [199,200] and VFA production [24,199] from CWW are presented in Tables 5.1 and 5.2, respectively. Further details of each selected model are available in the study by Morais [199].

Table 5.1 The kinetic models selected to describe the consumption of soluble organic matter during the AF of CWW.

Kinetic model	Kinetic model equation	Description of variables
First-order	$S_t = S_0 \exp(-K_B t)$	S_t: concentration of soluble organic matter over time (gCOD/L) S_0: initial soluble organic matter concentration (gCOD/L) K_B: soluble substrate degradation rate constant (d^{-1}) t: fermentation time (d) S_r: residual soluble organic matter concentration (gCOD/L) X_0: initial biomass concentration (gVSS/L) K_L: Logistic model constant (L/gCOD·d) μ_{max}: maximum microbial growth rate (d^{-1}) K_s: saturation constant/Monod constant (gCOD/L) X: final biomass concentration (gVSS/L) R_{max}: maximum substrate conversion rate (gCOD/L·d) S_{max}: maximum substrate conversion (gCOD/L)
First-order with residual	$S_t = S_r + (S_0 - S_r)\exp(-K_B t)$	
Logistic	$S_t = \dfrac{S_0 + X_0}{1 + \left(\frac{X_0}{S_0}\right)\exp[K_L(S_0 + X_0)t]}$ $K_L = \dfrac{\mu_{max}}{K_s}$ $K_B = K_L * X_0$	
Monod with growth	$S_t = \exp\left[\dfrac{(S_0 + X_0 + K_s)\ln\left(\frac{X}{X_0}\right) - (S_0 + X_0)\mu_{max}t + K_s\ln(S_0)}{K_s}\right]$	
Logarithmic	$S_t = S_0 + X_0[1 - \exp(R_{max}t)]$	
Modified Gompertz	$S_t = S_0 - S_{max}\exp\left\{-\exp\left[\frac{R_{max}e}{S_{max}}(\lambda - t) + 1\right]\right\}$	

Table 5.2 The kinetic models selected for describing VFA production during the AF of CWW.

Kinetic model	Kinetic model equation	Description of variables
First-order	$VFA_t = VFA_f[1 - \exp(-K_{VFA}t)]$	VFA_t: VFA concentration over time (gCOD/L) VFA_f: final concentration of VFAs (gCOD/L) K_{VFA}: first-order VFA production rate constant (d^{-1}) t: fermentation time (d) K''_{VFA}: second-order VFA production rate constant (L/gCOD·d) n: shape constant λ: lag phase time (d) μ_m: maximum VFA productivity (gCOD/L·d) e: Euler number v: constant of the Richards model
Second-order	$VFA_t = \dfrac{K''_{VFA}(VFA_f)^2 t}{1 + K''_{VFA}(VFA_f)t}$	
Fitzhugh	$VFA_t = VFA_f[1 - \exp(-K_{VFA}t)^n]$	
Cone	$VFA_t = \dfrac{VFA_f}{1 + (K_{VFA}t)^{-n}}$	
Monomolecular	$VFA_t = VFA_f[1 - \exp(-K_{VFA}(t - \lambda))]$	
Modified Gompertz	$VFA_t = VFA_f\exp\left\{-\exp\left[\frac{\mu_m e}{VFA_f}(\lambda - t) + 1\right]\right\}$	
Logistic	$VFA_t = \dfrac{VFA_f}{1 + \exp\left[\frac{4\mu_m(\lambda - t)}{VFA_f} + 2\right]}$	
Transference	$VFA_t = VFA_f\left\{1 - \exp\left[-\frac{\mu_m(t - \lambda)}{VFA_f}\right]\right\}$	
Richards	$VFA_t = VFA_f\left\{1 + v.\exp(1 + v).\exp\left[\frac{\mu_m}{VFA_f}(1 + v)\left(1 + \frac{1}{v}\right)(\lambda - t)\right]\right\}^{\left(-\frac{1}{v}\right)}$	

BPK	$VFA_t = VFA_f\left\{1 - \exp\left[(m-1)\left(\frac{t}{t_0}\right)^{\frac{1}{m}}\right]\right\}$ $\mu_m = \frac{VFA_f \exp(m)(1-m)}{e.m.t_0}$ $K_{VFA} = \frac{\exp(m)(1-m)}{e.m.t_0}$	m: constant of the BPK model t_0: time when VFA production rate is maximum (d)

A nonlinear least squares regression analysis was performed to estimate the parameters of the selected kinetic models. The estimation was carried out using the Generalized Reduced Gradient (GRG) Nonlinear optimization algorithm provided by Microsoft Excel's Solver tool (Version 2108). This optimization strategy determines whether an optimal solution has been reached by examining the gradient or slope of the objective function, verifying if the partial derivatives are equal to zero as the input values (or decision variables) vary [206]. This tool has also been used in other studies that analyzed the kinetics of acidogenic fermentation for VFA production [24,199].

In our study, the sum of squared errors (SSEs) was adopted as the objective function. In the first iteration, the SSEs was calculated based on the initial parameter values. In the second iteration, these parameters were slightly adjusted, and the SSEs was recalculated. This process was repeated multiple times until the lowest possible SSEs value was reached. The algorithm was executed using the forward differentiation method to compute the partial derivatives and a convergence criterion of 0.0001 was set to estimate the best possible values.

The appropriate model for describing the consumption of soluble organic matter and VFA production was initially chosen based on the coefficient of determination (R^2), calculated using Equation (5.4). R^2 assesses the dispersion of observations by examining the differences between theoretical and actual values. A value closer to 1 indicates a better model fit [207].

Additionally, the root mean square error (RMSE), defined in Equation (5.5), was used to quantify the difference between modeled and experimental data. The Akaike Information Criterion (AIC), calculated using Equation (5.6), was employed to prevent overfitting. The RMSE and AIC criteria help balance model complexity and predictive accuracy. Minimizing both RMSE and AIC is essential for achieving a better model fit.

R^2, RMSE, and AIC are widely recognized statistical indicators that help evaluate how well kinetic models fit experimental data [199,203,207].

$$R^2 = 1 - \frac{\sum_i \left(Y_{i\_exp} - Y_{i\_est}\right)^2}{\sum_i \left(Y_{i\_exp} - \bar{Y}\right)^2} \tag{5.4}$$

$$RMSE = \sqrt{\frac{\sum_i \left(Y_{i\_exp} - Y_{i\_est}\right)^2}{N}} \tag{5.5}$$

$$AIC = N \ln\left[\frac{\sum_i \left(Y_{i\_exp} - Y_{i\_est}\right)^2}{N}\right] + 2k + \frac{2k(k+1)}{N-k-1} \text{ for } \frac{N}{k} < 40 \tag{5.6}$$

where $Yi_{\_exp}$ is the experimental data value, $Yi_{\_est}$ is the value estimated by the model, $\bar{Y}$ is the average of the experimental data, N is the number of experimental observations, and k is the number of model parameters.

5.3 Results and Discussion

5.3.1 Physicochemical Characterization of Substrate and Inoculum

Tables 5.3 and 5.4 present the physicochemical characterization of the CWW and the solids concentration of the inoculum used in the acidogenic reactors. The measured values are consistent with those reported in previous studies [134,177].

Table 5.3 Physicochemical characterization of CWW used as substrate in experimental setup.

Parameter	Units	Value
pH	---	4.21
Total COD	mg/L	4975.00
Soluble COD	mg/L	4470.00
Total VFAs	mgHAc/L	1187.58
Carbohydrates	mg/L	1906.00
Total alkalinity	$mgCaCO_3/L$	0
Bicarbonate alkalinity	$mgCaCO_3/L$	0
Total acidity	$mgCaCO_3/L$	759.54
TS	mg/L	4705.00
VS	mg/L	3450.00
Ammonia nitrogen	$mgNH_4^+/L$	66.25
Orthophosphates	$mgPO_4^{3-}/L$	1.55

Table 5.4 Concentration of total and volatile solids in inoculum utilized in experimental setup.

Parameter	Units	Value
TS	g/L	54.11
VS	g/L	38.98
VS/TS	---	0.72

5.3.2 Yield, Productivity and VFA Distribution

Figure 5.1 shows the final VFA concentration and yield over the evaluated fermentation period.

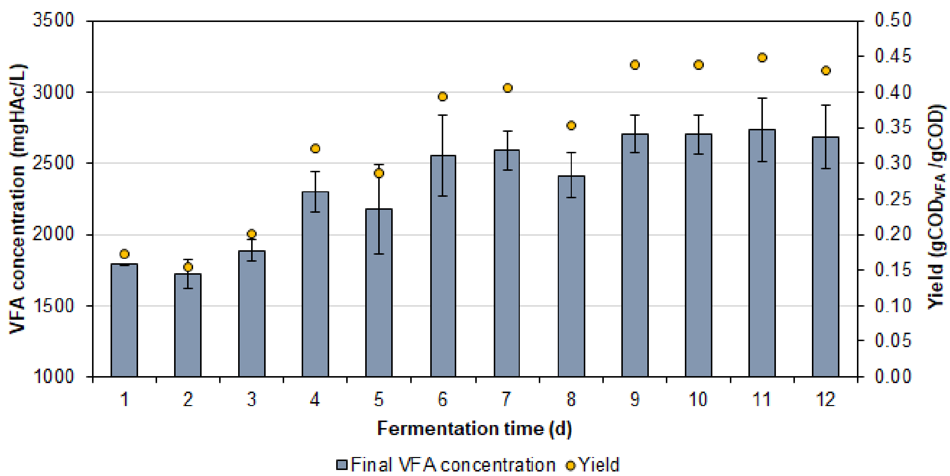


Figure 5.1 Final VFA concentration and yield by AF of CWW.

Figure 5.1 indicates that the final VFA concentration increases with fermentation time up to approximately 9 days, after which VFA production stabilizes and slightly declines. The yield increased from 0.17 $gCOD_{VFA}$/gCOD (1787.77 mgHAc/L) on day 1 to 0.44 $gCOD_{VFA}$/gCOD (2705.11 mgHAc/L) on day 9, and then decreased slightly to 0.43 $gCOD_{VFA}$/gCOD (2680.46 mgHAc/L) on day 12. The curve representing the VFA production from CWW shows an asymptotic increase over time, similar to that observed by Cheah et al. [208].

The yields obtained in this study align with those reported by Morais et al. [105] in the AF of swine wastewater. In their study, 40 ± 5% of the applied organic matter was converted into VFAs, yielding 0.40 mgCOD/$mgCOD_A$. Hasan et al. [14] evaluated the effect of temperature (30 to 50 °C) and alkalinity (2 to 4 g/L sodium bicarbonate) on the VFA production from CWW in batch reactors. Under conditions of 30 °C, 3 g/L sodium bicarbonate, 2 g/L reducing sugars, pH 5.9, and 45 h of fermentation, they reported yields of 0.45 gTVFA/gCOD (3400 mgTVFA/L).

In contrast, our yields were significantly lower than those reported in studies using different types of wastewaters. Coelho et al. [24] achieved yields of 0.83 $mgCOD_{CA}$/$mgCOD_A$ using dairy wastewater as a substrate over 7 days of fermentation. In another study, Coelho et al. [23] reported yields of 0.82 $mgCOD_{CA}$/$mgCOD_A$ over 14 days of fermentation using residual glycerol from biodiesel production, with 95% of the applied glycerol converted into VFAs.

The lower yields obtained with CWW are attributed to the specific characteristics of the substrate and inoculum used. Other influencing factors—such as pH, temperature, and S/M ratio—were assessed in our previous studies [177,205], and the optimal conditions were selected for kinetic evaluation. Specifically, the presence of cyanide in the substrate, the low concentrations of proteins and lipids, and the limited availability of essential nutrients (nitrogen and phosphorus) may have restricted VFA production from CWW compared to the yields observed for dairy wastewater and residual glycerol from biodiesel production.

Similarly, Morais et al. [25] reported a yield of 0.76 gCOD/$gCOD_A$ from bovine slaughterhouse wastewater. They attributed the high yield to the biodegradability of the substrate, adequate nutrient concentrations, and natural buffering, which favored acidogenic microorganism metabolism. This study highlights that the nutrient concentration and buffering capacity are key factors that can be adjusted in acidogenic reactors treating CWW to enhance yields. Hasan et al. [14] also concluded that increasing the medium alkalinity enhances VFA production in CWW fermentation.

For CWW, Niz et al. [65] reported acidification degrees of 75.8% on day 4 and 84.1% on day 15 of fermentation using adapted and unadapted inoculum which was pretreated thermally. Variations in yield, concentration, and productivity are largely attributed to differences in process operating parameters. Simonetti et al. [209] highlighted that the nature of the feedstock affects its biodegradability, while its concentration influences VFA levels. Liu et al. [93] note that variations in the initial carbon-to-nitrogen ratio can influence the conversion of carbohydrates and proteins, leading to differences in total VFA generation and its components. Additionally, sufficient concentrations of micronutrients and trace metals are essential for cell maintenance, metabolism, growth, and transport processes [210]. Wang et al. [211] studied the effect of the carbohydrate-to-protein (Car/Pro) ratio, ranging from 0.25 to 3, on VFA production using typical model substrates, including solubilized (bovine serum albumin-BSA and dextran), as well as hydrolyzed (amino acids and glucose) substrates. For solubilized substrates, the authors observed that as the Car/Pro ratio increased from 0.25 to 1, the maximum VFA yield rose from 0.64 to 0.71 mgCOD/mgCOD$_{substrate}$. However, when the Car/Pro ratio increased to 3, the maximum VFA yield significantly decreased to 0.50 mgCOD/mgCOD$_{substrate}$. A similar trend was observed for the hydrolyzed substrates, with maximum VFA production values of 0.60, 0.66, 0.72, 0.65, and 0.61 mgCOD/mgCOD$_{substrate}$ at Car/Pro ratios of 0.25, 0.5, 1, 2, and 3, respectively. The authors concluded that both the Car/Pro ratio and the type of organic substrate significantly affect the hydrolysis and acidogenesis processes during AF.

Figure 5.2 illustrates the productivity of VFAs from CWW. The highest productivity was observed on the first day of the experiment, followed by a gradual decline throughout the fermentation period. This trend can be explained by physiological and biochemical mechanisms that influence substrate utilization, microbial activity, and metabolic shifts throughout the acidogenic fermentation process.

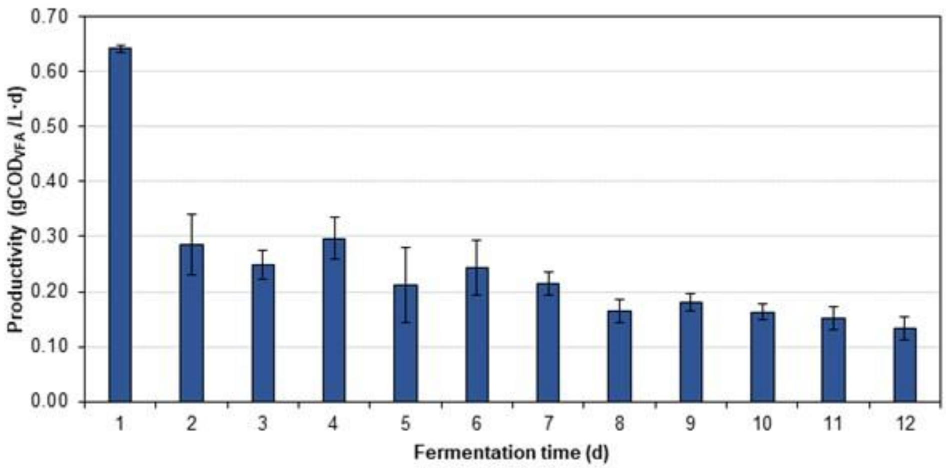


Figure 5.2 Productivity of VFAs from CWW.

Specifically, the productivities on days 1 and 12 were 0.642 and 0.133 gCOD$_{VFA}$/L·d, respectively. These results suggest that CWW is an easily fermentable substrate for acidogenic microorganisms, as substantial VFA production occurred within the first two days of fermentation. This finding aligns with the observations of Hasan et al. [14] during the AF of CWW.

The average productivity in our experiment was 0.245 gCODVFA/L·d, comparable to the value reported by Coelho et al. [23] for residual glycerol (0.27892 ± 0.06246 g/L·d). However, it differed significantly from the average VFA productivity observed by Coelho et al. [24] for dairy wastewater, which was 0.99965 ± 0.59735 g/L·d.

The productivity in AF is influenced by operational parameters, such as substrate concentration and residence time (both hydraulic and solids or run length for batch studies). In a review of the scientific literature, Simonetti et al. [209] found that higher substrate concentrations tend to increase productivity. Elevated substrate levels generally result in higher product concentrations, leading to greater productivity. The authors also concluded that productivity decreases as residence time increases. Shorter residence times correspond to smaller reactor volumes or shorter reaction times, which enhances productivity. They observed the highest productivities (above 10 g/L·d) at feed concentrations between 128 and 149 gCOD/L, with hydraulic residence times ranging from 1 to 5 days.

To optimize productivity, substrate concentration must be high enough to sustain maximum microbial activity, yet not exceed levels that could cause substrate inhibition, acidification, or the accumulation of toxic intermediates, which may slow microbial activity or compromise process efficiency. Similarly, the residence time should be short enough to maintain high yields, but if too short, it may lead to incomplete substrate conversion, reduced overall yields, or the loss of key microorganisms if their retention in the system is not ensured.

Figure 5.3 illustrates the distribution of VFAs produced throughout the fermentation period.

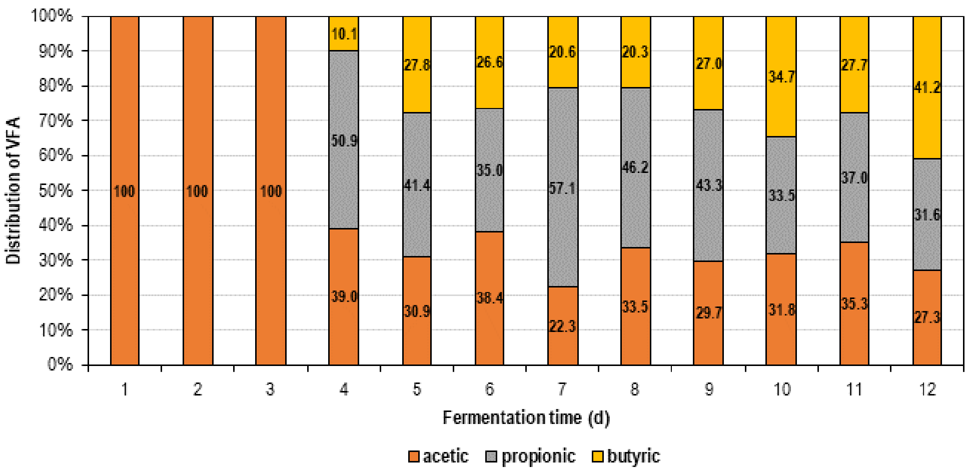


Figure 5.3 Distribution of VFA produced in AF of CWW.

During the first three days, only acetic acid was produced. From day 4 to day 12, a mixture of acetic, propionic, and butyric acids was observed. The proportions of butyric and propionic acids fluctuated slightly over time. As fermentation time varied from 4 to 12 days, the proportion of butyric acid increased from 10.1 to 41.2%, while the proportion of propionic acid decreased from 50.9 to 31.6%. Variations in VFA ratios during fermentation can be attributed to microbial, biochemical, and environmental factors that influence the metabolic pathways of acidogenic bacteria. Key factors, such as substrate availability and microbial preference, community dynamics, hydrogen partial pressure, and pH fluctuations with their inhibitory effects, can alter the microbial metabolism at different stages of fermentation, leading to fluctuations in acid ratios.

These results are qualitatively consistent with the findings of Coelho et al. [23] and Coelho et al. [24]. They observed a higher selectivity in the production and yield of acetic, propionic, and butyric acids during the AF of residual glycerol and dairy wastewater, respectively. Similarly, Morais et al. [25] reported a distribution of 59, 15, and 11% for acetic, butyric, and propionic acids, respectively, in the AF of bovine slaughterhouse wastewater. However, they also observed the production of caproic acid without the addition of electron donors.

For CWW, Amorim et al. [66] evaluated the effects of methanogenesis inhibition methods (acetylene at 1% v/v in the headspace and heat treatment at 120 °C for 30 min), inoculum types (bovine rumen and sludges from municipal and textile industrial wastewater treatment plants), and organic loading (10, 20, and 40 g/L in terms of COD) on hydrogen and carboxylic acid production in batch reactors. When assessing the pretreatment methods and the combined effects of different inoculum types and organic loads, the authors found that the main products were acetic, butyric, propionic, and caproic acids, along with ethanol. The same metabolites were detected by Amorim et al. [104] when evaluating caproic acid production through carbon chain elongation during fermentative hydrogen production, achieved by adding ethanol to the medium. The authors examined two initial substrate concentrations (10 and 20 g/L as COD) by diluting CWW with tap water. They set an initial pH of 5.5 for the acidogenic phase and a pH of 7.0 to promote carbon chain elongation. In previous studies, the conditions tested favored the production of caproic acid from CWW. However, in our study, this acid was not detected. This absence is likely due to the operating conditions, the microbial community, and the nature of the substrate used in the AF process. These factors may have favored the production of propionic acid over butyric acid. According to Undiandeye et al. [204], lactic, acetic and butyric acids, as well as ethanol, can be precursors of medium-chain carboxylic acids (those with six to twelve carbon atoms) through a chain elongation process. This process is a sequential reaction in which electron donors, such as lactic acid or ethanol, are utilized by microorganisms to elongate VFAs via a reverse β-oxidation pathway [212]. To produce medium-chain carboxylic acids with an even number of carbon atoms, butyric acid elongates to form caproic acid. This acid then elongates to form caprylic acid, and so on [204].

Additionally, Niz et al. [65] evaluated the potential for VFA production from the AF of CWW using both adapted and non-adapted inoculum sludge. They tested conditions with and without methanogenesis inhibition techniques in batch reactors (1 gTVS/gCOD and 32 ± 2 °C). The main product was butyric acid, which accounted for 50 and 64% of the total VFA produced when the inoculum was adapted and unadapted, respectively, as well as thermally pretreated. Hasan et al. [14] achieved the highest yield for CWW at 30 °C using 3 g/L sodium bicarbonate, 2 g/L reducing sugars, a pH of 5.9, and 45 h of fermentation. The primary products were acetic acid (63%), butyric acid (22%), and propionic acid (12%). According to Morais et al. [25], the kinetic parameters indicated that acetic acid was formed the fastest, while butyric acid formed the slowest during the AF of bovine slaughterhouse wastewater. These findings are consistent with those observed in our study (Figure 5.3).

5.3.3 Kinetic Model Fitting of AF

5.3.3.1 Modeling of Soluble Organic Matter Consumption

Table 5.5 presents the estimated kinetic parameters along with the R^2, RMSE, and AIC values for the models used to describe the consumption of soluble organic matter. This term refers to the soluble COD that is not in the form of VFAs but can be converted into VFAs.

Table 5.5 Kinetic parameters estimated for the conversion of soluble organic matter in the AF of CWW.

Kinetic model	Parameters and fitting criteria	Value
First-order	K_B (d^{-1})	0.068
	R^2	0.724
	RMSE	0.276
	AIC	-31.152
First-order with residual	K_B (d^{-1})	0.267
	R^2	0.847
	RMSE	0.204
	AIC	-38.915

Table 5.5 Kinetic parameters estimated for the conversion of soluble organic matter in the AF of CWW (continuation).

Logistic	K_L (L/gCOD·d)	0.011
	K_B (d^{-1})	0.060
	R^2	0.681
	RMSE	0.296
	AIC	-29.281
Monod with Growth	K_s (gCOD/L)	2.805
	X (gVSS/L)	5.423
	μ_{max} (d^{-1})	0.019
	R^2	0.753
	RMSE	0.260
	AIC	-26.322
Logarithmic	R_{max} (gCOD/L·d)	0.025
	R^2	0.509
	RMSE	0.367
	AIC	-23.683
Modified Gompertz	S_{max} (gCOD/L)	1.412
	R_{max} (gCOD/L·d)	0.396
	λ (d)	0.929
	R^2	0.885
	RMSE	0.177
	AIC	-36.291

The results indicated that the modified Gompertz model best described the conversion of soluble organic matter. The R^2, RMSE, and AIC values were 0.885, 0.177, and −36.291, respectively. Given the complexity of AF with mixed cultures—a bioprocess involving multiple metabolic pathways—R^2 values exceeding 0.80 were deemed satisfactory for describing the consumption of soluble organic matter in this study. An R^2 value of 0.885 suggests that the model explained 88.5% of the variability in soluble organic matter conversion, effectively capturing most of the underlying dynamics while leaving minimal unexplained variation. Additionally, an RMSE of 0.177 indicates a relatively low error, demonstrating that the model's predictions closely align with observed data, reinforcing its reliability in predicting the bioprocess. The low AIC (−36.291) reflects a well-balanced trade-off between model fit and complexity, confirming that the modified Gompertz model efficiently captured key process features without overfitting, outperforming alternative models.

According to this model, the R_{max}, λ, and S_{max} were 0.396 gCOD/L·d, 0.929 d, and 1.412 gCOD/L, respectively. The moderate maximum substrate conversion rate (R_{max}) suggests a steady degradation process, likely regulated by microbial activity and substrate availability. The short latency phase (λ) indicates that the microbial community adapted quickly to the environment, facilitating an efficient initiation of biodegradation. Additionally, the maximum substrate conversion (S_{max}) reflects the system's capacity to effectively process the organic load. These parameters further confirm that the modified Gompertz model accurately describes the conversion kinetics of soluble organic matter in this mixed-culture bioprocess.

The first-order with residual kinetic model also provided a good fit to the experimental data. The R^2, RMSE, and AIC values for this model were 0.847, 0.204, and −38.915, respectively. Although both models performed well, the modified Gompertz model proved superior in terms of fit and accuracy, making it more suitable for describing soluble organic matter conversion. However, the first-order with residue model is simpler and has a lower AIC, which may make it preferable in cases where a more straightforward process description is required.

In the first-order with residue model, the biodegradation rate constant (K_B of 0.267 d^{-1}) indicates moderate biodegradation efficiency (effective but not rapid). This value suggests that approximately 26.7% of the biodegradable substrate is degraded per day, following exponential kinetics.

In contrast, the first-order, Logistic, Monod with growth, and Logarithmic kinetic models did not fit the experimental data well ($R^2 \leq 0.753$, RMSE ≥ 0.260, AIC ≥ -31.152). As a result, the kinetic parameters from these models were excluded from further discussion.

Figure 5.4 illustrates the concentration of soluble organic matter throughout the fermentation period. It compares the experimentally measured values with those predicted by the best-fit models.

The results of our study are consistent with previous research. The modified Gompertz model, an unstructured approach, is widely applied to describe substrate consumption, particularly in AF for hydrogen production [200,213]. This study underscores the model's reliability as an alternative for characterizing substrate consumption in VFA production. Notably, the R_{max} and λ values derived from the modified Gompertz model have not yet been reported for CWW or other substrates used in VFA production.

Boshagh et al. [200] highlighted various models for predicting substrate degradation, including Logistic, ADM1, Michaelis–Menten, Luedeking–Piret, Gompertz, Stover-Kincannon, first-order, Grau second-order, and Logarithmic. The selection of an appropriate model often depends on the dataset's quality, level of detail, and the parameters analyzed. Similarly, Mu et al. [213] demonstrated that the modified Gompertz model effectively describes substrate utilization kinetics during hydrogen production from waste using mixed cultures.

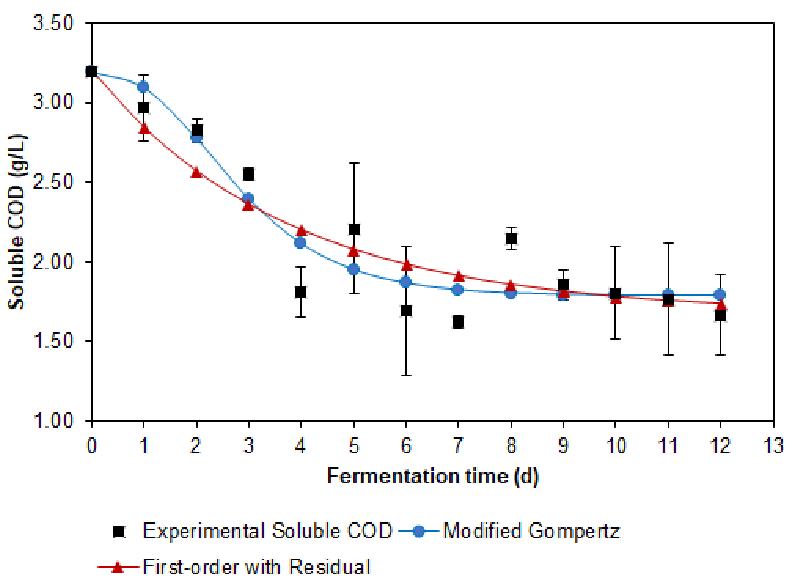


Figure 5.4 Concentration of soluble organic matter, as measured experimentally and predicted by kinetic models.

Morais et al. [25] reported that the first-order and first-order with residual models effectively described the consumption of soluble organic matter during the AF of bovine slaughterhouse wastewater. The first-order with residual model, with an R^2 of 0.963 and an AIC of −27.21, provided a superior fit by incorporating residual biodegradable organic matter. They determined a K_B value of $0.26 \pm 0.07\ d^{-1}$, closely matching the value obtained in our study for CWW. Similarly, de Sousa e Silva [73] identified the first-order with residual kinetic model as the most accurate for dairy wastewater, achieving R^2 values of 0.904 to 0.999 and AIC values between −69.82 and −22.77 for S/M ratios ranging from 0.8 to 1.9 gCOD/gVSS. The K_B values ranged from 0.24 to 0.57 d^{-1}. Higher S/M ratios (1.6 and 1.9 gCOD/gVSS) were associated with faster kinetics. Coelho et al. [24] also found the first-order with residual kinetic model most suitable for dairy wastewater. They reported an R^2 of 0.999, an AIC of −25.950, and a K_B value of $0.86 \pm 0.31\ d^{-1}$.

Nevertheless, our results differ slightly from other studies. Coelho et al. [19] investigated the AF of residual glycerol and observed that substrate consumption followed first-order kinetics. Among the three models tested—first-order, Monod with growth, and Logistic—the Logistic model provided the best fit, with the highest R^2 and lowest NRMSE. For this substrate, the K_B value was $0.05 \pm 0.01\ d^{-1}$. Additionally, Morais et al. [105] evaluated several models, including first-order, first-order with residual, Monod with growth, and Logistic, for the AF of swine wastewater but found that none adequately fit the experimental data.

To date, we have not found any KB values in the scientific literature for the AF of CWW aimed at VFA production.

The K_B in AF is influenced by several factors related to process conditions, such as temperature, pH, and stirring and mixing rates. Additionally, the nature of the substrate—including its concentration, composition, and complexity—as well as the microbial composition play crucial roles in the kinetics of substrate degradation.

5.3.3.2 Modeling of VFA Production from CWW

Table 5.6 presents the estimated parameters, along with the R^2, RMSE, and AIC values, for the models describing VFA production from CWW.

Table 5.6 Kinetic parameters estimated by modeling of VFA production in AF of CWW.

Kinetic model	Parameters and fitting criteria	Value
First-order	K_{VFA} (d^{-1})	0.297
	R^2	0.925
	RMSE	0.137
	AIC	-49.315
Second-order	K''_{VFA} (L/g.d)	0.402
	R^2	0.836
	RMSE	0.202
	AIC	-39.229
Fitzhugh	K_{VFA} (d^{-1})	0.240
	n	1.239
	R^2	0.925
	RMSE	0.137
	AIC	-47.479

Table 5.6 Kinetic parameters estimated by the modeling of VFA production in AF of CWW (continuation).

Cone	K_{VFA} (d^{-1})	0.432
	n	1.713
	R^2	0.892
	RMSE	0.164
	AIC	-41.826
Monomolecular	K_{VFA} (d^{-1})	0.297
	λ (d)	0
	R^2	0.925
	RMSE	0.137
	AIC	-46.479
Modified Gompertz	μ_m (g/L.d)	0.284
	λ (d)	0
	R^2	0.922
	RMSE	0.140
	AIC	-45.966
Logistic	μ_m (g/L.d)	0.263
	λ (d)	0
	R^2	0.920
	RMSE	0.141
	AIC	-45.648
Transference	μ_m (g/L.d)	0.474
	λ (d)	0
	R^2	0.925
	RMSE	0.137
	AIC	-46.479
Richards	μ_m (g/L.d)	0.028
	λ (d)	0
	v	0.039
	R^2	0.922
	RMSE	0.140
	AIC	-42.489
BPK	m	0.924
	t_0 (d)	0.319
	μ_m (g/L.d)	0.382
	K_{VFA} (d^{-1})	0.239
	R^2	0.926
	RMSE	0.136
	AIC	-46.718

The results indicate that all tested models provided a satisfactory fit to the experimental VFA production data. The R^2 values ranged from 0.836 to 0.926, RMSE values from 0.136 to 0.202, and AIC values from −49.315 to −39.229. Among these models, the exponential kinetic models—first-order, BPK, Fitzhugh, Monomolecular, and Transference—demonstrated the best fit. Notably, the BPK model achieved the highest R^2 value (0.926) and the lowest RMSE (0.136), while the first-order model recorded the lowest AIC value (−49.315).

The Logistic, modified Gompertz, and Richards models also exhibited high R2 values (≥0.920). However, their RMSE (≥0.140) and AIC (≥−45.966) values were higher than those of the best-fit models. In contrast, the second-order and Cone kinetic models did not fit the experimental data adequately, and their kinetic parameters were excluded from further discussion.

For models with a good fit, the estimated K_{VFA} values ranged from 0.239 to 0.297 d^{-1}. The highest values were observed in the first-order and Monomolecular models. The Fitzhugh model estimated an n value of 1.239, while all models predicted a λ value of 0 d. The μm values ranged from 0.263 to 0.474 g/L·d, except for the estimate from the Richards model, which was omitted due to its significant deviation from the predictions of the other models.

Figure 5.5 compares the experimentally measured VFA production values with those predicted by the best-fit models.

The curve exhibits an inverted L-shape, characteristic of processes with a high production rate during the initial incubation days and either no or a very short lag phase. A similar curve was reported by Morais [199] for VFA production from slaughterhouse wastewater. The rapid increase in VFA production during the early days indicates high microbial activity and substrate availability. In our study, the inoculum had a high biomass concentration (VS/TS = 0.72) and underwent progressive adaptation to the substrate before the experiment, facilitating rapid fermentation. Additionally, the organic matter was readily available and easily biodegradable, enabling swift microbial assimilation and conversion.

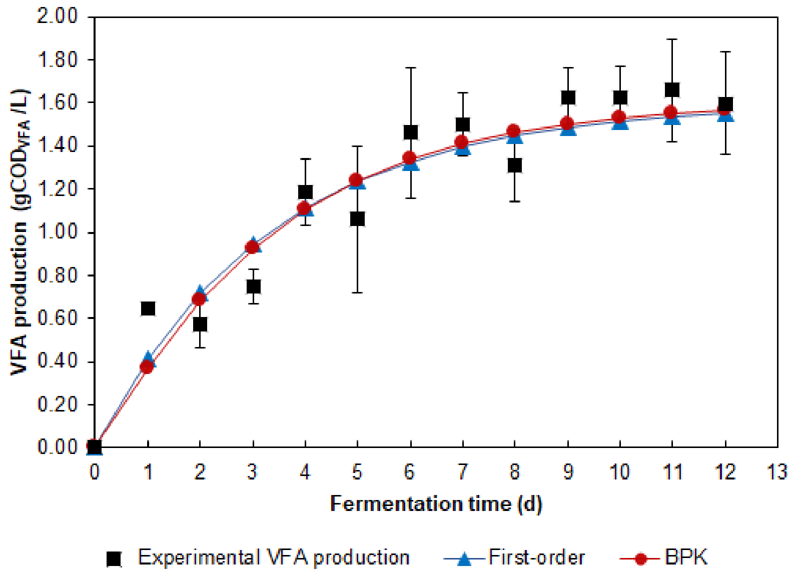


Figure 5.5 Comparison of experimentally measured VFA production in AF of CWW with values predicted by best-fit models.

Based on the adjusted curve (Figure 5.5), the VFA concentration at the end of the fermentation period was 1.564 gCOD/L. Within the first six days—half of the incubation period −86% of this concentration was achieved. Several biochemical and operational factors may have contributed to this accelerated VFA production, including the presence of easily degradable carbohydrates, the predominance of acidogenic bacteria in the microbial consortium, suitable pH and temperature conditions, the inhibition of methanogenic activity through thermal pretreatment, and a batch bioreactors operation.

Our findings align with previous studies on VFA production kinetics. Morais et al. [25] demonstrated that exponential kinetic models, including the Cone, first-order, and Fitzhugh models, effectively describe the VFA production from bovine slaughterhouse wastewater. Among these, the Cone model provided the best fit, with an R2 of 0.995 and an AIC of −37.08. The estimated K_{VFA} value (0.30 ± 0.07 d^{-1}) closely matched the value obtained in our study for CWW.

Similarly, de Sousa e Silva [73] evaluated various S/M ratios (0.8, 1.2, 1.6, and 1.9 gCOD/gVSS) for VFA production from dairy wastewater. They concluded that exponential models, particularly the Fitzhugh model (R^2 ranging from 0.985 to 0.999 and AIC from −60.450 to −31.027), were best suited for this process. Among the tested S/M ratios, 0.8 gCOD/gVSS yielded the most favorable results, with K_{VFA} values between 0.09 and 0.17 d^{-1}.

For dairy wastewater, Coelho et al. [24] found that exponential-phase models, such as the first-order and Fitzhugh models, were suitable for simulating VFA production. The first-order model provided the best fit (R^2 of 0.997 and AIC of −21.683), with an estimated K_{VFA} of 0.60 ± 0.06 d−1. Another study by Coelho et al. [23] on the AF of residual glycerol concluded that the Fitzhugh and modified Gompertz models best described the cumulative VFA production. The Fitzhugh model achieved a lower NRMSE and an estimated K_{VFA} of 0.61 ± 0.05 d^{-1}.

In contrast, Morais et al. [105] found that logistic growth models, such as the Richards and Logistic models, were more suitable for representing VFA production from swine wastewater. The Richards model provided the best fit, with an R^2 of 0.993 and an AIC of −41.072.

Research on modeling VFA production from cassava processing residues, particularly wastewater, remains scarce in the scientific literature. Undiandeye et al. [204] evaluated first-order, modified Gompertz, and dual pool models for medium-chain carboxylic acid production (caproic, heptanoic, and caprylic acids) from mixtures of cassava peel silage and wastewater. All models showed good fit ($R^2 > 0.974$), but the dual pool model had the lowest RMSE (≤0.004) and AIC (≤−37.09).

Several studies agree on the kinetic model that best describes the VFA production curve during the AF of wastewater. However, the K_{VFA} value results from a complex interaction of substrate characteristics, operating conditions, and microbial activity. Higher K_{VFA} values indicate faster VFA production for a given substrate, as reflected in the inverted L-shape of the production curve. In contrast, lower K_{VFA} values correspond to an elongated S-shaped production curve, signifying slower VFA generation.

In the Fitzhugh and Cone models, the form factor n indicates the presence ($n \geq 1$) or absence ($n < 1$) of a lag phase, reflecting the affinity of microorganisms for the soluble substrate. In our study, the Fitzhugh model estimated an n value of 1.239 (Table 5.6), suggesting the presence of a lag phase. During this phase, hydrolytic bacteria break down particulate material into soluble compounds, which acidogenic and acetogenic bacteria utilize to initiate VFA production (exponential phase) [25]. However, the lag phase in our study was likely very brief, as the λ value estimated by the Monomolecular, Transference, modified Gompertz, Logistic, and Richards models was 0 days.

This brief lag phase can be attributed to the rapid adaptation of microorganisms to the established environmental and operational conditions, as well as the high biodegradability of CWW. The absence of a lag phase suggests that the microorganisms were already metabolically active and prepared to convert organic matter into VFAs. The physicochemical characterization of the substrate (Table 5.3) showed that 89.8% of the total COD was in soluble form, indicating that hydrolysis was not a limiting step in the AF of CWW.

The absence or reduction of the lag phase significantly enhances process efficiency and scalability. A rapid fermentation onset increases yields in less time, reduces retention periods, and improves overall efficiency. Additionally, accelerated VFA production can facilitate integration with downstream processes, such as bioplastics or bioenergy production. In large-scale systems, a fast substrate conversion shortens operating times and optimizes reactor capacity. However, accelerated AF may require monitoring and control strategies for pH and other parameters to maintain stability in continuous reactors.

The observed discrepancies between the n and λ values, as noted by Coelho et al. [24], may result from differences in the parameters used by each mathematical model. Advanced optimization tools for estimating kinetic parameters could yield more accurate results, particularly for λ, which may approach but not reach zero. According to Fang et al. [214], various algorithms can be used to estimate parameters in biochemical reaction simulations, such as particle swarm optimization (PSO). Bai et al. [215] modeled substrate degradation and the influence of pH on VFA production using waste-activated sludge through a modified ADM1. They demonstrated that the improved PSO algorithm was the most effective method for parameter estimation.

After the lag phase, the VFA production curve exhibits an exponential growth pattern (Figure 5.5) and stabilizes toward the end of the evaluation period. The shape of the production curve may reflect the biodegradability characteristics of the substrate, the production of inhibitory compounds, and the kinetics of biological processes [216]. Morais et al. [25] observed a similar kinetic behavior in bovine slaughterhouse wastewater, where the VFA production curve also followed an exponential pattern. This indicated high VFA productivity in the initial days of incubation, a short lag time, and consequently a high yield of acidogenic microorganisms. VFA production stabilizes when the biodegradable fraction of organic matter is depleted and only the recalcitrant fraction remains [25]. In our study, the initial concentration of carbohydrates—the primary organic compound of the substrate—was 1906 mg/L (Table 5.3). By the end of the experimental period (day 12), this concentration had decreased to 38.42 mg/L.

The Modified Gompertz, Logistic, Transference, and BPK models (Table 5.6) were used to estimate the μ_m. The highest μ_m value (0.474 gCOD/L·d) was estimated by the Transference model, followed by the BPK (0.382 gCOD/L·d), modified Gompertz (0.284 gCOD/L·d), and Logistic (0.263 gCOD/L·d) models. The Transference model provided the closest approximation to the experimentally determined maximum productivity (0.642 gCODVFA/L·d), demonstrating its applicability for estimating this parameter. Similarly, Morais et al. [25] reported μ_m values for bovine slaughterhouse wastewater ranging from 0.33 ± 0.05 to 0.46 ± 0.09 gCOD/L·d, obtained using the Logistic and Transference models, respectively.

Finally, the poor fit of some models selected to represent VFA production from CWW may be attributed to their failure to account for a decrease in product concentration, which renders them unsuitable for accurately describing the experimental data.

5.4 Conclusions

CWW is a promising substrate for VFA production through AF. The maximum VFA yield, 0.44 $gCOD_{VFA}/gCOD$, was achieved on the ninth day of fermentation, with VFAs primarily consisting of propionic, acetic, and butyric acids. The highest experimentally calculated productivity, 0.642 $gCOD_{VFA}/L\cdot d$, occurred on the first day of fermentation.

The modified Gompertz and first-order with residual models provided the best fit for describing soluble organic matter consumption during the AF of CWW. The first-order kinetic model with residual estimated a K_B value of 0.267 d^{-1}. Additionally, the first-order and BPK models accurately described the VFA production data, with the selected models estimating a K_{VFA} value of 0.297 d^{-1}, a λ value of 0 d, and a μ_m value of 0.474 gCOD/L·d. The kinetic parameters derived from these models can guide the design of new acidogenic reactors and the development of strategies for controlling and simulating VFA production using CWW as a low-cost substrate.

Author Contributions: Conceptualization, L.M.S.-L., J.A.R.-V., and H.R.-M.; methodology, L.M.S.-L., J.A.R.-V., and H.R.-M.; validation, L.M.S.-L.; formal analysis, L.M.S.-L.; investigation, L.M.S.-L.; resources, L.M.S.-L.; data curation, L.M.S.-L.; writing—original draft preparation, L.M.S.-L.; writing—review and editing, J.A.R.-V. and H.R.-M.; visualization, L.M.S.-L.; supervision, J.A.R.-V. and H.R.-M.; project administration, H.R.-M.; funding acquisition, H.R.-M. and L.M.S.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universidad del Valle (project CI-21237) and the Ministerio de Ciencia, Tecnología e Innovación de Colombia—MinCiencias (convocatoria No. 933—2023).

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflicts of interest.

6 Conclusions and Future Perspectives

This doctoral thesis rigorously investigates the potential of AF to produce VFAs using wastewater generated during the cassava sour starch extraction. The research followed a structured approach encompassing bibliometric analysis, experimental trials evaluating key process parameters, and kinetic modeling.

Bibliometric analysis mapped the evolution, geographic distribution, thematic trends, and knowledge gaps in this field from a comprehensive perspective. It not only synthesizes decades of research but also offers strategic insights to guide scientific and technological development in both local and global contexts.

Findings from this analysis reveal a conceptual shift in the valorization of wastewater from a traditional water treatment focus to an emphasis on resource recovery. This transition, driven by advances in biotechnology, has enabled the development of technologies that position AF as a promising method for generating high-value products beyond biogas. However, research remains concentrated in a few countries (e.g., China, the United States, India, Italy, Spain, and Australia), raising concerns about scientific equity and technology transfer, particularly in Latin America, where research output remains limited.

One of the main contributions of this study lies in identifying and quantifying the effects of key variables influencing the AF process, such as fermentation time, pH, substrate-to-microorganism ratio, and temperature. The results demonstrated that CWW fermentation is highly sensitive to environmental and operational parameters, underscoring the need for precise control to maximize yield and selectivity. Under the evaluated conditions, VFA yields ranged from 0.44 to 0.58 $gCOD_{VFA}$/gCOD, values comparable to those reported for other organic liquid waste more widely studied in the literature. Individual evaluation of process variables revealed distinct effects on both the quantity and composition of VFAs—primarily acetic, butyric, and propionic acids—highlighting the complexity of fermentative processes involving agro-industrial substrates and mixed microbial cultures.

Although some interactions between variables (e.g., pH and fermentation time) were less significant, they still influenced dominant metabolic pathways and overall process efficiency. Future research should explore additional interactions (e.g., fermentation time and substrate-to-microorganism ratio, or pH and temperature) or adopt robust experimental designs that consider variables shown to significantly impact VFA production.

This study also contributed to the field by identifying kinetic models—including the modified Gompertz, first-order with residual, first-order, and BPK models—that effectively describe the dynamics of the AF, particularly in relation to soluble organic matter consumption and VFA production from CWW. The kinetic parameters estimated in this study (e.g., maximum substrate conversion, substrate conversion and VFA production rates, and lag phase duration) enabled a quantitative characterization of the process performance under controlled conditions. These parameters not only clarified substrate transformation capacity but also elucidated the timing and rates of the key biological processes involved.

Collectively, these kinetic insights support the development of predictive mathematical models, inform reactor design and control at larger scales, and facilitate the creation of simulation tools to optimize operating conditions. These contributions advance the engineering of biotechnological processes for treating agro-industrial wastewater.

Beyond the experimental findings, this thesis offers methodological and conceptual tools that can be adapted to other national or regional low-cost substrates with high organic loads.

The valorization of CWW for VFA production can diversify cassava-based product portfolios, reduce environmental impacts from improper effluent disposal, and create economic opportunities for rural communities engaged in this agro-industry. Thus, this thesis provides scientific evidence to support the transition toward more sustainable and resilient agro-industrial systems.

However, the inherent complexity of biological systems and the variability of substrates present significant challenges that must be addressed in future research. Transitioning to continuous and controlled systems will be essential to stabilize VFA production and accommodate feedstock fluctuations. Validating experimental results in continuous operations would enhance technological robustness. This progression requires not only appropriate reactor designs but also the integration of online sensors and automated control strategies. Pilot-scale evaluations will be crucial for assessing the technical and economic feasibility of real-world applications.

Moreover, the characterization and monitoring of the microbial community involved in AF are increasingly recognized as critical, given their direct influence on metabolic pathways and resulting VFA profiles. Integrating molecular biology tools with kinetic modeling offers a promising strategy to optimize inoculum selection and adaptation, tailored to specific process conditions and desired metabolic outputs.

Future research using CWW could prioritize the production of butyric acid, given its high market value and the observed predominance of butyric-type fermentation in acidogenic systems fed with this effluent. Furthermore, additional strategies such as chain elongation for medium-chain fatty acid production or the simultaneous recovery of hydrogen, ethanol, and other co-products should also be explored. These complementary approaches would enhance both the energetic and chemical valorization of the substrate, expand the product portfolio, and improve the overall profitability of the system.

The VFAs produced hold potential beyond conventional bioenergy, presenting a strategic opportunity to expand the reach of AF. Their application in the synthesis of bioplastics, biofertilizers, and other high-value bioproducts opens a multidisciplinary research avenue with strong potential for collaboration across scientific and industrial sectors. Realizing this potential will require policy frameworks that support the implementation of waste valorization technologies and promote collaboration among science, industry, and local communities.

Finally, advancing hybrid models that combine mechanistic approaches with artificial intelligence will be essential for accurate process prediction and the implementation of real-time, automated control systems. These advancements should be supported by techno-economic analyses to assess feasibility and scalability across diverse geographic and production contexts.

This thesis provides experimental, methodological, and conceptual evidence to support the continued exploration, development, and application of AF as a strategic tool in the transition toward more sustainable production systems. The knowledge generated offers a roadmap for researchers, engineers, and stakeholders in the agro-industrial sector to develop clean, efficient technologies aligned with the principles of sustainability and the circular economy.

List of Products

As part of this research, an undergraduate thesis was conducted, contributing to the formation of two students from the Sanitary and Environmental Engineering program at Universidad del Valle.

- Karen Daniela Díaz Bravo and Valentina Gutiérrez Domínguez: "Influencia de la temperatura en la producción de ácidos grasos volátiles a partir de agua residual del proceso de extracción de almidón agrio de yuca".

Additionally, four scientific articles were published in Q1-indexed journals.

- Sanchez-Ledesma, L. M., Ramírez-Malule, H., & Rodríguez-Victoria, J. A. (2023). Volatile Fatty Acids Production by Acidogenic Fermentation of Wastewater: A Bibliometric Analysis. Sustainability, 15(3), 2370. https://doi.org/10.3390/su15032370
- Sanchez-Ledesma, L. M., Rodríguez-Victoria, J. A., & Ramírez-Malule, H. (2024). Effect of Fermentation Time, pH, and Their Interaction on the Production of Volatile Fatty Acids from Cassava Wastewater. Water, 16(11), 1514. https://doi.org/10.3390/w16111514
- 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. https://doi.org/10.3390/w16233344
- Sanchez-Ledesma, L. M., Rodríguez-Victoria, J. A., & Ramírez-Malule, H. (2025). Volatile Fatty Acids Production Using Cassava Wastewater as Low-Cost Substrate. Water, 17(7), 991. https://doi.org/10.3390/w17070991

Finally, the research findings were presented at the following events:

- **WCCE11** – 11th World Congress of Chemical Engineering. Poster presentation tittle "Influence of retention time and substrate-microorganism ratio on the acidogenic fermentation of wastewater from the cassava starch extraction process for the production of volatile fatty acids". Buenos Aires (Argentina), June 4-8, 2023. Authors: Lina Marcela Sanchez Ledesma, Jenny Alexandra Rodríguez Victoria, Howard Ramírez Malule.
- **MICROBIOTECH2024** - 2nd International Congress of Microbiome Biotechnology. Oral presentation tittle "Efecto del pH y la temperatura en la fermentación acidogénica del agua residual proveniente del procesamiento de la yuca para la producción de ácidos grasos volátiles". Cali (Colombia), November 12-15, 2024. Authors: Lina Marcela Sanchez-Ledesma, Karen Daniela Díaz Bravo, Valentina Gutiérrez Domínguez, Daniela Cañón Rueda, Jenny Alexandra Rodríguez-Victoria, Howard Ramírez-Malule.

Supplementary Material

Volatile fatty acids production by acidogenic fermentation of wastewater: a bibliometric analysis

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Table 2S1 shows the top-10 cited studies in the field of VFA and wastewater fermentation.

Table 2S1. The top-10 cited publications in the field of VFA and wastewater fermentation

Rank	Title – Authors	Journal	Year	Cites	C/Y*	Comments
1	Enhancement of waste activated sludge protein conversion and volatile fatty acids Accumulation during Waste Activated Sludge Anaerobic Fermentation by Carbohydrate Substrate Addition: The Effect of pH Leiyu Feng, Yinguang Chen and Xiong Zheng	Environmental Science and Technology	2009	298	24.8	The authors investigated the effect of pH on waste activated sludge protein conversion and VFA production by anaerobic fermentation after the addition of carbohydrates. The results showed that the addition of carbohydrates resulted in improved protein conversion. In addition, pH influenced both total VFA production and the percentage of individual VFA. Maximum VFA production (520.1 mg COD per gram of volatile suspended solids) occurred at a pH of 8.0 and a fermentation time of 8 days.
2	Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater Simon Bengtsson, Alan Werker, Magnus Christensson and Thomas Welander	Bioresource Technology	2008	261	20.1	In this study, the production of PHA from activated sludge generated in the anaerobic treatment of wastewater from a paper industry was investigated. The authors carried out acidogenic fermentation to convert organic matter to VFA, enrichment of PHA-producing organisms and accumulation of PHA in batch experiments. Experimental results showed that 74% of the soluble COD was present as VFA (acetate, propionate, butyrate and valerate) and the resulting HAP after batch accumulation consisted of 31-47mol% hydroxybutyrate and 53-69mol% hydroxyvalerate.

Rank	Title – Authors	Journal	Year	Cites	C/Y*	Comments
3	Biohydrogen production from cheese processing wastewater by anaerobic fermentation using mixed microbial communities Peilin Yang, Ruihong Zhang, Jeffery A. McGarvey and John R. Benemannd	International Journal of Hydrogen Energy	2007	185	13.2	The authors investigated batch and continuous anaerobic fermentation processes for H_2 production from cheese whey wastewater using mixed microbial cultures. In batch experiments, they verified H_2 yields of 8 and 10 mM/gCOD when the bioreactors were fed with feed-to-microorganism ratios of 1.0 and 1.5, respectively. A pH range of 4.0 to 5.0 favored continuous fermentation of the wastewater studied. The maximum H_2 production yields were observed when Lactobacillus bacteria predominated in the bioreactor.
4	Anaerobic acidogenesis of a complex wastewater: I. The influence of operational parameters on reactor performance Georgia Dinopoulou, Thomasine Rudd and John N. Lester	Biotechnology and Bioengineering	1988	180	5.5	The objective of the study was to investigate the effect of different operating parameters (hydraulic retention time, influent substrate concentration, organic loading rate, pH and temperature) on the conversion of the substrate (beef extract-based) into volatile acids, on the rate of acid production per unit reactor and on the composition of the reactor effluent. The degree of acidification increased with hydraulic retention time and decreased with influent substrate concentration and organic loading rate. An opposite behavior was verified for the rate of product formation. For all operational conditions, the predominant fermentation products were acetic and propionic acid.
5	Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production Simon Bengtsson, Jakob Hallquist, Alan Werker and Thomas Welander	Biochemical Engineering Journal	2008	177	13.6	The objective of this study was to examine the acidogenic fermentation of four industrial wastewaters (three paper mill effluents and one whey effluent), as potential substrates for PHA production, through batch and continuous experiments. For the conditions applied in the batch (pH 6) and continuous (pH 3.5-6 and retention time 8-95 h) experiments, the main fermentation products were acetate, propionate and butyrate. Retention time and pH of the chemostat significantly affected the composition of VFA produced.

Rank	Title – Authors	Journal	Year	Cites	C/Y*	Comments
6	Olive oil mill effluents as a feedstock for production of biodegradable polymers D. Dionisi, G. Carucci, M. Petrangeli Papini, C. Riccardi, M. Majone and F. Carrasco	Water Research	2005	167	10.4	The objective of the study was to examine the production of PHA from olive oil mill effluent. The authors investigated anaerobic fermentation without pretreatment, with different pretreatments (centrifugation, bentonite addition and bentonite addition followed by centrifugation), at different COD concentrations. Additionally, PHA production was investigated through batch experiments with a mixed culture of an aerobic SBR. The results showed that at all initial concentrations, pretreatment by centrifugation (with or without prior bentonite addition) significantly increased the final VFA concentration and yield, while bentonite addition alone had no influence. The initial specific rate of PHA production obtained from the fermented effluent was approximately 420 mg COD $gCOD^{-1}$ h^{-1}.
7	Biological hydrolysis and acidification of sludge under anaerobic conditions: The effect of sludge type and origin on the production and composition of volatile fatty acids Ahmed Suheyl Ucisik and Mogens Henze	Water Research	2008	156	12.0	The objective was to examine the efficiency of acid fermentation (VFA production and composition) with a short SRT in various types of sludge, through batch and semi-continuous experiments. The results showed that primary sludge fermentation produced higher amounts of VFA and generated significantly higher COD and VFA yields compared to other types of sludge. COD yields for primary, activated and mixed sludge were 19.1%, 6.5% and 21.37%, respectively, in semi-continuous experiments operated with a SRT of 5 days and a temperature of 37 ºC.
8	Continuous fermentative hydrogen production from cheese whey wastewater under thermophilic anaerobic conditions Nuri Azbar, F. Tuba Çetinkaya Dokgöz, Tugba Keskin, Kemal S. Korkmaz and Hamid M. Syed	International Journal of Hydrogen Energy	2009	149	12.4	The objective of this study was to investigate continuous anaerobic fermentation processes for H_2 production from cheese whey under thermophilic conditions, varying TRH and OLR. The results showed a hydrogen content (5-82%) in the biogas and a hydrogen production rate ranging from 0.3 to 7.9 l H_2/l/day.

Rank	Title – Authors	Journal	Year	Cites	C/Y*	Comments
						The authors verified H_2 yields of 22, 15 and 5 mmol/g COD (at a constant influent COD of 40 g/l) with HRT values of 3.5, 2 and 1 day, respectively. In addition, H_2 yields of 3, 9 and 6 mmol/g COD, for OLR values of 47, 35 and 21 g COD/l/day (at a constant HRT in 1 day) were observed.
9	Microbial fuel cells operating on mixed fatty acids Stefano Freguia Ee Hoi The, Nico Boon, Kar Man Leung, Jurg Keller and Korneel Rabaey	Bioresource Technology	2010	141	12.8	The authors investigated the potential use of VFA mixtures from fermented sludge hydrolysates for power generation by microbial fuel cells. The results showed that acetate and propionate were preferred as electron donors in the mixed VFA system, generating a power density of 49±1 mW L^{-1} NAC. The other VFAs (butyrates/valerates/caproic acid) were also removed, although at a slower rate.
10	Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste Jun Wei Lim and Jing-Yuan Wang	Waste Management	2013	141	17.6	The objective of the study was to investigate the effect of microaeration pretreatment on the degradation of brown water and food waste in batch tests. Additionally, the authors examined the influence of inoculum on the effects of microaeration. The results showed that microaeration is an effective pretreatment for anaerobic co-digestion of wastewater and food waste. In addition to increasing solubilization and acidification efficiency, microaeration enhanced the conversion of other short-chain fatty acids to acetate. In addition, the authors demonstrated that the nature of the inoculum influences the effect of microaeration. A 21% and 10% increase in methane yield was verified when pretreatment was applied to inoculated substrates and to substrates without inoculum, respectively.

* Citations per year

References

1. Bastidas-Oyanedel, R.J. Thermodynamic Based Modelling of Biohydrogen Production by Anaerobic Fermentation, Université Montpellier 2, 2011.

2. de Sousa e Silva, A.; Morais, N.W.S.; Coelho, M.M.H.; Pereira, E.L.; dos Santos, A.B. Potentialities of Biotechnological Recovery of Methane, Hydrogen and Carboxylic Acids from Agro-Industrial Wastewaters. *Bioresour Technol Rep* **2020**, *10*, doi:10.1016/j.biteb.2020.100406.

3. CRA *Análisis de Impacto Normativo. Regulación Frente a Tarifas Por Actividad Del Servicio - Tratamiento de Vertimientos. Documento Final.*; Comisión de Regulación de Agua Potable y Saneamiento Básico–CRA: Bogotá, Colombia, 2019;

4. Aristizábal, J.; Sánchez, T. *Guía Técnica Para Producción y Análisis de Almidón de Yuca*; Roma, Italy, 2007; Vol. 163;.

5. Minagricultura Cadena Productiva de La Yuca: Cifras Sectoriales. *Dirección de Cadenas AgrÍcolas y Forestales* 2021, 23.

6. Taborda-Andrade, L.A. Determinación y Análisis Integral de Impactos de La Agroindustria Rural de Almidón de Yuca En Cauca Colombia. Tesis de Doctorado, Universidad Nacional de Colombia, 2018.

7. Canales, N.; Trujillo, M. *La Red de Valor de La Yuca y Su Potencial En La Bioeconomía de Colombia*; Estocolmo, Suecia, 2021;

8. Torres, P.; Rodríguez, J.; Rojas, O. Extracción de Almidón de Yuca. Manejo Integral y Control de La Contaminación Hídrica. *Livestock Research for Rural Development* **2005**, *17*, 1–11.

9. Howeler, R.H.; Oates, C.G. An Assessment of the Impact of Cassava Production and Processing on the Environment. *Research Gate* **2018**.

10. Howeler, R.H.; Oates, C.G.; Costa Allem, A. *Strategic Environmental Assessment. An Assessment of the Impact of Cassava Production and Processing on the Environment and Biodiversity*; 2001; Vol. 5;.

11. Pérez Vidal, A.; Torres Lozada, P.; Silva Leal, J. Tratamiento Anaerobio de Las Aguas Residuales Del Proceso de Extracción de Almidón de Yuca. Optimización de Variables Ambientales y Operacionales. *Dyna (Medellin)* **2009**, *76*, 139–148.

12. Sarria Núñez, H. Contaminación y Toxicidad de Las Aguas Residuales de Las Rallanderías Del Norte Del Cauca, Colombia, Universidad Complutense de Madrid, 2011.

13. Torres, P.; Rodríguez, J.A.; Uribe, I.E. Tratamiento de Aguas Residuales Del Proceso de Extracción de Almidón de Yuca En Filtro Anaerobio: Influencia Del Medio de Soporte. *Scientia et Technica Año IX* **2003**, *3*, 75–80.

14. Hasan, S.D.M.; Giongo, C.; Fiorese, M.L.; Gomes, S.D.; Ferrari, T.C.; Savoldi, T.E. Volatile Fatty Acids Production from Anaerobic Treatment of Cassava Waste Water: Effect of Temperature and Alkalinity. *Environ Technol* **2015**, *36*, 2637–2646, doi:10.1080/09593330.2015.1041426.

15. Bhatt, A.H.; Ren, Z. (Jason); Tao, L. Value Proposition of Untapped Wet Wastes: Carboxylic Acid Production through Anaerobic Digestion. *iScience* **2020**, *23*, doi:10.1016/j.isci.2020.101221.

16. Atasoy, M.; Owusu-Agyeman, I.; Plaza, E.; Cetecioglu, Z. Bio-Based Volatile Fatty Acid Production and Recovery from Waste Streams: Current Status and Future Challenges. *Bioresour Technol* **2018**, *268*, 773–786, doi:10.1016/j.biortech.2018.07.042.

17. Li, W.W.; Yu, H.Q. From Wastewater to Bioenergy and Biochemicals via Two-Stage Bioconversion Processes: A Future Paradigm. *Biotechnol Adv* **2011**, *29*, 972–982, doi:10.1016/j.biotechadv.2011.08.012.

18. Zacharof, M.P.; Lovitt, R.W. Complex Effluent Streams as a Potential Source of Volatile Fatty Acids. *Waste Biomass Valorization* **2013**, *4*, 557–581, doi:10.1007/s12649-013-9202-6.

19. Bastidas-Oyanedel, J.-R.; Bonk, F.; Thomsen, M.H.; Schmidt, J.E. Dark Fermentation Biorefinery in the Present and Future (Bio)Chemical Industry. *Rev Environ Sci Biotechnol* **2015**, *14*, 473–498, doi:10.1007/s11157-015-9369-3.

20. Fang, W.; Zhang, X.; Zhang, P.; Wan, J.; Guo, H.; Ghasimi, D.S.M.; Morera, X.C.; Zhang, T. Overview of Key Operation Factors and Strategies for Improving Fermentative Volatile Fatty Acid Production and Product Regulation from Sewage Sludge. *J Environ Sci (China)* **2020**, *87*, 93–111, doi:10.1016/j.jes.2019.05.027.

21. Bengtsson, S.; Hallquist, J.; Werker, A.; Welander, T. Acidogenic Fermentation of Industrial Wastewaters: Effects of Chemostat Retention Time and PH on Volatile Fatty Acids Production. *Biochem Eng J* **2008**, *40*, 492–499, doi:10.1016/j.bej.2008.02.004.

22. Khan, M.A.; Ngo, H.H.; Guo, W.; Liu, Y.; Nghiem, L.D.; Chang, S.W.; Nguyen, D.D.; Zhang, S.; Luo, G.; Jia, H. Optimization of Hydraulic Retention Time and Organic Loading Rate for Volatile Fatty Acid Production from Low Strength Wastewater in an Anaerobic Membrane Bioreactor. *Bioresour Technol* **2019**, *271*, 100–108, doi:10.1016/j.biortech.2018.09.075.

23. Coelho, M.M.H.; Morais, N.W.S.; Ferreira, T.J.T.; Silva, F.S.S.; Pereira, E.L.; dos Santos, A.B. Carboxylic Acids Production Using Residual Glycerol as a Substrate in Anaerobic Fermentation: A Kinetic Modeling Study. *Biomass Bioenergy* **2020**, *143*, doi:10.1016/j.biombioe.2020.105874.

24. Coelho, M.M.H.; Morais, N.W.S.; Pereira, E.L.; Leitão, R.C.; dos Santos, A.B. Potential Assessment and Kinetic Modeling of Carboxylic Acids Production Using Dairy Wastewater as Substrate. *Biochem Eng J* **2020**, *156*, doi:10.1016/j.bej.2020.107502.

25. Morais, N.W.S.; Coelho, M.M.H.; Ferreira, T.J.T.; Pereira, E.L.; Leitão, R.C.; dos Santos, A.B. A Kinetic Study on Carboxylic Acids Production Using Bovine Slaughterhouse Wastewater: A Promising Substrate for Resource Recovery in Biotechnological Processes. *Bioprocess Biosyst Eng* **2021**, *44*, 271–282, doi:10.1007/s00449-020-02440-3.

26. Cerrillo, M.; Viñas, M.; Bonmatí, A. Removal of Volatile Fatty Acids and Ammonia Recovery from Unstable Anaerobic Digesters with a Microbial Electrolysis Cell. *Bioresour Technol* **2016**, *219*, 348–356, doi:10.1016/j.biortech.2016.07.103.

27. González-Cabaleiro, R.; Lema, J.M.; Rodríguez, J. Metabolic Energy-Based Modelling Explains Product Yielding in Anaerobic Mixed Culture Fermentations. *PLoS One* **2015**, *10*, 1–17, doi:10.1371/journal.pone.0126739.

28. Ramos-Suarez, M.; Zhang, Y.; Outram, V. Current Perspectives on Acidogenic Fermentation to Produce Volatile Fatty Acids from Waste. *Rev Environ Sci Biotechnol* **2021**, *20*, 439–478, doi:10.1007/s11157-021-09566-0.

29. de Andrade, C.J.; Simiqueli, A.P.R.; de Lima, F.A.; da Silva, J.B.; de Andrade, L.M.; Fai, A.E.C. Cassava Wastewater as Substrate in Biotechnological Processes. In *Handbook on Cassava: Production, Potential Uses and Recent Advances*; Klein, C., Ed.; Nova Science Publishers, 2017; pp. 171–200 ISBN 9781536103076.

30. Preethi; Usman, T.M.M.; Rajesh Banu, J.; Gunasekaran, M.; Kumar, G. Biohydrogen Production from Industrial Wastewater: An Overview. *Bioresour Technol Rep* **2019**, *7*, doi:10.1016/j.biteb.2019.100287.

31. Lorenzo-Llanes, J.; Pagés-Díaz, J.; Kalogirou, E.; Contino, F. Development and Application in Aspen Plus of a Process Simulation Model for the Anaerobic Digestion of Vinasses in UASB Reactors: Hydrodynamics and Biochemical Reactions. *J Environ Chem Eng* **2020**, *8*, doi:10.1016/j.jece.2019.103540.

32. AIDIS *Uso Seguro Del Agua Para El Reúso*; Tello Espinoza, P., Mijailova, P., Chamy, R., Eds.; São Paulo, Brasil, 2016;

33. Lema Rodicio, J.M. Oportunidades y Retos Para La Digestión Anaerobia. In *Depuración de aguas residuales: digestión anaerobia*; Chiva Vicent, S., Berlanga Clavijo, J.G., Martínez Cuenca, R., Climent Agustina, J., Eds.; 2018; p. 256 ISBN 9788416546657.

34. Chernicharo, C.A. de L. *Anaerobic Reactors*; IWA Publishing: Minas Gerais, Brazil, 2007; Vol. 4; ISBN 9781843391647.

35. Pavlostathis, S.G. Kinetics and Modeling of Anaerobic Treatment and Biotransformation Processes. In *Comprehensive Biotechnology, Second Edition*; Elsevier B.V., 2011; Vol. 6, pp. 385–397 ISBN 9780080885049.

36. Cai, G.; Jin, B.; Monis, P.; Saint, C. Metabolic Flux Network and Analysis of Fermentative Hydrogen Production. *Biotechnol Adv* **2011**, *29*, 375–387, doi:10.1016/j.biotechadv.2011.02.001.

37. Karadag, D.; Köroğlu, O.E.; Ozkaya, B.; Cakmakci, M.; Heaven, S.; Banks, C. A Review on Fermentative Hydrogen Production from Dairy Industry Wastewater. *Journal of Chemical Technology and Biotechnology* **2014**, *89*, 1627–1636, doi:10.1002/jctb.4490.

38. Valentino, F.; Morgan-Sagastume, F.; Campanari, S.; Villano, M.; Werker, A.; Majone, M. Carbon Recovery from Wastewater through Bioconversion into Biodegradable Polymers. *N Biotechnol* **2017**, *37*, 9–23, doi:10.1016/j.nbt.2016.05.007.

39. Ramírez-Malule, H.; López-Agudelo, V.A.; Gómez-Ríos, D. Candida Auris: A Bibliometric Analysis of the First Ten Years of Research (2009-2018). *J Appl Pharm Sci* **2020**, *10*, 12–21, doi:10.7324/JAPS.2020.103002.

40. Ramírez-Malule, H.; Quiñones-Murillo, D.H.; Manotas-Duque, D. Emerging Contaminants as Global Environmental Hazards. A Bibliometric Analysis. *Emerg Contam* **2020**, *6*, 179–193, doi:10.1016/j.emcon.2020.05.001.

41. van Eck, N.J.; Waltman, L. Software Survey: VOSviewer, a Computer Program for Bibliometric Mapping. *Scientometrics* **2010**, *84*, 523–538, doi:10.1007/s11192-009-0146-3.

42. van Haandel, A.; van der Lubbe, J. *Handbook of Biological Wastewater Treatment: Second Edition*; IWA Publishing: London, UK, 2012; ISBN 9781780400808.

43. van Haandel, A.; Marais, G. *O Comportamento Do Sistema de Lodo Ativado: Teoria e Aplicações Para Projetos e Operação*; Campina Grande, 1999; ISBN 900847.

44. Kamm, B.; Kamm, M. Principles of Biorefineries. *Appl Microbiol Biotechnol* **2004**, *64*, 137–145, doi:10.1007/s00253-003-1537-7.

45. Ahmed, I.; Zia, M.A.; Afzal, H.; Ahmed, S.; Ahmad, M.; Akram, Z.; Sher, F.; Iqbal, H.M.N. Socio-Economic and Environmental Impacts of Biomass Valorisation: A Strategic Drive for Sustainable Bioeconomy. *Sustainability* **2021**, *13*, 1–32, doi:10.3390/su13084200.

46. Kamm, B.; Kamm, M. Biorefinery - Systems. *Chem Biochem Eng Q* **2004**, *18*, 1–6.

47. Leite, J.A.C.; Fernandes, B.S.; Pozzi, E.; Barboza, M.; Zaiat, M. Application of an Anaerobic Packed-Bed Bioreactor for the Production of Hydrogen and Organic Acids. *Int J Hydrogen Energy* **2008**, *33*, 579–586, doi:10.1016/j.ijhydene.2007.10.009.

48. Li, Z.; Zhu, L. The Scientometric Analysis of the Research on Microalgae-Based Wastewater Treatment. *Environmental Science and Pollution Research* **2021**, *28*, 25339–25348, doi:10.1007/s11356-021-12348-4.

49. Niknejad, N.; Nazari, B.; Foroutani, S.; Hussin, A.R. bin C. A Bibliometric Analysis of Green Technologies Applied to Water and Wastewater Treatment. *Environmental Science and Pollution Research* **2022**, doi:10.1007/s11356-022-18705-1.

50. Athanasopoulos, N.; Koutinas, A.A.; Papadimitriou, A. A Pilot Plant and Anaerobic Digestion Process for Raisin Finishing Wastewater Treatment. *Biological Wastes* **1987**, *22*, 129–138, doi:10.1016/0269-7483(87)90045-0.

51. Dinopoulou, G.; Rudd, T.; Lester, J.N. Anaerobic Acidogenesis of a Complex Wastewater: I. The Influence of Operational Parameters on Reactor Performance. *Biotechnol Bioeng* **1988**, *31*, 958–968, doi:10.1002/bit.260310908.

52. Chandrasekhar, K.; Kumar, S.; Lee, B.-D.; Kim, S.-H. Waste Based Hydrogen Production for Circular Bioeconomy: Current Status and Future Directions. *Bioresour Technol* **2020**, *302*, doi:10.1016/j.biortech.2020.122920.

53. Wu, J.H.; Lin, C.Y. Biohydrogen Production by Mesophilic Fermentation of Food Wastewater. *Water Science and Technology* **2004**, *49*, 223–228, doi:10.2166/wst.2004.0757.

54. Kim, W.; Shin, S.G.; Lim, J.; Hwang, S. Effect of Temperature and Hydraulic Retention Time on Volatile Fatty Acid Production Based on Bacterial Community Structure in Anaerobic Acidogenesis Using Swine Wastewater. *Bioprocess Biosyst Eng* **2013**, *36*, 791–798, doi:10.1007/s00449-013-0905-7.

55. Banks, C.J.; Wang, Z. Development of a Two Phase Anaerobic Digester for the Treatment of Mixed Abattoir Wastes. *Water Science and Technology* **1999**, *40*, 69–76, doi:10.1016/S0273-1223(99)00365-0.

56. Viturtia, A.Mtz.; Mata-Alvarez, J.; Cecchi, F.; Fazzini, G. Two-Phase Anaerobic Digestion of a Mixture of Fruit and Vegetable Wastes. *Biological Wastes* **1989**, *29*, 189–199, doi:10.1016/0269-7483(89)90130-4.

57. Wang, J.Y.; Xu, H.L.; Tay, J.H. A Hybrid Two-Phase System for Anaerobic Digestion of Food Waste. *Water Science and Technology* **2002**, *45*, 159–165, doi:10.2166/wst.2002.0422.

58. Liu, H.; Li, Y.; Fu, B.; Guo, H.; Zhang, J.; Liu, H. Recovery of Volatile Fatty Acids from Sewage Sludge through Anaerobic Fermentation. *Current Developments in Biotechnology and Bioengineering: Resource Recovery from Wastes* **2020**, 151–175, doi:10.1016/B978-0-444-64321-6.00008-2.

59. Rabinowitz, B.; Vassos, T.D.; Dawson, R.N.; Oldham, W.K. Upgrading Wastewater Treatment Plants for Biological Nutrient Removal. *Water Science and Technology* **1990**, *22*, 53–60, doi:10.2166/wst.1990.0229.

60. Martin Ruel, S.; Comeau, Y.; Ginestet, P.; Héduit, A. Modeling Acidogenic and Sulfate-Reducing Processes for the Determination of Fermentable Fractions in Wastewater. *Biotechnol Bioeng* **2002**, *80*, 525–536, doi:10.1002/bit.10410.

61. Comeau, Y.; Lamarre, D.; Roberge, F.; Perrier, M.; Desjardins, G.; Hade, C.; Mayer, R. Biological Nutrient Removal from a Phosphorus-Rich Pre-Fermented Industrial Wastewater. *Water Science and Technology* **1996**, *34*, 169–177, doi:10.1016/0273-1223(96)00507-0.

62. Drewnowski, J.; Makinia, J. Modeling Hydrolysis of Slowly Biodegradable Organic Compounds in Biological Nutrient Removal Activated Sludge Systems. *Water Science and Technology* **2013**, *67*, 2067–2074, doi:10.2166/wst.2013.092.

63. Münch, E.V.; Lant, P.; Newell, R.; Keller, J. Mathematical Modelling of Prefermenters-I. Model Development and Verification. *Water Res* **1999**, *33*, 2757–2768, doi:10.1016/S0043-1354%2898%2900516-8.

64. Garcia-Aguirre, J.; Aymerich, E.; González-Mtnez. de Goñi, J.; Esteban-Gutiérrez, M. Selective VFA Production Potential from Organic Waste Streams: Assessing Temperature and PH Influence. *Bioresour Technol* **2017**, *244*, 1081–1088, doi:10.1016/j.biortech.2017.07.187.

65. Niz, M.Y.K.; Formagini, E.L.; Boncz, M.À.; Paulo, P.L. Acidogenic Fermentation of Cassava Wastewater for Volatile Fatty Acids Production. *Int J Environ Waste Manag* **2020**, *25*, 245–261, doi:10.1504/IJEWM.2020.105353.

66. Amorim, N.C.S.; Amorim, E.L.C.; Kato, M.T.; Florencio, L.; Gavazza, S. The Effect of Methanogenesis Inhibition, Inoculum and Substrate Concentration on Hydrogen and Carboxylic Acids Production from Cassava Wastewater. *Biodegradation* **2018**, *29*, 41–58, doi:10.1007/s10532-017-9812-y.

67. da Silva, D.B.; Fenandes, B.S.; da Silva, A.J. Effect of Initial PH and Substrate Concentration on the Lactic Acid Production from Cassava Wastewater Fermentation by an Enriched Culture of Acidogenic Microorganisms. *Water Environment Research* **2021**, *93*, 1925–1933, doi:10.1002/wer.1467.

68. Arslan, D.; Steinbusch, K.J.J.; Diels, L.; Hamelers, H.V.M.; Strik, D.P.B.T.B.; Buisman, C.J.N.; De Wever, H. Selective Short-Chain Carboxylates Production: A Review of Control Mechanisms to Direct Mixed Culture Fermentations. *Crit Rev Environ Sci Technol* **2016**, *46*, 592–634, doi:10.1080/10643389.2016.1145959.

69. Steinbusch, K.J.J.; Hamelers, H.V.M.; Plugge, C.M.; Buisman, C.J.N. Biological Formation of Caproate and Caprylate from Acetate: Fuel and Chemical Production from Low Grade Biomass. *Energy Environ Sci* **2011**, *4*, 216–224, doi:10.1039/c0ee00282h.

70. Elain, A.; Le Grand, A.; Corre, Y.M.; Le Fellic, M.; Hachet, N.; Le Tilly, V.; Loulergue, P.; Audic, J.L.; Bruzaud, S. Valorisation of Local Agro-Industrial Processing Waters as Growth Media for Polyhydroxyalkanoates (PHA) Production. *Ind Crops Prod* **2016**, *80*, 1–5, doi:10.1016/j.indcrop.2015.10.052.

71. Karaca, S.; Yagci, N.; Randall, C.W. Polyhydroxyalkanoate Production Using Enriched Biomass and Acidogenic Fermentation Products of Dairy Wastewater and Organic Food Waste. *Desalination Water Treat* **2021**, *215*, 388–397, doi:10.5004/dwt.2021.26790.

72. Choi, G.; Kim, J.; Lee, C. Effect of Low PH Start-up on Continuous Mixed-Culture Lactic Acid Fermentation of Dairy Effluent. *Appl Microbiol Biotechnol* **2016**, *100*, 10179–10191, doi:10.1007/s00253-016-7871-3.

73. de Sousa e Silva, A.; Tavares Ferreira, T.J.; Sales Morais, N.W.; Lopes Pereira, E.; Bezerra dos Santos, A. S/X Ratio Impacts the Profile and Kinetics of Carboxylic Acids Production from the Acidogenic Fermentation of Dairy Wastewater. *Environmental Pollution* **2021**, *287*, 1–7, doi:10.1016/j.envpol.2021.117605.

74. Demirel, B.; Yenigun, O. Anaerobic Acidogenesis of Dairy Wastewater: The Effects of Variations in Hydraulic Retention Time with No PH Control. *Journal of Chemical Technology and Biotechnology* **2004**, *79*, 755–760, doi:10.1002/jctb.1052.

75. Fang, H.H.P.; Yu, H.Q. Effect of HRT on Mesophilic Acidogenesis of Dairy Wastewater. *Journal of Environmental Engineering* **2000**, *126*, 1145–1149, doi:10.1061/(ASCE)0733-9372(2000)126:12(1145).

76. Jankowska, E.; Chwialkowska, J.; Stodolny, M.; Oleskowicz-Popiel, P. Volatile Fatty Acids Production during Mixed Culture Fermentation – The Impact of Substrate Complexity and PH. *Chemical Engineering Journal* **2017**, *326*, 901–910, doi:10.1016/j.cej.2017.06.021.

77. Gameiro, T.; Sousa, F.; Silva, F.C.; Couras, C.; Lopes, M.; Louros, V.; Nadais, H.; Capela, I. Olive Oil Mill Wastewater to Volatile Fatty Acids: Statistical Study of the Acidogenic Process. *Water Air Soil Pollut* **2015**, *226*, doi:10.1007/s11270-015-2311-z.

78. Fra-Vázquez, A.; Pedrouso, A.; Val del Rio, A.; Mosquera-Corral, A. Volatile Fatty Acid Production from Saline Cooked Mussel Processing Wastewater at Low PH. *Science of the Total Environment* **2020**, *732*, 139337, doi:10.1016/j.scitotenv.2020.139337.

79. Vergine, P.; Sousa, F.; Lopes, M.; Silva, F.; Gameiro, T.; Nadais, H.; Capela, I. Synthetic Soft Drink Wastewater Suitability for the Production of Volatile Fatty Acids. *Process Biochemistry* **2015**, *50*, 1308–1312, doi:10.1016/j.procbio.2015.04.007.

80. Mato, T.; Ben, M.; Kennes, C.; Veiga, M.C. Valuable Product Production from Wood Mill Effluents. *Water Science and Technology* **2010**, *62*, 2294–2300, doi:10.2166/wst.2010.949.

81. Kim, W.; Hwang, K.; Shin, S.G.; Lee, S.; Hwang, S. Effect of High Temperature on Bacterial Community Dynamics in Anaerobic Acidogenesis Using Mesophilic Sludge Inoculum. *Bioresour Technol* **2010**, *101*, S17–S22, doi:10.1016/j.biortech.2009.03.029.

82. Yang, K.; Oh, C.; Hwang, S. Optimizing Volatile Fatty Acid Production in Partial Acidogenesis of Swine Wastewater. *Water Science and Technology* **2004**, *50*, 169–176, doi:10.2166/wst.2004.0512.

83. Silva, A.J.; Pozzi, E.; Foresti, E.; Zaiat, M. The Influence of the Buffering Capacity on the Production of Organic Acids and Alcohols from Wastewater in Anaerobic Reactor. *Appl Biochem Biotechnol* **2015**, *175*, 2258–2265, doi:10.1007/s12010-014-1424-y.

84. Wang, G.; Mu, Y.; Yu, H.Q. Response Surface Analysis to Evaluate the Influence of PH, Temperature and Substrate Concentration on the Acidogenesis of Sucrose-Rich Wastewater. *Biochem Eng J* **2005**, *23*, 175–184, doi:10.1016/j.bej.2005.01.002.

85. Ruel, S.M.; Comeau, Y.; Héduit, A.; Deronzier, G.; Ginestet, P.; Audic, J.M. Operating Conditions for the Determination of the Biochemical Acidogenic Potential of Wastewater. *Water Res* **2002**, *36*, 2337–2341, doi:10.1016/S0043-1354(01)00446-8.

86. Dahiya, S.; Lakshminarayanan, S.; Mohan, S.V. Steering Acidogenesis towards Selective Propionic Acid Production Using Co-Factors and Evaluating Environmental Sustainability. *Chemical Engineering Journal* **2020**, *379*, doi:10.1016/j.cej.2019.122135.

87. Lee, W.S.; Chua, A.S.M.; Yeoh, H.K.; Ngoh, G.C. A Review of the Production and Applications of Waste-Derived Volatile Fatty Acids. *Chemical Engineering Journal* **2014**, *235*, 83–99, doi:10.1016/j.cej.2013.09.002.

88. De Groof, V.; Coma, M.; Arnot, T.; Leak, D.J.; Lanham, A.B. Medium Chain Carboxylic Acids from Complex Organic Feedstocks by Mixed Culture Fermentation. *Molecules* **2019**, *24*, 398, doi:10.3390/molecules24030398.

89. Gallert, C.; Winter, J. Bacterial Metabolism in Wastewater Treatment Systems. In *Environmental Biotechnology: Concepts and Applications*; Jördening, H.J., Winter, J., Eds.; 2005; pp. 1–48 ISBN 3527305858.

90. Liu, H.; Chen, Y.; Wu, J. Municipal Wastewater Biological Nutrient Removal Driven by the Fermentation Liquid of Dairy Wastewater. *Environmental Technology (United Kingdom)* **2017**, *38*, 2639–2649, doi:10.1080/09593330.2016.1272638.

91. Atasoy, M.; Cetecioglu, Z. The Effects of PH on the Production of Volatile Fatty Acids and Microbial Dynamics in Long-Term Reactor Operation. *J Environ Manage* **2022**, *319*, 115700, doi:10.1016/j.jenvman.2022.115700.

92. Barana, A.C.; Cereda, M.P. Cassava Wastewater (Manipueira) Treatment Using a Two-Phase Anaerobic Biodigestor. *Ciência e Tecnologia de Alimentos* **2000**, *20*, 1–7, doi:10.1590/S0101-20612000000200010.

93. Liu, H.; Xie, L.; Chen, Y.G.; Zhou, Q. VFAs Production Potential of Brewery Industry Wastewater and Starch Wastewater. *Adv Mat Res* **2013**, *777*, 225–231, doi:10.4028/www.scientific.net/AMR.777.225.

94. Dareioti, M.A.; Vavouraki, A.I.; Kornaros, M. Effect of PH on the Anaerobic Acidogenesis of Agroindustrial Wastewaters for Maximization of Bio-Hydrogen Production: A Lab-Scale

Evaluation Using Batch Tests. *Bioresour Technol* **2014**, *162*, 218–227, doi:10.1016/j.biortech.2014.03.149.

95. Lv, N.; Cai, G.; Pan, X.; Li, Y.; Wang, R.; Li, J.; Li, C.; Zhu, G. PH and Hydraulic Retention Time Regulation for Anaerobic Fermentation: Focus on Volatile Fatty Acids Production/Distribution, Microbial Community Succession and Interactive Correlation. *Bioresour Technol* **2022**, *347*, 126310, doi:10.1016/j.biortech.2021.126310.

96. Guo, X.M.; Trably, E.; Latrille, E.; Carrère, H.; Steyer, J.P. Hydrogen Production from Agricultural Waste by Dark Fermentation: A Review. *Int J Hydrogen Energy* **2010**, *35*, 10660–10673, doi:10.1016/j.ijhydene.2010.03.008.

97. Wainaina, S.; Lukitawesa; Kumar Awasthi, M.; Taherzadeh, M.J. Bioengineering of Anaerobic Digestion for Volatile Fatty Acids, Hydrogen or Methane Production: A Critical Review. *Bioengineered* **2019**, *10*, 437–458, doi:10.1080/21655979.2019.1673937.

98. Ben, M.; Mato, T.; Lopez, A.; Vila, M.; Kennes, C.; Veiga, M.C. Bioplastic Production Using Wood Mill Effluents as Feedstock. *Water Science and Technology* **2011**, *63*, 1196–1202, doi:10.2166/wst.2011.358.

99. Azbar, N.; Tuba, F.C.; Keskin, T.; Korkmaz, K.S.; Syed, H.M. Continuous Fermentative Hydrogen Production from Cheese Whey Wastewater under Thermophilic Anaerobic Conditions. *International Journal of Hidrogen Energy* **2009**, *34*, 7441–7447, doi:10.1016/j.ijhydene.2009.04.032.

100. Jiang, J.; Zhang, Y.; Li, K.; Wang, Q.; Gong, C.; Li, M. Volatile Fatty Acids Production from Food Waste: Effects of PH, Temperature, and Organic Loading Rate. *Bioresour Technol* **2013**, *143*, 525–530, doi:10.1016/j.biortech.2013.06.025.

101. Infantes, D.; Gonzáles del Campo, A.; Villaseñor, J.; Fernández, F.J. Kinetic Model and Study of the Influence of PH, Temperature and Undissociated Acids on Acidogenic Fermentation. *Biochem Eng J* **2012**, *66*, 66–72, doi:10.1016/j.bej.2012.04.017.

102. Khan, M.A.; Ngo, H.H.; Guo, W.S.; Liu, Y.; Nghiem, L.D.; Hai, F.I.; Deng, L.J.; Wang, J.; Wu, Y. Optimization of Process Parameters for Production of Volatile Fatty Acid, Biohydrogen and Methane from Anaerobic Digestion. *Bioresour Technol* **2016**, *219*, 738–748, doi:10.1016/j.biortech.2016.08.073.

103. Chogi, M.A.N.; Araujo, A.C.V.; Do Prado, P.F.; Da Silva, D.B.; Da Silva, A.J.; Da Silva, G.F.; Duarte, I.C.S. Production of Biofuel and Organic Acids for Adding Value to Cassava Wastewater. *Revista Virtual de Quimica* **2020**, *12*, 89–98, doi:10.21577/1984-6835.20200009.

104. Amorim, N.C. dos S. Produção de Ácido Capróico Associado á Produção de Hidrogênio Utilizando a Manipueira, Universidade Federal de Pernambuco, 2018.

105. Morais, N.W.S.; Coelho, M.M.H.; Silva, A. de S. e.; Pereira, E.L.; Leitão, R.C.; dos Santos, A.B. Kinetic Modeling of Anaerobic Carboxylic Acid Production from Swine Wastewater. *Bioresour Technol* **2020**, *297*, doi:10.1016/j.biortech.2019.122520.

106. Yu, H.Q.; Fang, H.H.P. Anaerobic Acidification of a Synthetic Wastewater in Batch Reactors at 55°C. *Water Science and Technology* **2002**, *46*, 153–157, doi:10.2166/wst.2002.0731.

107. Policastro, G.; Luongo, V.; Frunzo, L.; Fabbricino, M. A Comprehensive Review of Mathematical Models of Photo Fermentation. *Crit Rev Biotechnol* **2021**, *41*, 628–648, doi:10.1080/07388551.2021.1873241.

108. Ortega Quintana, F.A.; Alvarez Zapata, H.; Botero Castro, H.A. Enfrentando El Modelado de Bioprocesos: Una Revisión de Las Metodologías de Modelado Facing Bioprocess Modeling: A Review of the Methodologies of Modeling Enfrentando a Modelagem de Bioprocesos: Uma Revisão de Metodologias de Modelagem. *ION, Investigación, Optimización y Nuevos procesos en Ingeniería* **2017**, *30*, 74, doi:10.18273/revion.v30n1-2017006.

109. Mendes, Á.J.B.; Valdman, B.; de Souza Júnior, M.B. Uma Revisão de Modelagem Matemática Em Bioprocessos. Parte I: Fundamentos Básicos e Classificacao. *Ciência e Tecnologia* **2011**, 40–59.

110. Membere, E.; Sallis, P. Effect of Temperature on Kinetics of Biogas Production from Macroalgae. *Bioresour Technol* **2018**, *263*, 410–417, doi:10.1016/j.biortech.2018.05.023.

111. Fernández, F.J.; Villaseñor, J.; Infantes, D. Kinetic and Stoichiometric Modelling of Acidogenic Fermentation of Glucose and Fructose. *Biomass Bioenergy* **2011**, *35*, 3877–3883, doi:10.1016/j.biombioe.2011.06.052.

112. Pérez-Morales, J.; B.-Arroyo, C.; Morales-Zarate, E.; Hernández-García, H.; Méndez-Acosta, H.O.; Hernández-Martínez, E. Mathematical Modeling of Volatile Fatty Acids Production from Cheese Whey: Evaluation of PH and Substrate-Inoculum Ratio Effects. *Fuel* **2021**, *287*, doi:10.1016/j.fuel.2020.119510.

113. Batstone, D.J.; Keller, J.; Angelidaki, I.; Kalyuzhnyi, S. V.; Pavlostathis, S.G.; Rozzi, A.; Sanders, W.T.; Siegrist, H.; Vavilin, V.A. The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Sci Technol* **2002**, *45*, 65–73, doi:10.2166/wst.2002.0292.

114. Shi, E.; Li, J.; Zhang, M. Application of IWA Anaerobic Digestion Model No. 1 to Simulate Butyric Acid, Propionic Acid, Mixed Acid, and Ethanol Type Fermentative Systems Using a Variable Acidogenic Stoichiometric Approach. *Water Res* **2019**, *161*, 242–250, doi:10.1016/j.watres.2019.05.094.

115. Arzate, J.A.; Kirstein, M.; Ertem, F.C.; Kielhorn, E.; Ramirez Malule, H.; Neubauer, P.; Cruz-Bournazou, M.N.; Junne, S. Anaerobic Digestion Model (AM2) for the Description of Biogas Processes at Dynamic Feedstock Loading Rates. *Chem Ing Tech* **2017**, *89*, 686–695, doi:10.1002/cite.201600176.

116. Zaatri, A.; Kelaiaia, R. Analysis and Simulation of AM2 Model for Anaerobic Digesters. *Banats J Biotechnol* **2020**, *11*, 4738–12, doi:10.7904/2068.

117. Yogeswari, M.K.; Dharmalingam, K.; Mullai, P. Implementation of Artificial Neural Network Model for Continuous Hydrogen Production Using Confectionery Wastewater. *J Environ Manage* **2019**, *252*, doi:10.1016/j.jenvman.2019.109684.

118. Tufaner, F.; Demirci, Y. Prediction of Biogas Production Rate from Anaerobic Hybrid Reactor by Artificial Neural Network and Nonlinear Regressions Models. *Clean Technol Environ Policy* **2020**, *22*, 713–724, doi:10.1007/s10098-020-01816-z.

119. Peres, J.; Oliveira, R.; Feyo de Azevedo, S. Hybrid Modelling of Fermentation Processes: A Study on the Use of Modular Neural Networks for Modelling Cells Reaction Kinetics. *IFAC Proceedings Volumes* **2004**, *37*, 293–298, doi:10.1016/s1474-6670(17)32596-x.

120. Regueira, A.; Lema, J.M.; Carballa, M.; Mauricio-Iglesias, M. Metabolic Modeling for Predicting VFA Production from Protein-Rich Substrates by Mixed-Culture Fermentation. *Biotechnol Bioeng* **2020**, *117*, 73–84, doi:10.1002/bit.27177.

121. Almquist, J.; Cvijovic, M.; Hatzimanikatis, V.; Nielsen, J.; Jirstrand, M. Kinetic Models in Industrial Biotechnology - Improving Cell Factory Performance. *Metab Eng* **2014**, *24*, 38–60, doi:10.1016/j.ymben.2014.03.007.

122. Yin, X.; Li, J.; Shin, H. dong; Du, G.; Liu, L.; Chen, J. Metabolic Engineering in the Biotechnological Production of Organic Acids in the Tricarboxylic Acid Cycle of Microorganisms: Advances and Prospects. *Biotechnol Adv* **2015**, *33*, 830–841, doi:10.1016/j.biotechadv.2015.04.006.

123. FAOSTAT Cultivos y Productos de Ganadería Available online: https://www.fao.org/faostat/es/#data/QCL (accessed on 14 December 2023).

124. Nnaji, C.C.; Akanno, C.C. Assessment of Environmental Degradation Due to Processing of Cassava into Garri Flakes Using Pollution Indices. *Environmental Processes* **2022**, *9*, 1–18, doi:10.1007/s40710-022-00594-8.

125. De Amorim, M.C.C.; De Souza Silva, P.T.; Barbosa, P.S.; Montefusco, N.E. Anaerobic Biodegradation of Cassava Wastewater under Different Temperatures and Inoculums. *Comunicata Scientiae* **2019**, *10*, 65–76, doi:10.14295/cs.v10i1.3009.

126. Sawatdeenarunat, C.; Saipa, S.; Suaisom, P.; Mai, C.; Mai, C. Methane Recovery from Cassava Starch Wastewater via Anaerobic Digestion: Effect of Inoculum Source and Kinetic Study. *Asia-Pacific Journal of Science and Technology* **2021**, *26*, 1–9, doi:10.14456/apst.2021.27.

127. Dionisi, D.; Silva, I.M.O. Production of Ethanol, Organic Acids and Hydrogen: An Opportunity for Mixed Culture Biotechnology? *Rev Environ Sci Biotechnol* **2016**, *15*, 213–242, doi:10.1007/s11157-016-9393-y.

128. Sun, L.; Gong, M.; Lv, X.; Huang, Z.; Gu, Y.; Li, J.; Du, G.; Liu, L. Current Advance in Biological Production of Short-Chain Organic Acid. *Appl Microbiol Biotechnol* **2020**, *104*, 9109–9124, doi:10.1007/s00253-020-10917-0.

129. Dahiya, S.; Lingam, Y.; Venkata Mohan, S. Understanding Acidogenesis towards Green Hydrogen and Volatile Fatty Acid Production – Critical Analysis and Circular Economy Perspective. *Chemical Engineering Journal* **2023**, *464*, 141550, doi:10.1016/j.cej.2023.141550.

130. Nizzy, A.M.; Kannan, S. A Review on the Conversion of Cassava Wastes into Value-Added Products towards a Sustainable Environment. *Environmental Science and Pollution Research* **2022**, 69223–69240, doi:10.1007/s11356-022-22500-3.

131. Liu, H.; Wang, F.; Wang, Z.; Wu, D.; Xing, T.; Kong, X.; Sun, Y. Impact of PH, Temperature, and Hydraulic Residence Time on the Acidogenic Fermentation of Fruit and Vegetable Waste and Microbial Community Analysis. *Journal of Chemical Technology and Biotechnology* **2023**, *98*, 819–828, doi:10.1002/jctb.7289.

132. Lu, Y.; Chen, R.; Huang, L.; Wang, X.; Chou, S.; Zhu, J. Acidogenic Fermentation of Potato Peel Waste for Volatile Fatty Acids Production: Effect of Initial Organic Load. *J Biotechnol* **2023**, *374*, 114–121, doi:10.1016/j.jbiotec.2023.08.003.

133. Tamis, J.; Joosse, B.M.; van Loosdrecht, M.C.M.; Kleerebezem, R. High-Rate Volatile Fatty Acid (VFA) Production by a Granular Sludge Process at Low PH. *Biotechnol Bioeng* **2015**, *112*, 2248–2255, doi:10.1002/bit.25640.

134. Mañunga, T. Acople Entre Un Reactor Anaerobio de Medio Suspendido y Un Reactor Anaerobio de Crecimiento Adherido Para La Producción de Hidrógeno y Metano a Partir de Agua Residual Del Proceso de Extracción de Almidón de Yuca, 2019.

135. Sanchez-Ledesma, L.M.; Ramírez-Malule, H.; Rodríguez-Victoria, J.A. Volatile Fatty Acids Production by Acidogenic Fermentation of Wastewater: A Bibliometric Analysis. *Sustainability (Switzerland)* **2023**, *15*, doi:10.3390/su15032370.

136. Bolaji, I.O.; Dionisi, D. Acidogenic Fermentation of Vegetable and Salad Waste for Chemicals Production: Effect of PH Buffer and Retention Time. *J Environ Chem Eng* **2017**, *5*, 5933–5943, doi:10.1016/j.jece.2017.11.001.

137. APHA; AWWA; WEF Standard Methods for the Examination of Water and Wastewater 2005.

138. Dubois, M.; Gilles, K.; Hamilton, J.K.; Rebers, P.A.; Smith, F. A Colorimetric Method for the Determination of Sugars. *Anal Chem* **1956**, *28*, 350–356, doi:10.1038/168167a0.

139. DiLallo, R.; Albertson, O. Volatile Acids by Direct Titration. *Water Pollution Control Federation* **1961**, *33*, 356–365.

140. Jankowska, E.; Chwiałkowska, J.; Stodolny, M.; Oleskowicz-Popiel, P. Effect of PH and Retention Time on Volatile Fatty Acids Production during Mixed Culture Fermentation. *Bioresour Technol* **2015**, *190*, 274–280, doi:10.1016/j.biortech.2015.04.096.

141. Horiuchi, J.I.; Shimizu, T.; Tada, K.; Kanno, T.; Kobayashi, M. Selective Production of Organic Acids in Anaerobic Acid Reactor by PH Control. *Bioresour Technol* **2002**, *82*, 209–213, doi:10.1016/S0960-8524(01)00195-X.

142. Colin, X.; Farinet, J.L.; Rojas, O.; Alazard, D. Anaerobic Treatment of Cassava Starch Extraction Wastewater Using a Horizontal Flow Filter with Bamboo as Support. *Bioresour Technol* **2007**, *98*, 1602–1607, doi:10.1016/j.biortech.2006.06.020.

143. Alibardi, L.; Cossu, R. Effects of Carbohydrate, Protein and Lipid Content of Organic Waste on Hydrogen Production and Fermentation Products. *Waste Management* **2016**, *47*, 69–77, doi:10.1016/j.wasman.2015.07.049.

144. Castilla-Archilla, J.; Papirio, S.; Lens, P.N.L. Two Step Process for Volatile Fatty Acid Production from Brewery Spent Grain: Hydrolysis and Direct Acidogenic Fermentation Using Anaerobic Granular Sludge. *Process Biochemistry* **2021**, *100*, 272–283, doi:10.1016/j.procbio.2020.10.011.

145. Cohen, A.; Van Gemert, J.M.; Zoetemeyer, R.J.; Breure, A.M. Main Characteristics and Stoichiometric Aspects of Acidogenesis of Soluble Carbohydrate Containing Wastewaters. *Process Biochemistry* **1984**, *19*, 228–232.

146. Liu, C.; Ren, L.; Yan, B.; Luo, L.; Zhang, J.; Awasthi, M.K. Electron Transfer and Mechanism of Energy Production among Syntrophic Bacteria during Acidogenic Fermentation: A Review. *Bioresour Technol* **2021**, *323*, doi:10.1016/j.biortech.2020.124637.

147. Schuchmann, K.; Müller, V. Energetics and Application of Heterotrophy in Acetogenic Bacteria. *Appl Environ Microbiol* **2016**, *82*, 4056–4069, doi:10.1128/AEM.00882-16.

148. Lee, H.S.; Salerno, M.B.; Rittmann, B.E. Thermodynamic Evaluation on H2 Production in Glucose Fermentation. *Environ Sci Technol* **2008**, *42*, 2401–2407, doi:10.1021/es702610v.

149. Yan, Y.; Feng, L.; Zhang, C.; Wisniewski, C.; Zhou, Q. Ultrasonic Enhancement of Waste Activated Sludge Hydrolysis and Volatile Fatty Acids Accumulation at PH 10.0. *Water Res* **2010**, *44*, 3329–3336, doi:10.1016/j.watres.2010.03.015.

150. de Oliveira Schmidt, V.K.; de Vasconscelos, G.M.D.; Vicente, R.; de Souza Carvalho, J.; Della-Flora, I.K.; Degang, L.; de Oliveira, D.; de Andrade, C.J. Cassava Wastewater Valorization for the Production of Biosurfactants: Surfactin, Rhamnolipids, and Mannosileritritol Lipids. *World J Microbiol Biotechnol* **2023**, *39*, 1–17, doi:10.1007/s11274-022-03510-2.

151. Yu, H.G.; Fang, H.H. Acidogenesis of Dairy Wastewater at Various PH Levels. *Water science and technology* **2002**, *45*, 201–206, doi:10.2166/wst.2002.0331.

152. Arslan, D.; Steinbusch, K.J.J.; Diels, L.; De Wever, H.; Buisman, C.J.N.; Hamelers, H.V.M. Effect of Hydrogen and Carbon Dioxide on Carboxylic Acids Patterns in Mixed Culture Fermentation. *Bioresour Technol* **2012**, *118*, 227–234, doi:10.1016/j.biortech.2012.05.003.

153. Yuan, Y.; Hu, X.; Chen, H.; Zhou, Y.; Zhou, Y.; Wang, D. Advances in Enhanced Volatile Fatty Acid Production from Anaerobic Fermentation of Waste Activated Sludge. *Science of the Total Environment* **2019**, *694*, 133741, doi:10.1016/j.scitotenv.2019.133741.

154. Pabón Pereira, C.P.; Castañares, G.; Van Lier, J.B. An OxiTop® Protocol for Screening Plant Material for Its Biochemical Methane Potential (BMP). *Water Science and Technology* **2012**, *66*, 1416–1423, doi:10.2166/wst.2012.305.

155. Khanal, S.K.; Chen, W.H.; Li, L.; Sung, S. Biological Hydrogen Production: Effects of PH and Intermediate Products. *Int J Hydrogen Energy* **2004**, *29*, 1123–1131, doi:10.1016/j.ijhydene.2003.11.002.

156. Zhou, M.; Zhou, J.; Tan, M.; Du, J.; Yan, B.; Wong, J.W.C.; Zhang, Y. Enhanced Carboxylic Acids Production by Decreasing Hydrogen Partial Pressure during Acidogenic Fermentation of Glucose. *Bioresour Technol* **2017**, *245*, 44–51, doi:10.1016/j.biortech.2017.08.152.

157. Martinez-Burgos, W.J.; Sydney, E.B.; de Paula, D.R.; Medeiros, A.B.P.; de Carvalho, J.C.; Soccol, V.T.; de Souza Vandenberghe, L.P.; Woiciechowski, A.L.; Soccol, C.R. Biohydrogen Production in Cassava Processing Wastewater Using Microbial Consortia: Process Optimization and Kinetic Analysis of the Microbial Community. *Bioresour Technol* **2020**, *309*, 123331, doi:10.1016/j.biortech.2020.123331.

158. Souto, T.F.; Aquino, S.F.; Silva, S.Q.; Chernicharo, C.A.L. Influence of Incubation Conditions on the Specific Methanogenic Activity Test. *Biodegradation* **2010**, *21*, 411–424, doi:10.1007/s10532-009-9311-x.

159. Hilkiah Igoni, A.; Ayotamuno, M.J.; Eze, C.L.; Ogaji, S.O.T.; Probert, S.D. Designs of Anaerobic Digesters for Producing Biogas from Municipal Solid-Waste. *Appl Energy* **2008**, *85*, 430–438, doi:10.1016/j.apenergy.2007.07.013.

160. Dinamarca, S.; Aroca, G.; Chamy, R.; Guerrero, L. The Influence of PH in the Hydrolytic Stage of Anaerobic Digestion of the Organic Fraction of Urban Solid Waste. *Water Science and Technology* **2003**, *48*, 249–254, doi:10.2166/wst.2003.0409.

161. Norli, I.; Ho, Y.C.; Fischer, K.; Kranert, M.; Boley, A. Biodegradability Assessment of Bio-Flocculant via Anaerobic and Aerobic Test.; 2011; Vol. 7, pp. 189–193.

162. Barrios Pérez, J.D. Influencia Del PH Sobre La Producción Biológica de Hidrógeno a Partir de Agua Residual Agroindustrial, Universidad del Valle, 2015.

163. Polettini, A.; Pomi, R.; Rossi, A.; Zonfa, T.; De Gioannis, G.; Muntoni, A. Continuous Biohydrogen Production from Cheese Whey - Part 1: New Insights into Process Stability. *J Clean Prod* **2022**, *(submitted*, 21044–21059, doi:10.1016/j.ijhydene.2022.04.229.

164. Torres Lozada, P.; Pérez Vidal, A.; Cajigas, Á.A.; Otero, A.M.; González, M. *Selección de Acondicionadores Químicos Para El Tratamiento Anaerobio de Aguas Residuales Del Proceso de Extracción de Almidón de Yuca*; Cali, Colombia, 2008;

165. Huang, Y.X.; Guo, J.; Zhang, C.; Hu, Z. Hydrogen Production from the Dissolution of Nano Zero Valent Iron and Its Effect on Anaerobic Digestion. *Water Res* **2016**, *88*, 475–480, doi:10.1016/j.watres.2015.10.028.

166. Atasoy, M.; Eyice, O.; Schnürer, A.; Cetecioglu, Z. Volatile Fatty Acids Production via Mixed Culture Fermentation: Revealing the Link between PH, Inoculum Type and Bacterial Composition. *Bioresour Technol* **2019**, *292*, 121889, doi:10.1016/j.biortech.2019.121889.

167. Temudo, M.F.; Kleerebezem, R.; van Loosdrecht, M. Influence of the PH on (Open) Mixed Culture Fermentation of Glucose: A Chemostat Study. *Biotechnol Bioeng* **2007**, *98*, 69–79, doi:10.1002/bit.21412.

168. Eng, F.; Fuess, L.T.; Bovio-Winkler, P.; Etchebehere, C.; Sakamoto, I.K.; Zaiat, M. Optimization of Volatile Fatty Acid Production by Sugarcane Vinasse Dark Fermentation Using a Response Surface Methodology. Links between Performance and Microbial Community Composition. *Sustainable Energy Technologies and Assessments* **2022**, *53*, 102764, doi:10.1016/j.seta.2022.102764.

169. Puyol, D.; Batstone, D.J.; Hülsen, T.; Astals, S.; Peces, M.; Krömer, J.O. Resource Recovery from Wastewater by Biological Technologies: Opportunities, Challenges, and Prospects. *Front Microbiol* **2017**, *7*, 1–23, doi:10.3389/fmicb.2016.02106.

170. Khatami, K.; Atasoy, M.; Ludtke, M.; Baresel, C.; Eyice, Ö.; Cetecioglu, Z. Bioconversion of Food Waste to Volatile Fatty Acids: Impact of Microbial Community, PH and Retention Time. *Chemosphere* **2021**, *275*, doi:10.1016/j.chemosphere.2021.129981.

171. Xiao, B.; Han, Y.; Liu, J. Evaluation of Biohydrogen Production from Glucose and Protein at Neutral Initial PH. *Renew Energy* **2010**, *35*, 6152–6160, doi:10.1016/j.ijhydene.2010.03.084.

172. Sittijunda, S.; Reungsang, A.; O-Thong, S. Biohydrogen Production from Dual Digestion Pretreatment of Poultry Slaughterhouse Sludge by Anaerobic Self-Fermentation. *Int J Hydrogen Energy* **2010**, *35*, 13427–13434, doi:10.1016/j.ijhydene.2009.11.116.

173. Pittmann, T.; Steinmetz, H. Influence of Operating Conditions for Volatile Fatty Acids Enrichment as a First Step for Polyhydroxyalkanoate Production on a Municipal Waste Water Treatment Plant. *Bioresour Technol* **2013**, *148*, 270–276, doi:10.1016/j.biortech.2013.08.148.

174. Espinosa Chávez, B. Evaluación Del Desarrollo de La Actividad Sulfatorreductora En Un Lodo Granular Metanogénico de Diferentes Tamaños, Instituto Potosino de Investigación Científica y Tecnológica, 2007.

175. Koottatep, T.; Khamyai, S.; Pussayanavin, T.; Kunsit, U.; Prapasriket, P.; Polprasert, C. Meso-Thermophilic Acidogenic Biotreatment of Mixed Wastewater from Toilets and Coffeeshop: Effect of Temperature on the Efficiency of Organic Removal and VFA Productions. *Biomass Convers Biorefin* **2022**, 1–6, doi:10.1007/s13399-021-02141-1.

176. Vilpoux, O.F.; Santos Silveira Junior, J.F. Global Production and Use of Starch. *Starchy Crops Morphology, Extraction, Properties and Applications: Vol 1: Underground Starchy Crops of South American Origin: Production, Processing, Utilization and Economic Perspectives* **2022**, *1*, 43–66, doi:10.1016/B978-0-323-90058-4.00014-1.

177. Sanchez-Ledesma, L.M.; Rodríguez-Victoria, J.A.; Ramírez-Malule, H. Effect of Fermentation Time, PH, and Their Interaction on the Production of Volatile Fatty Acids from Cassava Wastewater. *Water (Switzerland)* **2024**, *16*, doi:https://doi.org/10.3390/w16111514.

178. Arevalo Ortiz, H.F.; Arias Arroyo, G.C. Determinación de La Concentración de Inoculo y Tiempo de Fermentacion, Utilizando Microbiota de Los Granos de Kefir Como Agente Biológico y Suero de Leche Como Sustrato. *Cienc Invest* **2008**, *11*, 16–22.

179. Silva, F.C.; Serafim, L.S.; Nadais, H.; Arroja, L.; Capela, I. Acidogenic Fermentation towards Valorisation of Organic Waste Streams into Volatile Fatty Acids. *Chem Biochem Eng Q* **2013**, *27*, 467–476.

180. Wang, J.; Wan, W. Effect of Temperature on Fermentative Hydrogen Production by Mixed Cultures. *Int J Hydrogen Energy* **2008**, *33*, 5392–5397, doi:10.1016/j.ijhydene.2008.07.010.

181. Yu, H.Q.; Fang, H.H.P. Acidogenesis of Gelatin-Rich Wastewater in an Upflow Anaerobic Reactor: Influence of PH and Temperature. *Water Res* **2003**, *37*, 55–66, doi:10.1016/S0043-1354(02)00256-7.

182. Valentino, F.; Chitharanjan, A.; Micolucci, F.; Pavan, P.; Gottardo, M. Valuable Routes for Sewage Sludge Utilization : Effect of Temperature and Hydraulic Retention Time in the Acidogenic Fermentation Process. *Chem Eng Trans* **2022**, *93*, 193–198, doi:10.3303/CET2293033.

183. Fernández-Domínguez, D.; Astals, S.; Peces, M.; Frison, N.; Bolzonella, D.; Mata-Alvarez, J.; Dosta, J. Volatile Fatty Acids Production from Biowaste at Mechanical-Biological Treatment Plants: Focusing on Fermentation Temperature. *Bioresour Technol* **2020**, *314*, 123729, doi:10.1016/j.biortech.2020.123729.

184. Wang, X.; Zhang, S.; Wang, J.; Yu, X.; Lu, X. Exploring Optimal Feed to Microbes Ratio for Anaerobic Acidogenic Fermentation of Cassava Residue from Brewery. *Bioresources* **2012**, *7*, 1111–1122, doi:10.15376/biores.7.1.1111-1122.

185. Casero-Díaz, T.; Castro-Barros, C.; Taboada-Santos, A.; Rodríguez-Hernández, L.; Mauricio-Iglesias, M.; Carballa, M. Turning Fish Canning Wastewater into Resources: Effluents and

Operational Conditions Selection for Volatile Fatty Acids Production. *Journal of Water Process Engineering* **2024**, *64*, 2–9, doi:10.1016/j.jwpe.2024.105738.

186. Song, X.; Chen, G.; Wang, F.; Zhang, J.; Liu, Y.; Zhao, J. Potential of Volatile Fatty Acids Production from Banana Waste Juice: Impacts of Storage, Substrate Phase, Operation Conditions, and Microbial Community Analysis. *Fuel* **2024**, *366*, 131294, doi:10.1016/j.fuel.2024.131294.

187. Eng Sánchez, F.; Tadeu Fuess, L.; Soares Cavalcante, G.; Ângela Talarico Adorno, M.; Zaiat, M. Value-Added Soluble Metabolite Production from Sugarcane Vinasse within the Carboxylate Platform: An Application of the Anaerobic Biorefinery beyond Biogas Production. *Fuel* **2021**, *286*, doi:10.1016/j.fuel.2020.119378.

188. Perez-Esteban, N.; Vives-Egea, J.; Peces, M.; Dosta, J.; Astals, S. Temperature-Driven Carboxylic Acid Production from Waste Activated Sludge and Food Waste: Co-Fermentation Performance and Microbial Dynamics. *Waste Management* **2024**, *178*, 176–185, doi:10.1016/j.wasman.2024.02.026.

189. Jung, K.; Kim, W.; Park, G.W.; Seo, C.; Chang, H.N.; Kim, Y.C. Optimization of Volatile Fatty Acids and Hydrogen Production from Saccharina Japonica: Acidogenesis and Molecular Analysis of the Resulting Microbial Communities. *Appl Microbiol Biotechnol* **2015**, *99*, 3327–3337, doi:10.1007/s00253-015-6419-2.

190. Ceron, C.; Andres, A.; Vidal, P.; Lozada, T.; Del, I.; La, Y.; En, A.; Tratamiento, E.L.; Las, A.D.E. Importancia Del PH y La Alcalinidad En El Tratamiento Anaerobio de Las Aguas Residuales Del Proceso de Extracción de Almidón de Yuca. *Scientia Et Technica* **2005**, *XI*, 243–248.

191. Dahiya, S.; Sarkar, O.; Swamy, Y. V.; Venkata Mohan, S. Acidogenic Fermentation of Food Waste for Volatile Fatty Acid Production with Co-Generation of Biohydrogen. *Bioresour Technol* **2015**, *182*, 103–113, doi:10.1016/j.biortech.2015.01.007.

192. Wu, H.; Scheve, T.; Dalke, R.; Holtzapple, M.; Urgun-Demirtas, M. Scaling up Carboxylic Acid Production from Cheese Whey and Brewery Wastewater via Methane-Arrested Anaerobic Digestion. *Chemical Engineering Journal* **2023**, *459*, 140080, doi:10.1016/j.cej.2022.140080.

193. Rafay, R.; Allegue, T.; Fowler, S.J.; Rodríguez, J. Exploring the Limits of Carbohydrate Conversion and Product Formation in Open Mixed Culture Fermentation. *J Environ Chem Eng* **2022**, *10*, doi:10.1016/j.jece.2022.107513.

194. Agler, M.T.; Wrenn, B.A.; Zinder, S.H.; Angenent, L.T. Waste to Bioproduct Conversion with Undefined Mixed Cultures: The Carboxylate Platform. *Trends Biotechnol* **2011**, *29*, 70–78, doi:10.1016/j.tibtech.2010.11.006.

195. Trancone, G.; Policastro, G.; Spasiano, D.; Race, M.; Parrino, F.; Fratino, U.; Fabbricino, M.; Pirozzi, F. Treatment of Concrete Waste from Construction and Demolition Activities: Application of Organic Acids from Continuous Dark Fermentation in Moving Bed Biofilm Reactors. *Chemical Engineering Journal* **2025**, *505*, doi:10.1016/j.cej.2025.159536.

196. Trancone, G.; Spasiano, D.; Race, M.; Luongo, V.; Petrella, A.; Pirozzi, F.; Fratino, U.; Piccinni, A.F. A Combined System for Asbestos-Cement Waste Degradation by Dark Fermentation and Resulting Supernatant Valorization in Anaerobic Digestion. *Chemosphere* **2022**, *300*, doi:10.1016/j.chemosphere.2022.134500.

197. Li, W.; Huang, J.; Shi, Z.; Han, W.; Lü, T.; Lin, Y.; Meng, J.; Xu, X.; Hou, P. Machine Learning Enabled Prediction and Process Optimization of VFA Production from Riboflavin-Mediated Sludge Fermentation. *Front Environ Sci Eng* **2023**, *17*, doi:10.1007/s11783-023-1735-8.

198. Atasoy, M.; Scott, W.T.; Regueira, A.; Mauricio-Iglesias, M.; Schaap, P.J.; Smidt, H. Biobased Short Chain Fatty Acid Production - Exploring Microbial Community Dynamics and Metabolic Networks through Kinetic and Microbial Modeling Approaches. *Biotechnol Adv* **2024**, *73*, 108363, doi:10.1016/j.biotechadv.2024.108363.

199. Morais, N.W.S. Recuperacao de Subprodutos (Metano e Ácidos Carboxílicos) Em Sistemas Anaeróbios Tratando Residuos Agroindustriais, 2019.

200. Boshagh, F.; Rostami, K.; van Niel, E.W.J. Application of Kinetic Models in Dark Fermentative Hydrogen Production–A Critical Review. *Int J Hydrogen Energy* **2022**, *47*, 21952–21968, doi:10.1016/j.ijhydene.2022.05.031.

201. Mu, Y.; Wang, G.; Yu, H.Q. Kinetic Modeling of Batch Hydrogen Production Process by Mixed Anaerobic Cultures. *Bioresour Technol* **2006**, *97*, 1302–1307, doi:10.1016/j.biortech.2005.05.014.

202. Ramos, R.E.; Márquez, M.C. Volatile Fatty Acids from Household Food Waste: Production and Kinetics. *ChemEngineering* **2024**, *8*, 84, doi:10.3390/chemengineering8050084.

203. Trisakti, B.; Turmuzi, M.; Taslim, T.; Irvan, I. Determination of Kinetic Models in Acidogenesis Process of Palm Oil Mill Effluent. *Ecological Engineering & Environmental Technology* **2024**, *25*, 118–125, doi:10.12912/27197050/191418.

204. Undiandeye, J.; Kiman, S.; Abubakar, A.M.; Dahunsi, S.O. Medium Chain Carboxylate Production from Cassava Wastes Pretreated by Ensiling. *Biofuels, Bioproducts and Biorefining* **2023**, *17*, 933–943, doi:10.1002/bbb.2485.

205. Sanchez-Ledesma, L.M.; Rodríguez-Victoria, J.A.; Ramírez-Malule, H. Acidogenic Fermentation of Cassava Wastewater: Effect of the Substrate-to-Microorganism Ratio and Temperature on Volatile Fatty Acids Production. *Water (Switzerland)* **2024**, *16*, doi:10.3390/w16233344.

206. Karne, H.; Bendre, A.; Singh, A. Mathematical Modelling of a Pilot Scale Biogas Plant. *Mater Today Proc* **2023**, doi:10.1016/j.matpr.2023.01.303.

207. Pererva, Y.; Miller, C.D.; Sims, R.C. Existing Empirical Kinetic Models in Biochemical Methane Potential (BMP) Testing, Their Selection and Numerical Solution. *Water (Switzerland)* **2020**, *12*, 216–218, doi:10.1093/acprof:oso/9780195371833.003.0024.

208. Cheah, Y.K.; Vidal-Antich, C.; Dosta, J.; Mata-Álvarez, J. Volatile Fatty Acid Production from Mesophilic Acidogenic Fermentation of Organic Fraction of Municipal Solid Waste and Food Waste under Acidic and Alkaline PH. *Environmental Science and Pollution Research* **2019**, *26*, 35509–35522, doi:10.1007/s11356-019-05394-6.

209. Simonetti, S.; Saptoro, A.; Martín, C.F.; Dionisi, D. Product Concentration, Yield and Productivity in Anaerobic Digestion to Produce Short Chain Organic Acids: A Critical Analysis of Literature Data. *Processes* **2020**, *8*, 1–17, doi:10.3390/pr8121538.

210. Lucas, S.D.M.; Peixoto, G.; Mockaitis, G.; Zaiat, M.; Gomes, S.D. Energy Recovery from Agro-Industrial Wastewaters through Biohydrogen Production: Kinetic Evaluation and

Technological Feasibility. *Renew Energy* **2015**, *75*, 496–504, doi:10.1016/j.renene.2014.10.025.

211. Wang, L.; Hao, J.; Wang, C.; Li, Y.; Yang, Q. Carbohydrate-to-Protein Ratio Regulates Hydrolysis and Acidogenesis Processes during Volatile Fatty Acids Production. *Bioresour Technol* **2022**, *355*, 127266, doi:10.1016/j.biortech.2022.127266.

212. Sarkar, O.; Rova, U.; Christakopoulos, P.; Matsakas, L. Ethanol Addition Promotes Elongation of Short-Chain Fatty Acids to Medium-Chain Fatty Acids Using Brewery Spent Grains as Substrate. *J Environ Chem Eng* **2021**, *9*, 105990, doi:10.1016/j.jece.2021.105990.

213. Mu, Y.; Yu, H.Q.; Wang, G. A Kinetic Approach to Anaerobic Hydrogen-Producing Process. *Water Res* **2007**, *41*, 1152–1160, doi:10.1016/j.watres.2006.11.047.

214. Fang, F.; Ni, B.J.; Yu, H.Q. Estimating the Kinetic Parameters of Activated Sludge Storage Using Weighted Non-Linear Least-Squares and Accelerating Genetic Algorithm. *Water Res* **2009**, *43*, 2595–2604, doi:10.1016/j.watres.2009.01.002.

215. Bai, J.; Liu, H.; Yin, B.; Ma, H. Modeling of Enhanced VFAs Production from Waste Activated Sludge by Modified ADM1 with Improved Particle Swarm Optimization for Parameters Estimation. *Biochem Eng J* **2015**, *103*, 22–31, doi:10.1016/j.bej.2015.06.015.

216. Labatut, R.A.; Angenent, L.T.; Scott, N.R. Biochemical Methane Potential and Biodegradability of Complex Organic Substrates. *Bioresour Technol* **2011**, *102*, 2255–2264, doi:10.1016/j.biortech.2010.10.035.