

AN ASSESSMENT OF AGRO-INDUSTRIAL WASTEWATERS AS A RESOURCE FOR BIOHYDROGEN RECOVERY POTENTIAL USING DARK FERMENTATION BIOREFINERIES

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by

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EXECUTIVE SUMMARY

South Africa's agro-industrial sector generates substantial volumes of wastewater, presenting both environmental challenges and opportunities for renewable energy production, particularly through the development of biohydrogen. Valorising these waste streams aligns with national strategies promoting the circular economy, the Hydrogen Society Roadmap (DSI, 2021) and the Bioeconomy Strategy.

This study assessed the agro-industrial sector's potential for biohydrogen production via dark fermentation, focusing on waste-stream quantification, spatial mapping, identification of high-potential streams, and technical feasibility. In this regard, a mixed-methods approach was applied, including literature review, industry surveys, geospatial analysis, and laboratory-scale experimental work. Data from various sources were used to estimate wastewater volumes, organic loads, and nutrient composition. Substrate suitability for hydrogen production was assessed based on wastewater characteristics obtained from literature data, while geospatial mapping identified provincial and district-level hotspots for implementing resource recovery interventions, including biohydrogen production.

The study findings indicate that agro-industrial wastewater streams exhibit a wide range of Chemical oxygen demand (COD) values depending on feedstock and processing type. Beverage and fruit processing effluents have been identified as carbohydrate-rich, with COD typically between 3,000 and 15,000 mg L⁻¹, demonstrating high suitability for hydrogen production via dark fermentation. The sugar mill and wet corn milling streams also exhibited high COD, ranging from 5,000–20,000 mg L⁻¹ and 6,000–18,000 mg L⁻¹, respectively and this represents abundant fermentable sugars. However, the pulp and paper mill effluents show moderate COD (4,000–12,000 mg L⁻¹) and these may require pre-treatment to optimise hydrogen yields.

Regions with high concentrations of beverage, sugar, fruit-processing, pulp and paper, and wet corn milling facilities were identified as priority hotspots for biohydrogen recovery. These include KwaZulu-Natal (sugar, paper and pulp mills, beverages, dairy), Western Cape (beverages, fruit processing, and juicing factories), Gauteng (beverages, wet corn mills), and Mpumalanga (sugar mills, paper and pulp, and juicing factories). These clusters offer the most significant potential for establishing pilot-scale dark fermentation systems and the development of regional agro-industrial symbiosis networks, enabling shared infrastructure and maximised substrate utilisation.

Among **biological hydrogen production routes evaluated and experimental work conducted**, dark fermentation has been identified as the most practical and scalable option for processing carbohydrate-rich agro-industrial wastewaters. It offers high hydrogen yields and operational simplicity compared to light-dependent methods, such as photo-fermentation, photocatalysis, or photoelectrochemical systems.

However, most South African agro-industrial facilities continue to rely on conventional wastewater treatment systems such as primary sedimentation, aerated lagoons, and activated sludge. These systems are primarily designed to meet regulatory compliance rather than to recover resources, limiting opportunities for energy or nutrient capture. Currently, anaerobic digestion is applied in only a few beverage-processing facilities, leaving significant potential for the adoption of dark fermentation and other waste-to-energy technologies in other sub-sectors.

Overall, agro-industrial wastewater represents a strategic, renewable feedstock for low-carbon hydrogen production. Integrating dark fermentation biorefineries within wastewater management systems is therefore critical as it can reduce pollution, generate energy, and advance the circular economy and decarbonisation objectives.

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GLOSSARY

AGHA	Africa Green Hydrogen Alliance
ARC	Agricultural Research Council
BOD	Biological Oxygen Demand
BPP	Biohydrogen Production Potential
BHP	Biohydrogen Yield Potential
CDP	Carbon Disclosure Program
COD	Chemical Oxygen Demand
DALRRD	Department of Agriculture, Land Reform and Rural Development
DFFE	Department of Forestry, Fisheries and the Environment
DWS	Department of Water and Sanitation
EPA	Environmental Protection Agency
ESG	Environmental, Social, and Governance
FAO	Food and Agriculture Organisation of the United Nations
HFCT	Hydrogen and Fuel Cells Technologies
HySA	Hydrogen South Africa
IoT	Internet of Things
ISCI	Industry Standard Coding Identification
NATSURV	National Survey
NCIMS	National Compliance Information Management System
NDP	National Development Plan
NEMA	National Environmental Management
NGO	Non- governmental organisation
NIWIS	National Integrated Water Information System
NWA	National Water Act
NWand SMP	National Water and Sanitation Master Plan
NWRS	National Water Resource Strategy
OECD	Organisation for Economic Co-operation and Development
PEC	PEC — Photoelectrochemical
PNSB	PNSB — Purple non-sulphur bacteria
SAF	Sustainable Aviation Fuel
SAB	South African Breweries
SASA	South African Sugar Association
SAWIS	SA Wine Industry Information and Systems
SDG	Sustainable Development Goals
SEVsub	Specific Effluent Volume or Waste Generating Factor
STATSSA	Statistics South Africa
SWI	Specific Water Intake
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
UN	United Nations
UNEP	United Nations Environment Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNSD	United Nations Statistics Division
WARMS	Water use Authorisation and Registration Management System
WHO	World Health Organization
WRC	Water Research Commission
WSA	Water Services Act
WWTP	Wastewater Treatment Plant

1 INTRODUCTION

Worldwide, the industrial sector generates significant volumes of wastewater containing various pollutants, resulting from processes such as manufacturing, mining, chemical production, and agro-processing (UNEP, 2021). In many cases, these high-strength industrial wastewaters are inadequately treated before discharge into the environment or municipal sewer systems, placing a burden on already constrained wastewater treatment infrastructure, particularly in developing countries (OECD, 2020). Inadequate pre-treatment adds to environmental degradation, including eutrophication, oxygen depletion, and damage to aquatic ecosystems in receiving water bodies (UNEP, 2021). According to the UN-Water report, *Progress on Wastewater Treatment – 2024 Update*, among member countries that report industrial wastewater data (representing 8% of the global population), only 38% of industrial wastewater undergoes treatment, and just 27% complies with national or local safety standards (UN-Water, 2024).

Gradually, attention is being directed toward recovering resources from industrial wastewater as a key element of environmental sustainability, energy security, and resource efficiency, directly supporting Sustainable Development Goals (SDGs) and global climate action objectives (UNEP, 2021; UN-Water, 2024). This paradigm shift repositions wastewater not as waste but as a strategic resource that can promote sustainable development and support climate change mitigation and adaptation, particularly through reduced greenhouse gas emissions, improved water reuse, and the recovery of renewable energy (OECD, 2020; IEA, 2023). Wastewater resource recovery supports SDG 6 (Clean Water and Sanitation) by encouraging safe treatment, reuse, and pollution reduction; SDG 7 (Affordable and Clean Energy) via the recovery of renewable energy carriers like biogas and hydrogen; and SDG 12 (Responsible Consumption and Production) by closing material and nutrient loops within circular economy frameworks (UNEP, 2021; UN-Water, 2024). Overall, these efforts highlight the vital role of wastewater resource recovery in sustainable development and fostering low-carbon economies (IEA, 2023).

Under the broader umbrella of industrial wastewater, agro-industrial effluents are of particular concern due to their high organic load, elevated nutrient content, and high biodegradability, which sets them apart from other industrial waste streams and creates both substantial treatment challenges and opportunities for resource recovery, particularly energy and value-added products (UNEP, 2021; IEA, 2023). The sector is a major wastewater generator as it utilises water-intensive processing operations, including sanitation, heating, cooling, and cleaning-in-place. While agro-industrial activities, such as beverage production, sugar milling, dairy processing, and fruit and vegetable processing, are central to economic growth, employment, and food security, especially for developing nations; they generate large volumes of wastewater characterised by high COD, Biochemical oxygen demand (BOD), suspended solids, and variable nutrient concentrations, which remain difficult and costly to manage (FAO, 2020; UN-Water, 2024).

1.1 Project Contextualisation

The agro-processing sector is a key contributor to South Africa's economy, underpinning food security, rural employment, and export competitiveness. However, it is one of the most water- and energy-intensive industrial sub-sectors and subsequently generates large volumes of wastewater. On average, nearly 50% of the water used in food and beverage processing ends up as wastewater (Akratos et al., 2021). Based on an estimated annual consumption of approximately 130 million kL, the agro-processing sector potentially generates up to 65 million kL of wastewater per year (Mpfung et al., 2020). Much of this wastewater is discharged untreated or partially treated, resulting in surface-water pollution, eutrophication, and public health concerns (Mpfung et al., 2020).

Agro-industrial wastewater treatment challenges in the country are exacerbated by chronic water scarcity, constrained municipal wastewater treatment capacity, rising energy costs, and increasingly stringent discharge regulations, exposing the limitations of compliance-driven wastewater management (DWS, 2022; DWS, 2023). Successive Green Drop Reports consistently document declining treatment performance, infrastructure backlogs, and widespread non-compliance across many municipalities, underscoring the need for integrated wastewater management approaches that extend beyond pollution control.

Conventional agro-industrial wastewater practices in South Africa treat effluent as an environmental liability that requires pre-discharge treatment for disposal purposes only. This approach results in the loss of valuable resources in the effluent. However, in line with global trends, South Africa is increasingly promoting wastewater resource recovery within national policy frameworks, including the National Water Act (1998), the National Water and Sanitation Master Plan, the National Strategy for Water Reuse, and the National Waste Management Strategy (NWMS 2020), all of which explicitly promote pollution prevention, wastewater reuse, and recovery as central to sustainable agro-industrial development.

Waste-to-energy technologies play a critical role in implementing circular and resource-efficient wastewater management in the agro-industrial sector (Smith et al. 2023). Low-energy biological conversion routes that utilise microbial processes to transform organic matter into energy carriers and value-added products offer significant potential for treating high-strength agro-industrial effluents (Patel and Kumar, 2022). Anaerobic digestion represents the dominant microbial waste-to-energy technology, producing methane-rich biogas for heating, electricity generation, and vehicular fuel applications (Jones and Lee, 2022). Recent investigations have examined alternative pathways for producing high-value energy carriers (Chen et al. 2020), including dark fermentation, microbial electrolysis cells, photo-fermentation, and photo-catalysis.

Dark fermentation presents a low-energy alternative for treating agro-industrial effluents, yielding biohydrogen and fermentative by-products such as volatile fatty acids (Kumar and Singh, 2023). Hydrogen has received considerable attention as a clean energy vector due to its combustion characteristics and high energy density (Sharma et al. 2021). The compound demonstrates greater market value than methane and serves multiple functions as a chemical feedstock (IEA, 2024; Zhang et al., 2023). Achieving net-zero emissions by 2030 requires establishing a low-carbon global economy, with industrial decarbonisation representing a critical component (IPCC, 2023). Current biomass conversion facilities are being configured as integrated biorefineries to maximise resource utilisation and product diversification (Lee and Wong, 2022). Integration of dark fermentation into biorefinery frameworks enables simultaneous hydrogen generation and recovery of co-products, thereby improving the economic viability and environmental performance of circular agro-industrial wastewater management systems (Rodriguez and Martinez, 2023).

South Africa is actively promoting green hydrogen as an enabler of industrial decarbonisation and economic development through policy instruments such as the Hydrogen Society Roadmap (DSI, 2021; IEA, 2024). Current initiatives, however, are largely centred on renewable–electricity–driven water electrolysis, with limited attention to alternative hydrogen-production pathways, particularly low-energy biological routes. Biohydrogen production from agro-industrial wastewater remains underexplored despite its strong alignment with circular-economy and water–energy nexus objectives (UNEP, 2021; OECD, 2020). This study seeks to address this gap by conducting a systematic resource assessment to evaluate the potential for biohydrogen generation from agro-industrial wastewater streams in South Africa.

1.2 Project Aim

The aim of this study is to conduct a national resource assessment of agro-industrial wastewaters in South Africa by quantifying generation rates, spatial distribution, and physicochemical characteristics, and to evaluate their suitability for biohydrogen production and other bio-products via dark-fermentation-centred biorefineries.

To achieve this aim, the study sought to:

- Review existing wastewater management practices in South Africa and identify innovative technologies for wastewater resource recovery that are in use or under development globally.
- Conduct a national agro-industrial wastewater resource assessment to quantify, map, and characterise South Africa's agro-industrial wastewater streams.
- Calculate South Africa's theoretical biohydrogen production using dark fermentation technology.
- Demonstrate and evaluate biohydrogen production at laboratory scale using selected agro-industrial wastewater streams. from selected agro-industrial wastewater streams through literature and laboratory investigations.
- Propose scale-up strategies for translating study findings into practical applications.

1.3 Project Scope and Limitations

The study focuses on high-strength, biodegradable agro-processing wastewater streams, with a primary emphasis on dark fermentation as a biological pathway for hydrogen production. The research is limited to wastewater resource assessment, theoretical assessment of biohydrogen production, and laboratory-scale demonstration of biohydrogen production.

2 WASTEWATER MANAGEMENT PRACTICES IN THE SOUTH AFRICAN AGRO-PROCESSING INDUSTRY

The National Water Act (Act 36 of 1998) defines “waste” as including any solid, liquid, or gaseous material discharged into water. Thus, wastewater generated from agro-processing activities falls within this definition. The Act further recognises biodegradable industrial wastewater as a distinct effluent category which encompasses wastewater from milk processing, fruit and vegetable manufacturing, sugar mills, beverage production, breweries, wineries, abattoirs, fish processing, feedlots, and animal feed manufacturing. Therefore, management of wastewater generated by the agro-processing sector in South Africa is governed by the regulatory framework detailed in the National Water Act (National Water Act, 1998)

2.1 Key Framework of the National Water Act for Agro-processing Wastewater Management

The Act establishes that any person who disposes of waste must do so in a manner that does not violate water quality standards or negatively affect the basic water needs of people and the environment. Section 19 of the NWA assigns responsibility for taking reasonable measures to prevent water resource pollution to the owner of the land or the person in charge of the potentially polluting activity; ergo, the agro-process. The responsible party is liable for taking reasonable measures that will prevent the pollution of water resources from occurring, continuing, or recurring. **Figure 2.1** depicts the framework that governs wastewater management in South Africa.

Section 21 defines the discharge of wastewater into a water resource, such as a river, dam, or even on land, that may result in groundwater seepage, as a “water use”. The NWA mandates that this water use

be licensed or authorised. A person, the agro-processor, may not discharge treated or untreated wastewater without holding a Water Use Licence (WUL). WULs are site-specific and set strict limits within which the volume and quality (for example, COD, pH, suspended solids, and fats/oils/greases) of the wastewater to be discharged must fall. Therefore, in practice, this framework means an agro-processing facility cannot lawfully discharge its process water, wash-down water, or effluent into the environment without having satisfied the following conditions:

- Applying for and receiving a WUL.
- Implementing reasonable pollution control measures.
- Monitoring and reporting its discharge quality to the regulatory body.

The NWA provides a legal framework that holds agro-processing facilities accountable for their wastewater. The Act mandates that agro-processors obtain a WUL, adhere to discharge standards, and bear responsibility for preventing pollution of water resources in South Africa (National Water Act, 1998).

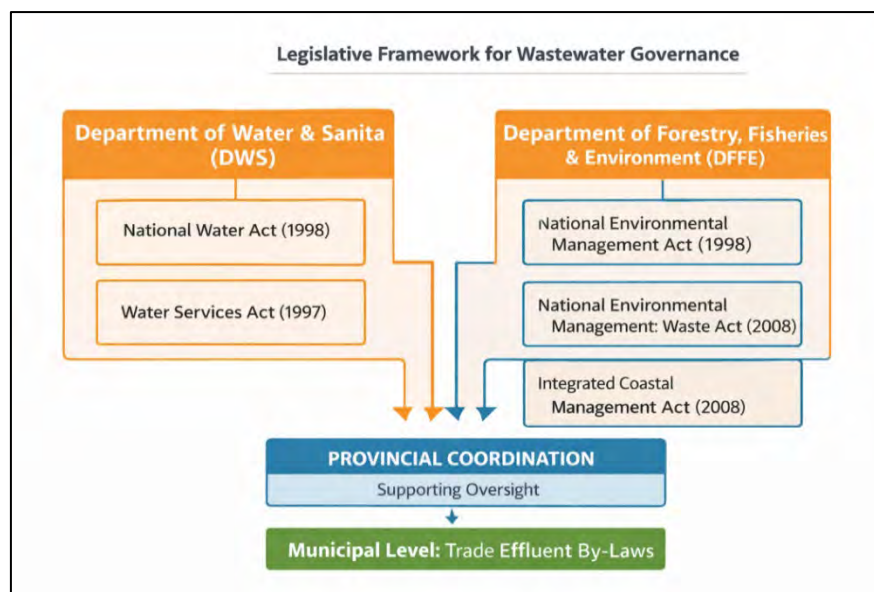


Figure 2.1 The framework that governs wastewater in South Africa

2.2 Regulatory Authorities Governing Agro-processing Wastewater Management in South Africa

The Department of Water and Sanitation (DWS) is the national authority responsible for regulating water resources in South Africa. Its function includes the allocation, protection, use, development, conservation, management and control of water resources. Per the NWA, the responsibility to ensure that water is protected, used, developed, conserved, and controlled sustainably and equitably for the benefit of all persons is that of the Minister of Water and Sanitation. The Act authorises the Minister and the DWS to set and enforce water use conditions, including waste discharge requirements, which include agro-processing wastewater discharges. The DWS acts as both the custodian and regulator of water resources, assessing and issuing WULs and monitoring compliance with licence conditions (SAFLI, 2024).

In addition to national management of water resources by the DWS, the NWA allows for the establishment of decentralised bodies called Catchment Management Agencies (CMAs). The role of the CMAs is to support the equitable and sustainable management of water resources at the regional scale across defined Water Management Areas (WMAs). Even though the role of CMAs is still evolving, they are important for local water resource planning and protection and making decisions about water

use. They also contribute to meeting water quality objectives and the administration of local licenses. The CMAs are not completely independent economic regulatory bodies that create and enforce rules on their own. Rather, they are statutory bodies created under the NWA to implement national water policies at the catchment level and to support decentralised management of water resources (Weston and Goga, 2016).

At the local and municipal level, the function of regulating wastewater overlaps with wastewater service delivery under the Water Services Act (Act 108 of 1997). Municipalities act as Water Services Authorities (WSAs) and are responsible for reticulated wastewater services. They often enforce bylaws on industrial effluent discharge requirements into sewer systems. The bylaws are linked to national water quality standards and licensing requirements under the NWA. **Figure 2.2** illustrates the hierarchical institutional framework for wastewater governance in South Africa.

The WSAs are not the primary licensors of water use under the NWA. However, they are vital for enforcing standards for wastewater infrastructure and quality at point of discharge into public systems, ensuring that municipalities support and align with national water resource protection objectives (Redelinghuis et al. 2025; Slabbert et al. 2025).

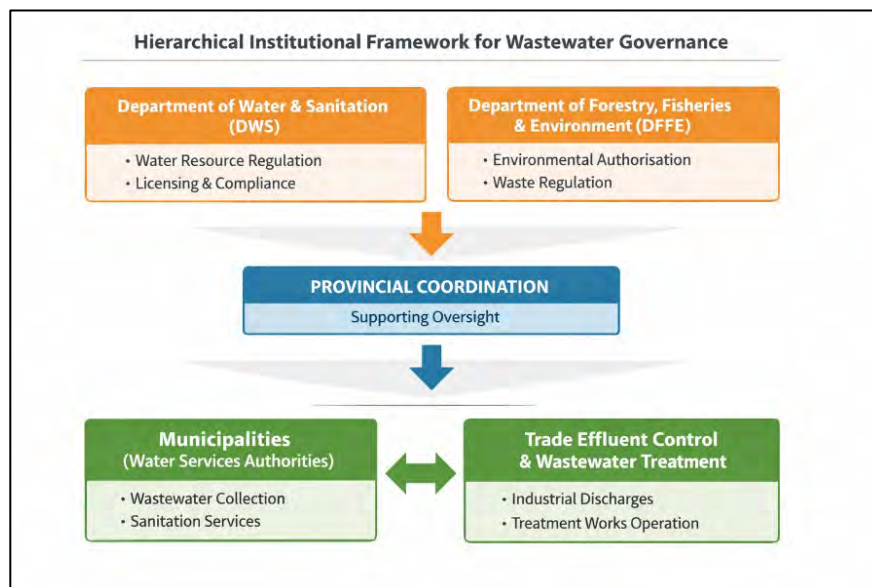


Figure 2.2 The hierarchy of wastewater governance in South Africa

2.3 Enforcement and Penalties for Wastewater Quality Infringements in South Africa

The framework for managing wastewater quality infringements is anchored in the NWA as well as the National Environmental Management Act (Act 107 of 1998) (NEMA). Contravention of the Acts' pollution and water use provisions can result in criminal prosecution and penalties. Discharging wastewater without a licence or failing to comply with licence conditions are examples of contraventions that could result in agro-processors incurring fines or facing imprisonment. The DWS often cooperates with the National Prosecuting Authority (NPA) to enforce retribution. The DWS can issue compliance notices and directives to entities that contravene the Acts. Where an entity repeatedly ignores the directives, regulators may initiate criminal proceedings to compel compliance and punish non-compliance (Redelinghuis et al. 2025; Rangata and Odeku, 2014).

When an agro-processing facility fails to comply with its WUL conditions or discharges wastewater unlawfully, the DWS initiates enforcement action. The process typically begins with administrative enforcement measures, which may involve the DWS conducting inspections and, where non-compliance is found, issuing a directive that requires the polluter to take specific remedial steps within a specified timeframe. The directives are the primary compliance tools used before prosecution and are legally binding. The directives give the offender an opportunity to rectify pollution and prevent any further harm. If the offender complies with the directive, the matter may be resolved with no further legal escalation. However, if the offender ignores the compliance notices and directives, the DWS may lay criminal charges and refer the matter to the NPA for prosecution. Once the matter is in the criminal justice system, the offender may face fines and/or imprisonment if convicted for the offences (DWS, 2025; Feris 2017).

2.4 Agro-processing Wastewater Treatment Practices

Ultimately, the effective management of wastewater depends on a clear understanding of the effluent characteristics. Agro-processing wastewater properties vary significantly depending on several factors such as the type of raw material-, the type and degree of processing-, and the types of cleaning agents used, as well as seasonal volume fluctuations. But, generally, agro-processing wastewater contains a varying combination of high levels of organic matter, high concentrations of suspended and dissolved solids, variable pH, detergents, and other constituents that make it complicated to manage.

To achieve effective treatment, agro-processing wastewater treatment is often done in stages. These stages include pre-treatment, primary-, secondary-, and tertiary treatment (Shrivastava et al. 2022; Aderibigbe, 2017). The main purpose of pre-treating wastewater is to protect downstream equipment and to reduce peak loads. Processes such as coarse particle screening, grit removal, oil-water separation, and use of grease traps are commonly used in the pre-treatment of agro-processing wastewater. Equalisation tanks or basins can also be used to stabilise flow and effluent strength fluctuations, as well as to homogenise effluent pH before primary treatment (Kato and Kansha, 2024; Hubbard et al., 2021; Englande et al., 2015).

Primary treatment is done to physically or physico-chemically remove suspended organic solids and fats, oils, and grease. This is typically achieved using sedimentation or clarifiers and coagulation-flocculation. These processes are then followed by clarification or dissolved air flotation. Coagulation-flocculation is often used when the wastewater contains small colloidal solids or colloidal organic matter that do not settle well. Dissolved air flotation is typically used to remove low-density fats, oils and grease as well as fine solids. After primary treatment, suspended solids and a portion of particulate biological oxygen demand are reduced, which improves the efficiency of the downstream treatment processes. The sludge which results from primary treatment is often thickened and dewatered before it is disposed of on land or composted (Nikolić et al. 2025; Shrivastava et al. 2022; Nayyar et al. 2021).

In secondary treatment, biological methods are used to remove dissolved organic matter in agro-processing wastewaters. Commonly used full-scale systems in the agro-processing industry include activated sludge, sequencing batch reactors, aerated lagoons and facultative or anaerobic ponds, anaerobic treatments, and membrane bioreactors. Activated sludge treatment is widely used in moderate to large plants, such as breweries, dairies and beverage manufacturers because of its high efficiency at removing biological oxygen demand. Standard components of the process include aeration basins, secondary clarifiers, and return activated sludge. Sequencing batch reactors are used in facilities that have variable flow or have a constrained footprint. They provide flexible control of aeration and settling phases. They can also handle variable loads that are common in agro-processing (Nayyar et al. 2021; Barbera and Gurnari, 2018; Englande et al. 2015).

Aerated lagoons and facultative or anaerobic ponds are generally used in smaller or rural agro-processing facilities, such as fruit and vegetable processing facilities, small dairies, and sugar mills. These lagoons and ponds are low cost and have low operational complexity. However, they need large land area and long detention times (Kato and Kansha, 2024; Shrivastava et al., 2022; Nayyar et al., 2021). Anaerobic treatment processes are used to treat high-strength, warm, and organic material-rich effluents. Anaerobic treatment can facilitate energy recovery (biogas) and is typically less energy intensive than aerobic treatment. It can be used to effectively treat dairy-, fruit and vegetable processing-, meat processing-, and beverage and brewery wastewater. The systems are especially suited where high COD and constant warm temperatures improve performance (Barbera and Gurnari, 2018; Kato and Kansha, 2024).

Tertiary treatment is applied to meet discharge standards or facilitate wastewater reuse. Tertiary treatment is rarely applied to agro-processing wastewater, especially in developing countries. Some of the methods used in tertiary treatment include constructed wetlands, polishing ponds, nutrient removal, disinfection, membrane processes and filtration processes (Shrivastava et al. 2022; Nayyar et al. 2021).

Following on-site treatment, agro-processing wastewater is disposed of using several avenues or it is reused where possible. Treated effluent can be discharged to rivers, streams, lakes, or coastal waters where permitted. Many agro-processing facilities discharge treated or, sometimes, only pretreated effluent to municipal wastewater treatment plants. Discharging to sewers can be simpler for agro-processing facilities, but municipal systems commonly require industrial pretreatment (screening, grit removal, FOG control, pH adjustment, limits on toxicants or high loads) to protect the sewer network and municipal treatment works.

Some facilities use adequately treated effluent for irrigation. However, regional standards outline quality classes, crop restrictions, application methods, and risk-management measures to protect human health and the environment. Treated wastewater is also often reused within the facility or by nearby industries for non-potable purposes such as cooling towers, boiler feed (after appropriate polishing), equipment washing, or process water where potable quality is not required (Shrivastava et al. 2022; Nayyar et al. 2021; Barbera and Gurnari, 2018).

2.5 Agro-processing Wastewater Treatment in South Africa

Finding reliable, comprehensive information on wastewater treatment practices specifically for the agro-processing sector in South Africa is challenging. Companies are often reluctant to fully disclose their practices for several reasons, such as protecting intellectual property, managing regulatory and legal risks, mitigating reputational risk, and safeguarding brand protection, among others. This lack of transparency limits the availability of consistent, verifiable data across the sector and makes it difficult to compare treatment performance or evaluate the effectiveness of implemented technologies. Consequently, most accessible information comes from academic studies, industry case reports, and Water Research Commission (WRC) technical reports, which focus on specific sub-sectors or facilities rather than on the industry. Despite these limitations, the available literature provides valuable insights into the types of treatment systems currently in use and the general approaches adopted for managing and disposing of agro-processing wastewater in South Africa.

South African abattoirs commonly use naturally aerated-, mechanically forced aerated-, facultative-, and anaerobic ponds for secondary treatment of wastewater. Dissolved air flotation cells and septic tanks are also commonly used for secondary treatment of abattoir wastewater (Pocock and Joubert, 2017). Abattoirs often discharge inadequately treated wastewater into municipal sewage systems or natural water resources. Therefore, most abattoir wastewater does not comply with the quality

requirements that are prescribed by national by-laws. On-site septic tanks are also often used to dispose of wastewater in areas that are not connected to sewage infrastructure. Septic collectors then collect the effluent and dispose of it in the nearest municipal wastewater treatment plants. Although uptake is very slow, anaerobic digestion of abattoir waste has been explored in some abattoirs across the country (Mutisi and Moreroa, 2025; Moreroa and Basitere, 2022).

The South African sugarcane sector implements several wastewater treatment and disposal methods. Treatment methods include aerobic and anaerobic ponds, dilution, physicochemical treatment, activated sludge systems, natural percolation through sand and treatment wetlands, electrochemical systems, and in a few cases, no treatment is applied before discharge. Irrigation, river discharge, marine outfall, evaporation dams and municipal sewers are some of the ways in which treated wastewater is disposed of. Irrigation is the most commonly used method of disposal (Welz and Ndobeni, 2017).

Fruit and vegetable processing companies in South Africa use a variety of wastewater treatment practices. In primary treatment, the focus is on the initial removal of solids and pH adjustment. To achieve this, facilities use filtration and screening, settling and sedimentation, neutralisation, and other methods such as flocculation and chemical treatment. Some facilities do not practise primary treatment. Secondary treatment uses biological processes to improve effluent quality. Aerobic and anaerobic treatment and septic systems are some of the methods used for secondary treatment. Some facilities do not conduct secondary treatment. Although it is the least applied stage, tertiary treatment is used to further improve effluent quality. Chemical treatment and advanced filtration are some of the methods observed in the South African fruit and vegetable processing sector. The facilities dispose of treated or partially treated wastewater through irrigation (the most frequently used disposal method), municipal wastewater treatment works, and discharge into the marine environment (Swartz et al., 2021)

In the dairy processing sector, wastewater treatment practices depend primarily on location and access to municipal infrastructure. The majority of dairy processors surveyed in NATSURV 4 discharge their effluent into municipal sewer systems. They implement pre-treatment in the form of pH adjustment, screening, and dissolved air flotation before discharge to comply with municipal discharge limits and to avoid financial penalties. The participants located in rural areas manage their wastewater using strategies such as man-made wetlands or land irrigation. Full wastewater recycling is generally not practiced due to high implementation costs compared to municipal tariffs, some participants have begun adopting cleaner production methods to reduce both water consumption and the associated costs of wastewater disposal (Visser et al., 2023).

According to Jaiyeola and Bwapwa (2016), historically, the brewery sector had limited wastewater treatment options, and as a result, breweries simply disposed of their effluent into the environment without treatment. The resulting environmental pollution necessitated pre-treatment before disposal into municipal wastewater treatment plants, where it is subjected to primary, secondary, and tertiary treatment. The high volume of brewery effluent in municipal wastewater treatment plants has adversely affected treatment plants' overall performance. To promote sustainability, South African Breweries uses biogas digesters to treat its wastewater and generate electricity at the Alrode and Newlands Breweries.

Wastewater management practices across South Africa's agro-processing industries reveal a diverse yet uneven implementation of treatment technologies. While some sectors, such as brewing, have begun integrating resource recovery solutions, such as anaerobic digestion, others still rely heavily on low-cost or outdated systems that offer limited environmental protection. The predominance of partial treatment and direct discharge into municipal systems or the environment highlights persistent infrastructural, financial, and regulatory challenges. Addressing these gaps through improved

enforcement, technology adoption, and sector-specific guidance will be crucial to achieving consistent compliance and advancing sustainable water management within the country's agro-processing sector.

2.6 Current State of Wastewater Treatment Infrastructure in South Africa

Overall, wastewater treatment infrastructure in South Africa is currently under significant strain. The DWS has repeatedly reported declining levels of compliance at wastewater treatment works (WWTWs) through its Green Drop assessments. The WWTWs are plagued with widespread non-compliance with effluent discharge standards, inadequate infrastructure maintenance, and limited technical capacity. Aging infrastructure, skills shortages, and financial mismanagement at the municipal level have led to poor WWTWs' performance across many municipalities (Graham et al. 2025; Phungela et al, 2021). The most recent Green Drop progress report found that the majority of WWTWs in the country were in high- or critical-risk condition, with a high likelihood of partially treated or untreated effluent being discharged into rivers and other receiving water bodies (DWS, 2023).

The agro-processing sector generates high-strength wastewaters that place additional pressure on the already fragile wastewater treatment infrastructure, as some facilities are connected to municipal sewer systems. Agro-processing wastewater is typically very rich in organic matter and, as a result, has a high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentration. Depending on the sector, agro-processing wastewater may contain substantial amounts of dissolved and/or suspended solids, such as minerals, salts, nutrients, fats, oils, and grease, plant material, food particles, and biological organisms, among other components. When agro-processing wastewater is not treated adequately, it exacerbates the deterioration of municipal wastewater infrastructure by overloading treatment processes that were largely designed for domestic sewage rather than high-strength industrial effluent. Ongoing organic and nutrient overloading can accelerate equipment wear and shorten infrastructure lifespan (Abdullah et al. 2023; Nayyar et al. 2021).

Management of agro-processing wastewater in South Africa falls within regulatory frameworks that emphasise pollution control, compliance, and risk mitigation. This reinforces the current perception of agro-processing effluent as a liability that needs treatment before disposal. As such, agro-processing wastewater is often managed with the aim of minimising harm rather than maximising value. The sector is largely compliance-driven, prioritising meeting discharge standards over resource recovery. This problem-centred approach obscures the potential of recovering compliance-driven resources from wastewater. Agro-processing wastewater is an important resource that can support water security, energy generation, and nutrient recovery. Recognising its value can transform agro-processing wastewater from an environmental burden to a productive input. Therefore, it is critical to understand wastewater composition, variability, and treatment responsiveness to facilitate this paradigm shift.

2.7 Chapter Summary and Conclusion

This chapter reviewed the regulatory and institutional frameworks governing wastewater management in South Africa, including agro-processing wastewaters. The principal law governing wastewater management in the country is the National Water Act, which requires agro-processing facilities to obtain a Water Use Licence (WUL) before discharging effluent that could harm water resources. Enforcement of these regulations falls to the Department of Water and Sanitation, which oversees them, with support from Catchment Management Agencies and Municipal Water Services Authorities. Under the National Water Act and the National Environmental Management Act, non-compliance with discharge requirements or licence conditions can result in directives, financial penalties, or legal action against facilities.

The chapter also examined treatment methods for agro-processing wastewater, including pre-treatment, primary, secondary, and sometimes tertiary processes such as screening, sedimentation, dissolved air flotation, activated sludge systems, anaerobic digestion, and lagoons. Treatment practices in the sector vary widely, with some facilities using low-cost systems or discharging partially treated effluent into municipal sewer networks. In general, wastewater management practices in the agro-processing sector have overlooked opportunities to recover resources, focusing more on compliance and pollution control than on generating energy and recovering nutrients from wastewater.

The agro-processing sector requires a shift from linear wastewater discharge practices to circular approaches that harness value from wastewater. While current frameworks emphasise pollution control and compliance, the high organic and nutrient content of agro-industrial effluents presents significant opportunities for resource recovery. Therefore, the next chapter focuses on quantifying and characterising agro-industrial wastewater streams in South Africa, providing a national-scale resource assessment that evaluates their potential for energy generation, water reuse, and bioproduct recovery within emerging biorefinery and industrial symbiosis systems.

3 A NATIONAL AGRO-PROCESSING WASTEWATER RESOURCE ASSESSMENT

3.1 Sectoral Context and Scope of Study

According to the International Standard Industrial Classification (ISIC Rev. 4) and the Food and Agriculture Organization (FAO), agro-processing is a manufacturing sub-sector that converts raw materials from agriculture, fisheries, and forestry into value-added products (Chitonge, 2021). This definition is adopted by both the Department of Agriculture, Forestry and Fisheries (DAFF) and the Department of Trade and Industry (Chitonge, 2021). While the FAO/ISIC definition includes 11 sub-sectors, this report adopts South Africa's Industrial Policy Action Plan (IPAP 2016/17–2018/19) definition, which limits agro-processing to the food and beverage (F and B) sector. South Africa's F and B sector accounts for 60% of the country's agro-processing sector under the FAO/ISIC definition (DTI, 2016). The paper and pulp industry was also included in the assessment, as previous studies have identified it as a high wastewater-generating industry (Harding, et al., 2020). **Figure 3.1** displays the output contributed by each agro-processing sector, expressed as percentages, between 2011 and 2018, as reported by Chitonge (2021).

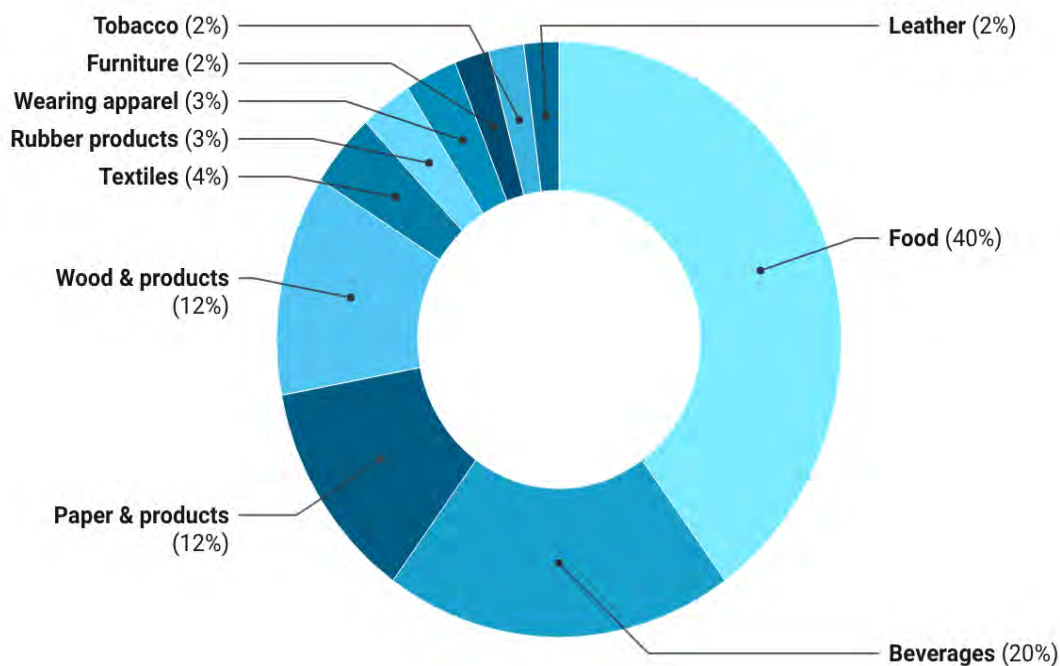


Figure 3.1 Percentage contribution of South Africa's Agro-Processing Industry by sub-sector average for 2011-2018. Source: Chitonge, 2021

3.2 Industry structure

South Africa's agro-processing sector has a dual structure, comprising numerous small, medium, and micro enterprises (SMMEs) and a small number of large, vertically integrated corporations. A 2017 SETA study on the food and beverage sector reports that the sector comprised approximately 11,000 registered companies, of which small firms accounted for 91%, medium-sized firms for 5%, and large corporations for only 4% (SETA, 2017). Despite small and medium-sized enterprises dominating numerically, large vertically integrated companies hold the largest market share and control most production (Nhundu, et al., 2017). Large-scale F and B companies often have vertically integrated supply chains that encompass raw material sourcing, processing, packaging, and distribution (Chitonge, 2021). Large-scale, vertically integrated companies often operate high-volume facilities that run continuous production schedules and use automated cleaning systems, which contribute to high water use intensity and wastewater generation (Nikolić, et al., 2025). Consequently, owing to the scale of production and production practices, a few large-scale companies account for a large share of a sector's wastewater generation.

Industry sub-clusters

The industry can be divided into five main clusters based on raw materials and products. Table 3.1 summarises the main clusters, key products, each cluster's relative output share, and examples of the key large-scale producers under each cluster (Chitonge, 2021).

Table 3.1 Major clusters of South Africa's food and beverage sector and their percentage share (Chitonge, 2021)

Cluster	Representative Products	Output Share (%)	Major Producers
Fresh & Perishable Foods	Meat, poultry, fish, fruit & vegetable processing, edible oils	28	Astral Foods, RCL Foods, Karan Beef, Beef Master, Oceana Group
Dairy Processing	Milk, cheese, butter, yogurt, milk powder	8	Clover SA, Lactalis South Africa, Woodlands Dairy
Grain Milling & Starch Products	Wheat and maize milling, starch, animal feed	14	Ingrain
Confectionery & Bakery	Bread, sugar refining, chocolate, pasta, syrups	22	Illovo, Tongaat, RCL Foods, UCL
Beverages	Beer, wine, spirits, soft drinks, juices, bottled water	28	Coca-Cola Beverages, AB-InBev, Heineken

**Percentages based on aggregated 2018 manufacturing data from FAO (2020) and Chitonge (2021)*

Geographic distribution

Agro-processing facilities in the country are mainly located near major urban areas or in farming areas to facilitate access to markets, infrastructure, labour, and raw materials. The primary clusters are found near metropolitan centres, including Gauteng (Ekurhuleni, Johannesburg, Tshwane), KwaZulu-Natal (eThekweni), the Western Cape (Cape Town, Drakenstein, Stellenbosch), and the Eastern Cape (Nelson Mandela Bay). These metropolitan areas host a variety of industries, such as grain milling, beverages, meat processing, sugar refining, pulp and paper, wine, and fruit processing, near transport hubs and large city markets. Secondary clusters are typically found in agricultural production zones such as the Breede Valley, Greater Tzaneen, and Nkomazi, where processing facilities are located near raw-material supply (IFC, 2019). Some of the agro-processing facilities are also located in regions that experience chronic water shortages, such as the Western Cape, Mpumalanga, and parts of Limpopo. Water scarcity and high energy costs pose high operational risks. Creating a need to invest in water reuse, turning wastewater into energy, and using technologies that save resources within these agro-processing clusters (IFC, 2019).

3.3 Study scope

The study focused on large-scale agro-processing facilities, which generate the largest share of F and B industrial wastewater. Secondly, high-volume generating facilities were preferred because they are ideal candidates for piloting or implementing resource recovery technologies, given their consistent effluent supply, financial resources, and, in most cases, a commitment to ESG performance. The number of sub-sectors covered by this study and the number of agro-processing facilities reviewed during the assessment are summarised in Table 3.2.

Table 3.2 Sub-sectors included in the resource assessment and the number of agro-processing facilities analysed or identified by the study

Cluster	Sub-sectors Included in the Study N=12	Number of Facilities analysed n~60
Fresh & Perishable Foods	Red meat abattoirs (high throughput)	80*
	Poultry abattoirs	27*
	Fish processing	4
	Fruit juice / fruit juice concentrate	11
Secondary Dairy Processing	Cheese, butter, whey	129*
	Grain Milling & Starch	4
Products	Confectionery & Bakery	12
	Beverages	13
		8
		3
		8
Paper & Pulp	Paper and pulp processing	8

** Agro-processing facilities identified, but data not analysed or facilities not mapped

3.4 Methodology for Agro-processing Wastewater Resource Assessment

This section outlines the study's methodological framework for conducting a national-level assessment of agro-processing wastewater resources using secondary data. The assessment primarily relied on publicly available documents, including national statistics, sector reports, commodity association publications, and peer-reviewed literature. The resource assessment addressed three principal aspects:

- Wastewater quantification (i) at the national level, looking at the total wastewater generated by all 12 sub-sectors and (ii) at the provincial level, looking at the wastewater generated by sub-sectors located in that province.
- Wastewater characterisation to assess the wastewater quality by compiling literature data on the physicochemical composition of agro-processing wastewater, with emphasis on parameters relevant to biological resource recovery. These parameters informed the screening and identification of streams most suitable for biohydrogen production.
- Geospatial analysis to map large-scale agro-processing facilities at the provincial level. This enabled the identification of geographic hotspots based on facility density and sub-sector representation.

3.4.1 Quantifying agro-processing wastewater volumes

The amount of wastewater generated by the selected agro-processing sectors was estimated using annual production statistics and sector-specific effluent generation rates, either specific effluent volume (SEV) or specific water intake (SWI), depending on data availability. The stepwise, systematic computations first calculated the amount of wastewater generated by each sub-sector, then summed the sub-sector totals to obtain the overall national wastewater generation volume. The total wastewater generated at the province level was determined by summing each sub-sector's proportional contribution in that province.

Production data aggregated to national totals for each agro-processing sub-sector were used when available, e.g., from StatsSA (2021) manufacturing data or commodity associations. When national totals were unavailable for a given sub-sector, facility-level production data were compiled and aggregated to derive sub-sector national totals. The equations used to calculate the amount of wastewater generated are presented in **Table 3.3**.

Table 3.3 Equations used to estimate the wastewater generated by each sub-sector.

	Solid products (e.g., sugar, grain mill, paper and pulp)	Liquid products (beverages, fruit juice, dairy liquids)
Equation 1: Quantifying the volume of wastewater generated per sub-sector per annum	$WWV_{si} = P_{si} \times SEV_{si}$ <p>Where SEV was not available:</p> $WWV_{si} = P_{si} \times (SWI_{si} \times WW_{GR,si})$	$WWV_{li} = P_{li} \times SEV_{li}$ <p>Where SEV was not available:</p> $WWV_{li} = P_{si} \times (SWI_{li} \times WW_{GR,li})$
Equation 2: National wastewater generated by all sub-sectors.	$NWWV = \sum_{i=1}^n WWV_{si} + \sum_{i=1}^n WWV_{li}$	
Equation 3: Provincial wastewater generated by sub-sectors located in that province	$\sum_{i=1}^n WWV_{si} \times Share_{si} + \sum_{i=1}^n WWV_{li} \times Share_{si}$	

Parameter Definitions and Assumptions

Where

Specific Effluent Volume (SEV) is the volume of wastewater generated per unit of product output. For solid products, this was expressed as $\text{m}^3 \cdot \text{t}^{-1}$ and for liquid products, as $\text{m}^3 \text{m}^{-3}$.

Specific Water Intake (SWI) is the volume of water consumed per unit of product output ($\text{m}^3 \cdot \text{t}^{-1}$ or $\text{m}^3 \text{m}^{-3}$). Where SEV data were unavailable, SEV was estimated from SWI using a wastewater generation rate (WWGR):

$$\text{SEV} = \text{SWI} \times \text{WWGR}$$

Wastewater Generation Rate (WWGR) is the fraction of water intake that ends up discharged as wastewater. A uniform value of $\text{WWGR} = 0.5$ (50%) was applied across agro-processing sub-sectors, which lies within the 50–80% range reported in the literature for water consumed in food and beverage production that ends up as wastewater (UNIDO, 2017; FAO, 2011). The 50% was chosen as a conservative assumption to avoid overestimating wastewater generation. While assuming a uniform rate introduces inaccuracies, it provided a conservative baseline for national-scale estimation.

Data Sources

Annual production data were compiled from multiple national and sectoral sources to maximise coverage and reliability. These sources included Statistics South Africa manufacturing production statistics (2021), commodity association reports (such as those for sugar, red meat, wine, and dairy), industry annual and sustainability reports, and published sector studies. Where more than one data source was available for a sub-sector, data were cross-checked and reconciled against national production totals. SEV and SWI coefficients were sourced from the NATSURV series, company sustainability and annual reports, and, lastly, journal articles and peer-reviewed literature when sector data gaps existed. The sources for annual production data and SEV for the different sub-sectors are contained in **Table 3.4**.

Table 3.4 Data sources for annual production data and SEV

Cluster	Sub-sector	Annual National Production Statistic	SEV
Cluster 1	Fruit Juice	Statistics South Africa (Stats SA, 2021)	NATSURV 14, Edition 2. 2021
	Fish Processing	StatsSA, 2023 (Ocean marine) fisheries	NATSURV 18, Edition 2 (2021)
	Poultry Abattoir		Local Journal Articles
	Red Meat Abattoir	Red Meat Abattoir Association	NATSURV 7, Edition 2 (2017)
Cluster 2	Dairy: Cheese, whey, butter	Milk South Africa	NATSURV 4, Edition 2 (2023)
Cluster 3	Wet corn mill	Ingrain Website	(Ndlovhu, 2013)
Cluster 4	Sugar mill	StatsSA, 2021; SASA	NATSURV 11, Edition 1 (2017)
Cluster 5	Carbonated Soft Drink	StatsSA, 2021	NATSURV 2, Edition 2 (2015)
	Clear Beer (Malt)	StatsSA, 2021	SABMiller Annual Report
	Sorghum Beer	StatsSA, 2021	NATSURV 5 Edition 2 (2016)
	Winery	SAWIS	(Mulidzi, 2001)
Cluster 6	Paper and Pulp	SAPPI and Mondi websites, NATSURV	NATSURV 12 Edition 2 (2017)

Limitations

The assessment was limited by its reliance on publicly available secondary data, which was sometimes outdated, incomplete, covered short time periods, was fragmented across many sources, and had inconsistent reporting formats. Access to facility-level and compliance documentation, such as discharge and trade effluent permits and monitoring reports, could have enhanced the assessment. However, confidentiality restrictions prevented access to these datasets.

3.4.2 Geo-spatial analysis

A Microsoft Excel database of large-scale agro-processing facilities in South Africa was compiled to support spatial analysis of wastewater-relevant industrial activity. The database contained georeferenced location data (latitude and longitude), province, sub-sector classification (e.g., wineries, fruit juice concentrate, dairy processing, abattoirs), production scale indicators, and wastewater treatment practices. Facilities were selected based on operational scale and market share to represent the majority of national processing capacity; for example, only the major clear-beer producers, AB InBev and Heineken, were included, as they account for over 90% of production.

The Excel database served as the primary platform for data collation, filtering, and aggregation, and was linked to a GIS environment to generate georeferenced facility distribution maps. Provincial facility accounts were computed through geo-referencing of agro-processing plants to administrative areas using GIS methods (de Smith, et al., 2019; Longley, et al., 2015). Facility density, normalised to provincial area, was obtained, and a clustering index based on a Location Quotient–type ratio of provincial to national mean density was applied to evaluate relative concentration (Miller & Blair, 2012; Isserman, 1977). Together, these metrics describe the spatial distribution, intensity, and clustering of agro-processing facilities (Duranton & Overman, 2005; Feser & Bergman, 2000).

3.4.3 Wastewater characterisation

A comprehensive literature review was conducted to collate data on physicochemical characteristics of agro-processing wastewater streams generated in South Africa. The review focused solely on South African datasets to ensure national representativeness. However, when local datasets were unavailable, values from the international literature were incorporated. Data were primarily obtained from the respective NATSURV reports for each sub-sector.

When data from the NATSURV reports were incomplete, outdated, or no NATSURV report was available, peer-reviewed journal publications and technical reports were used, identified using search tools such as Scopus, Web of Science, Science Direct, and Google Scholar. The search was limited to sources reporting on agro facilities located in South Africa. Only literature sources reporting measured composition characteristics of untreated agro-processing wastewater (pH, COD, BOD₅, TSS, TN, TP, etc) were included.

3.5 Results

3.5.1 Sub-sector National Wastewater Volumes

The total annual wastewater volume generated by the eleven agro-processing sub-sectors was estimated at approximately 130 000 ML yr⁻¹. **Figure 3.2** displays the annual wastewater volumes generated by the different agro-processing sub-sectors. The national wastewater generation profile shows that South Africa's agro-processing sector is strongly dominated by pulp and paper and sugar milling, both highly water-intensive industries.

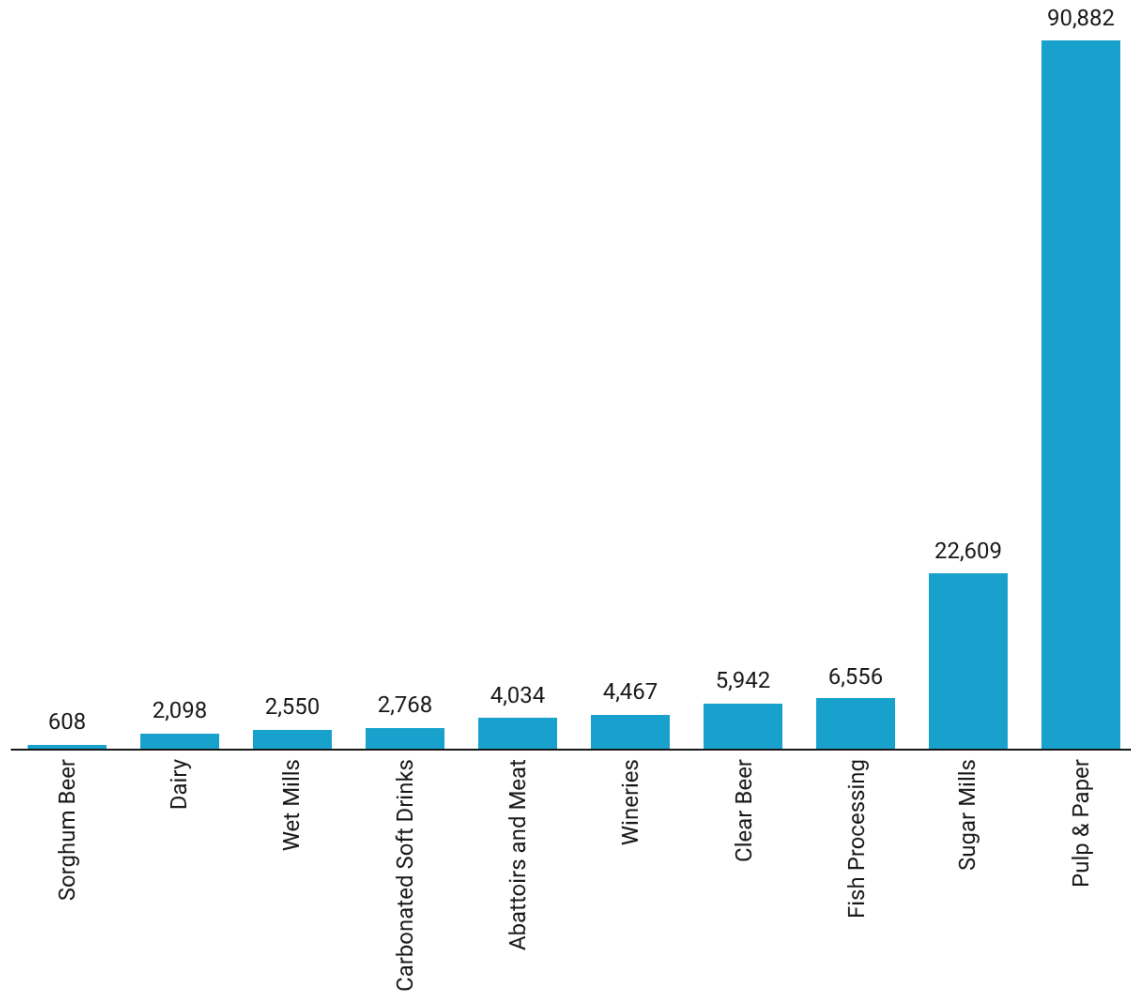


Figure 3.2 Estimated annual wastewater volumes generated by selected agro-processing industries in South Africa. Expressed in megalitres per year (ML yr⁻¹)

Pulp and paper mills are the dominant wastewater sources, contributing about 70% of the total ($\approx 90\,882$ ML yr⁻¹), largely from approximately ten major mills concentrated in KwaZulu-Natal. Sugar mills are the second-largest contributors, generating $\approx 22\,609$ ML yr⁻¹ ($\approx 17\%$). The remaining food and beverage sub-sectors individually produce $< 7\,000$ ML yr⁻¹, with fish processing ($\approx 6\,556$ ML yr⁻¹), clear beer ($\approx 5\,942$ ML yr⁻¹), and red-meat abattoirs ($\approx 4\,034$ ML yr⁻¹) being the next most significant. Beverage streams such as wineries, fruit juice, and sorghum beer generate comparatively modest volumes ($\approx 1\,000$ – $2\,000$ ML yr⁻¹), while carbonated soft drinks and dairy contribute only minor amounts, as reflected in Figure 3.3. The strong dominance and geographic clustering of pulp and paper and sugar processing indicate that these sectors should be prioritised for wastewater-to-energy or integrated biorefinery interventions to maximise national resource-recovery impact.

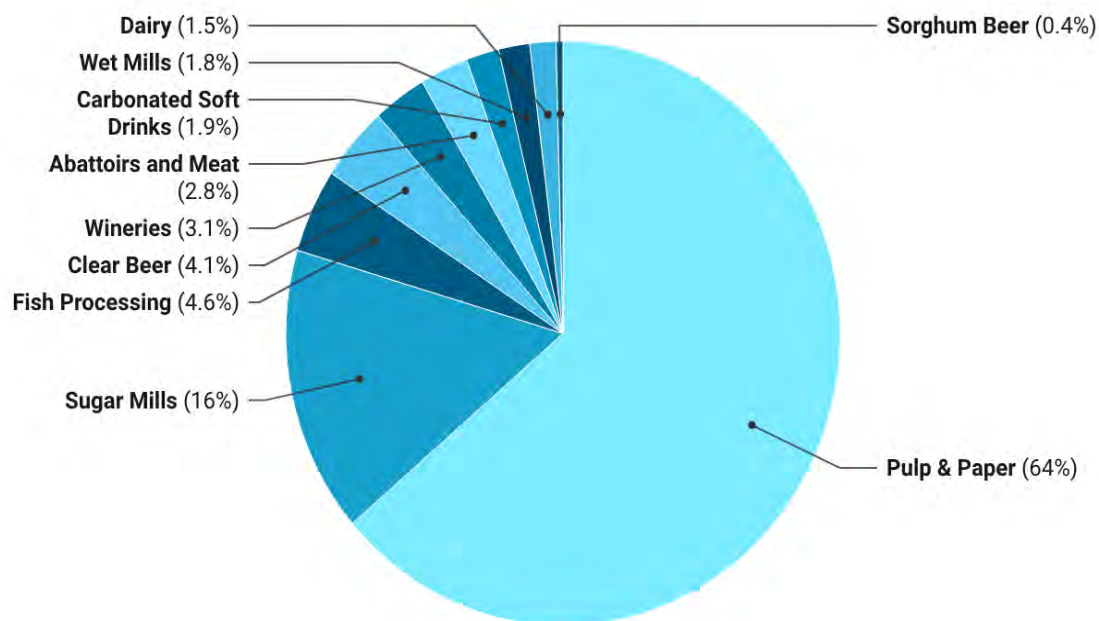


Figure 3.3 Sub-sectoral contribution to annual agro-processing wastewater generation in South Africa

Provincial wastewater generation

Figure 3.4 shows an uneven spatial distribution of agro-processing wastewater generation across South Africa, with KwaZulu-Natal (KZN) and Mpumalanga (MPU) accounting for the majority of national volumes. KZN, where most of the country's pulp-and-paper and sugar mills are located, accounts for the largest share ($\approx 90\,000\text{ ML yr}^{-1}$), driven by these highly water-intensive industries with substantial effluent loads. This aligns with national water-use assessments that identify the pulp-and-paper and sugar sectors among the largest industrial water users and wastewater generators in South Africa (Harding, et al., 2020; Cloete, et al., 2010; Van der Merwe, et al., 2009). MPU is the second-largest contributor ($\approx 24\,000\text{ ML yr}^{-1}$). It is also closely associated with pulp-and-paper and sugar-mill operations, consistent with the geographic clustering of forestry-based industries in eastern South Africa.

The concentration of wastewater within large single industrial sites in these sectors means that individual mills typically generate sufficient flow and organic loading to support mill-integrated anaerobic conversion or biorefinery systems, a configuration widely reported in the pulp and sugar industries globally (Harrison, et al., 2023; Haile, et al., 2021; de Jong & Jungmeier, 2015).

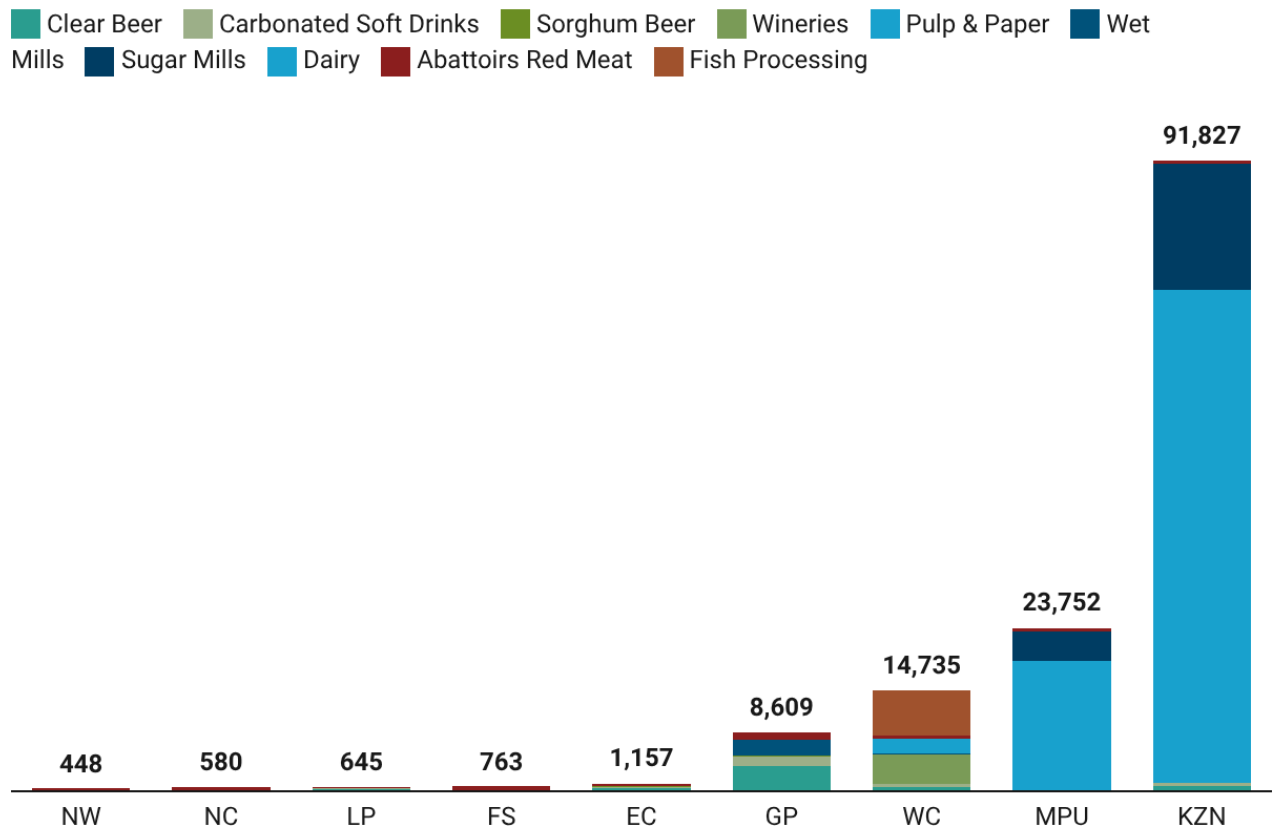


Figure 3.4 The wastewater generated by the different agro-processing sub-sectors per province. Expressed in megalitres per year (ML yr⁻¹)

Gauteng Province, the third-largest contributor, generates a much smaller volume ($\approx 7\,000\text{ ML yr}^{-1}$) but has a diversified range of agro-processing activities and high facility density, creating opportunities for co-digestion of heterogeneous feedstocks and shared treatment or valorisation infrastructure. Similar regional-aggregation opportunities have been noted in industrial symbiosis studies, where dense clusters of medium-scale agro-industries favour shared anaerobic digestion platforms. The Western Cape (WC) and Eastern Cape (EC) contribute comparatively modest volumes ($\approx 2\,000\text{--}3\,000\text{ ML yr}^{-1}$), mainly from fruit processing, wineries, and beverage industries aligned with their horticultural economies. In these regions, wastewater arises from numerous smaller and spatially dispersed facilities, limiting the feasibility of large single-site recovery plants. The remaining provinces (FS, LP, NC, NW) contribute minimal quantities ($<1\,000\text{ ML yr}^{-1}$ each), reflecting limited agro-processing concentration.

From a volumetric and spatial configuration perspective, the high wastewater generation in KZN and MPU indicates strong potential for mill-integrated wastewater valorisation and biorefinery deployment, given the availability of continuous, high-strength effluent streams from the pulp-and-paper and sugar industries. This supports broader findings that large, single-facility effluent sources favour economies of scale and stable operation in anaerobic conversion systems (Kumar, et al., 2025; Harrison, et al., 2023).

However, wastewater quality characteristics (e.g., COD fractionation, biodegradability, inhibitory compounds) remain critical in determining suitability for specific conversion pathways, as shown in comparative agro-processing effluent studies for bioenergy and biohydrogen production (Azbar, et al., 2009). In contrast, the smaller and more dispersed beverage and fruit-processing streams in provinces

such as the Western Cape are better suited to decentralised on-site systems at individual facilities or cluster-scale recovery systems serving groups of nearby plants, rather than a single large centralised biorefinery, consistent with distributed biorefinery concepts for regions with fragmented feedstock availability (Otto & Drechsel, 2018; IEA, 2015).

3.5.2 Geospatial analysis

There is a regional clustering of large-scale agro-processing facilities in South Africa, located in areas with high agricultural production and along processing corridors, as shown in Figure 3.5. Facilities are primarily concentrated in KwaZulu-Natal, Gauteng, Western Cape, and Mpumalanga. This shows the close relationship among agro-processing infrastructure, raw-material supply regions, and established logistics networks. This kind of spatial concentration is typical of industrial clusters, where firms benefit from being close to suppliers, infrastructure, and markets (Duranton & Overman, 2005; Feser & Bergman, 2000).

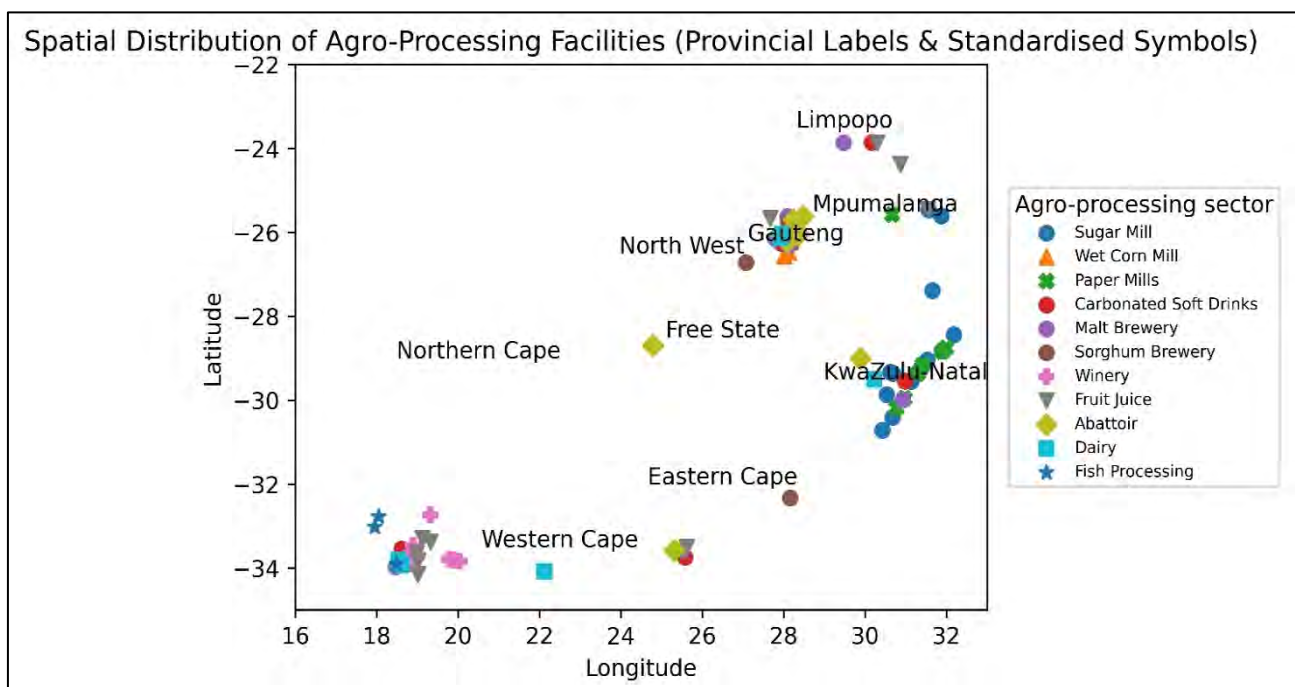


Figure 3.5 The geospatial distribution of agro-processing facilities across the different provinces in South Africa

Within these clusters, there is also notable sub-sector diversity. The Western Cape and Gauteng host the widest range of agro-processing activities, including wineries, fruit-processing plants, dairies, abattoirs, corn wet mills, and fish-processing facilities. In contrast, KwaZulu-Natal and Mpumalanga exhibit more specialised clusters dominated by sugar milling and pulp-and-paper industries linked to regional sugarcane and forestry production. Other provinces, including Eastern Cape, Free State, Limpopo, and North-West, show fewer facilities and lower sub-sectoral diversity.

The combination of facility clustering and sub-sector diversity provides an important indicator for identifying priority locations for industrial symbiosis and shared infrastructure. Industrial symbiosis works best when industries are close to each other because it makes it easier for facilities to exchange materials, energy, and waste streams (Kumar, et al., 2025; Harrison, et al., 2023). Consequently,

diverse clusters such as those in the Western Cape and Gauteng present strong opportunities for shared wastewater treatment, waste-to-energy systems, and biorefinery platforms, while specialised clusters in KwaZulu-Natal and Mpumalanga may support sector-based symbiotic networks centred on dominant industries. Spatial clustering analysis, therefore, helps identify optimal locations for circular resource recovery and shared industrial infrastructure (Lombardi & Laybourn, 2012; Feser & Bergman, 2000). To calculate facility density, the number of facilities in each province was normalised by provincial land area. The result was expressed as facilities per 100 000 km², enabling comparisons across provinces of varying geographic sizes. The clustering index was derived by dividing provincial facility density by the national mean facility density. This provided a relative measure of spatial concentration. Values greater than 1 indicate above-average clustering, while values below 1 indicate lower-than-average concentration. These metrics capture the absolute presence of facilities and the relative spatial intensity of agro-processing across the country. The large-scale agro-processing facility count, density, and clustering index across the different provinces is reflected in **Table 3.5**.

Table 3.5 The count, density and clustering index of large-scale agro-processing facilities across the different provinces

Province	Facility count (Np)	Facility density (per 100,000 km ²)	Clustering index
Eastern Cape	6	3.55	0.43
Free State	2	1.54	0.19
Gauteng	19	104.55	12.76
KwaZulu-Natal	32	33.93	4.14
Limpopo	4	3.18	0.39
Mpumalanga	8	10.46	1.28
North-West	2	1.91	0.23
Western Cape	27	20.86	2.55

The provincial facility distribution metrics show that there is a difference in how large-scale agro-processing facilities are concentrated in the different provinces across South Africa. The highest number of large-scale agro-processing facilities is found in KwaZulu-Natal (n = 32), followed by the Western Cape (n = 27) and Gauteng (n = 19). This indicates that agro-processing activity is concentrated primarily in these three provinces. However, when normalised for land area, Gauteng has the highest facility density (104.6 facilities per 100 000 km²) and the strongest clustering index (CI = 12.76). From this result, it is clear that Gauteng has a high concentration of agro-processing activity within a relatively small geographic footprint. KwaZulu-Natal also shows considerable clustering (CI = 4.14), while the Western Cape presents moderate but above-average concentration (CI = 2.55). Mpumalanga has moderate clustering (CI = 1.28), whereas Eastern Cape, Limpopo, Free State, and North -West remain below the national mean (CI < 1), which indicates that the latter provinces have dispersed processing activity.

3.5.3 Wastewater composition data

The compiled wastewater characterisation dataset for the agro-processing sectors is shown in **Table 3.6** and **Table 3.7**. The wide range of parameter values reported indicates differences in effluent quality within a sector and across sectors. Across the 12 sub-sectors, effluents can generally be classified as medium- to high-strength, organic-rich streams with acidic to near-neutral pH (≈ 3.6 – 8.2). This is typical of the food and beverage effluents, which are dominated by carbohydrate and protein waste streams. While the literature search was not exhaustive, the compiled dataset reveals gaps across all sectors, particularly in parameters critical to assessing the suitability of valorisation. The most reported variables across sectors are pH and total COD, followed by bulk solids indicators (TDS, TSS). These parameters allow only basic classification of wastewater strength. Key biodegradability and process-design metrics-

soluble COD fraction, volatile fatty acids (VFA), detailed nitrogen speciation (NH_4^+ -N, TKN), alkalinity, and nutrient data (TN, PO_4^{3-} -P) are rarely reported.

Table 3.6 Wastewater composition data for the mills and beverage sectors

Parameter N = sample size /data sets Reference	Unit	Pulp & paper Mills N=13		Sugar Mills N=5		Wet Corn Mills N=4		CSD N=7		Fruit Juice N=2		Malt Breweries N=5		Sorghum Breweries N=4		Wineries N=10	
		NATSURV		NATSURV		**Thesis		NATSURV		NATSURV		Local Journals		NATSURV			
		Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
pH	-	6.5-8.5	7.46	5.1-6.7	5.8	3.7-11.4	6.5	2.8-12.2	7.7	6.1-11	7.25	4.4-8.7	6.66	3.5-8.4	6.1	2.8-12.2	7.67
Temperature	°C											24-37	29.45	25-35	28		
COD (total)	mg/L	165-3853	1773	3400-6820	4796.8			752-29570	7442.4	176-17670	3652	457-11813	6321	1233-20000	5913.25	752-29570	7442.43
COD (soluble)	mg/L																
COD (particulate)	mg/L																
BOD ₅	mg/L											1609-3980	3026.4233		1629		
BOD/COD ratio				0.26-0.44	0.35	1157-455000	6846.25										
TS	mg/l											1289-14981	5688	560-8750	4500.5		
TDS				1008-1480	829.3	32-1611	384.3	9.9-3545	1494.5	610-18878	5050	2198-7400	3016	2020-5940	2482.5	9.9-3545	1494.50
TSS /solids	mg/L	38-2260	561	344-790	500.2					56-759	243	200-3728	1933.3333	2901-3000	1759.5		
VSS	mg/L											804-2572	1444.5				
PO ₄ ³⁻ -P	mg/L			0.8-5.9	3.35	0.1-46	43.35					229-424	179.17				
NH ₃ -N	mg/L											0.84-15.31	6.3775				
TKN	mg/L											6.24-94.7	29.3		59.7		
TN	mg/L											13.7-106	38.6				
Total Organic Nitrogen	mg/L											0-39.1	8.92	0.0196-0.0336	0.023		
Total Inorganic Nitrogen	mg/L					90-2903	726					7.78-93	34.4				
NO ₃ +NO ₂ -N	mg/L						7.2					2.87-49.4	10				
Conductivity	µS/cm	210-4970	2070									1893-6017	2613	35-165	86		
Turbidity	NTU											303-1039	570	82.5-5306.7	1393.92		
VFA	mg/L																
Fixed VS	mg/l					2128-21189	6363					825-4975	2327				

Table 3.7 Wastewater composition data for dairies, abattoirs, and fish processing sectors

Parameter N = sample size /data sets Reference	Unit	Poultry Abattoirs N=13		Red Meat Abattoirs N=5		Fish Processing N=4		Dairy N=7	
		Range	Average	Range	Average	Range	Average	Range	Average
pH	-	6-8.0		7.83				2.9-10.7 6.6	
Temperature	°C			21.03					
COD (total)	mg/L	1423 – 5280	4684.5	2784.95		60-3800		121-8631 3827.5	
COD (soluble)	mg/L								
COD (particulate)	mg/L								
BOD ₅	mg/L	850 – 6125	2635.4			52-4494	2200	55-5034 2224.8	
BOD/COD ratio									
TS	mg/l								
TDS		237-1740	1041.5						
TSS /solids	mg/L	60-8920	1583.2	1630.27				36-4953 1195.3	
VSS	mg/L								
PO ₄ ³⁻ -P	mg/L			94.58					
NH ₃ -N	mg/L	16-95	53			118.45			
TKN	mg/L								
TN	mg/L	62-313	176						
Total Organic Nitrogen	mg/L								
Total Inorganic Nitrogen	mg/L								
NO ₃ +NO ₂ -N	mg/L	124							
Conductivity	µS/cm	17.2-2450	957.5	321					
Turbidity	NTU	99-2405	957.5	1285.58		25-3000	311.6		
VFA	mg/L	71-838	390.75						
Fixed VS	mg/l								
Fats, Oil & Grease	ppm					0-17566	0.57		

Sectoral composition and comparative parameter analysis

A comparative analysis of wastewater composition was conducted using COD, TSS, pH, and conductivity because these were the only physicochemical parameters consistently reported across all sectors in the compiled dataset. The box-and-whisker plots in **Figure 3.6** illustrate compositional differentiation across agro-processing sectors. The COD distributions show that corn wet milling and malt brewery have the highest organic strength, with medians around 6,000–9,000 mg/ L⁻¹ and upper ranges exceeding 10,000–14,000 mg/ L⁻¹. This indicates that they are the strongest energy-bearing streams. Sorghum brewery and wineries have an intermediate COD range (~3,000–9,000 mg/ L⁻¹). Sugar and CSD cluster have lower ranges (~3,000–6,000 mg/ L⁻¹). Pulp and paper have the lowest COD levels (<3 000 mg L⁻¹).

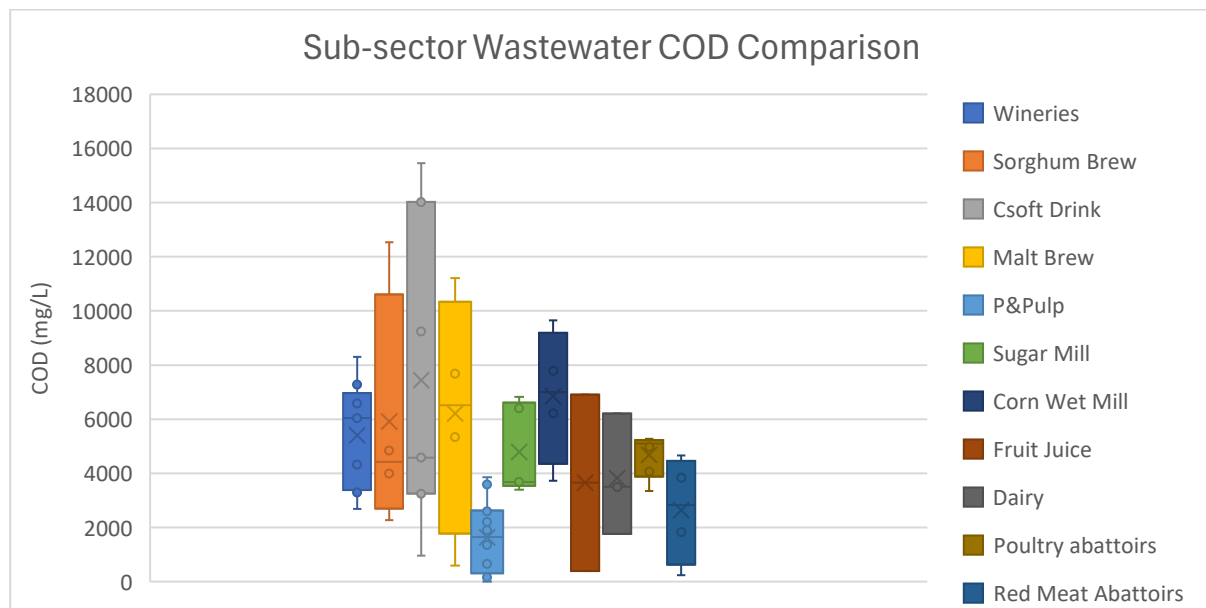


Figure 3.6 Comparison of COD values across the different agro-processing sectors

The TSS distributions in **Figure 3.7** differentiate between particulate-dominated and soluble wastewaters. Sorghum and malt brewery have the highest suspended solids (~800–3 500 mg L⁻¹) because of the cereal fibre and starch residues that require hydrolysis. Pulp and paper also show elevated TSS (~600–1 500 mg L⁻¹) due to fibre fines. On the other hand, corn wet milling and CSD have low TSS (<400 mg L⁻¹) which indicates that the substrates are predominantly soluble.

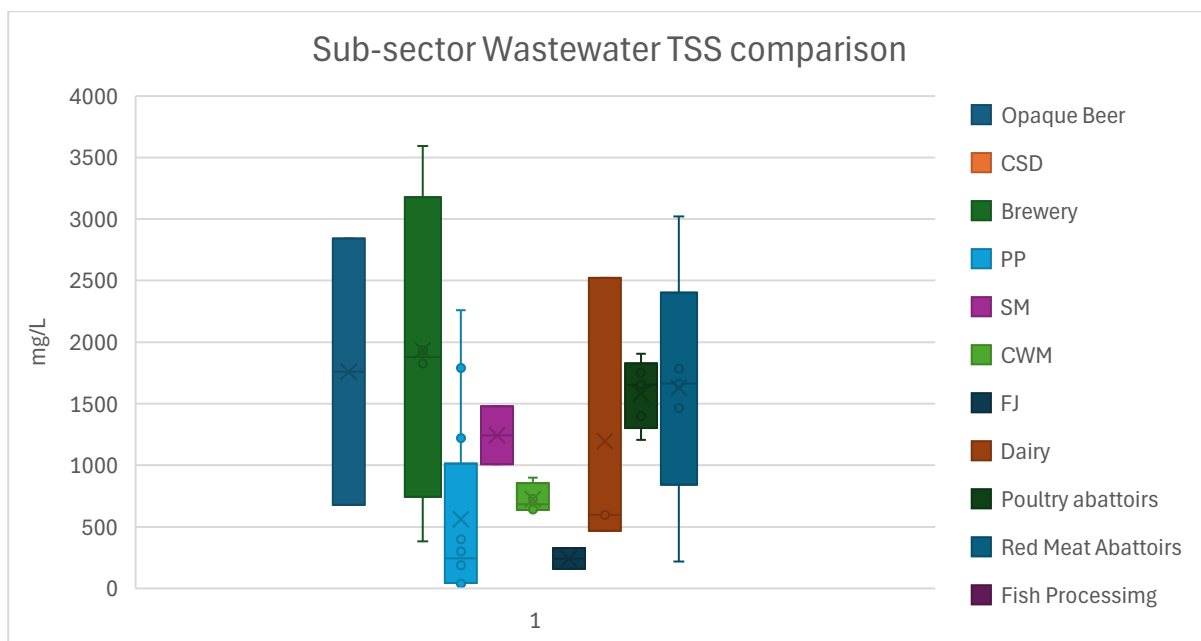


Figure 3.7 Comparison of TSS values across the different agro-processing sectors

The pH profiles in **Figure 3.8** indicate that most sectors fall within the biological tolerance range (~4–9). Wineries and sorghum show more acidic medians (~4.5–6.5) which is consistent with fermentation acids. Sugar effluent is mildly acidic (~5–6), while malt brewery and CSD cluster are near neutral (~6.5–8). Pulp and paper wastewater tend towards slight alkalinity (~7–9) due to pulping chemicals.

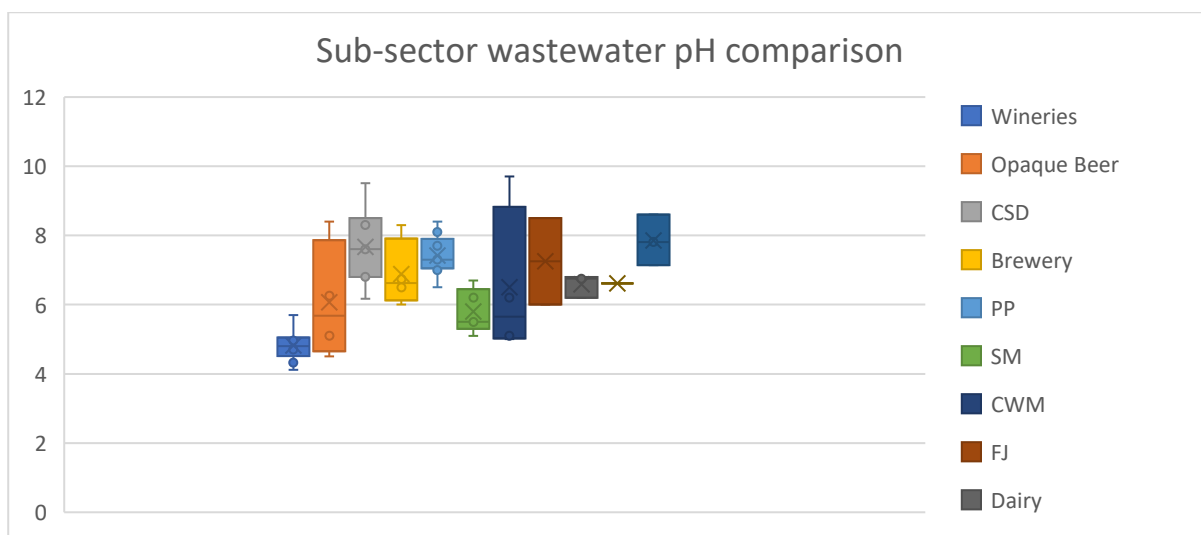


Figure 3.8 Comparison of pH values across the different agro-processing sectors

Conductivity patterns in **Figure 3.9** further differentiate ionic environments. Malt brewery and pulp exhibit the highest conductivity (~1.3–2.7 $\mu\text{S cm}^{-1}$), indicating substantial dissolved ions and favourable electrolyte conditions for bio-electrochemical systems. Corn wet milling shows moderate conductivity (~0.25–0.5 $\mu\text{S cm}^{-1}$), while wineries remain the lowest (<0.3 $\mu\text{S cm}^{-1}$).

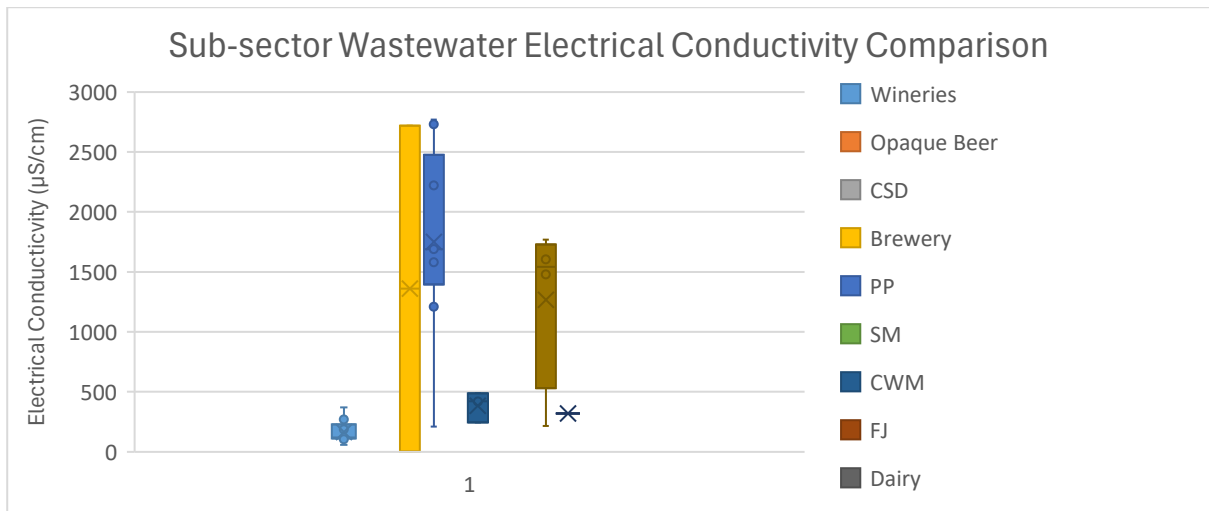


Figure 3.9 Comparison of EC values across the different agro-processing sectors

The agro-processing wastewater streams were further classified by their main organic fractions, based on compositional characteristics reported in the literature. Food and agro-processing effluents are often described as mixtures of carbohydrates (sugars, starch, pectin), proteins, lipids, or lignocellulosic fibres in proportions determined by raw materials and processing operations (Bustillo-Lecompte and Mehrvar, 2015; Carvalho et al., 2013). These biochemical constituents govern biodegradability, phase behaviour, and the potential for recovery as a resource, thereby defining the most suitable valorisation routes (Metclaf, et al. 2014). **Table 3.8** summarizes wastewater types, industry sources, and chemical conversion pathways for resource recovery.

Table 3.8 Classification of industrial wastewater by organic composition, representative sub-sectors, and potential valorisation pathways

Wastewater classification	Representative sub-sectors	Main organic components	Suitable conversion pathways	Product spectrum
Carbohydrate-rich	Sugar mills, breweries, fruit & vegetable processing	Glucose, sucrose, starch, cellulose, pectin	Fermentation; anaerobic digestion; bio-electrochemical systems; biochemical production	VFAs, ethanol, biogas, organic fertilizer
Protein-rich	Slaughterhouses; dairy processing; fish processing; rendering	Proteins, peptides, amino acids, casein, urea	Anaerobic digestion; protein hydrolysis/fermentation; ammonia recovery; co-digestion	Ammonia, VFAs, bioplastics (PHA/PHB), single-cell protein
Lactose-rich	Cheese; whey; milk; yoghurt; ice-cream processing	Lactose, whey proteins, fats	Fermentation (lactic/VFA); anaerobic digestion; membrane lactose recovery; BES	Lactic acid, ethanol, biogas
Organic-acid-rich	Fermentation industries; wineries; citrus processing; digestate streams	Acetic, lactic, citric and other organic acids	Chain elongation; anaerobic digestion; biochemical upgrading; PHA synthesis	Succinic acid, medium-chain acids, PHA, methane
FOG-rich	Dairy; meat processing; edible oil processing	Long-chain fatty acids, triglycerides, phospholipids	Lipid recovery; anaerobic digestion; biodiesel production; hydrothermal conversion	Biodiesel, long-chain fatty acids, biogas, biosurfactants

Wastewater streams from the beverage sectors and from sugar and corn milling were identified as the most suitable candidates for biohydrogen production based on their physicochemical characteristics and macronutrient classification. These streams have high concentrations of readily fermentable carbohydrates and relatively low protein and ammonia contents, conditions that favour stable hydrogenogenic fermentation and high hydrogen yields (Show, et al., 2011).

In contrast, pulp and paper effluents were excluded from further analysis due to their lignocellulosic and recalcitrant organic composition, which limits fermentability and typically requires intensive physicochemical or enzymatic pre-treatment prior to biological conversion (Pokhrel & Viraraghavan, 2004). Wastewaters from meat-processing sectors were also excluded because their high protein and ammonia content is associated with ammonia inhibition, pH imbalance, and instability in fermentative hydrogen production systems (Guo, et al., 2010; Chen, et al., 2008).

3.6 Rationale and Motivation for the Establishment of a National Database

The resource assessment that is presented in the preceding sections highlighted substantial challenges in accessing and compiling agro-processing wastewater data in South Africa. As discussed in Sections 3.2–3.4, the assessment relied heavily on fragmented secondary sources because facility-level discharge volumes and composition data were difficult to obtain, often confidential, incomplete, or reported in inconsistent formats. Several sub-sectors lacked recent production or wastewater coefficients, while available NATSURV and industry datasets were frequently outdated or based on limited voluntary participation. These data constraints introduced uncertainties in national volume estimates, limited sub-sector coverage, and created gaps in wastewater characterisation. Ultimately, this constrained the robustness and spatial completeness of the resource assessment.

These problems mirror a deeper systemic problem: South Africa's industrial wastewater information is still fragmented between several compliance-oriented platforms (e.g., NIWIS, WARMS, NCIMS, NATSURV) with limited information availability, inconsistent reporting standards, and no unified national repository. As a result, complete data that is available at the facility level, which connects wastewater generation, composition, treatment, and location, is not easily accessible for research or planning purposes. The data limitations encountered throughout Chapter 3 emphasise the need to establish a national agro-processing wastewater database to consolidate dispersed information into a standardized, regularly updated, and accessible evidence base. Such a database would address the access, completeness, and fragmentation limitations outlined in earlier sections, as well as enable a more reliable national assessment of wastewater resources and integrated circular-economy planning in South Africa's agro-processing sector.

3.7 Chapter Summary and Conclusion

This chapter presented a national-scale assessment of agro-processing wastewater resources in South Africa. The assessment quantified wastewater volumes, characterised physicochemical composition, and mapped the spatial distribution of major agro-processing facilities using secondary data. Results showed that wastewater generation is highly concentrated in pulp and paper and sugar milling, with strong geographic clustering in KwaZulu-Natal and Mpumalanga, where large single-site mills produce continuous high-volume effluents. In contrast, Gauteng and the Western Cape have more diversified but lower-volume and spatially dispersed food and beverage processing. These volumetric and spatial patterns indicate that facility-integrated recovery systems are most appropriate for high-volume processing industries. Decentralised or cluster-scale systems are better suited to dispersed beverage and food sectors. Wastewater characterisation confirmed that most agro-processing effluents are medium- to high-strength organic streams compatible with biological conversion. Carbohydrate-rich wastewaters from sugar, breweries, fruit processing, and corn milling were the most favourable candidates for fermentative biohydrogen production. Whereas pulp and paper and protein-rich meat and dairy streams are less suitable due to recalcitrance or ammonia inhibition.

However, major data gaps and fragmented, inaccessible datasets constrained the assessment. There is thus a need for a centralised national agro-processing wastewater database that integrates standardised facility-level volume, composition, and spatial information. Overall, Chapter 3 establishes the national wastewater resource baseline and identifies priority fermentable streams and regions for energy recovery. These quantified wastewater volumes and composition profiles provide the feedstock basis for Chapter 4, which conducts a theoretical assessment of biohydrogen production potential across South Africa's agro-processing subsector.

4 A THEORETICAL ASSESSMENT OF SOUTH AFRICA'S BIOHYDROGEN PRODUCTION FROM AGRO-PROCESSING WASTEWATER

4.1 South Africa's Green Hydrogen Landscape and Strategic Drivers

South Africa has set targets to develop a green hydrogen economy. The country's national development plan (NDP) and international sustainable development goals and environmental commitments guide these targets (DSI, 2021; DTIC, 2022). Furthermore, international goals and obligations are accelerating the transition to a global green hydrogen economy, with many developing countries, such as South Africa, developing green hydrogen roadmaps to attract investment and position themselves as key players in the global hydrogen market (IEA, 2023; IRENA, 2022).

The G20 Energy Transition Working Group, which promotes cooperation on hydrogen between developed and developing nations, is advocating for the development of global standards, certification schemes, and investment frameworks for green hydrogen under the G20 High-Level Voluntary Principles on Hydrogen (Olwagen, 2025). At a regional level, the Africa Green Hydrogen Alliance (AGHA) established in 2022 by South Africa, Egypt, Kenya, Namibia, Morocco, and Mauritania, aims to develop Africa's hydrogen economy and position Africa as a leading exporter of green hydrogen to Europe and Asia by supporting infrastructure investments, policy alignment, and capacity building for hydrogen development (AGHA, 2022).

In line with international trends, as detailed in the country's Hydrogen Society Roadmap (DSI, 2021), South Africa intends to develop a green hydrogen economy to support its decarbonisation goals and transition to a low-carbon economy, as well as to stimulate economic growth. The country aims to produce approximately 500 kilotons of green hydrogen per year by 2030 and to create 30,000 jobs annually by 2040 (DSI, 2021; DTIC, 2022).

Nationally, several initiatives underpin hydrogen development. For example, the Hydrogen South Africa (HySA) Programme was launched in 2008 under the National Hydrogen and Fuel Cell Technologies (HFCT) strategy. The programme supports research and innovation through three competency centres – HySA Infrastructure, HySA Catalyst, and HySA Systems (Bessarabov, et al., 2012). The South African government has set a target of 10 GW of electrolyser capacity in the Northern Cape region by 2030, increasing to 15 GW by 2040 (PRISM, 2024). Additionally, the Hydrogen Valley Feasibility Study, led by the Department of Science and Innovation in partnership with Anglo American Platinum, SANEDI, Bambili Energy, and ENGIE, identified a hydrogen development corridor extending from Mokopane in Limpopo through Gauteng to Durban. The corridor will link mining, industrial, and export hubs (Engie, 2021). The Northern Cape province also plans to install 10 gigawatts (GW) of electrolyser capacity by 2030 (Salma & Tsafos, 2022).

The use of green hydrogen in industry is also developing. Several major industrial companies in South Africa are implementing projects to integrate green hydrogen into their operations. For example, Sasol operates electrolysers powered by a 3MW solar farm at its Sasolburg facility with a green hydrogen production capacity of 150 kg/day (SASOL, 2023). On the other hand, Anglo American Platinum is developing the Mogalakwena Solar Power Station, which includes green hydrogen production to power the mine's rail system and, potentially, its transport trucks and buses (Anglo-American, 2022). These projects demonstrate an emerging domestic market for low-carbon hydrogen and industrial readiness to adopt hydrogen technologies. **Figure 4.1** maps other important green hydrogen initiatives at various stages of development across the country, including steel, synfuels, and ammonia production

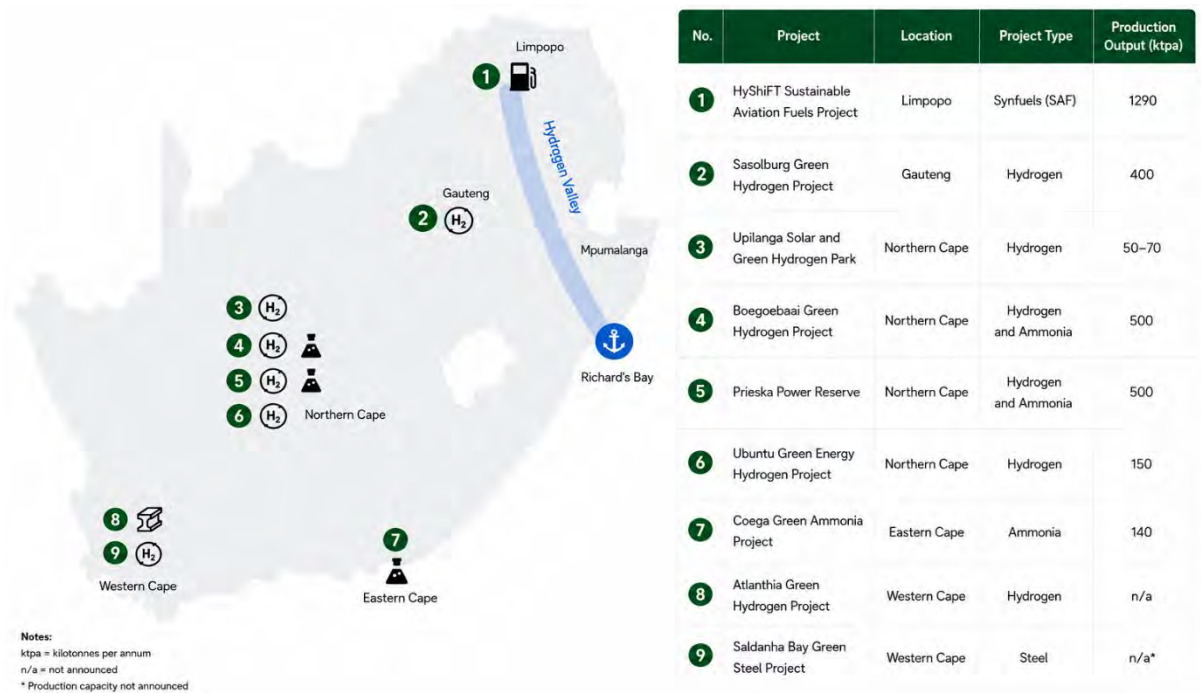


Figure 4.1 Key green hydrogen projects under development in South Africa (After Kalt, et al., 2023).

Even though the projects in **Figure 4.1** only record hydrogen produced through water electrolysis, they demonstrate an emerging market and an appetite among industries to adopt green hydrogen into their operations. Despite this momentum, current national strategies focus primarily on hydrogen produced through renewable energy-powered water electrolysis. Biological hydrogen, defined as hydrogen produced from biomass, including agro-processing wastewater (Sivaranjani, et al., 2023) remains unexplored within South Africa's renewable hydrogen development matrix.

4.2 Wastewater as a Feedstock for Biohydrogen Production in South Africa

Chapter 3 demonstrated that the South African agro-processing industry generates large volumes of organic-rich wastewater, spread across the country. Organic-rich wastewaters, particularly carbohydrate and sugar-rich wastewaters, are good candidates for hydrogen recovery because they have high concentrations of readily biodegradable compounds, which are represented by high COD levels (D'Silva, et al., 2023; Mutsvene, 2020). The assessment also identified industries in South Africa that generate carbohydrate and sugar-rich streams, such as corn wet mills, beverages, fruit processing, and sugar manufacturing, and earmarked them for hydrogen production.

Using carbohydrate-rich wastewater streams for hydrogen production supports South Africa's national priorities under the Green Hydrogen Commercialisation Strategy and the National Water Resource Strategy (NWRS-3). Additionally, coupling wastewater treatment with renewable energy generation aligns with South Africa's Circular Economy Science, Technology, and Innovation (STI) Strategy (DSTI, 2024). South Africa's dispersed agro-processing facilities offer a promising avenue for decentralised hydrogen production systems and could integrate with existing on-site or centralised wastewater treatment infrastructure, increasing resource efficiency and decreasing transport costs. The geo-mapping exercise revealed hot-spots, provinces where wastewater generation is high, linked to industries that are intensive water users, such as pulp and paper in KZN, or high population of diverse

agro-industrial activities, as in the case of Gauteng and Western Cape. Provinces such as KZN, Western Cape, and Gauteng, which have high cluster density, are ideal for establishing regional biohydrogen production hubs. The carbohydrate-rich composition and large volumes of South Africa's agro-processing effluents, therefore, position wastewater as a technically viable and locally available feedstock for biohydrogen production

4.3 Overview of biohydrogen production technologies

Biohydrogen production technologies can broadly be divided into biochemical and thermochemical conversion routes (Sharma, et al., 2023; Ahmed, et al., 2021), which can also be classified as high energy or low energy-intensive processes as shown in **Figure 4.2**.

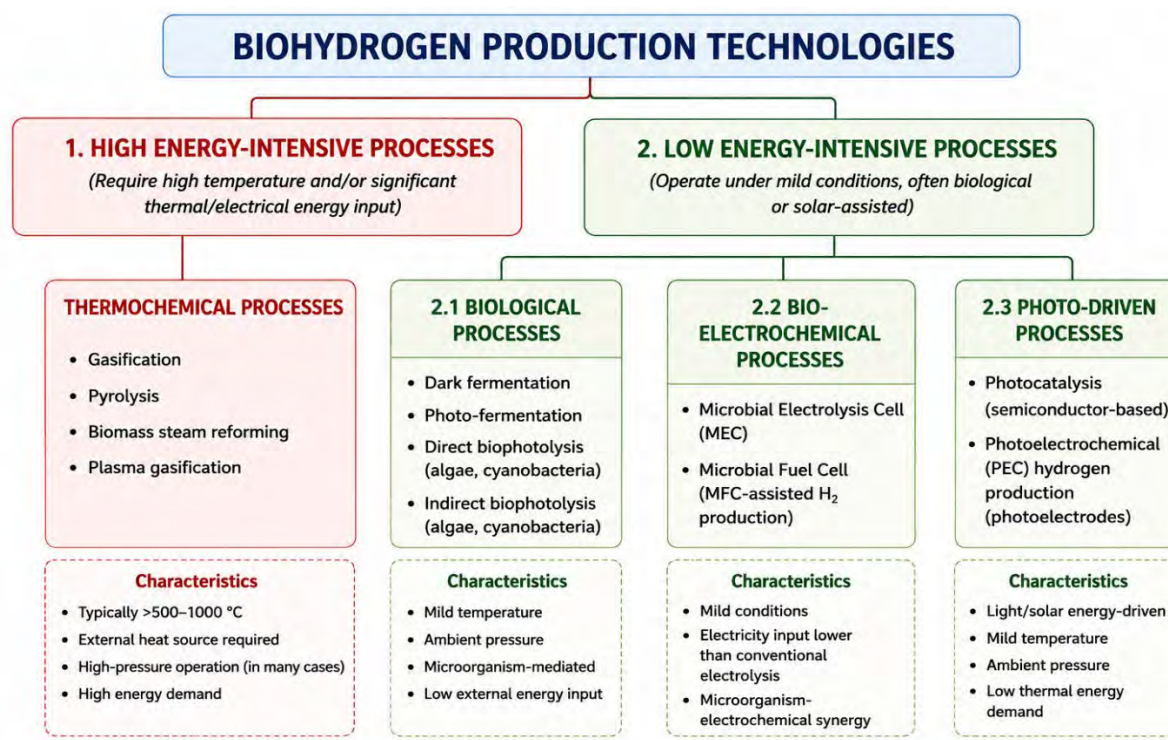


Figure 4.2 General classification of biohydrogen production technologies.

Thermochemical hydrogen production routes, such as gasification, pyrolysis, and biomass steam reforming, are classified as energy intensive. These routes require enormous amounts of external thermal energy, typically ranging above 200 MJ/kg of H₂ (Sharma, et al., 2023). They are more suited for dry biomass with low moisture content, such as lignocellulosic solids or sludge cake (Shahzad, et al., 2024; Islam & Nafees, 2025). In contrast, low-energy routes, such as biological and bio-electrochemical pathways, operate under mild conditions and are well-suited to wet substrates (e.g., wastewater) (Sharma, et al., 2023; Kumar & Fiori, 2024; Islam & Nafees, 2025). Photochemical hydrogen production routes use light and semiconductor catalysts to convert photon energy into chemical energy, producing hydrogen from water or biomass-derived organic compounds (Zheng, et al., 2025). Overall, low-energy biological and photochemical routes contrast with thermochemical processes by relying primarily on substrate-bound or photonic energy rather than large external thermal inputs, making them more compatible with dilute, aqueous waste streams (Sharma, et al., 2023).

Technologies for producing biohydrogen from wastewater primarily rely on biological routes that use microorganisms to generate hydrogen from wet biomass (Rosa e Silva, et al., 2024; Adekanbi, et al., 2023). These processes are more environmentally friendly and energy-efficient than thermochemical methods, but are constrained by low yields, production rates, and gas purity, which limit their large-scale application (Kumar & Fiori, 2024; Osman, et al., 2020). Continued research to enhance yields and reduce production costs is therefore critical to unlocking the hydrogen potential of wastewater streams. Current efforts focus on low-energy technologies that can be classified according to their dependence on light energy input (Islam, et al., 2021) as described in **Figure 4.3**.

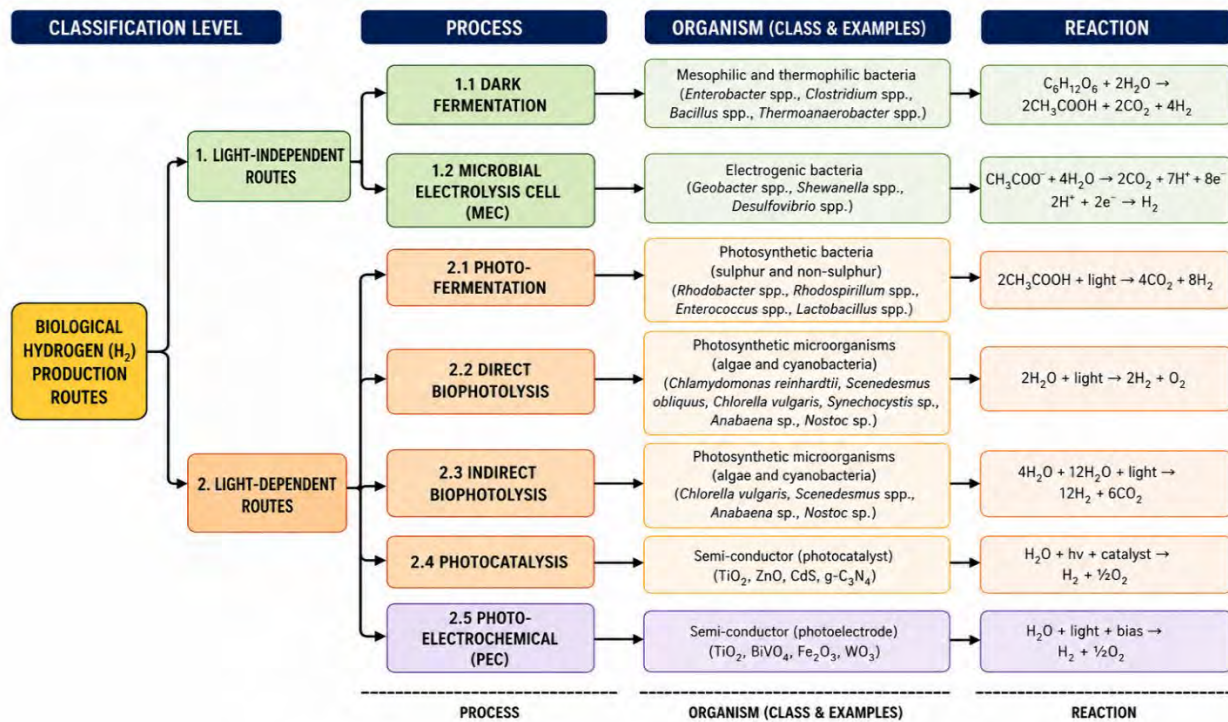


Figure 4.3 Classification of low-energy biological hydrogen production processes based on light dependency (Islam, et al., 2021)

4.3.1 Biohydrogen production technologies for wastewater valorisation

Light-independent routes

Light-independent processes cover biological pathways that use microorganisms to convert biomass into hydrogen under anaerobic conditions, without relying on light. These include dark-fermentation and microbial-electrolysis cells.

- **Dark fermentation**, also known as acidogenic fermentation, is an anaerobic fermentation process that uses anaerobic bacteria to break down organic compounds into hydrogen and other intermediates. It is a series of biochemical reactions conducted by various bacterial groups under anaerobic conditions, as shown in **Figure 4.4**. (Ahmed, et al., 2021). **Figure 4.5** shows the laboratory batch reactions used for dark fermentation. Dark fermentation occurs in the first three stages (hydrolysis, acidogenesis, and acetogenesis) of anaerobic digestion, whereas the complete anaerobic digestion process proceeds to the fourth stage to produce methane-rich biogas. In dark fermentation, the conversion of acidogenic fermentation products into biogas is prevented by inhibiting methanogenic activity through inoculum pre-treatment or altering process conditions (fourth stage) (Islam, et al., 2021).

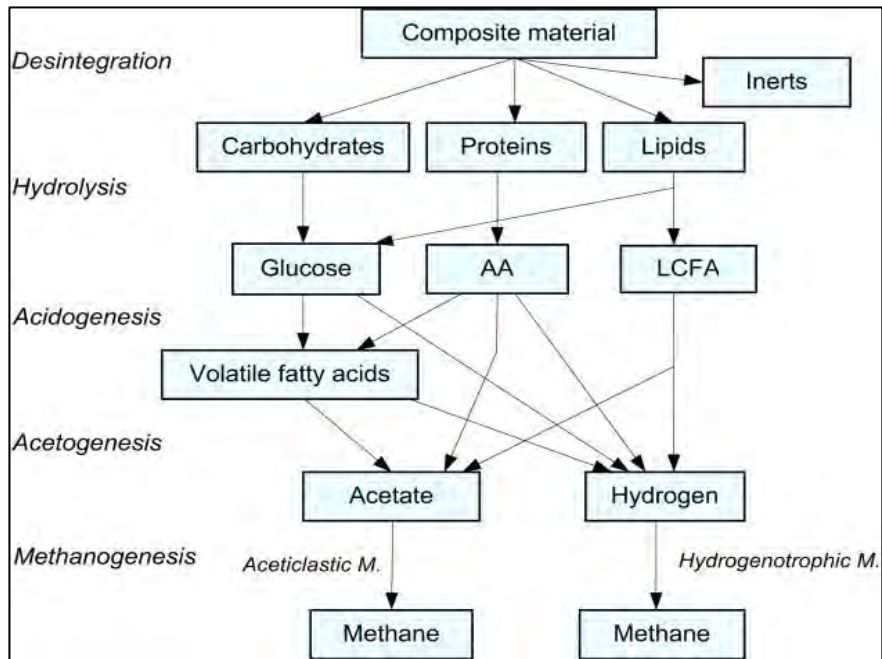


Figure 4.4 Anaerobic digestion process according to ADM1 model



Figure 4.5 Laboratory batch reactors for conducting dark fermentation tests

- **Microbial electrolysis** is a bio-electrochemical process that takes place in reactors known as microbial electrolysis cells, where exoelectrogenic bacteria oxidise organic substrates to release electrons and protons that are further reduced to form hydrogen gas. Like dark fermentation, it relies on microorganisms to break down organic matter under anaerobic conditions (Wrana, et al., 2010).

An MEC reactor typically consists of:

- 1) An anode and cathode electrode pair.
- 2) Electrogenic microbes that form a biofilm at the anode.
- 3) An external power source to drive the process.
- 4) In the case of dual chamber reactors, an ion membrane to separate the two chambers.
This will prevent the mixing of hydrogen and oxygen gases, thus improving hydrogen purity (Koul, et al., 2022).

Microbial electrolysis is best described by the chemical reactions that occur at the anode and cathode, as follows: at the anode, electrogenic microbes such as *Geobacter* and *Shewanella* species form a biofilm at the negative anode electrode; these microbes oxidise organic matter and release electrons and protons (Equation 7.1). The released electrons are transferred to the anode by exoelectrogenic bacteria through direct contact, nanowires or mediators. The electrons then flow through an external circuit to the cathode, energized by a small externally applied voltage (0.2-0.8 V) to overcome the thermodynamic barrier. The protons move through the liquid and or membrane to the cathode. At the cathode, the electrons and protons combine to produce hydrogen biomass (Rosa e Silva, et al., 2024; Adekanbi, et al., 2023; Islam, et al., 2021) . This process is depicted in **Figure 4.6**.

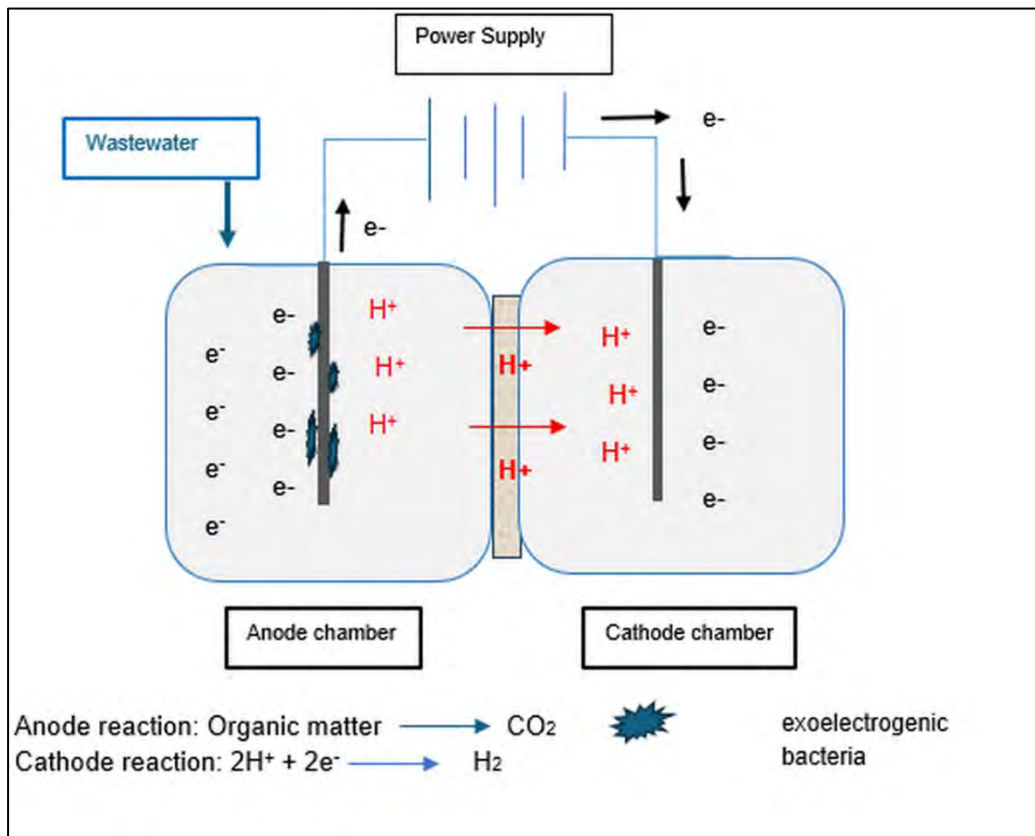


Figure 4.6 Schematics of MEC reaction processes

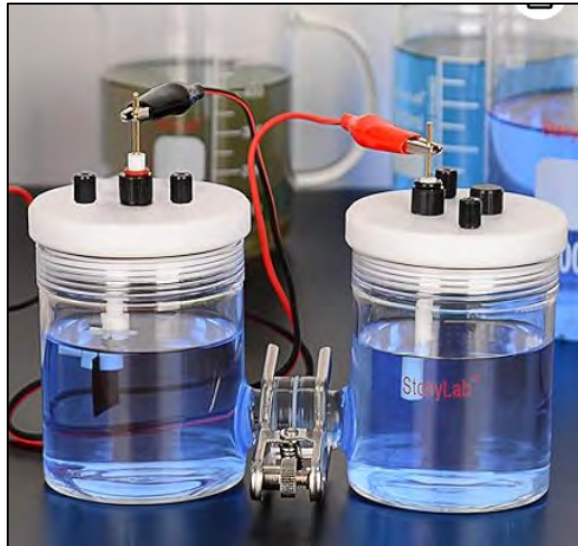


Figure 4.7 Laboratory-scale MEC batch reactors

Light-dependent biohydrogen production routes

Light-dependent processes that are used to produce biohydrogen from wastewater include photo-fermentation and other pathways that are still at early stages of investigation, including photocatalysis and microbial photoelectrochemical.

- Photo-fermentation is a biological process that uses purple non-sulphur bacteria (PNSB) and other photosynthetic bacteria, such as green sulphur bacteria and purple sulphur bacteria, to convert organic matter, mainly volatile fatty acids, into hydrogen (H_2) and carbon dioxide (CO_2) under anaerobic conditions using natural or artificial light as an energy source (Pandey, et al., 2023; Melitos, et al., 2021). **Figure 4.8** depicts the process. Photo-fermentation occurs in a nitrogen-deficient medium, where light energy reduces the nitrogenase in photosynthetic bacteria. The reduced nitrogenase then uses the hydrolysis of adenosine triphosphate (ATP) to reduce protons to biohydrogen. However, under nitrogen-rich conditions, the nitrogenase reduces nitrogen to ammonia instead (Song, et al., 2022).

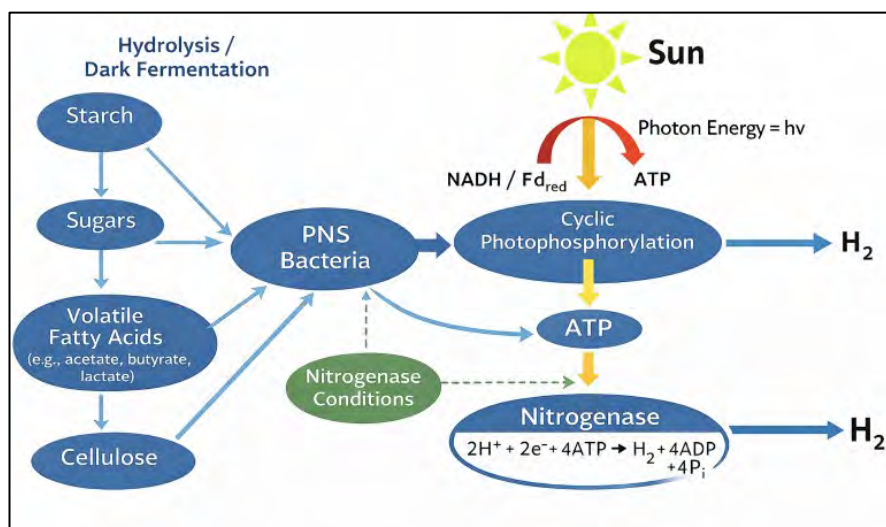


Figure 4.8 Mechanism for hydrogen production via photo-fermentation. Source: (Pandey, et al., 2023)



Figure 4.9 Laboratory setup for hydrogen production through photo-fermentation

- Photocatalysis is an emerging technology for biohydrogen production and wastewater treatment, currently in early stages of development. A light-driven process that uses solar or artificial light and semi-conductor photocatalysts to produce hydrogen from the breakdown of water or organic compounds (Islam, et al., 2021; Cabanillas, et al., 2021). **Figure 4.10** summarizes the step-by-step mechanism of hydrogen production via photocatalysis.

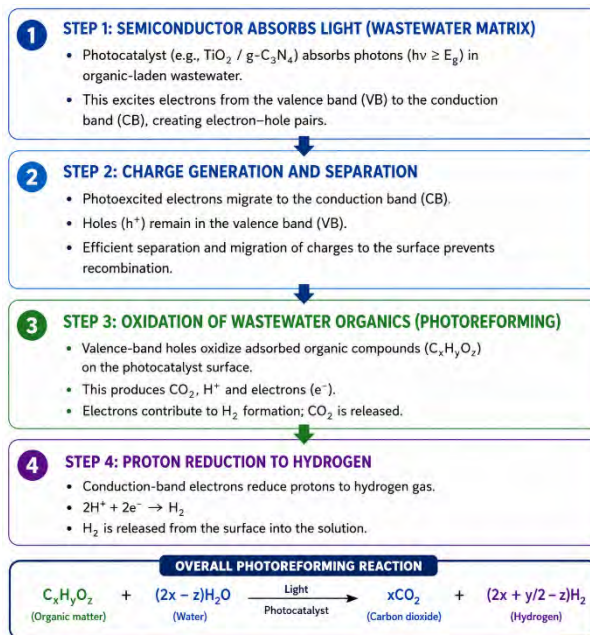


Figure 4.10 Photocatalysis mechanism for biohydrogen production from water

- Photoelectrochemical (PEC) methods combine photocatalysis and electrochemical reactions. PEC systems use semiconductor materials, such as titanium dioxide (TiO_2), as photocatalysts that absorb light and produce electron-hole pairs (Cabanillas, et al., 2021). At the photoanode, the positively charged holes oxidise organic matter in the wastewater, while the negatively charged electrons flow to the photocathode through an external circuit, where they reduce water molecules (H_2O) into hydrogen gas (H_2) and oxygen gas O_2 (Zheng, et al., 2025).

4.4 Comparative Assessment of Wastewater-Based Biohydrogen Production Technologies

A comparative analysis of the five technologies based on reviews by (Aydin, et al., 2021) (Ghasemi, et al., 2024) (Emetere, 2025) and (Liang & Shahabuddin, 2026) was conducted. The assessment evaluated hydrogen yields, unit production costs, carbon footprint, and the maturity of each technology.

The theoretical maximum hydrogen yield obtainable from any wastewater stream via any biohydrogen conversion process is roughly 1.4 L H₂/gCOD at standard conditions. This maximum yield is determined by the electron equivalence of chemical oxygen demand (COD) and is achieved only when complete electron recovery occurs (Logan, et al., 2008; Rabaey & Rozendal, 2010). Dark fermentation (DF) has the lowest hydrogen yield, ranging from 0.5 to 0.7 L H₂ per gram COD, because some electrons are consumed during the formation of by-products, including volatile fatty acids and ethanol (Lee, et al., 2022; Show, et al., 2011). In contrast, other conversion platforms, such as photofermentation (PF) and microbial electrolysis cells (MECs), can theoretically achieve close to the maximum yield, ranging between 1.2 and 1.4 L H₂ per gram of COD, as they can almost completely oxidize volatile fatty acids through nitrogenase-driven or electrochemical pathways (Hulsen, et al., 2016; Kadier, et al., 2016; Escapa, et al., 2016). Similarly, photocatalytic and photoelectrochemical (PEC) systems have a theoretical maximum hydrogen yield for treating organic substrates; their actual hydrogen yields are much lower due to factors such as low quantum efficiency and electron–hole recombination (Hisatomi, et al., 2014).

Unit production costs vary widely across the biohydrogen pathways under development owing to differences in system complexity, energy requirements, and maturity. With production costs reported at \$2.50–3.20 kg⁻¹ H₂, dark fermentation currently offers one of the most cost-competitive biohydrogen production pathways (Sharma, et al., 2020; Ngo, et al., 2020), owing to its simple reactor design, lack of external energy input, and ability to convert complex carbohydrate-rich wastewaters without pretreatment. Although the production costs for photo-fermentation (PF) are close to those of dark fermentation, at \$2.50–3.70 kg⁻¹ H₂, its economic viability is constrained by the need for an external light source, the requirement for specialised photobioreactors, and its applicability to volatile fatty acids.

Microbial electrolysis cells (MECs), on the other hand, display a wider range of unit production costs, reported at \$1.70–4.51 kg⁻¹ H₂ (Kadier, et al., 2016; Cusick, et al., 2010). The lower unit costs reflect optimised, integrated configurations, while the higher costs are due to the use of costlier electrode materials and the type of external power source. Among the biohydrogen production paths reviewed, photocatalytic systems are currently the most expensive route; unit production costs are generally above 3 USD (\$3.28–4.98 kg⁻¹ H₂). Higher production costs are linked to high semiconductor prices and low efficiency (Parthasarathy & Narayanan, 2014; Ratlamwala & Dincer, 2018). Production costs for photoelectrochemical (PEC) systems also vary widely, ranging from \$1.60 to \$10.40 kg⁻¹ H₂. For PEC systems, reactor design, semiconductor selection, and energy consumption strongly influence the final production cost (A. Grimm, et al., 2020; Bicer & Dincer, 2019; Pinaud, et al., 2013). Overall, dark fermentation and photo-fermentation are the least expensive, followed by MEC, then the more costly photocatalytic systems, with PEC showing the greatest variability.

Using the technology readiness level (TRL) scale to compare technology maturity shows that, currently, the most mature technologies are dark fermentation (DF) and photofermentation (PF), which are reported to be at TRL 4-5. Both DF and PF have progressed from proof-of-concept to preliminary stages of pilot-level demonstrations but have not fully exited the “valley of death” or “innovation gap.” (Vikash & Basak, 2026). The innovation gap, as described by Nwaka (2021), refers to the difficulty of advancing from TRL 4 to TRL 7 due to the high costs or lack of funding required to move from laboratory-scale to real-world demonstration.

Microbial electrolysis cells (MECs) are at a lower maturity level, TRL 3–4, constrained by scaling and operational limitations such as high electrode costs (Vikash & Basak, 2026). At TRL 3-4 (nearing TRL

5), photoelectrochemical (PEC) cells are at a similar level of development to MECs (Pitchaimuthu, et al., 2022). At the lower end are Photocatalytic (PC) systems, which have only reached TRL 2–3 and are at early catalyst studies (Merabet, et al., 2024; Alqahtani, 2024). In summary, biological pathways are closer to implementation than electrochemical or solar-driven pathways.

A review paper by Aydin et al. (2021), reports that Microbial Electrolysis Cells (MEC) have the lowest emissions ($\approx 0.17\text{--}0.22$ kg CO₂e/kg H₂ but can exceed 2.17 kg CO₂e/kg H₂ depending on the energy supply (Chen, et al., 2019; Shemfe, et al., 2018). Slightly higher emissions are reported for photocatalytic and photoelectrochemical pathways, ranging from 0.48 to 0.53 kg CO₂e/kg H₂ (Acar & Dincer, 2015; Muñoz, et al., 2005), followed by photofermentation ($\approx 0.55\text{--}0.80$ kg CO₂e/kg H₂) (Ngo, et al., 2020). Dark fermentation has the highest emission values, ranging from 0.70 to 1.11 kg CO₂e/kg H₂, linked to carbon losses during metabolic conversion (Ortigueira, et al., 2020; Ngo, et al., 2020). Overall, MEC has the best environmental performance, while DF has the lowest environmental efficiency, yet is technically a more mature system.

Dark fermentation (DF) converts raw carbohydrate-rich agro-processing wastewaters, such as sugar, beverage, and starch effluents, directly into hydrogen without the need for expensive upstream pretreatment. Hydrolysis, the first step of dark fermentation, generates soluble monomers that are primarily converted, via acidogenesis, into short-chain fatty acid intermediates such as acetate, propionate, and butyrate, also known as volatile fatty acids (Aydin, et al., 2021; Osman, et al., 2020). These first two critical steps of DF eliminate the need for feedstock pretreatment and directly influence hydrogen yield.

In contrast, Microbial electrolysis cell (MEC) systems produce hydrogen by oxidizing low-molecular-weight VFAs, particularly acetate. Consequently, treatment of carbohydrate-rich wastewaters via MEC systems requires prior fermentation or substrate conversion to provide suitable substrates for MECs. Like MEC, photo-fermentation processes also depend on VFAs, such as acetate and butyrate, requiring clarified, low-turbidity substrates to achieve effective light penetration and optimal photobioreactor performance. Photocatalytic and photoelectrochemical hydrogen production routes are also not suited for the direct conversion of carbohydrate-rich wastewaters into hydrogen (Chen et al. 2023). Accordingly, DF was selected as the primary conversion route. **Table 4.1** summarises the comparative analysis of the different biohydrogen production technologies.

Table 4.1 Comparison of different hydrogen production technologies

Parameter	Dark Fermentation (DF)	Photofermentation (PF)	Microbial Electrolysis Cells (MEC)	Photocatalytic (PC)	Photoelectrochemical (PEC)	Ref.
Hydrogen Yield (L H₂/gCOD)	0.5 – 0.7	1.2 – 1.4	1.2 – 1.4	Theoretical high, actual low	Theoretical high, actual low	[1–6]
Key Yield Limitation	Electron loss to by-products	Light dependency, substrate limits	External voltage, electrode losses	Low quantum efficiency	Semiconductor losses	[2,3,4,6]
Production Cost (USD/kg H₂)	2.50 – 3.20	2.50 – 3.70	1.70 – 4.51	3.28 – 4.98	1.60 – 10.40	[9–16]
Cost Drivers	Simple reactors, no energy input	Photobioreactors, light	Electrodes, electricity	Semiconductor cost	Reactor + semiconductor design	[9–13,14]
Technology Readiness Level (TRL)	4 – 5	4 – 5	3 – 4	2 – 3	3 – 4	[17–20]
Maturity Status	Pilot-stage	Pilot-stage	Lab, early pilot	Early research	Lab, early pilot	[17]
Emissions (kg CO₂e/kg H₂)	0.70 – 1.11	0.55 – 0.80	0.17 – 0.22 (up to 2.17)	0.48 – 0.53	0.48 – 0.53	[21–27]
Feedstock Suitability	Direct carbohydrate wastewater	Solids-free, VFA-rich effluent (post-dark fermentation clarification)	VFA-rich DF effluent	Dilute refractory organics	Dilute organics + water	[21,28–3]

1. Lee et al., 2022
 2. Show et al. 2011
 3. Hulsen et al.2016
 4. Kadir et al. 2016
 5. Escapa et al.2016

6.Hisatomi et al.2016
 7. Logan et al,2008
 8. Rabaey and Rozenda, 2010
 9.Sharma et al,2020
 10.Ngo et al.2020

11.Cusick et al.2010
 12. Parthasarathy and Narayanan
 13.Ratlamwala and Dincer,2018
 14. Grimm et al.2020
 15.Bicer and Dincer

16. Pinaud et al,2013
 17. Vikash and Basak, 2026
 18. Merabet et al.2013
 19.Alqahtani,2024
 20. Pitchaimuthu et al.2022

21. Aydin et al.2021
 22. Chen et al.2019
 23. Shmfie et al.2018
 24. Acar and Dincer,2015
 25. Munoz et al.2005

26.Ortiqueira et al.2020
 27. Ngo et al.2020
 28.Osman et al.2020
 29.Hulsen et al.2016
 30.Chen et al.2023

4.5 Theoretical Biohydrogen Potential from South African Agro-processing Wastewater

Chapter 3 quantified the national agro-processing wastewater resource in terms of both volumetric availability and physicochemical composition, thereby establishing the recoverable organic substrate base expressed as COD. Following the comparative evaluation of available hydrogen production technologies, dark fermentation (DF) was identified as the most suitable primary conversion pathway due to its compatibility with carbohydrate-rich, high-COD wastewaters and its comparatively advanced technology readiness. The subsequent step is therefore to translate the quantified wastewater resource into a theoretical hydrogen production potential. Section 4.5 thus presents a theoretical assessment of biohydrogen production via dark fermentation, providing an upper-bound estimate of its potential contribution to the country's overall energy supply.

Equations for estimating theoretical hydrogen production

A stepwise approach was adopted to estimate the theoretical hydrogen production. Firstly, the total organic load generated by each sub-sector (COD_{TOTAL}), was quantified from the annual wastewater volumes and composition characteristics compiled in Chapter 3. The total organic load generated by each agro-processing sub-sector was calculated as follows:

$$COD_{TOTAL} = V \times C_{COD}$$

Where

V = is annual wastewater volume (ML/ annum),

C_{COD} = the wastewater stream COD concentration (mg/l).

The theoretical hydrogen potential ($H_{2,POT}$) in cubic meters for each sub-sector wastewater stream was then determined using:

$$H_{2,POT} = COD_{TOTAL} \times \eta_{DF} \times Y_{H_2}$$

Where η_{DF} is dark fermentation COD removal efficiency (~ 20 -40%), and Y_{H_2} = hydrogen yield per unit COD (m^3/ kg COD). Reported dark fermentation yields typically range between **0.35 to 0.9 L H₂ g⁻¹ COD removed**, depending on substrate composition and process conditions (Show et al. 2012; Guo et al. 2010),

The energy equivalent was calculated using the LHV for hydrogen (3kWh/ m³ H₂) as follows:

$$E_{H_2} = \sum_i^N H_{2,POT} \times HHV_{H_2}$$

Table 4.2 summarises the hydrogen yield for seven wastewater streams.

Table 4.2 Hydrogen yield data for the 7 selected wastewater streams selected for their dark fermentation suitability (corn mill, sugar mill, carbonated soft drinks, fruit juice, malt beer, sorghum beer, winery) were obtained from literature.

Sector	Substrate	Temp (°C)	Mode	Yield Reported	Standardized Yield (mL H ₂ / g COD _{added})	Reference
Corn wet mill	Starch WW	55	Batch	92 mL/ g starch _{added}	78	(Zhang, et al., 2003)
	Starch WW	30	Continuous	67 mL H ₂ /g COD _{removed}	40.2 (η =60%)	(Nasr, et al., 2015)
	Starch WW	30	Continuous	105 mL H ₂ /g COD _{removed}	63 (η =60%)	(Nasr, et al., 2015)
	Starch WW	37	Batch	186 mLH ₂ /g starch _{added}	158	(Wei, et al., 2010)
Sugar mill	Starch WW	35	Batch	194 mL H ₂ /g starch _{added}	165	(Liu & Shen, 2004)
	Molasses wastewater	35	Batch	200 mL H ₂ /gCOD	200	(Ren, et al., 2018)
	Molasses wastewater	30	Continuous	0.6 mol H ₂ /molhexose	70	(Ferreira, et al., 2020)
Sugar mill	Molasses wastewater	55	Continuous	0.52 H ₂ /mol hexose	61	(Ferreira, et al., 2020)
Malt brewery	Brewery WW	36	Batch	1.67 mol H ₂ / mol glucose	195	(Estevam, et al., 2018)
Malt brewery	Brewery WW	35	Batch	169 mL H ₂ / gCOD	169	(Garduno-Ibarra, et al., 2025)
	Brewery WW	35	Batch	117 mL H ₂ / gCOD	117	(Garduno-Ibarra, et al., 2025)
Malt brewery	Brewery	35.9	Batch	147 ml H ₂ /g COD	147	(Shi, et al., 2010)
Malt brewery	Brewery	37	Batch	1.5 mol H ₂ /mol fructose	175	(Pachiega, et al., 2019)
CSD		--	Continuous	5.87 mol H ₂ /mol substrate		(Menezes, et al., 2019)
Fruit juice	Citrus wastewater	37	Batch	85.3 mmol H ₂ /L		(Torquato, et al., 2017)
Fruit juice	Apple wastewater	--	Batch	0.100 L H ₂ /gCOD	100	(Van Ginkel, et al., 2005)
Winery	Winery wastewater	37	CSTR	2.75 mL H ₂ / COD	2.75	(Mejia-Saucedo, et al., 2022)

All calculations were performed in an Excel spreadsheet, as shown in the Excel screenshot in **Figure 4.11**.

1. Annual Wastewater Generaion (Graph , Chapter 3)	Carbonated Soft Drink	Fruit Juice	Malt Brewery	Sorghum Brewery
Annual Wastewater Generation 2021 **(ML)	750.00	1364	5197	1064
Annual wastewater generation L	750 000 000.00	1 364 000 000.00	5 197 000 000.00	1 064 000 000.00
Annual wastewater generation billion L	0.75			
2. Wastewater composition (Table 5, Chapter 3)	Carbonated Soft Drink	Fruit Juice	Malt Brewery	Sorghum Brewery
COD concentration range (mg/L)	752-29570	176-17670	457-11813	1233-20000
COD concentration average (mg/L)	7442.4	3652	6321	5913.25
COD conc (g/L)	7.4424	3.652	6.321	5.91325
Annual COD production (g): COD conc x Annual wastew	5 581 800 000.00	4 981 328 000.00	32 850 237 000.00	6 291 698 000.00
3. Hydrogen gas production	Carbonated Soft Drink	Fruit Juice	Malt Brewery	Sorghum Brewery
Hydrogen yield (ml H ₂ /gCOD) (Table , Chapter 4)	120	133	80	80
Annual COD generated (g/annum)	5581800000	4981328000	32850237000	6291698000
Hydrogen produced (ml H ₂ /annum) η=0.35	234 435 600 000.00	231 880 818 400.00	919 806 636 000.00	176 167 544 000.00
Hydrogen produced (m ³ /annum)	234 435.60	231 880.82	919 806.64	176 167.54
Hydrogen produced (thousand m ³ /annum)	234.44	231.88	919.81	176.17
Energy recovery @ /3kWh/m ³ (GWh)	0.70	0.70	2.76	0.53

Figure 4.11 Screenshot of the Excel spreadsheet used for the calculations.

4.6 Results

4.6.1 Hydrogen Production Potential

Figure 4.12 shows that the estimated annual hydrogen production potential from agro-processing wastewater is approximately 78.4 million m³ per annum, equivalent to about 7,000 tonnes of hydrogen per year and roughly 235 GWh of energy on a lower heating value basis. In the context of South Africa's energy system, annual electricity generation is on the order of 230–250 TWh per year, making the wastewater hydrogen equivalent to less than 0.1% of national electricity production. Under the national Hydrogen Society Roadmap (DSI, 2021), South Africa's medium-term green hydrogen ambitions include targets such as 500,000 tonnes per year of production by 2030, supported by gigawatt-scale electrolysis deployment and potentially much larger scale up to several million tonnes per annum by mid-century. When compared to these targets, the 78.4 million m³ (≈7 kt) of hydrogen from wastewater represents only a fraction of a percent of the envisaged national green hydrogen output, underscoring that wastewater-derived hydrogen is relatively small in magnitude. Looking at the different sectors, the graph highlights that sugar mills contribute approximately 96% of the wastewater-derived hydrogen potential, with much smaller contributions from corn wet milling and beverage sectors. The highly concentrated distribution suggests that wastewater-derived hydrogen is most relevant as a site-level decarbonisation opportunity and circular resource utilisation pathway rather than as a core component of national green hydrogen supply or export-focused targets.



Figure 4.12 The estimated annual hydrogen production potential from agro processing wastewater (thousand m³/annum)

The baseline assessment indicates that hydrogen production from agro-processing wastewater (~7 kt H₂ per annum nationally) is modest in scale and highly concentrated within the sugar sector. Expansion through inclusion of additional wastewater streams is unlikely to materially increase overall hydrogen output. In particular, while pulp and paper mills generate large, continuous wastewater volumes and operate year-round, the largely lignocellulosic and recalcitrant nature of these effluents limits the availability of fermentable substrate. Even with pre-treatment, their inclusion would not be expected to significantly alter national hydrogen magnitude, though such facilities may offer stable industrial platforms for pilot deployment.

More substantive improvements in viability may arise from valorisation of fermentation-derived volatile fatty acids (VFAs). Dark fermentation typically leaves a considerable fraction of COD as intermediate acids. Integration of secondary conversion pathways, such as microbial electrolysis cells (MECs), could increase overall hydrogen recovery per unit COD. Alternatively, selective recovery and upgrading of VFAs as chemical intermediates may provide greater economic value than maximising hydrogen production alone, improving overall project economics.

The most meaningful scale-up opportunity lies in integrating solid agro-processing residues (e.g., bagasse, press mud, spent grain, and fibre residues). These materials generally contain higher energy density than wastewater streams and could be incorporated into hybrid biological–thermochemical systems. While existing uses, such as cogeneration, must be considered, integrated solid–liquid biorefinery configurations have the potential to materially increase hydrogen production beyond wastewater-only estimates. Overall, future development pathways should focus on resource integration and value optimisation rather than expansion of wastewater volumes alone.

4.7 Conclusion

Chapter 4 evaluated wastewater-based biohydrogen production technologies through a structured comparative assessment using unit production cost ($\$ \text{kg}^{-1} \text{H}_2$), technology readiness level (TRL), energy intensity ($\text{kWh kg}^{-1} \text{H}_2$), hydrogen yield ($\text{L H}_2 \text{gCOD}^{-1}$), and carbon footprint as key decision criteria. This multi-criteria analysis identified dark fermentation (DF) as the most suitable primary conversion technology for South African agro-processing wastewater due to its compatibility with high-COD, carbohydrate-rich streams, relatively low external energy requirement, moderate cost range, and comparatively advanced TRL relative to other biological and photo-driven routes. The subsequent theoretical resource evaluation translated the quantified national wastewater COD base into an estimated hydrogen potential of approximately 78.4 million $\text{m}^3 \text{H}_2$ per annum ($\approx 7 \text{ kt H}_2$; $\approx 235 \text{ GWh LHV}$). Although technically feasible, this magnitude constitutes only a small fraction of national green hydrogen ambitions, positioning wastewater-derived hydrogen as a decentralised circular resource recovery pathway rather than a central contributor to export-scale hydrogen production.

Nevertheless, there is clear scope to enhance viability through multi-feedstock, integrated biorefinery configurations capable of producing multiple value streams in addition to hydrogen. Cascaded systems such as DF coupled with microbial electrolysis cells (DF–MEC) offer the potential to improve overall electron recovery, increase COD removal, and maximise energy extraction from wastewater substrates. However, progression toward conceptualisation and design of integrated dark fermentation biorefineries requires robust foundational data generated under locally relevant conditions. Basic laboratory research remains necessary to characterise process kinetics, establish achievable yields, evaluate COD conversion efficiencies, and develop optimisation strategies prior to pilot-scale implementation. Accordingly, Chapter 5 presents laboratory-scale biohydrogen experiments demonstrating hydrogen production from two selected wastewater streams and systematically evaluating the influence of key process parameters on hydrogen yield and process performance.

5 DEMONSTRATING LABORATORY BIOHYDROGEN PRODUCTION USING SA'S AGRO-INDUSTRIAL WASTEWATER

In Chapter 4, dark fermentation was selected as the most promising route for converting carbohydrate-rich agro-industrial wastewaters into biohydrogen. Additionally, Chapter 4 estimated the theoretical biohydrogen volume that can be generated from carbohydrate-rich wastewater, including corn and sugar mills, and beverage wastewaters, namely, carbonated soft drinks, fruit juice, malt beer, sorghum beer, and wine. Chapter 5 demonstrates the production of biohydrogen via dark fermentation using 5 agro-industrial wastewater streams. All experiments were performed at the Agricultural Research Council's (ARC) Sustainable Energy and Bioeconomy Laboratory, in Pretoria, South Africa

5.1 Materials and Methods

5.1.1 Substrates used

Five wastewater samples were used: two real wastewater streams and three synthetic ones. Real wastewater samples were difficult to collect from agro-processing facilities because most facilities were unwilling to have their wastewater sampled. Long-term sample storage was also challenging, resulting in some collected wastewater samples being discarded. As a result, only 2 real wastewater samples were used. The real wastewater samples were collected from a sugar mill and a fruit juice concentrate plant. Sampling was conducted using clean, labelled containers to prevent contamination. The containers were tightly sealed immediately after collection and transported to the laboratory, where they were stored at 4 °C until use.



Figure 5.1 Sugar mill and fruit juice wastewater used for the experiments

Beverage synthetic wastewaters, including carbonated soft drinks, malt beer, and white wine, were prepared by diluting store-bought products with distilled water. The synthetic wastewater was prepared by a dilution factor of 1:10. The carbonated soft drink, white wine, and beer malt were purchased from

the store (Figure 1). For each wastewater stream, 1 L of synthetic wastewater was prepared by adding 100 mL of the beverage to 900 mL of distilled water.

5.1.1.1 Wastewater characterisation

Physicochemical parameters of the wastewater samples, including pH, dissolved oxygen (DO), and electrical conductivity, were measured using a portable multi-parameter water quality meter. The instrument was calibrated prior to each analytical session according to the manufacturer's instructions to ensure accurate and reliable measurements.

For each measurement, the probe was immersed directly into the homogenised wastewater sample, and readings were recorded once the values stabilised. All measurements were conducted at room temperature to maintain consistency among samples. Measurements were performed in triplicate.



Figure 5.2 Portable Multi-Parameter Water Quality Meter

The COD concentrations of the wastewater samples and inoculum were measured using the H1839800 COD Reactor and H183399 Multiparameter Photometer from Hanna Instruments. The COD dichromate method was used, which involved cod high-range reagent containing mercury (III) sulphate, sulphuric acid, potassium dichromate and silver sulphate in vials. 0.2 ml of the diluted mixtures from the four centrifuge tubes was pipetted at a 45° angle in four vials and the mixtures were shaken until diluted. The diluted vials were heated in the Hanna H1839800 COD reactor at 150°C for two hours. After two hours, the diluted vials were then left to cool down for 15 minutes. The diluted vials were put in the Hanna H183399 Multi-Parameter Photometer to measure the COD concentration.



Figure 5.3: Hanna Instruments COD Reactor and Multi-Parameter Photometer

5.1.2 Inoculum

The study used a mixed microbial consortium comprising fermentative bacteria and methanogenic archaea. The inoculum was collected from an operational biogas digester plant that operates on multiple feedstocks. To promote hydrogen production, two inoculum pre-treatment methods were employed, namely heat pre-treatment and combined heat and base pre-treatment. These approaches are widely reported as effective methods for inhibiting methanogens while enriching spore-forming hydrogen-producing bacteria, particularly *Clostridium* species (Logan et al. 2002; Wang and Wan, 2009).

- Heat Pre-treatment

For heat pre-treatment, the inoculum was homogenised and transferred into 1 L reactor bottles. The bottles were placed in a thermostatically controlled water bath and held at 80 °C for 30 minutes, as shown in Table 5.4. After the heating period, the bottles were removed from the water bath and allowed to cool naturally to room temperature. Heat pre-treatment selectively inactivates methanogenic archaea, which are highly sensitive to elevated temperatures, while allowing heat-resistant and spore-forming hydrogen-producing bacteria to survive (Logan et al. 2002; Kim et al. 2006).



Figure 5.4 Reactor bottles containing inoculum were subjected to heat pre-treatment at 80 degrees Celsius

- Heat and Base Pre-treatment

For the combined heat and base pre-treatment, the initial pH of the inoculum was measured at 7.75 and adjusted by adding 5 M sodium hydroxide (NaOH) dropwise under continuous stirring until the pH reached ~10 to 10.5. The pH was measured using a calibrated Hanna Instruments HI9126 pH meter (Figure 5.5). The alkalinised inoculum was then transferred into 1 L reactor bottles and subjected to heat treatment at 80 °C for 30 minutes in a water bath. After heat exposure, the inoculum was allowed to cool to room temperature and kept at pH 10.5 for 24 hours, after which the pH was adjusted to 6.5 prior to use.

Alkaline pre-treatment disrupts microbial cell membranes and enhances the inactivation of methanogenic archaea, while combined heat and alkaline stress further favours selection for hydrogen-producing bacteria (Chen et al. 2002; Wang and Wan, 2009). This dual pre-treatment approach has been reported to significantly improve hydrogen yield compared to single pre-treatment methods.



Figure 5.5 Measuring inoculum pH adjustment for alkaline pre-treatment

5.1.3 Batch fermentation reactors

Two testing platforms were utilised: the Bioprocess Control Instruments (BPC Instruments), Automatic Methane Potential Test System (AMPTS II), operated under mesophilic conditions (35 °C), and the Gas Endeavour III system (BPC Instruments, Sweden), operated under thermophilic conditions (55 °C) (Bioprocess Control, 2023; BPC Instruments, 2023). The use of these two systems enabled a comparative evaluation of substrate performance under different temperature regimes, providing insight into the effect of mesophilic and thermophilic operation on gas production from agro-industrial wastewater, as temperature is a key parameter influencing microbial activity and gas yield in anaerobic digestion processes (Lin et al. 2011; Alexandropoulou et al. 2017).

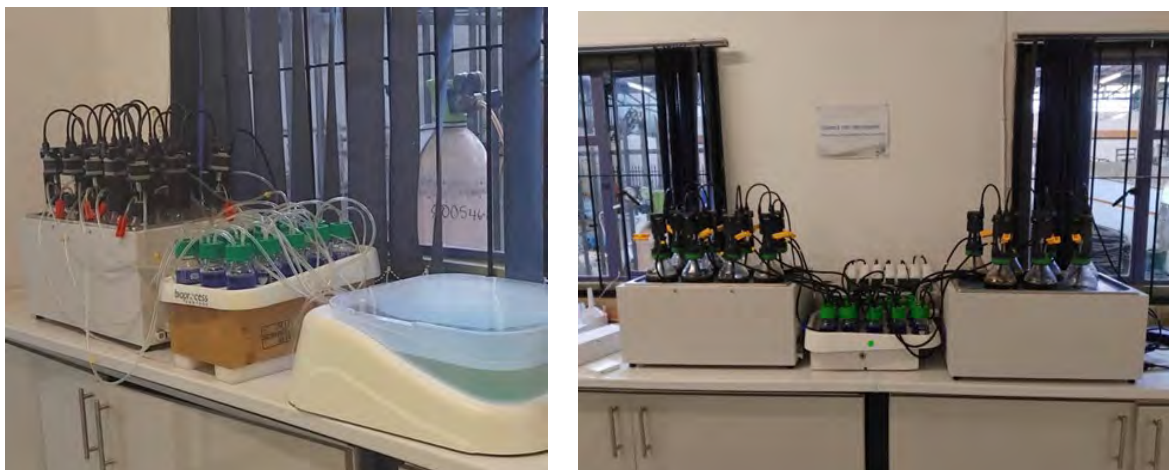


Figure 5.6: The AMPTS II and Gas Endeavour Bioprocess Control Instruments that were used for batch dark fermentation experiments

Both platforms operate on similar principles and comprise 15 sealed Schott glass reactors (0.5 L or 1 L working volume), placed in thermostatically controlled water baths, integrated with carbon dioxide absorption units and automated gas measurement devices. Each reactor operates independently and is equipped with an overhead mechanical stirrer to maintain a homogeneous mixture throughout the experiment. This configuration enables precise and continuous quantification of gas production under strictly controlled anaerobic conditions. The systems incorporate automated gas monitoring, precise temperature control, and standardized reactor configurations, allowing reproducible laboratory-scale evaluation of microbial gas production processes under different temperature regimes.

Biogas produced in the reactors is first directed through a carbon dioxide scrubbing unit containing an alkaline sodium hydroxide solution (3 M NaOH), which selectively absorbs CO₂ from the gas stream. The remaining gas is then measured volumetrically using automated multi-channel gas counters connected to BPC's dedicated data acquisition software, enabling continuous monitoring of cumulative gas production and reactor performance.

5.2 Experimental Procedure

Three experiments were set up with a running duration of between 7 and 14 days. For both units, reactors were loaded with inoculum and wastewater substrate at ratios determined by COD concentration, a standard approach to ensure comparable organic loading and process stability (Raghunath et al., 2016). Prior to reactor loading, the 5 wastewater samples and inoculum were characterised for key parameters, including pH and chemical oxygen demand (COD), which were used to determine appropriate inoculum-to-substrate loading ratios, as COD is a critical indicator of substrate biodegradability and gas production potential (Raghunath et al., 2016; Balachandar et al. 2020).

Once loaded, all reactors were sealed and placed into their respective incubation units. Afterward, an 80-20% carbon dioxide/nitrogen mixture was used to purge the reactors and establish an anaerobic environment. Operating parameters, such as temperature and mixing speed, were set using the BPC Instrument system software. Gas production was monitored continuously throughout the experimental period. All experiments were conducted in triplicate to ensure data reliability and reproducibility (Alexandropoulou et al., 2017). The details of each experiment trial are as follows:

- Experiment 1 used sugar mill wastewater (SMW) as a substrate, investigating hydrogen yield under inoculum heat pre-treatment as well as inoculum alkali pretreatment at mesophilic and thermophilic temperatures. The experiment also investigated the impact of microwave pre-treatment of wastewater on the gas yield. A constant inoculum-to-substrate ratio (I/S) of 0.5 was applied to all reactors, except the control. Table 5.1 records the experimental conditions for experiment 1 conducted at both mesophilic (35 °C) and thermophilic (55 °C) conditions.

Table 5.1: Experimental Design for Experiment 1 using Sugar Wastewater

Reactor conditions	Reactor 1-3	Reactors 4-6	Reactors 7-9	Reactors 10-12	Reactors 13-15
Reactants	Inoc alone	Inoc+ SMW	Inoc + SMW	Inoc +SMW	Inoc + SMW
I/S ratio	Inoc alone	0.5	0.5	0.5	0.5
Inoc-pre-treatment	None	Heat	Heat+ alkali	Heat	Heat + alkali
Substrate pre-treatment	None	None	None	Microwave, 180 sec at 600W	Microwave 180 sec at 600W
Experiment duration	6 days				

The thermophilic set-up replicated the process conditions for Reactors 1-3, 10-12, and 13-15. Only the combined inoculum pre-treatment and substrate pre-treatment were investigated.

- Experiment 2 used fruit juice wastewater (FJW) as a substrate. For this experiment, only the inoculum heat pre-treatment was applied, so inoculum pre-treatment was not a variable; instead, the (I/S) ratio was varied as shown in Table 5.2, which summarises the test conditions for the second experiment. As in the previous experiment, Experiment 2 was run at mesophilic and thermophilic temperatures. Like Experiment 1, the thermophilic run duplicated the reactor conditions for Reactors 1-3, 10-12, and 13-15.

Table 5.2 Experimental Design for Experiment 2 using Fruit Juice Wastewater

Reactor conditions	Reactor 1-3	Reactors 4-6	Reactors 7-9	Reactors 10-12	Reactors 13-15
Reactants	Inoc alone	Inoc+ SMW	Inoc + SMW	Inoc +SMW	Inoc + SMW
I/S ratio	Inoc alone	0.5	1.0	0.5	1.0
Inoc pre-treatment	None	Heat	Heat	Heat	Heat
Substrate pre-treatment	None	None	None	Microwave, 180 sec at 600W	Microwave 180 sec at 600W
Experiment duration	13 days				

- Experiments 1 and 2 did not include a direct comparison of hydrogen yields for sugar wastewater and fruit juice, as the samples had been collected at different times and storage times affected sample integrity; hence, only the impact of process parameters on hydrogen production was investigated.
- Experiment 3 used synthetic beverage wastewaters prepared on the same day to compare the hydrogen yields of carbonated soft drink wastewater (CSDW), wine wastewater (WW), and malt beer (MB). This experiment was run only at mesophilic temperatures. **Table 5.3** presents the experimental design for synthetic beverage waters

Table 5.3: Experimental Design for Experiment 3 Synthetic Beverage Waters

Reactor conditions	Reactor 1-3	Reactors 4-6	Reactors 7-9	Reactors 10-12
Reactants	Inoc alone	Inoc+ CSDW	Inoc + WW	Inoc +MB
I/S ratio	Inoc alone	3	3	3
Inoc pre-treatment	None	Heat	Heat	Heat
Substrate pre-treatment	None	None	None	None
Experiment duration	10 days			

5.3 Analytical Methods and Data Analysis

Hourly gas production was automatically logged by the Gas Endeavour/AMPTS II software interface, which also enabled real-time monitoring and data export for calculations. In-line gas composition analysis was done for Experiments 1 and 2, but not for Experiment 3. The gas composition was measured every 2 days using the Geotech Biogas 5,000 Analyzer during the experimental run. The biogas analyser measures only the percentages of methane (CH₄), carbon dioxide (CO₂), oxygen (O₂), and other trace gases (ammonia, carbon monoxide, and hydrogen sulphide) in the biogas and cannot measure hydrogen. The analysis was conducted to confirm a reduction in the methane fraction following inoculum pre-treatment, which, in turn, could be interpreted as indicating hydrogen production.



Figure 5.7 Geo-Tech 5000 Analyser used for gas composition analysis

The digestate/effluent at the end of the experimental duration was supposed to be analysed for VFA composition and quantity; however, as both the laboratory GC and HPLC were not operational, VFA analysis was not conducted.

5.4 Results

5.4.1 Biohydrogen yields.

Experiment 1: Sugar wastewater at mesophilic and thermophilic temperatures

Experiment 1 was discarded due to operational issues with the AMPTS II gas measurement system operating under mesophilic conditions. Some gas counters failed to record data during the experiment, particularly for the alkali-heat pretreatment reactors (R9–R11), which produced no measurable gas readings as shown in Figure 5.8a. As a result, hydrogen yields could not be determined for these treatments.

Furthermore, under both mesophilic and thermophilic regimes, the overall gas yields observed were extremely low ($<10 \text{ Nm}^3\text{H}_2/\text{gCOD}$). The mesophilic experiments ceased after three days, while the thermophilic experiments continued until day six; however, very low hydrogen yields were still observed. The low inoculum-to-substrate (I/S) ratio of 0.5 was identified as the likely cause of the low yields observed, as this might have limited microbial activity and suppressed fermentative hydrogen production. The trial could not be repeated because the remaining sugar mill effluent was insufficient to reset and rerun the experiment.

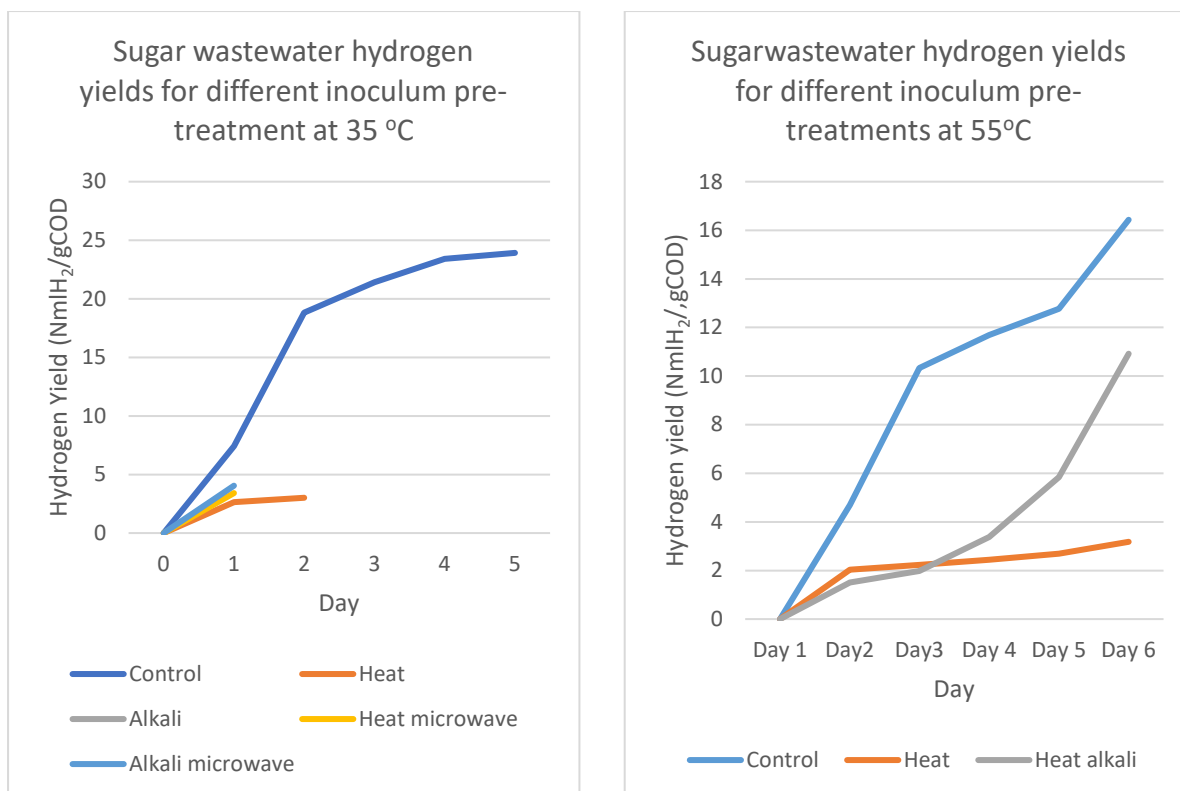


Figure 5.8 Graphs of hydrogen yield for sugar mill wastewater at mesophilic and thermophilic temperatures

Experiment 2: Fruit juice wastewater at mesophilic and thermophilic temperatures

At 35 °C, heat pretreatment combined with microwave substrate treatment at I/S = 1.0 produced the highest hydrogen yield of 60 NmL H₂/g COD. In contrast, the lowest hydrogen yield of 13 NmL H₂/gCOD was observed for heat pretreatment alone at I/S = 0.5. Likewise, heat + microwave at an I/S of 0.5 produced negligible hydrogen gas, suggesting that the lower I/S ratio limited hydrogen production.

At 55 °C, only microwave-assisted heat pretreatments were evaluated. The hydrogen yields were ~25 NmL H₂/gCOD at I/S = 0.5 and 26–27 mL H₂/gCOD at I/S = 1, indicating relatively similar performance at both substrate loadings. The lower hydrogen yields observed at 55 °C likely reflect insufficient thermophilic adaptation of the microbial community and shifts in fermentation pathways, which reduced the efficiency of hydrogen-producing metabolism compared with the better-adapted mesophilic system at 35 °C.

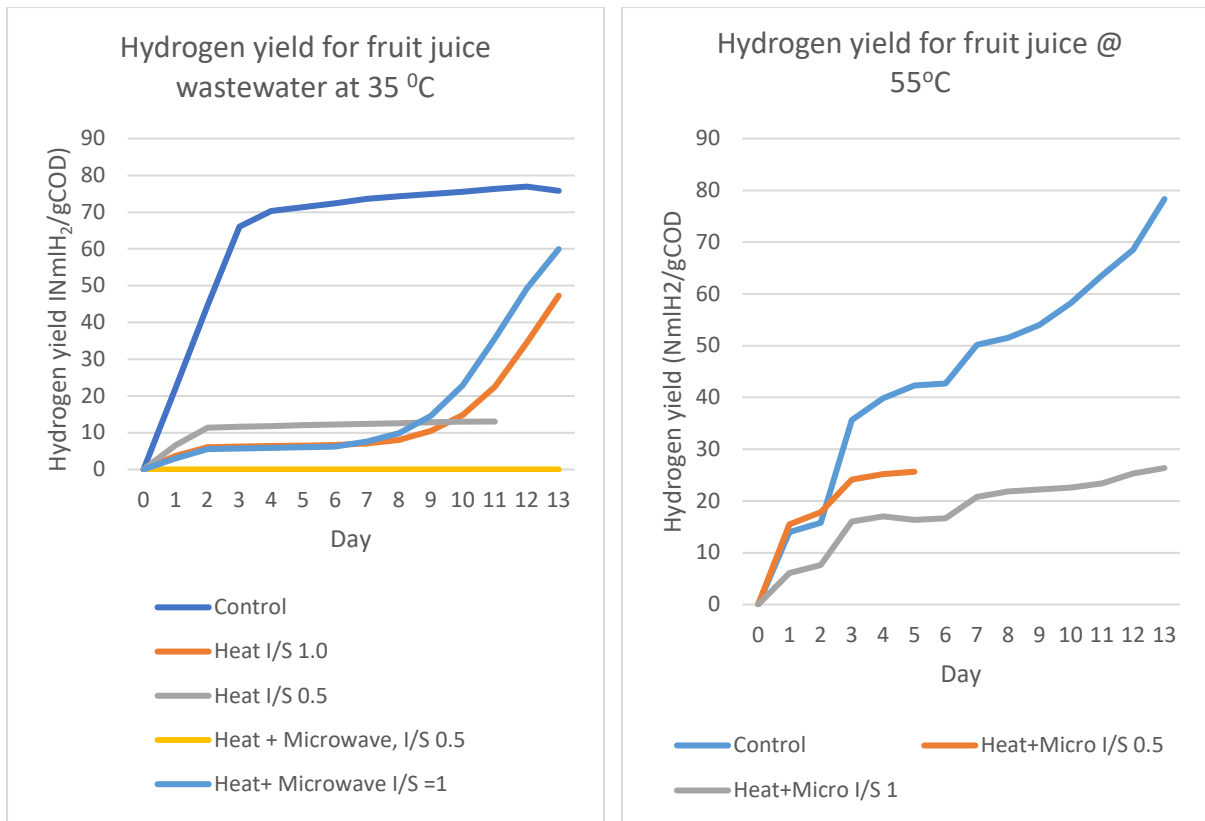


Figure 5.9 Graphs of hydrogen yield for fruit juice wastewater at mesophilic and thermophilic temperatures

Overall, higher I/S ratios generally resulted in greater hydrogen yields, and microwave-assisted pretreatment enhanced hydrogen production compared with heat pretreatment alone under mesophilic conditions, while thermophilic yields remained moderate and relatively unaffected by I/S ratio.

Experiment 3: Synthetic beverage wastewater at mesophilic temperature

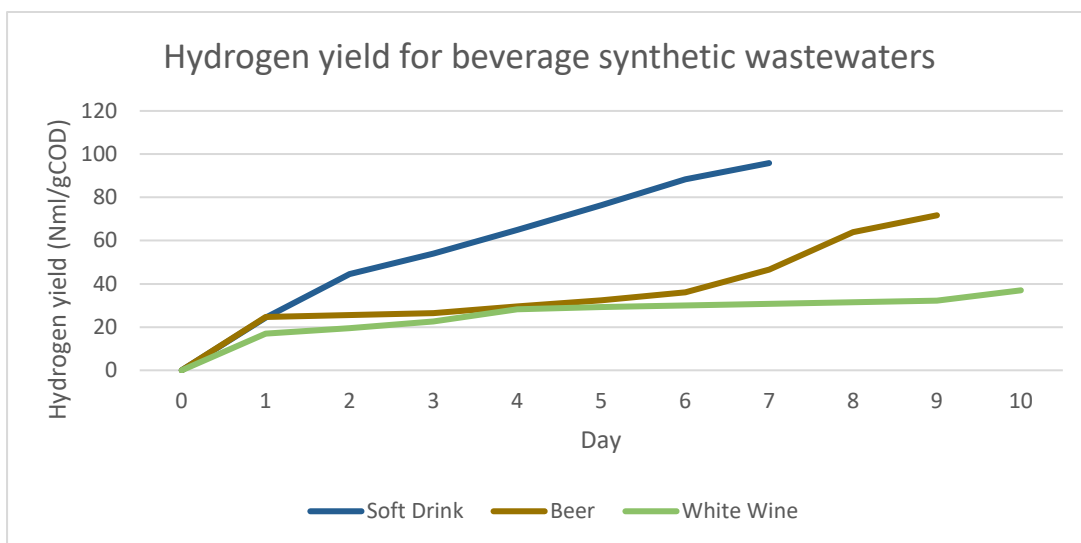


Figure 5.10 Graphs of hydrogen yield for synthetic beverage wastewater at mesophilic temperatures

The hydrogen yields varied among the three synthetic wastewater substrates, with soft drink wastewater yielding the highest, followed by beer wastewater, while white wine wastewater yielded the lowest across the tested synthetic wastewater streams.

Soft drink wastewater displayed the greatest hydrogen yields of approximately 95 NmL H₂/ gCOD⁻¹, indicating better hydrogen production compared to the other synthetic wastewater streams. Beer wastewater produced lower hydrogen yields, at 70 NmL H₂/g COD. In contrast, white wine wastewater exhibited the lowest hydrogen yields, with a maximum observed yield of about 37 NmLH₂/gCOD.

5.4.2 Biogas Composition

Biogas analysis at different time points of the experiments, using the Geotech 5000 Biogas Analyser, showed that inoculum pre-treatment was effective in inhibiting methane production. For both Experiment 1 and 2, the control reactors (R1-R3) recorded methane percentages above 60% as shown in Figure 11. All inoculum pre-treatments, namely heat and alkali+heat, resulted in biogas with significantly reduced methane content. Recorded methane content for the inoculum pre-treatments was typically less than 5%. The reduction in methane content was inferred to indicate the production of hydrogen gas. However, to validate hydrogen production, gas analysis using a GC that detects hydrogen gas still needs to be carried out.

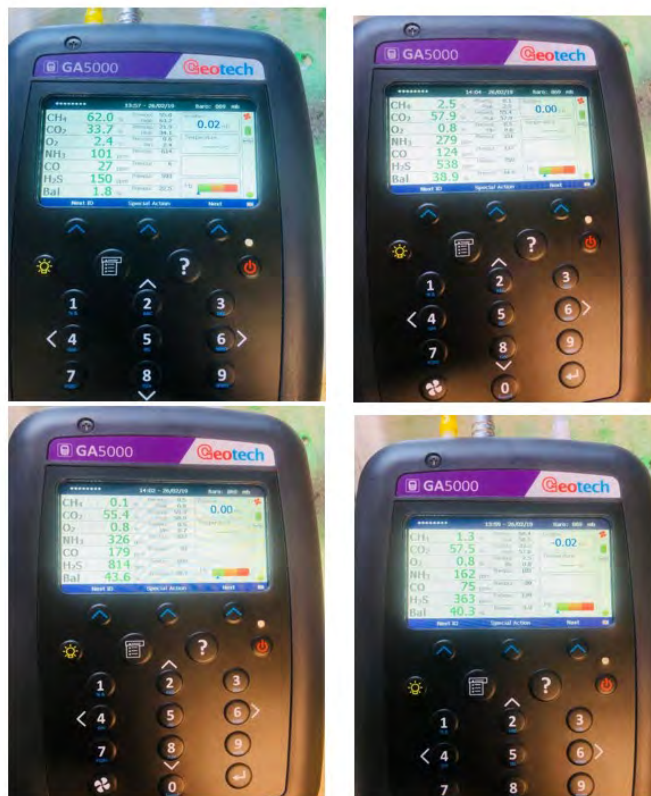


Figure 5.11 Geotech 5000 Biogas Analyser showing gas composition readings for the different treatments.

Inoculum pre-treatment also affected the carbon dioxide levels of the produced gas. For the two experimental runs in which the biogas produced was analysed, the carbon dioxide fraction increased compared to the controls. In the past, very little focus was directed towards the use or valorisation of carbon dioxide produced during anaerobic fermentation. Traditionally, carbon dioxide from biogas plants has been used directly in industrial applications, such as food-grade CO₂. Emerging biological, chemical, and mineralisation-based valorisation methods are now being researched to produce a range of products, including microalgae biomass, single-cell protein, biofuels, synthetic methane and methanol, polymers, and construction materials such as calcium carbonates. Converting CO₂ into these products can improve resource recovery from wastewater and enhance the economic viability of anaerobic biorefinery systems, including those based on dark fermentation.

5.5 Chapter Summary and Conclusion

This chapter demonstrated the production of biohydrogen from agro-industrial wastewater through dark fermentation. The first two experiments showed, to a limited extent, that process parameters influence hydrogen yield, with the inoculum-to-substrate (I/S) ratio having the strongest effect. Thermophilic temperatures did not increase hydrogen yields as expected, and microwave substrate pretreatment did not significantly enhance hydrogen production.

Among the synthetic beverage wastewaters, carbonated soft drink wastewater achieved the highest hydrogen yield ($\approx 95 \text{ NmL H}_2 \text{ gCOD}^{-1}$), followed by beer wastewater ($\approx 70 \text{ NmL H}_2 \text{ gCOD}^{-1}$), while wine wastewater produced the lowest yield ($\approx 37 \text{ NmL H}_2 \text{ gCOD}^{-1}$), confirming that substrate type influences hydrogen production performance. Biogas composition analysis further confirmed the effectiveness of inoculum pre-treatment in suppressing methanogenesis, with methane concentrations decreasing from above 60% in control reactors to below 5% in pre-treated systems. Although hydrogen concentrations were not directly measured due to analytical limitations, reductions in methane production and cumulative gas production trends indicate hydrogen-dominated fermentation. The increased carbon dioxide fraction also highlights an additional product stream that could potentially be valorised.

However, the experiments were limited in their ability to fully assess the impact of process parameters and to generate the detailed data required for process upscaling. Instrument limitations also prevented a comprehensive analysis of both the gas and liquid fractions, indicating the need for further laboratory investigations.

6 PATHWAYS TO UPSCALING STUDY RESULTS

The chapter proposes upscaling potential pathways based on three key result areas of the study, as a large-scale application was not within the original scope of the research. The pathways explain how the study results can be expanded and made more deployable for agro-industrial wastewater valorisation. The upscaling pathways integrate the study's main findings: wastewater resource assessment, estimated theoretical hydrogen potential, and laboratory-scale biohydrogen production.

6.1 Upscaling of Result 1 for Wastewater Resource Assessment

Several agro-industrial wastewater streams were identified as potential substrates for biological hydrogen production based on the resource assessment conducted in this paper. Although the proposed dataset provides a good baseline for wastewater availability, scaling to support industrial adoption would require establishing a comprehensive, detailed database of organic waste resources with a consistent quality and reliable compositional data. An organic waste database that addresses three central aspects of resource availability: location, amount produced, quality, and possibly seasonal availability patterns. These parameters are important for assessing the feasibility of waste conversion systems in operation and for identifying suitable locations for setting up resource recovery facilities.

Firstly, the theoretical hydrogen production assessment in Chapter 4 showed that agro-industrial wastewater streams alone produce only limited amounts of biohydrogen relative to the country's targets. Therefore, the dataset can be expanded to include other organic streams, not just wastewater. Agricultural residues, food processing by-products, and municipal organic waste streams are often much higher in organic carbon than wastewater and thus constitute important additional feedstocks for bio-based conversion systems (Wang et al. 2023). Integration of organic resources into a single database would accelerate the development of multi-feedstock biorefineries capable of processing heterogeneous organic waste streams.

The resource assessment in Chapter 3 highlighted significant challenges in accessing and compiling agro-industrial wastewater data in South Africa, including the lack of accurate composition data and limited access. South Africa's industrial wastewater information is fragmented across multiple compliance-oriented platforms, including NIWIS, WARMS, NCIMS, and NATSURV, some of which have restricted access and inconsistent reporting standards. As a result, integrated facility-level datasets linking wastewater generation, composition, treatment, and location are not readily available for research or planning. Data constraints and fragmentation, therefore, motivate the development of a centralized, comprehensive national agro-industrial organic database that consolidates dispersed information into a standardized, regularly updated, and accessible system, thereby enabling more reliable assessments of wastewater and solid organic waste resources to support circular-economy planning in South Africa's agro-industrial sector.

Additionally, the compiled composition database in Section 3.4 showed that only a limited number of variables, such as pH, chemical oxygen demand (COD), electrical conductivity, and suspended solids, are frequently reported. However, biomass conversion systems generally require more comprehensive compositional specifications for selecting feedstock types (Li et al. 2016). Recently, machine learning methods have been applied to model wastewater treatment plant performance using monitoring data. This showcases the use of artificial intelligence in predicting water quality characteristics and process behaviour (Mahanna, et al. 2024). Similar machine learning techniques can be applied to organic waste databases to predict key parameters involved in biomass conversion processes, such as carbohydrate fractions and fermentation potential, using limited datasets.

The basic geo-spatial mapping conducted by the study can be expanded using GIS techniques, such as kernel density heat maps, to identify clusters of sites where waste generation occurs and to inform the siting of centralised waste conversion facilities. Also, mapping of neighbouring industrial facilities that may serve as sinks for products produced by waste conversion facilities must be included, as this will contribute to the establishment of industrial symbiosis networks, with waste flows from one facility serving as feedstocks for products demanded by adjacent industries.

In summary, upscaling of resource assessment includes expanding the feedstock to include solid organic fractions generated by the agro-industry, applying machine learning to generate expanded composition datasets using available data, and using GIS mapping techniques to enable industrial symbiosis, all culminating in the development of a comprehensive and centralized agro-industry organic waste database.

6.2 Upscaling of Result 2 for the Theoretical Hydrogen Production Assessment

The theoretical assessment of hydrogen production via dark fermentation of agro-industrial wastewater estimated that South Africa produces roughly 78.4 million m³ of hydrogen per annum, equivalent to about 7,000 tonnes of hydrogen per year. This is relatively small compared to the 500,000 tonnes the country targets for green hydrogen by 2030. This result indicates that upscaling of hydrogen production should not focus on agro-industrial wastewater alone as a feedstock, but expand to other organic residues applying co-digestion of organic liquid and solid streams. These systems have been extensively studied in the context of anaerobic digestion research and can greatly improve substrate utilisation and system performance (Mata-Alvarez et al. 2014).

Additionally, given the low estimated volumes, hydrogen should not be the sole output; instead, waste conversion systems must target multiple products. Most biological fermentation processes convert only a portion of organic carbon to hydrogen; the balance is converted into various intermediate materials, such as volatile fatty acids (VFAs), which are highly valuable biochemical intermediates that may be marketed as feedstocks to bio-based chemicals. As a result, waste conversion systems should be structured according to multiproduct biorefinery concepts, which do not always have to target hydrogen production. Instead, other products can be considered, such as organic acids, biogas, biosorbents, and nutrient recovery products. Biorefineries based on such integrated product portfolios have been widely proposed as a pathway for maximising the value recovered from biomass resources (Cherubini, 2010).

Product selection in these systems should be guided not only by feedstock composition or a technology bias, but also by market demand. Linking waste resource databases with information on nearby industrial activity can enable the identification of local product markets, thereby enhancing industrial symbiosis networks and improving the economic viability of waste conversion systems.

In summary, upscaling of result 2 focuses on co-digestion of organic solid and liquid streams, multi-product biorefineries, and the use of market demand to guide product portfolio selection. A study conducted by Filho *et al.* (2024) lays a good framework for applying market demand to product selection and biorefinery design.

6.3 Upscaling Result 3 for Laboratory Experiments

The laboratory experiments presented in Chapter 5 of this report demonstrated, at the laboratory level, the technical feasibility of hydrogen production using two samples of South Africa's agro-industrial wastewater. Additionally, the experimental design provided comparative insights into the effects of substrate pretreatment and operational conditions, such as temperature and the inoculum-to-substrate ratio, on hydrogen yield. For industrial-level application of findings requires a combination of technical,

operational, and economic strategies to ensure that processes remain efficient, reliable, and economically viable at larger scales. Key strategies would include:

6.3.1 Pilot-Scale Validation of Laboratory Results

To scale up the findings of the study, the biohydrogen yield results should be tested at a pilot or demonstration scale to bridge the gap between laboratory experiments and industrial production. Pilot studies help evaluate process stability, performance under continuous operation, and potential operational challenges. In particular, a 100 – 1000L pilot-scale facility could be established for the wastewater conversion to biohydrogen and other value-added products. Within pilot studies, co-digestion experiments involving wastewater and solid organic wastes, instead of using wastewater alone. Pilot-scale experiments would also require determining optimal substrate ratios and evaluating the influence of mixed feedstocks on hydrogen production and microbial activity. Furthermore, value-added products such as VFA characterisation and recovery will be prioritized as this might represent one of the most profitable product streams. Investigating the feasibility of VFA extraction could significantly improve the economic viability of fermentation-based biorefineries.

Pilot-scale would investigate the feasibility of biorefinery configurations, such as a combination of Dark fermentation and Microbial Electrolytic Cells (MEC), to evaluate potential pathways for increasing hydrogen yields and improving resource recovery.

6.3.2. Process Optimisation

Pilot-Scale protocols must be optimized and standardised to ensure consistent performance and product quality at larger scales. This includes refining operating conditions such as temperature, pH, residence time, and organic loading rate to maintain efficiency. This phase will generate the operational datasets required for reactor scale-up, including hydrogen productivity rates, substrate conversion efficiencies, and optimal operating conditions for continuous hydrogen production systems.

6.3.3. Process Modelling and Simulation

Globally, mathematical modelling and process simulation tools are used to predict system behaviour at larger scales, allowing engineers to assess mass and energy balances, mixing efficiency, and reactor performance before implementation. The report suggests laboratory results could be utilised for this purpose. Process simulation and modelling will enable the evaluation of alternative technology combinations, energy balances, and product recovery pathways, providing the basis for the design of adaptive multi-feedstock biorefineries capable of responding to variations in feedstock availability and market demand. Evaluation of integrated biorefinery configurations combining multiple conversion technologies using modelling software such as ASPEN and DWSIM. Including techno-economic studies.

6.3.4. Appropriate Reactor and Equipment Design

Since scaling up requires the design and selection of reactors and equipment suitable for industrial throughput, this study will make considerations the laboratory studies in the selection of the type of reactor. Parameters including mixing characteristics, mass transfer rates, heat transfer, and material compatibility are critical in the scale-up reactor selection. A subsequent choice of pilot-scale reactor systems (100–1000 L) to validate process stability, assess scale-dependent phenomena, and experimentally verify integrated biorefinery configurations prior to industrial demonstration.

6.3.5. Feedstock Supply and Wastewater Resource Management

Ensuring a reliable and consistent supply of raw materials or feedstock is essential for large-scale operations for this project. This study will require further investigation of the database sets per province and the development of robust supply chains, appropriate storage systems, and preprocessing methods

to maintain feedstock quality and uniformity. This will also require the establishment of logical framework to ensure access to raw materials.

6.3.6. Process Integration and Automation

Since industrial-scale systems benefit from automation and process control technologies to maintain stable operating conditions, reduce human error, and improve efficiency, the scaling-up strategy will have to include these aspects of automation. From South Africa's manufacturing and processing sectors' perspective, there are a lot of systems integrators and automation firms, such as PCISA, Process Solutions Automation (P-S-A), Wecon PLC and GMC Automation, that provide PLC-, SCADA-, and integrated control solutions to request assistance from local firms to improve performance, reliability, and safety of the pilot-scale systems.

6.3.7. Techno-Economic Assessment of Wastewater to biological hydrogen

A techno-economic analysis (TEA) is always key to evaluate capital costs, operating expenses, profitability, and return on the would-be investments. The ARC team of agricultural economists can provide expertise in evaluating the commercial viability of scaling up the process. This would require pulling sub-sector national wastewater volume results (Section 3.5.2), wastewater compositional data (Section 3.5.3), theoretical biohydrogen results (Section 4.6.1) and laboratory -scale results in Chapter 5.

6.3.8. Regulatory and Environmental Compliance

Commercial operations would need to comply with environmental regulations, safety standards, and industry guidelines. Life cycle assessments (LCA) and environmental impact analyses are often required. In South Africa, developers of green hydrogen facilities must comply with several national regulatory and environmental requirements before the commencement of commercial operation. For example, the project would be required to register with the National Energy Regulator of South Africa (NERSA) under the Gas Act, which governs the registration, licensing, and safety of piped-gas activities and related infrastructure. To obtain environmental authorisation, the project needs to undergo the National Environmental Management Act (NEMA) and its Environmental Impact Assessment (EIA) Regulations is essential, as these ensure that potential environmental impacts are assessed and mitigated before approval. The Department of Forestry, Fisheries and the Environment (DFFE) is the primary authority responsible for environmental permitting and oversight in South Africa, and recent sector-specific EIA guidelines and planning tools have been developed to provide clearer pathways for green hydrogen developments.

6.3.9. Industrial Partnerships and Transfer integration

For large-scale biohydrogen production, strategic collaboration with industrial partners, technology centres, academic institutions and investors is critical. These partnerships would then facilitate the transfer of proven technologies from the proposed pilot scale to commercial operations, provide access to essential infrastructure, and assist in securing the right financial and technical resources needed for a successful commercialisation venture. In the South African context, collaboration with local companies and research institutions, including Sasol, South African Renewable Energy Technology Centre (SARETEC), Bambili Energy and HySA, also supports compliance with national regulations, enhances local capacity building, and ensures alignment with government initiatives promoting renewable energy and green hydrogen production. A proposed merging of the study with, for instance, Bambili Energy's fuel cell systems, which use hydrogen to generate clean energy power can be groundbreaking.

6.4 Integrated up-scaling framework

The upscaling components described in the previous sections can be integrated into a single framework linking resource assessment, industrial symbiosis, and biorefinery design. The framework involves developing a centralised database that quantifies and maps both solid and liquid agro-industrial waste streams and applying geospatial analysis to identify opportunities for industrial symbiosis networks that combine complementary waste resources. These networks can support the design of integrated biorefinery facilities that valorise the waste streams and link industrial facilities to nearby users capable of utilising the recovered products. Laboratory experimentation and process modelling provide the technical data required to support the design and optimisation of these smart biorefineries.

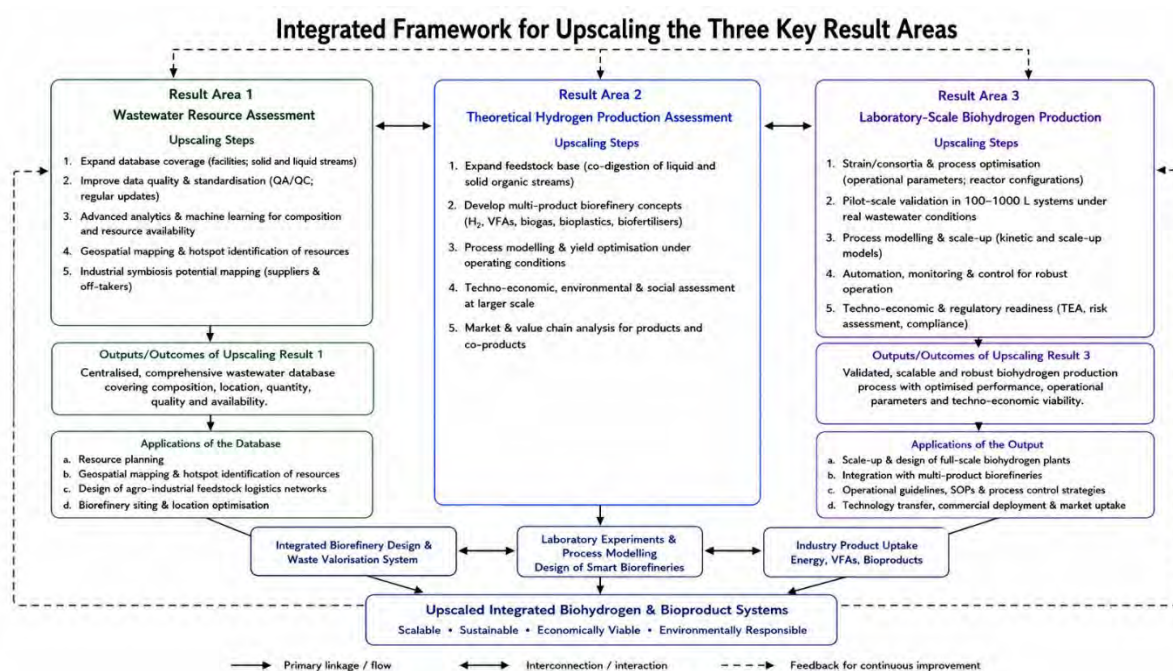


Figure 6.1 Integrated Upscaling Framework Linking Resource Assessment, Laboratory Experiments and Biorefinery Design

6.5 Summary and Conclusion

This chapter outlined pathways to upscale the study's results from resource assessment and laboratory experimentation toward the development of integrated waste-to-bioprocess pilot-scale systems. The findings highlight the need to expand resource datasets into a centralised organic waste database, incorporate geospatial analysis to identify opportunities for industrial symbiosis, and integrate both liquid and solid organic waste streams to support multi-feedstock biorefineries. A phased upscaling strategy involving bench-scale experiments, continuous reactor studies, and system modelling was proposed as a route to pilot-scale laboratory demonstration. Together, these components form a framework that links resource assessment, industrial symbiosis, and adaptive biorefinery design, providing a pathway to translate laboratory research into practical waste valorisation systems that support circular bioeconomy development.

7 CONCLUSION

The aim of this study was to conduct a national resource assessment of agro-industrial wastewaters in South Africa by quantifying generation rates, spatial distribution, and physicochemical characteristics, and to evaluate their suitability for biohydrogen production and other bio-products via dark-fermentation-centred biorefineries. Overall, this study prompted the re-evaluation of agro-industrial wastewater in South Africa from an environmental liability to a valuable resource within a circular economy. The research highlighted the need for integrated resource recovery rather than only complying with disposal practices. Based on South Africa's challenges related to water scarcity, infrastructure constraints, and rising energy demands, agro-industrial effluents represent a significant untapped opportunity for renewable energy production.

Chapter 2 provided an overview of wastewater management practices in the South African agro-processing industry. Whilst the agro-processing sector is central to food security and economic development, it is among South Africa's most energy and water-intensive industries. As such, it generates substantial volumes of high-strength, biodegradable wastewater, placing increasing pressure on wastewater treatment infrastructure as well as limited water resources. Although South Africa has robust policy and legislative frameworks that support pollution prevention, water reuse, circular economy principles, and low-carbon development, wastewater management in the agro-processing sector remains largely compliance-driven in practice. Conventional treatment systems are employed across sub-sectors, with very limited uptake of technologies for resource recovery. Many facilities continue to rely on municipal wastewater treatment works for effluent disposal, further burdening systems that are already affected by aging infrastructure, operational inefficiencies, and declining compliance performance. As a result, valuable organic carbon, nutrients, and energy stored in agro-industrial effluents are inadvertently lost. An additional issue is the fragmented, incomplete and limited nature of agro-industrial wastewater data. Information on the volume of wastewater generated, its composition, treatment practices, and spatial distribution is spread across regulatory platforms, voluntary surveys, and isolated studies. This limits strategic planning and the identification of high-potential opportunities for wastewater valorisation, thereby hampering a circular economy approach to wastewater use. Addressing these challenges requires a systematic, data-driven national assessment. Hence, this study quantified, characterised, and spatially mapped agro-industrial wastewater streams to provide a strong analytical basis for evaluating their suitability for biohydrogen production and integrated biorefinery-based resource recovery pathways.

Chapter 3 established a national wastewater resource baseline and identified priority fermentable streams and regions for energy recovery. Using publicly available secondary data, the assessment quantified wastewater volumes across 12 sub-sectors, characterised effluent composition, and mapped the spatial distribution of large agro-processing facilities. The findings demonstrate that pulp and paper, as well as sugar milling, dominated national wastewater volumes, comprising the majority of the total estimated $\pm 130\ 000$ ML of generated wastewater per annum, which was mostly prevalent in KwaZulu-Natal and Mpumalanga. These large milling operations generate continuous, high-volume effluent streams, making them suitable for integrating wastewater valorisation. In contrast, provinces such as Gauteng and the Western Cape exhibited more diversified, spatially dispersed, but comparatively lower-volume food and beverage wastewater. While these regions generate smaller accumulated wastewater volumes, their higher facility density supports alternative models such as decentralised treatment, co-digestion, or shared infrastructure. The spatial differentiation of facilities, therefore, highlights that wastewater valorisation strategies must align with wastewater volume, availability and type.

Wastewater characterisation further revealed that most agro-industrial effluents are medium- to high-strength, biodegradable organic streams. Carbohydrate-rich wastewaters from sugar milling, breweries,

fruit processing, and corn wet milling were identified as the most favourable candidates for biohydrogen production due to high chemical oxygen demand (COD) and readily fermentable substrates. Conversely, pulp and paper effluents as well as protein-rich abattoir and dairy streams would require significant pre-treatment to improve suitability for biohydrogen production.

A major finding when attempting to establish a wastewater database was the substantial data fragmentation, limited accessibility to facility-level discharge records and samples, as well as inconsistent reporting across existing platforms. These constraints introduced uncertainty into national estimates and directly motivated the proposal for a centralised national agro-industrial wastewater database. Such a system would integrate standardised volume, composition, treatment, and geospatial data, thereby strengthening future resource assessments and investment planning. Overall, Chapter 3 provides the quantitative and spatial agro-processing wastewater distribution for subsequent biohydrogen potential analyses, anchoring the study's broader conclusions on wastewater valorisation feasibility in South Africa's agro-industrial sector.

Chapter 4 conducted a theoretical assessment of biohydrogen production potential across South Africa's agro-industrial sub-sectors. As a first step, various biological hydrogen production technologies were evaluated, and dark fermentation was identified as the most feasible technology for South African agro-industrial wastewater due to its compatibility with elevated COD and carbohydrate-rich wastewater streams. Moreover, its relatively low external energy requirement, moderate cost range, and comparatively advanced technology readiness level (TRL), relative to other hydrogen production technologies, further support its implementation in SA. Additionally, compared with light-dependent routes, dark fermentation offers greater ease of operation and compatibility with conventional wastewater treatment systems, making it suitable for large-scale applications. With the aid of the wastewater composition data that was compiled in Chapter 3, the study estimated the theoretical biohydrogen potential of selected agro-industrial wastewater streams. Sugar mill effluents were identified as the wastewater stream with the highest potential for hydrogen production due to their large wastewater volumes and high concentrations of readily fermentable carbohydrates. Additionally, other carbohydrate-rich wastewaters, including those from fruit processing, beverage production, and corn wet milling, were also identified in literature as favourable substrates for dark fermentation. Overall, the findings demonstrated that agro-industrial wastewater streams in South Africa provide a viable feedstock for hydrogen production through dark fermentation and could therefore contribute to emerging waste-to-energy strategies and circular bioeconomy initiatives within the agro-processing sector.

Chapter 5 presents laboratory-scale biohydrogen experiments demonstrating hydrogen production from five selected wastewater streams, three of which were synthetic wastewaters. The experiments evaluated the influence of key process parameters on hydrogen yield and biogas composition. Laboratory experiments were used to demonstrate the technical feasibility of biohydrogen production from carbohydrate- and sugar-rich agro-industrial wastewaters via dark fermentation.

Although operational and time constraints hindered the generation of complete experimental datasets, the limited results still provided important insights into how process parameters influence hydrogen generation. For example, the results showed that the inoculum-to-substrate ratio (I/S) significantly affected hydrogen yield. In general, a higher I/S ratio of 1 increased hydrogen yields, whereas a lower I/S ratio of 0.5, in some cases, resulted in zero or negligible gas production.

Hydrogen yields comparative experiments using synthetic beverage wastewaters demonstrated that substrate composition strongly influences hydrogen production potential, with carbonated soft drink wastewater producing the highest yields, followed by beer and wine wastewaters. These results

emphasise the importance of selecting suitable substrates as substrate type influences overall hydrogen productivity. Volatile fatty acid analysis and direct hydrogen measurement were omitted due to nonfunctional equipment, limiting a full evaluation of the product spectrum of dark fermentation reactions; however, the experiments are still useful for demonstrating and validating the concept of the valorisation of agro-industrial wastewaters into biohydrogen. The laboratory work also assisted in identifying additional experimental work to be conducted before process scale-up, such as kinetic datasets, co-digestion of feedstocks, and upstream valorisation of intermediate fermentation products and by-products, such as carbon dioxide.

Chapter 6 proposed pathways for translating the study's results into real-world implementation of wastewater-based biorefineries. The chapter proposed an integrated framework linking resource assessment, laboratory experimentation, and industrial deployment through phased upscaling strategies. The first upscale pathway recommended the expansion of the national resource assessment into a comprehensive organic waste database that includes both liquid and solid organic waste streams generated across South Africa's agro-industrial sector. Such a database would improve resource assessment and support the identification of suitable locations for waste valorisation facilities through geospatial analysis and industrial symbiosis mapping. Secondly, the theoretical hydrogen potential assessment showed that, while the agro-industrial sector generates substantial amounts of wastewater, these quantities are insufficient to meet national green hydrogen production targets, producing far less than 5% of the country's 500,000 tonne annual green hydrogen target. As such, additional agro-industrial solid organic streams must be included and a multi-feedstock conversion system adopted. Consequently, the study proposed a shift from single-feedstock systems toward multi-product biorefinery concepts capable of producing not only energy products but also other potentially more economically viable biobased products, such as volatile fatty acids, upgraded carbon dioxide products, and other bio-based chemicals. The design of such biorefineries should not only be informed by technology selection but also be market-driven, producing products with strong, clear market demand. The laboratory results also informed the design of a phased experimental roadmap that guides progression from bench-scale experimentation to continuous reactor studies, process modelling, and, finally, laboratory pilot-scale demonstration. This phased approach limits upscaling risks. Together, the various up-scaling components form a comprehensive upscaling framework that integrates resource mapping, experimental work, and adaptive biorefinery design. This framework provides a pathway to translate laboratory research into scalable waste valorisation systems that support the development of the circular bioeconomy and improve resource efficiency in South Africa's agro-industrial sector.

Overall, the study demonstrates that agro-industrial wastewater in South Africa represents a largely underutilised resource with significant potential to contribute to renewable energy generation and the development of a circular bioeconomy. By combining national-scale resource assessment, theoretical estimates of hydrogen potential, laboratory experimentation, and forward-looking upscaling pathways, the research provides a comprehensive framework for rethinking wastewater management beyond conventional treatment and disposal. While the estimated hydrogen production potential from agro-industrial wastewater alone remains modest relative to national green hydrogen targets, the study highlights the broader opportunity to develop integrated waste-to-bioproduct systems that recover multiple valuable outputs from organic waste streams. Realising this potential will require improved data availability, stronger collaboration between industry and research institutions, and continued technological development to optimise fermentation-based biorefineries. Nevertheless, the findings provide an important foundation for future research and policy development aimed at transforming agro-industrial waste streams into productive resources that support sustainable industrial development, resource efficiency, and climate mitigation in South Africa.

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