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Analysis of the Sustainable VFAs Production Using Anaerobic Digestion Through the Biorefinery Concept

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Análisis de la producción sostenible de AGV mediante digestión anaerobia a través del concepto de biorrefinería

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Dedicatoria

A Dios, mi madre, hermana, leo, mi amor y, mis ángeles (mi padre, tía y, Tango), por amarme, apoyarme y demostrarme su presencia en todo momento.

Resumen

La digestión anaerobia (AD) convencional es una tecnología ampliamente aplicada para la generación de energía renovable en forma de biogás. a partir de residuos orgánicos. La simplicidad del proceso y el consorcio microbiano existente permite utilizar diferentes tipos de residuos como sustrato. Se han utilizado residuos de diferentes cadenas de valor (VC), por ejemplo, agrícolas, agroindustriales y alimentarias, procedentes de distintos eslabones. La AD tiene múltiples vías metabólicas presentes en cada etapa del proceso. Por ello, se ha demostrado que la AD podría diseñarse para producir ácidos grasos volátiles mixtos a través de la AD modificada. La AD modificada corresponde a la variación de las condiciones operativas del proceso para promover rutas metabólicas específicas. Se propuso la integración de la AD convencional y modificada como pilar sostenible para la valorización de tres residuos generados en diferentes eslabones de VC a través del concepto de biorrefinería. Se realizó un análisis funcional (FA) para determinar los cuellos de botella de las VC y la posible integración de las biorrefinerías. Así mismo, se evaluó la sostenibilidad (considerando las dimensiones técnica, económica, ambiental y social) de diferentes escenarios de biorrefinerías para las tres materias primas. Se realizó una evaluación experimental de la AD convencional y modificada considerando diferentes técnicas. Las biorrefinerías fueron evaluadas a nivel de simulación usando los resultados experimentales como datos de entrada. Se propuso un compendio de procesos aguas abajo para incrementar la valorización de las fracciones obtenidas en el proceso de AD convencional y modificada.

Palabras clave: Digestión anaerobia convencional, digestión anaerobia modificada, biorrefinería, biogás, ácidos grasos volátiles, cadena de valor, sostenibilidad

Abstract

Conventional anaerobic digestion (AD) is a widely applied technology for generating renewable energy (biogas) from organic waste. The simplicity of the process and the existing microbial consortium allows the use of different types of waste as substrate. Waste from different value chains (VC) has been used, e.g., agricultural, agro-industrial, and food. AD has multiple metabolic pathways present at each stage of the process. Therefore, it was demonstrated that AD could be designed to produce mixed volatile fatty acids through modified AD. Modified AD corresponds to varying the operating conditions of the process to promote specific metabolic pathways. The integration of conventional and modified AD was proposed as a sustainable pillar for the valorization of three wastes generated in different VC links through the biorefinery concept. A functional analysis (FA) was performed to determine the VC bottlenecks and the possible integration of biorefineries. Likewise, the sustainability (considering technical, economic, environmental, and social dimensions) of different biorefinery scenarios for the three feedstocks was evaluated. An experimental evaluation of conventional and modified AD using different techniques was conducted. The biorefineries were evaluated at the simulation level using the experimental results as input data. A compendium of downstream processes was proposed to increase the valorization of the fractions obtained in the conventional and modified AD process.

Keywords: Conventional anaerobic digestion, modified anaerobic digestion, biorefinery, biogas, mixed volatile fatty acids, value chain, sustainability.

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International conferences participation

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"Investigación y desarrollo experimental de un modelo sostenible de generación de biocombustibles y productos de valor agregado a partir de residuos agrícolas y agroindustriales (biomasa residual) de la industria porcícola en el Valle del Cauca"

Introduction

In recent decades, Anaerobic Digestion (AD) to produce energy (in the form of biogas) has been attracting great interest based on its potential applications and the simplicity of the process [1]. Energy production from AD increased by more than 90% between 2010 and 2018 and was successfully implemented in European countries, the United States, and some Latin American countries [2]. AD presents multiple advantages compared to conventional fermentation. For example, no strict operating conditions are required, and raw materials from different origins can be used [3]. AD is based on organic matter degradation through four stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis). In each stage of AD, a consortium of microorganisms is involved [4]. These microorganisms act synergistically. The "waste" generated in the first stage of the process is consumed by the microorganisms in the next stage until methanogenesis is reached [5]. Waste from different value chains (VC), e.g., agricultural, agro-industrial, and others, have been used in the AD process [6].

Conventional AD produced a biogas composed mainly of CH_4 and CO_2 [7]. The biogas units are implemented for the generation of thermal and electrical energy. In addition, it can be purified for injection into fuels [8]. AD has multiple metabolic pathways at each stage of the process. Therefore, recent research studies have shown that AD could be designed to produce mixed volatile fatty acids, fertilizers, and hydrogen, among others. To Promote these products it is necessary the implementation of modified AD [9]. Modified AD corresponds to the variation of the operating conditions of the process to inhibit the methanogenic microorganisms and promote other routes (e.g., the acidogenic stage) [10]. Volatile fatty acids (VFAs) are low molecular weight carboxylic acids containing six or fewer carbon atoms, including acetic, propionic, butyric, and other acids. The mixed VFAs generated during the AD process are dissolved in the liquid fraction of the digestate [11]. Mixed VFAs can be separated by techniques such as distillation, membrane separation, and liquid-liquid extraction. Most of these methods have disadvantages, such as co-production of other products or additional process steps [12]. Consequently, different strategies have been evaluated to use mixed VFAs as substrates for producing high-value products such as polyhydroxyalkanoates (e.g., PHB), biodiesel, and others [13]. On the other hand, the digestate in AD is a mixture of partially degraded organic matter, microbial biomass, and inorganic compounds [14]. Digestates can contain many undigested materials, nutrients, and trace elements [15]. Digestate directly to soil is currently considered an economically attractive process. During AD, most of the labile organic components are degraded, increasing the stability of the remaining organic matter in the digestate. However, the prevalence of efficiency criteria for energy production (biogas) on an industrial scale may lead to a limited residence time of the material in the digester, producing a digestate that is not completely depleted in readily degradable organic compounds [16].

The integral valorization of all fractions generated in the digestion process (products, by-products, and waste) in a network of facilities results in the biorefinery concept [17]. A biorefinery is a well-designed complex system where biomass is integrally processed or fractionated to obtain more than one product, including bioenergy (i.e., direct energy), biofuels, chemicals, and high-value-added compounds that can only be extracted from biological sources [18]. These characteristics are reached only after the analysis of several valorization routes [19]. The biorefinery concept encompasses the utilization of all fractions generated during the AD process. Multiple production lines are possible from this process. Conventional AD can be performed to obtain biogas and valuable compounds from the remaining fractions [20]. Modified AD (varying operating conditions) can promote other metabolic pathways and generate valuable compounds. Several reviews describe the best routes (according to operating conditions, substrate types, purification techniques, economic analysis, or environmental analysis) for conventional and modified AD. However, the analyses are performed only on a stand-alone basis.

Based on the above, the main objective of this work is to highlight the role of AD as a sustainable pillar for generating energy and producing high-value products through the biorefinery concept. Likewise, to evaluate the potential of implementing this technology in different links of agro-industrial VC (ethanol VC), agricultural VC (cassava VC), and food VC (food VC) through a functional analysis. For this purpose, the sustainability (considering the technical, economic, environmental, and social dimensions) of different biorefinery scenarios was evaluated. Experimental evaluation of conventional and modified AD was performed considering different techniques to promote biogas and mixed VFAs production. The biorefineries were designed considering the technological approach of the context where they would be applied. Likewise, a compendium of downstream processes was proposed to increase the valorization of the fractions obtained in the conventional and modified AD process.

Hypothesis

The mixed volatile fatty acid and biogas production through the biorefinery concept is more sustainable than other waste valorization routes in the cassava, food, and ethanol value chains.

Objectives

General objective

To analyze the sustainability of the mixed volatile fatty acids and biogas production through anaerobic digestion under the biorefinery concept.

Specific objectives

- To characterize the value chains.
- To evaluate the mixed volatile fatty acid and biogas production from raw materials at an experimental level.
- To evaluate mass and energy indicators of different raw material biorefinery scenarios.
- To perform an economic and environmental analysis of the biorefinery scenarios.
- To evaluate the sustainability of the biorefinery scenarios.

1. Chapter 1: Theoretical Framework

1.1. Overview of anaerobic digestion system

1.1.1. Biochemical reactions and steps

AD is a biological and degradative process where a substrate (organic waste) generates biogas without oxygen in the medium [6]. The AD process of biodegradable organic resources consists of four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as seen in **Figure 1.1** [21]. AD is a complex process involving several groups of bacteria and substrates and takes place under strict anaerobic conditions to transform organic matter mainly into methane (CH_4) and carbon dioxide (CO_2), and a minor amount of hydrogen sulfide (H_2S), ammonia (NH_3), and other gases [22]. Each degradation step is carried out by different microorganisms acting in interrelation and require different environmental conditions [23].

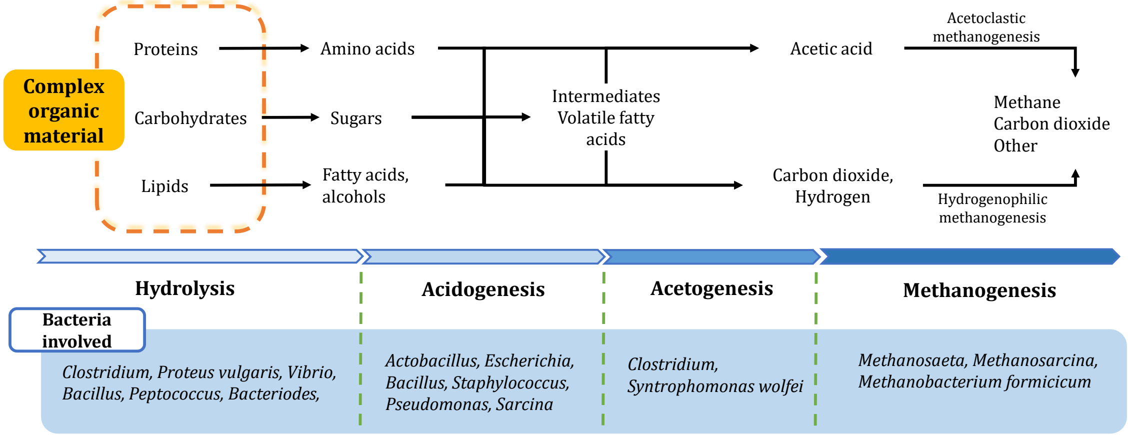


Figure 1.1. Stages of the AD process.

The first stage of the AD process is the hydrolysis. In this stage, complex organic polymers are converted into simple soluble molecules [24]. During the hydrolysis stage, lipids (fats) are converted into fatty acids, carbohydrates (polysaccharides) into simple sugars (monosaccharides), and proteins into amino acids. Different groups of bacteria carry out the hydrolysis step through the excretion of extracellular enzymes (see **Table 1.1**) [25]. Lipases convert lipids to long-chain fatty acids, proteases convert proteins to amino acids, and polysaccharides such as cellulose, starch, and pectin are hydrolyzed to monosaccharides by cellulases, amylases, and pectinases, respectively. Generally, the hydrolysis of carbohydrates takes a few hours, but the degradation of proteins and lipids takes a few days [26].

In the second stage, the soluble compounds produced by hydrolysis diffuse into other bacterial cells (i.e., acidogenic bacteria). These new substrates are converted into mixed VFAs, hydrogen, CO_2, ethanol, and some organic nitrogen and sulfur compounds [27]. The predominant acids produced at this stage are acetic, propionic, butyric, and valeric acids [28]. The acetic acid formed in this stage is directly taken to the last stage, and the other products are taken to the third stage for further degradation by acetogens [29]. Alcohols and mixed VFAs can be decomposed into acetic acid and hydrogen in the process of acetogenesis. As these two processes are very rapid, a sudden drop in pH might occur. In the case of AD based on food waste, the process involves a high rate of hydrolysis indicating that more substrate is available for the acidogenesis bacteria [30].

The third stage of the AD process corresponds to acetogenesis. In this stage, mixed VFAs having more than two carbon atoms (from the acidogenesis stage) are converted into acetic acids, hydrogen, and carbon dioxide by the action of acetogens [31].

Table 1.1. Chemical reactions and some bacteria involved in the AD process.

Stage	**Reactions**	**Process conditions**	**Involved bacteria**	**From-To Conversion**
Hydrolysis	$(C_6H_{10}O_5)_n + nH_2O \rightarrow n(C_6H_{12}O_6)$	T:25–30 ◦C; pH 5.2–6.8; C/N ratio: 10–45; Required C:N:P:S ratio: 500: 15: 5: 3; facultative microorganisms	*Clostridium, Proteus vulgaris, Vibrio, Bacillus, Peptococcus, Bacteriodes,*	Carbohydrates-soluble sugars. Proteins-soluble peptides and amino acids. Lipids-fatty acids or alcohols
Acidogenesis	$C_6H_{12}O_6 + H_2O \rightarrow CH_3COOH + 4H_2 + CO_2$ $C_6H_{12}O_6 + H_2 \rightarrow CH_3CH_2COOH + 2H_2O$ $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2$ $C_6H_{12}O_6 \rightarrow 2CH_3CH_2COH + 2CO_2$ $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$	T: 25–30 ◦C; pH: 5.2–6.5; C/N ratio: 10–45; Generation time:24–36 h; facultative microorganisms	*Actobacillus, Escherichia, Bacillus, Staphylococcus, Pseudomonas, Sarcina, Desulfovibrio, Streptococcus,Veollone, Desulforomonas*	Amino acids-fatty acids, acetate, and others. Sugars-intermediary fermentation products
Acetogenesis	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 2H_2$ $2CH_3CH_2COH + 2CO_2 \rightarrow 2CH_3COOH + CH_4$ $CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$ $CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$ $CH_3CHOHCOOH + H_2O \rightarrow CH_3COOH + CO_2 + 2H_2$	Generation time: 80– 90 h	*Clostridium, Syntrophomonas wolfeii, Syntrophomonas wolfei*	Higher fatty acids or alcohols-hydrogen and acetate. Volatile fatty acids and alcohols- acetate or hydrogen
Methanogenesis	$CH_3COOH \rightarrow CH_4 + CO_2$ $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	Mesophilic: 32–42 ◦C; Thermophilic: 50–58 ◦C; pH: 6.0–8; C/N ratio: 20–30, Generation time: 5–16	*Methanosaeta, Methanosarcina, Methanobacterium formicicum, Methanobrevibacterium*	Acetate-methane and carbon dioxide. Hydrogen and carbon dioxide- methane

		d; Obligate anaerobes microorganisms		

In the last stage, methanogenic bacteria (methanogens) produce methane by consuming acetic acid, hydrogen, and carbon dioxide. Almost 70% of methane is formed from acetic acids by decarboxylation of acetate (Acetotrophic methanogens) [34]. In this type of methanogenesis, acetic acid is decomposed into a carbon dioxide molecule and a methyl group. CO is gradually oxidized and coincides with the release of electrons necessary to reduce the methyl group to methane [32]. The remaining 30% of methane is formed from reducing carbon dioxide with the participation of hydrogen as an electron donor (hydrogenotrophic methanogens) [33].

1.1.2. Microorganisms involved in anaerobic digestion.

The conversions of complex organic compounds into CH_4 and CO_2 are possible due to the cooperation of four different groups of microorganisms [34]. These microorganisms can be counted among primary fermentation bacteria, secondary fermentation bacteria (syntrophic and acetogenic bacteria), and two types of methanogens belonging to the *Archaea* domain. These microorganisms are found in the natural environment and perform various functions during the anaerobic degradation of waste. Cooperation in the population of microorganisms allows the synthesis of certain products later used as food by another group of bacteria [35]. Interspecific transfer of hydrogen during the acetogenic stage allows the growth of syntrophic bacteria (a species living on the metabolic products of another species such as *Syntrophomonas* and *Syntrophospora*). These microorganisms oxidize compounds such as propionate and butyrate [36]. Syntrophic bacteria cannot grow in the form of pure cultures and are only accompanied by microorganisms that use the hydrogen produced by them, for example, mutagenic archaeons [37]. Therefore, syntrophy is an essential process during the stages of digestion in which the decomposition of a compound occurs by the participation of two or more microorganisms, and none of them can use this compound separately. Methanogenic archaeons use the CH_3COOH and H_2 produced by these bacteria to produce methane. Methanogens process a limited amount of simple organic substrates, the most important of which are CH_3COOH, H_2, and CO_2 [38]. De Vrieze et al. [39] analyzed the effect of four different inoculums on the methane production potential. The authors reported a high free ammonia concentration in the mainly animal manure inoculum and increased residual VFAs concentrations in the energy crops and manure inoculum indicating an unstable methanogenic community.

1.1.3. Parameters in anaerobic digestion

1.1.3.1. Feedstock

Any source of organic matter is considered suitable for being implemented in AD [40]. The preference lies in using raw materials of residual origin. This contributes to mitigating waste and adverse effects (economic, social, and environmental) [41]. In addition, conflicts related to using crops for food consumption and generating high-value products are avoided [42]. The most solid wastes used in AD include agricultural, livestock, sewage sludge, municipal solid waste (organic fraction), and food waste. For liquid wastes, wastewater, agro-industrial, chemical, food processing, and pharmaceutical industries have been used [43]. The biochemical characteristics of the raw material to obtain the previously mentioned products should favor the development and microbial activity of the system. **Table 1.2** presents some results of biogas, mixed VFAs, and hydrogen production from different feedstocks.

Lignocellulosic waste comprises mainly crop residues. These residues are difficult for microorganisms to digest due to the chemical composition (high cellulose, hemicellulose, and lignin content) [44]. Then, the residues could be submitted to previous processes (pretreatments) in order to favor hydrolysis [45]. Moreover, due to the high C/N ratio, several studies have evaluated the co-digestion processes of lignocellulosic waste and other organic materials.

The constant economic growth, urbanization, industrialization, and accelerated obsolescence of products and consumer waste have led to a progressive increase in MSW (Municipal solid waste) [46]. MSW consists mainly of food waste, paper and cardboard, yard trimmings, wood, plastic, metal, and glass. Almost 60% of MSW is composed of organic matter, followed by paper-cardboard (13%), and plastics (10%), among others. Organic wastes are a unique case within biomass where valorization into renewable energy and high-value products through AD represents one of the most attractive processes for utilization [47].

Table 1.2. Results for biogas, mixed VFAs, and hydrogen production from different feedstock.

Mixed VFAs production							
Feed stock	**Operation condition**	**Yield (g VFAs/g VSS)**	**VFAs %vol**			**Remarks**	**Ref**
			Acetic	**Propionic**	**Butyric**		
Organic solid waste	pH:10, TR: 10 days, T: 30°C, Scale: Laboratory	0.832	70	7	13	Seven organic waste streams were treated. Slaughterhouse wastewater produced the highest mixed VFAs yield.	[48]
Food waste	pH:6, TR: 20 days, T: 30°C, Scale: Laboratory	0.918	70	5	17	Mixed VFAs were significantly improved using anaerobic activated sludge to inoculate food waste.	[24]
Food waste	pH:6, TR: 17 days, T: 30°C, Scale: Laboratory	0.79	30	2	60	The effects of redox potential (ORP) and inoculum on the production of mixed VFAs were evaluated.	[49]
Livestock and poultry waste	pH:5.5, TR: 4 days, T: 35°C, Scale: Batch reactor	0.67	-	-	-	The effect of pretreatment and feed-to-microorganism ratios on the rapid generation of mixed VFAs was investigated.	[50]
Waste activated sludge	pH:9, TR: 6 days, T: 55°C, Scale: Semi-continuous reactor	0.423	-	-	-	The sludge was subjected to a gradual increase in pH from 7 to 10. Maximum acidification was obtained at pH 8.9.	[51]

Municipal organic waste	pH= 4.8-5.7, TR: 10 days, T: 55°C, Scale: CSTR reactor	0.28	31-41	2-7	18-65	Hydrogen production was evaluated under thermophilic acidogenic conditions. In addition, the best operating conditions for the process were evaluated.	[52]
Food waste	pH:6, TR: 5 days, T: 35°C, Scale: CSTR reactor	0.31	31	7	42	Different operating conditions (pH, temperature, and OLR) were evaluated in producing mixed VFAs from food waste to achieve maximum yields.	[53]
Food waste	pH:6, TR: 17 days, T: 30°C, Scale: Batch reactor	0.79	15	26	50	Mixed VFAs were produced from three different substrates (glucose, peptone and, glycerol).	[54]
Starch industrial Wastewater	pH:6, TR: 10 days, T: 25°C, Scale: Batch reactor	0.78	40	-	25	The effect of varying the ratio of starch-rich wastewater to municipal wastewater on the production of mixed VFAs was studied.	[55]
Vinasses	pH:5.5, TR: 10 days, T: 25°C, Scale: Batch reactor	0.621	25	-	54	The potential of vinasse as a substrate for producing biohydrogen and mixed VFAs was evaluated.	[56]
Biogas production							
Feed stock	**Operation condition**	**Yield (m^3/kg VS)**	**Comments**				**Ref**

Potato waste	pH:7.64, TR: 35 days, T: 37°C, Scale: CSTR reactor	435.7	Gradually increasing the organic loading rate from 1.0 to 5.0 kg VS/m^3-d improved methane yield.	[57]
Kitchen waste	pH:7.5, TR: 45 days, T: 35°C, Scale: Laboratory	179.8	The effect of different initial pH (6.0, 7.0, 7.5, and 8.0) on laboratory-scale AD of kitchen waste was investigated.	[58]
Food waste	pH:7.1-7.5, TR: N.R, T: 35°C, Scale: CSRT reactor	344	The effects (temperature and substrate characteristics) on process stability and microbial community structure were studied.	[59]
Food waste	pH:6.8, TR: 302 days, T: 35°C, Scale: Batch reactor	388	The effects of organic loading rate (OLR) and temperature on the co-digestion of food waste and residual activated sludge were evaluated.	[60]
Municipal food waste	pH:7.64, TR: 17,5 days, T: 37°C, Scale: CSRT	444.7	The yield and kinetic constants of mesophilic anaerobic reactors operated at increasing organic loading rates were evaluated.	[61]
Fruits and vegetables waste	pH:7.4, TR: 30 days, T: 35°C, Scale: CSRT, co-digestion: slaughterhouse waste + manure: 11-8-7	320	The co-digestion process (slaughterhouse waste + manure) was evaluated to reduce the volatile solids content of fruit and vegetable waste.	[62]
Cow manure	pH:7.5, TR: 38 days, T: 35°C, Scale: CSRT, co-digestion of grass	188	A 1:4 ratio of manure to crop residues promotes biogas production.	[23]

	silage, sugar beet tops and oat straw			
Bio hydrogen production				
Feed stock	**Operation condition**	**Yield**	**Comments**	**Ref**
Food waste and brown water	pH:5-5.5, TR: 133 days, T: 37°C, Scale: two-phase CSTR	99.8 mL H_2/g Vs_{added}	The optimum Hydraulic Retention Time (HRT) of the two-stage anaerobic digester system for hydrogen and methane production was determined.	[63]
Cassava wastewater	pH:5.5, TR: 40 days, T: 37°C, Scale: two-phase continuous UASB	39.83 L H_2/kg $COD_{removed}$	Hydrogen production from wastewater cassava starch production was maximized using two stages of anaerobic up-flow anaerobic sludge blanket sludge (UASB) reactors.	[64]
Sugarcane juice	pH:4-5, TR: 213 days, T: 30°C, Scale: Continuous EGBS	0.73 mol H_2/mol hexose	The influence of hydraulic retention time (HRT) on hydrogen production in three expanded granular sludge bed reactors (GSLRs) was evaluated.	[65]
Food waste	pH:5.5, TR: 18 days, T: 37°C, Scale: Semi-continuous	14.66 mL/VS_{added}	The production and recovery performance of mixed VFAs and hydrogen using food waste through a submerged membrane was investigated.	[66]

Generally, in most countries, livestock farming is in constant development and manure is mostly used as fertilizer. Nevertheless, the manure abundance exceeds the demand for fertilizer production [67]. Several studies have been put forward to use animal manure as a substrate to be implemented in AD [68], [69]. Manure mono-digestion generates low biogas yields due to nutrient imbalance and ammonia inhibition (low C/N ratio). Generally, livestock manure contains high nitrogen content: chicken manure (1.03%), cow manure (0.35%), fresh goat manure (1.01%), and pig manure (0.24%) [70]. In this sense, co-digestion techniques of animal manure with other organic matter have been proposed to solve these limitations [71], [72].

1.1.3.2. Inoculum

As mentioned above, AD of organic matter is carried out by a consortium of microorganisms in sequential stages, resulting in a synergistic action [73]. The quality and quantity of inoculum added to the digestion process are key factors determining the product's quality. In addition, selecting the waste-to-inoculum ratio is crucial, as well as evaluating the anaerobic biodegradability of solid wastes [74]. Thus, several inoculums have been used for biogas production. For instance, Forster et al. [75] determined swine wastewater, rumen, and sewage sludge as promising inoculums for biogas production due to the high methanogenic bacteria content.

1.1.3.3. Operational parameters

Several studies have evaluated the effects of operating conditions, such as pH, temperature, organic loading rate, retention time, substrate, and inoculum, among others, on AD process for generating energy and high-value products [76]. In this section, the main differences in the operating conditions to favor some routes of the AD process are described. **Figure 1.2** shows the main differences between the operating conditions for the analyzed routes.

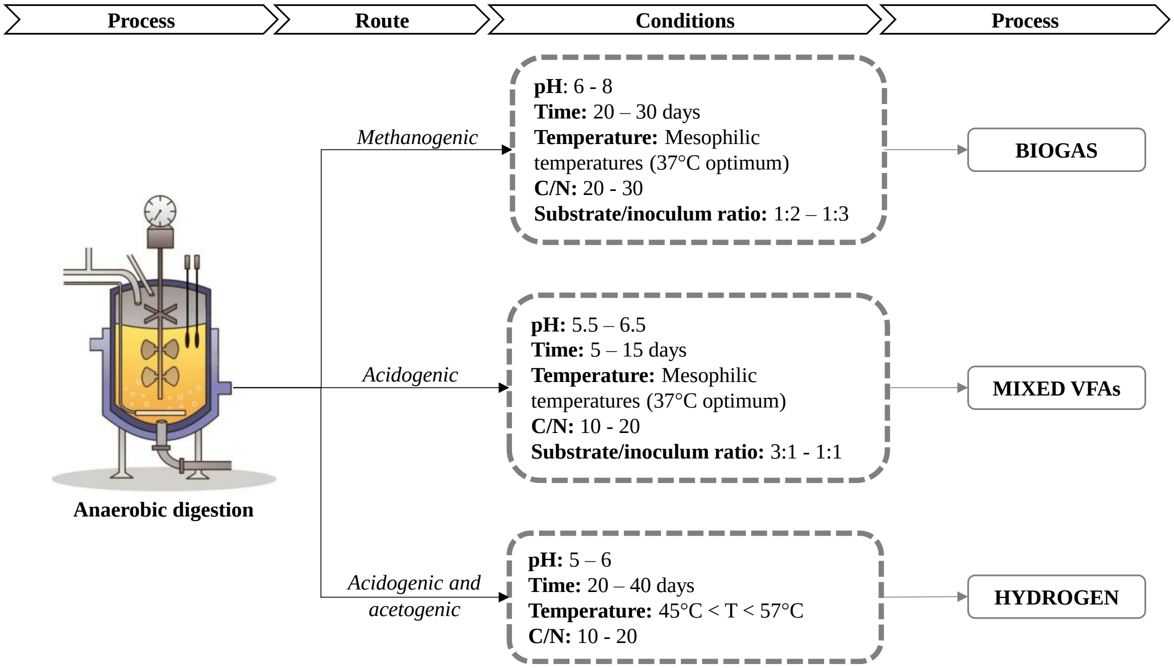


Figure 1.2. Main differences between the operating conditions for the analyzed routes.

Small changes in pH levels affect the anaerobic process. Methanogenic microorganisms are more susceptible to pH variations than other microorganisms in the anaerobic microbial community [77]. The different bacterial groups in the AD process have optimal activity levels around neutrality [78]. The pH value in the biodigester not only determines the biogas production but also the composition [22]. Low pH values reduce the activity of methanogenic microorganisms, causing the accumulation of acetic acid and hydrogen. Consequently, propionic acid degrading bacteria might be severely inhibited, causing excessive accumulation of mixed VFAs [79]. The optimum pH for hydrolysis and acidogenesis is in the range of 5.2 to 6.3 [80]. Most acidogens could not survive at either very low pH (<pH 3) or very high pH (>pH 12) [51].

The AD process is strongly temperature dependent. The reaction rate of biological processes depends on the growth rate of the microorganisms involved and the medium temperature [81]. As the temperature increases, the growth rate of the microorganisms increases, and the digestion process is accelerated, resulting in higher biogas yields [78]. Nevertheless, abrupt temperature variations in the biodigester can generate destabilization of the process. Anaerobic microorganisms can tolerate three temperature ranges:

psychrophilic (below 25°C, not very applicable), mesophilic (between 25 and 45°C, most commonly used), and thermophilic (between 45 and 65°C) [22]. Temperature affects mixed VFAs production because of the effect on microbial growth. Many acidogens thrive optimally at mesophilic temperatures. According to studies presented in open literature, increasing the temperature to 45 °C for biohydrogen production improves the production of H_2 from potato peel waste [82]. Likewise, a higher temperature of 57 °C promotes the maximum hydrogen production from palm oil mill effluent [83].

Hydraulic retention time (HRT) refers to the time the substrate is stored in the digester [84]. Generally, the HRT varies between 10 and 40 days for mesophilic microorganisms. For thermophilic microorganisms, the retention can last 14 days [85]. Short retention times are preferred for hydrogen-producing bacteria since volatile fatty acids and hydrogen are produced in the exponential phase and alcohols in the stationary phase. As methanogenic bacteria consume hydrogen to produce methane and carbon dioxide, a higher hydrogen yield is obtained when inhibited. Conversely, there is a decrease in methane production by methanogenic bacteria at short retention times [86].

Most organic matter is potentially applicable to AD processes [1]. The yield and quality of the final product might be influenced by the composition and nature of the feedstock. Carbon and nitrogen are the main energy sources and feed for forming new cells of methanogenic microorganisms. These microorganisms consume approximately 30 times more carbon than nitrogen, so the optimal ratio reported for these two elements is 30:1 [87]. The decomposition of organic matter with high carbon content (>35:1) occurs slowly because the multiplication and development of bacteria is low due to the absence of nitrogen, but the biogas production period is longer. On the other hand, with a C/N ratio lower than 8:1, bacterial (methanogenic) activity is inhibited due to the formation of excessive ammonium content, reducing the pH of the medium and consequently favoring the production of mixed VFAs [80].

1.1.4. Products derived from anaerobic digestion.

A promising alternative to produce high value products (e.g., mixed VFAs, hydrogen) is using modified AD to minimize the release of carbon dioxide and methane [88]. There are several techniques to achieve this objective, mainly by changing the operating conditions of the process (pH, temperature, agitation speed, time, raw material, etc.) [89]. These techniques consist of inhibiting methane-producing microorganisms and favoring other routes or stages to the process. This means it is prevented the methanogenesis process from occurring to ensure that only the desired products are obtained in higher volumes [90].

1.1.4.1. Biogas

The biomass-to-energy conversion has been constantly increasing to reduce the environmental impact generated by exploiting and consuming non-renewable energy sources [91]. Biogas is generated in natural media or specific devices by biodegradation reactions of organic matter through the action of microorganisms in the absence of oxygen [92]. Currently, most methane consumption and utilization come from natural gas resources, but biomethane production from waste recovery approaches has increased significantly. The production potential has improved by 4% in 9 years (from 2010 to 2018) [93]. Developed countries use large-scale advanced plants to utilize biogas. Biogas is regularly applied to generate heat, power, and electricity. In addition, several industrial applications are being developed in biogas plants as a substitute for natural gas [94]. In the European Union and North America, biogas plants have been more developed than in other continents during the last 40 years. The main advantages of the units located in the mentioned regions are industrial scale, energy efficiency, and high level of complexity [95]. Academic centers and governments considered biogas production because of the potential to respond to different global challenges [96]. Moreover, biogas technologies allow industries to reduce greenhouse gas (GHG) emissions and pollution from waste disposal. Due to the renewable nature, these technologies also provide a broad spectrum of energy utilization, such as heat, electricity, and transportation. Another advantage of biogas production is the applicability in rural areas with limited access to energy sources [97]. Thus, the calorific value of biogas is estimated to be around 5300 kcal/m^3 and is associated with the methane content. The presence of inhibitors during the process (e.g., volatile fatty acids) and compounds such as CO_2 H_2S, NH_3, H_2O, N_2, and siloxanes in the product decreases the product yield and calorific value when compared to natural gas (see **Table 1.3)** [98].

Table 1.3. Composition of biogas and natural gas.

Compound	**Unit**	**AD Biogas**	**Natural gas**	**Biogas utilization impact**
CH_4	%vol	53-70	81-89	
CO_2	%vol	30-50	0.67-1	Decreasing calorific value, antiknock properties of engines and corrosion
N_2	%vol	2-6	0.28-14	Decreasing calorific value, antiknock properties of engines and corrosion
O_2	%vol	0-5	0	Corrosion, fooling in cavern storage, risk of explosion
H_2	%vol	N. R	N. R	
Higher hydrocarbons	%vol	N. R	3.5-9.4	
H_2S	ppm	0-2000	0-2.29	Corrosion, catalytic converter poison, emission, and health hazards.
NH_3	ppm	<100	N. R	Emission, anti-knock properties of engines and corrosion when dissolved
LHV	MJ/Nm^3	23	40	
Density	kg/Nm^3	1.1	0.84	

LHV: Low heating value; N.R no report

Due to biogas applicability as a fuel, in addition to complying with the specific regulatory standards for each country, various biogas refining methods and different techniques or methodologies have been developed to control these inhibitors [99]. **Figure 1.3** shows some biogas upgrading technologies. To date, the most widely used technology is adsorption (water washing) [100]. Moreover, biogas has the potential to generate electricity in power plants where the most used generation methods are internal combustion engines or gas turbines [101].

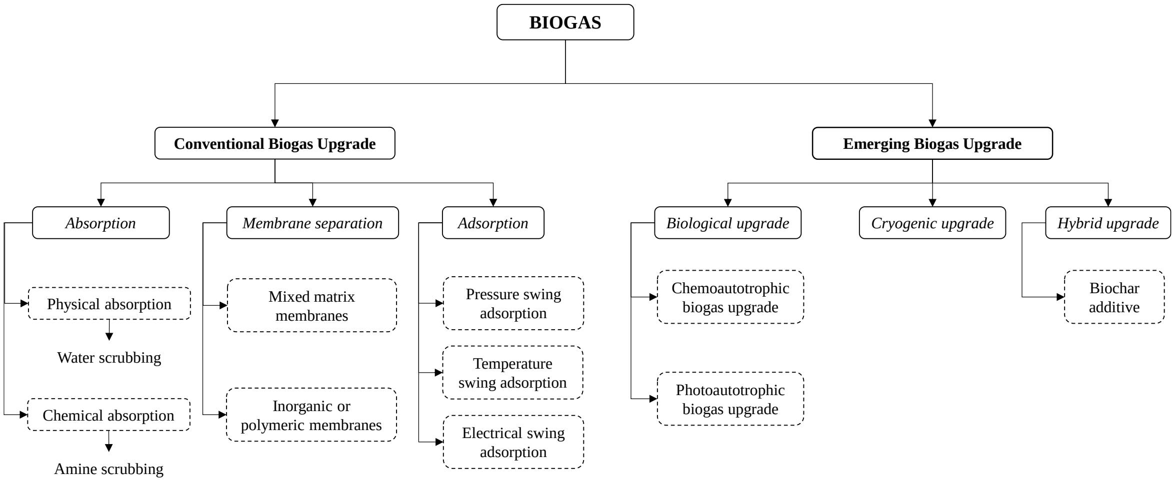


Figure 1.3. Biogas upgrading technologies.

1.1.4.2. Hydrogen

The third stage of the digestion process (acetogenesis) plays a key role in biohydrogen production [102]. In this stage, the mixed VFAs are converted to acetate and hydrogen. When hydrogen production is promoted, the specific gas can be generated during treatment or after digestion [103]. Currently, about 85% of the hydrogen produced in the world is obtained from reforming fuels, and the remaining 15% is obtained through electrolysis or electrolysis of water. In 2019, the industry produced and consumed approximately 70 tons of hydrogen. Generally, the hydrogen produced currently is consumed in hydrocracking and desulfurization processes for the crude oil refining industry or for ammonia production when combined with nitrogen in chemical industries [104]. Pure hydrogen is used in many applications and can be found in the production of many common industries. Hydrogen is also used in hydrogen fuel cells, producing energy for vehicles and other systems [105]. Moreover, hydrogen fuel cells are a developing industry due to the incapability to minimize size concerning energy potential when using hydrogen successfully. Nevertheless, the energy-to-mass ratio in the process is extremely high, and the energy-to-volume ratio is extremely low [106].

1.1.4.3. Bio-hythane

Hydrogen production can also be carried out through fermentative routes. Dark fermentation is considered to be the most efficient method [107]. Experimental yields of H_2

production fail to reach the theoretical yields due to problems such as high operating costs and the formation of inhibitory metabolites (e.g., mixed VFAs) [108]. AD is the best way to utilize mixed VFAs for CH_4 production in the form of biogas. This process also promotes H_2 production during the first three steps of digestion due to the coexistence of methanogens with H_2 producing bacteria in the microbial consortium [109]. However, the conventional (one-step) AD process is structured to result in only CH_4 as a major part of the biogas with only trace H_2. By designing the AD as a two-stage process for the co-production of H_2 and CH_4 simultaneously instead of the production, the thermal efficiency of the biogas could be improved [110]. H_2 and CH_4 could complement each other and the production in the form of "hitane" is gaining attention as a valuable fuel. Hythane is significantly advantageous over biogas in terms of high flammability range due to the presence of H_2, as the flame speed is equal to seven times that of CH_4. The term "hythane" is being replaced by "bio-hythane" since organic waste is used as a substrate in the production process [111]. Bio.hytane is reported to be composed of 5-30% hydrogen and 50-60% methane [112].

1.1.4.4. Bio-based products

Digestate, in addition to biogas, is a mixture of microbial biomass and undigested material produced in large quantities as a by-product of AD [113]. Digestate is usually separated mechanically into liquid (70%vol) and solid fractions (30%vol) to be stored separately for easy handling and transport. The liquid fraction contains a large part of N and K, while the solid fraction comprises many residual fibers and phosphorus [114]. Thus, digestate has been implemented as a soil improver or fertilizer during the last decades. Digestate application as a fertilizer represents an economic and environmental opportunity due to the generation of a value-added by-product [115]. In addition, this solid fraction represents an opportunity to substitute chemical fertilizers that have proven to be a source of significant environmental pollution. For instance, Walsh et al. indicated that, unlike commercial fertilizers, liquid digestate can maintain or improve grassland crop yields and, at the same time, reduce nutrient losses to the environment [116]. Nevertheless, the use of digestate for land application has also posed certain drawbacks. For example, since digestate must be stored as the immediate implementation is not feasible, the consequent gases loss (CH_4, CO_2, NH_3, and N_2O) contribute to environmental issues [117]. Dragicevic et al. mention that digestate has a low nutrient retention capacity and groundwater could be contaminated due to possible leach [118]. Different digestate valorization routes have been evaluated, showing obtaining products through various thermochemical technologies such as gasification and pyrolysis, leading to the production of biofuels and biochar [119]. These alternatives promote a circular economy, close production cycles, and maximize economic and environmental benefits [120]. **Table 1.4** presents the digestate composition under different feedstock sources for biogas generation.

Table 1.4. Digestate composition obtained from different feedstock sources.

Parameter	**Unit**	**Grass**	**Organic waste**	**Food waste**	**Poultry manure**
Ms	%	8.12	14.05	3.83	7.8
pH	-	7.8	7.8	7.9	7.9
N total	kg/mg	5.56	6.64	6.29	6.7
C total	% wt	36.2	29.1	36.2	35.1
C/N	-	5.29	6.15	2.18	4.09
P	%wt	0.906	0.604	1.5	1.83
K	%wt	5.59	2.48	4.1	4.9
S	g/kg rm	0.906	0.604	1.5	1.83
Mg	g/kg rm	1.86	3.87	3.62	2.76
Ca	g/kg rm	0.541	0.71	0.286	0.879
Na	g/kg rm	0.592	8.26	50.3	3.83

rm: Raw material

1.1.4.5. Byproducts: Volatile Fatty Acids

Microbial processes have been categorized as possible routes to produce mixed VFAs through pure cultures to obtain a specific fatty acid or through mixed cultures by the AD process [121]. The production of mixed VFAs from microbial cultures allows the utilization of renewable feedstocks, representing an advantage compared to conventional routes. In addition, mixed VFAs production generates safer products for human health and offers high product selectivity. Consecutively, mixed culture microbial processes present certain advantages compared to pure cultures in terms of utilization of several feedstocks (e.g., food waste, agricultural waste, sewage sludge). In addition, this process allows energy savings by operating in non-sterile conditions [122]. AD is a constituted used technology in various countries for waste valorization in biogas production [123]. Several investigative approaches have been proposed to finalize the AD process at the acidogenic stage for mixed VFAs production (termed acidogenic fermentation) [124]. The different reports of mixed VFAs production from AD have started to improve in terms of increasing efficiency, optimizing operating conditions, providing a renewable and sustainable source as substrate, defining and evaluating microbial communities with the respective interactions, and new separation techniques [50].

1.2. Volatile Fatty Acids production

Pure VFA production is generated from conventional thermochemical processes (90%) [125]. Chemical synthesis has high production yields. Nevertheless, by using non-renewable sources as raw materials, this process presents high environmental pollution. Alternative bio-based routes cover the remaining percentage. These routes have lower yields compared to conventional routes [126] The wide industrial interest in pure VFAs is due to the various applications offered [127]. Moreover, the most marketed pure VFAs are acetic, butyric, propionic, valeric and caproic acid.

1.2.1. Acetic acid

Acetic acid (CH_3COOH) is the most widely used organic acid and one of the most commercially important pure VFAs [128]. Acetic acid is a product with a wide range of applications in, for instance, the pharmaceutical, food, and textile industries. Over 65% of production is directed to manufacture polymers derived from vinyl acetate or cellulose acetate [129]. On the other hand, the world demand for 2020 was estimated at 16.1 million tons [130]. The production of acetic acid can be carried out by different technologies. Traditionally, thermo-catalytic routes have been the predominant ones in the chemical industry, prevailing in methanol carbonylation, where methanol with excess carbon monoxide from synthesis gas is used [131]. Regarding biological production routes, several microbial strains have been investigated for acetic acid production, including *Acetobacter Thermoanaerobacter*, among others [132].

1.2.2. Propionic acid

Propionic acid ($CH_3CH_2C_2H$) is a colorless water-soluble organic acid with a characteristic odor. The propionic acid market generated USD 1200 million in 2018 [133]. Propionic acid has a high commercial level and is used in various industries. This chemical is widely used to manufacture herbicides; and regarding the food industry, this acid is used for emulsions or as a preservative because of the various bacteria growth inhibition [134]. Commercial production of propionic acid is mainly through chemical synthesis. Generally, three routes are used: (i) carboxylation of ethylene with carbon monoxide and water, (ii) ethylene hydroformylation/ethylene oxidation, and (iii) direct hydrocarbon oxidation. On the other hand, propionic acid biosynthesis is mainly carried out by using bacteria of the genus *Propionibacterium*. Several strains, such as *P. acidipropionici* and *P. freudenreichii*, produced propionate from hexoses and pentoses [135].

1.2.3. Butyric acid

Butyric acid ($C_4H_8O_2$) is a colorless oily liquid with an unpleasant odor. This acid is naturally found esterified in animal fats and vegetable oils [136]. The global market for butyric acid derivatives is estimated to reach USD 170 million by 2026 [137]. Butyric acid and the derivatives have many applications in different industrial sectors (pharmaceutical, food, polymeric). Industrial production of butyric acid is mainly carried out by chemical synthesis during the oxidation of butyraldehyde obtained from propylene by oxo synthesis. This route is the most attractive from an economic point of view. Nevertheless, the food industry does not use chemically obtained butyric acid [134]. Thus, biological production is performed through fermentation using different microorganisms (e.g., *p.Butyrivibrio, Butyribacterium, Clostridium, Eubacterium*). *Clostridium* bacteria is the most used industrially due to the high productivity and ability to use different substrates as carbon sources [138].

1.2.4. Mixed Volatile fatty acids by anaerobic digestion

1.2.4.1. Upstream process of mixed volatile fatty acids production

The performance of the modified AD process to produce VFAs can be improved. Pretreatments have proven an interesting approach to increase the mixed VFAs production yield [139]. Pretreatment is generally performed when feedstocks are difficult for microorganisms to degrade (e.g., lignocellulosic wastes). The application of this approach focused on VFAs production is scarce. **Table 1.5** presents report on the pretreatment of different feedstocks to obtain better VFAs production yields. The main pretreatments involve physical, chemical, physicochemical, and biological processes. The pretreatment type selection must address not only the performance of the process but also the economic feasibility of the process to be implemented and applied. Techno-economic analyses of biomass pretreatment systems applied to digestion processes often lack a basis for direct comparison due to different feedstock properties and system designs. Roger Kim et al. [140] evaluated various thermochemical pretreatment strategies (acid, alkaline, sulfite) techno-economically for anaerobic manure digestion. Moreover, three biogas utilization scenarios (electricity, biomethane, liquefied biomethane) were considered to determine the break-even price of each biogas byproduct at which the technology becomes economically viable in a North American context. The techno-economic analysis revealed that pretreatment with moderate acid works best for larger facilities (≥5000 animal units), while very alkaline pretreatment is preferred for smaller facilities. Rufino et al. [141] evaluated the technical and economic feasibility of alkaline pretreatment (NaOH) to improve AD of activated sludge. The economic analysis performed in this work showed that if the pretreatment was performed with an alkali dose of 0.08 g NaOH/g TS, only an increase in methane yield of 60 % could compensate for the cost of the chemicals.

Table 1.5. Results of different pretreatment techniques for VFAs production.

Pretreatment		**Feedstock**	**Results**	**Remarks**	**Ref**
Chemical	Alkaline	Activated sludge	12.5-fold increase in VFAs recovery	NaOH was used to adjust the pH to 10	[142]
	Acid		15.3-fold increase in VFAs recovery	HCl was used to adjust the pH to 3	

	Nitrous acid	Activated sludge	3.7-fold increase in VFAs recovery	Reduced fermentation times were achieved by improving hydrolysis.	[143]
	Alkali	Primary sludge	4-fold increase in VFAs recovery	Pretreatments with three alkalis ($NaHCO_3$, Na_2CO_3 and NaOH) were applied.	[144]
Physical	Heat treatment	Activated sludge	VFAs recovery was increased 6.8-fold.	It was determined that sludge pretreated at 100 °C for 60 min can achieve maximum hydrolyzation.	[145]
	Heat treatment	Food waste	30.53% increase in VFAs production was achieved.	The heat treatment was performed in an autoclave at a temperature of 121 °C for 30 min.	[146]
	Microwave		A 4.74% increase in VFAs production was achieved.	For microwave pretreatment 700 W; 170 C; 30 min were chosen.	
Physico-chemical	Thermal-alkaline	Food waste	VFAs production increased by more than 60%.	The raw material was exposed to alkaline treatment at pH 12 for 30 minutes using NaOH.	[146]

	Expansion/ explosion of ammonia fiber	Lignocellulosic waste (bagasse)	Achieved 21% increase in VFAs production	An ammonia/raw material ratio of 1.5, a temperature of 93 °C, and a time of 15 minutes were used.	[147]

1.2.4.2. Downstream process of mixed volatile fatty acids production

One of the main challenges for using mixed VFAs from AD is the recovery process since mixed VFAs form an azeotropic mixture with H_2O. A multi-phase enrichment and separation process is generally required to obtain marketable products from biomass transformation effluents [148]. Separation and recovery are even more difficult for individual VFAs, rather than mixed VFAs. Then, some studies cover these issues. Lopez-Garon and Straathof [149] have provided a detailed review of the recovery of individual carboxylic acids from pure culture fermentations. While the general processing steps are likely similar, recovery of mixed VFAs is more complicated due to the mixture of acids that must be separated for sale as individual chemicals. Before choosing a separation process, using the mixed VFAs as a product should be considered, as this will influence downstream processing [132]. The recovery process should selectively focus on mixed VFAs over other fermentation broth components. Many methods of mixed VFAs recovery have been evaluated. Among these methods, liquid-liquid extraction, electrodialysis, nanofiltration, adsorption, and ion exchange are analyzed. **Table 1.6** presents some results reported in the literature. Pure VFAs recovery should consider those mentioned above in the previous section. Few reports of the economic analysis of the recovery process of mixed VFAs obtained from AD have been found in the open literature. The main difficulties reported in the literature are related to the range of VFAs produced and their separation routes. Bonk et al. [150] attempted to solve this problem by assuming a selling price for the VFAs, indicating the maximum allowable purification cost. Mixed VFAs was assumed to separate into their acids, creating numerous product streams. Considering organic waste as raw material, a maximum production cost of US\$14.96/m^3 of effluent was achieved. On the other hand, Fasahati [151] performed an economic evaluation of VFAs production by AD from algae and the separation of VFAs from the fermentative broth through different distillation columns. In addition, the economic variation of the process by integrating membrane distillation to increase the concentration of recovered VFAs was evaluated. This analysis was carried out with Aspen Plus v8.4 software. The selling price of VFAs was determined to reach a break-even point after 10 years of plant operation of 384 USD/ton. These results are compared to the current price of acetic acid (1200-1500 USD/ton), thus showing the economic advantages of this process.

Table 1.6. Some reports on mixed VFAs separation techniques

Separation technique	**Feedstock**	**Characteristics of the separating agent**	**Mixed VFAs selectivity**	**Ref**
Absorption and ion exchange	Food waste	Amberlite IRA-67 and activated carbon were used as sorbents.	Predominant to recovery of butyric acid followed by acetic acid	[152]
Distillation	Liquid effluent from palm oil production	Pilot scale distillation unit	Predominant to butyric acid recovery followed by acetic acid	[153]
Electrodialysis	Sucrose solution	Anionic and cation exchange membrane stack (AEM and CEM)	Predominant to acetic acid recovery followed by butyric acid	[154]
Liquid-liquid extraction	Sugar solution	-	Predominant to butyric acid recovery followed by acetic acid	[10]
	Sewage sludge	TOA in n-octanol	Predominant to butyric acid recovery followed by acetic acid	[155]
Membrane extraction	Synthetic VFAs solution	Commercial membrane	Predominant in the recovery of acetic acid	[148]

1.2.4.3. Mixed volatile fatty acids applications

VFAs generated from AD are characterized by being in the liquor interacting with each other. Due to the difficulty and limitations of the methods described above in separating the acids, several alternatives have been proposed to use the mixed VFAs as substrates to generate high-value products such as bioplastics and biofuels.

1.2.4.3.1. Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates (PHA) are biodegradable polyesters that can be produced from biological routes using renewable resources [156]. This plastic represents a promising alternative for the substitution of plastics derived from non-renewable resources. The production of PHA has the limitation that it requires high production costs, between 5 to 10 times compared to conventional technology [157]. PHA production by biological routes is conventionally performed by pure microbial cultures. The production yield of PHA is significant, however, it requires sterilization pretreatments, the selection of a specific substrate and, subsequent purification processes that raise production costs considerably [148]. In this sense, the production of PHA by residual organic sources represents a promising alternative. In recent years, mixed microbial cultures have been used to reduce the cost of PHA production. Several microorganisms such as *Alcaligenes eutrophus, Bacillus megaterium, Rhizobium*, among others, can consume VFAs as carbon sources to produce PHA [158]. Moreover, PHA production using organic wastes does not require sterility, making it much more attractive than pure microbial culture. Reis M et al. [11] mention that a 50% reduction in production cost is achieved.

1.2.4.3.2. Lipids

Several studies have shown that waste derived mixed VFAs can be converted into microbial lipids for biodiesel production [159].. Fei et al. [160] investigated the use of mixed VFAs for microbial lipid accumulation in *C. albidus* cultures and achieved a lipid content of up to 27,8 % with a lipid yield in mixed VFAs of 0,167 g/g of C. albidus at acetic, propionic, and butyric levels. On the other hand, Park et al. [161] used rice straw residues in AD for mixed VFAs production and subsequent conversion to major compounds for biodiesel production. Within the results, they identified that mixed VFAs derived from rice straw waste resulted in a yield of 0.43 g VFAs/g substrate and a 40 % higher specific growth rate (0,305 h-1) than synthetic VFAs. VFAs as a carbon source resulted in a cetane number of 56-59, which is suitable for biodiesel production.

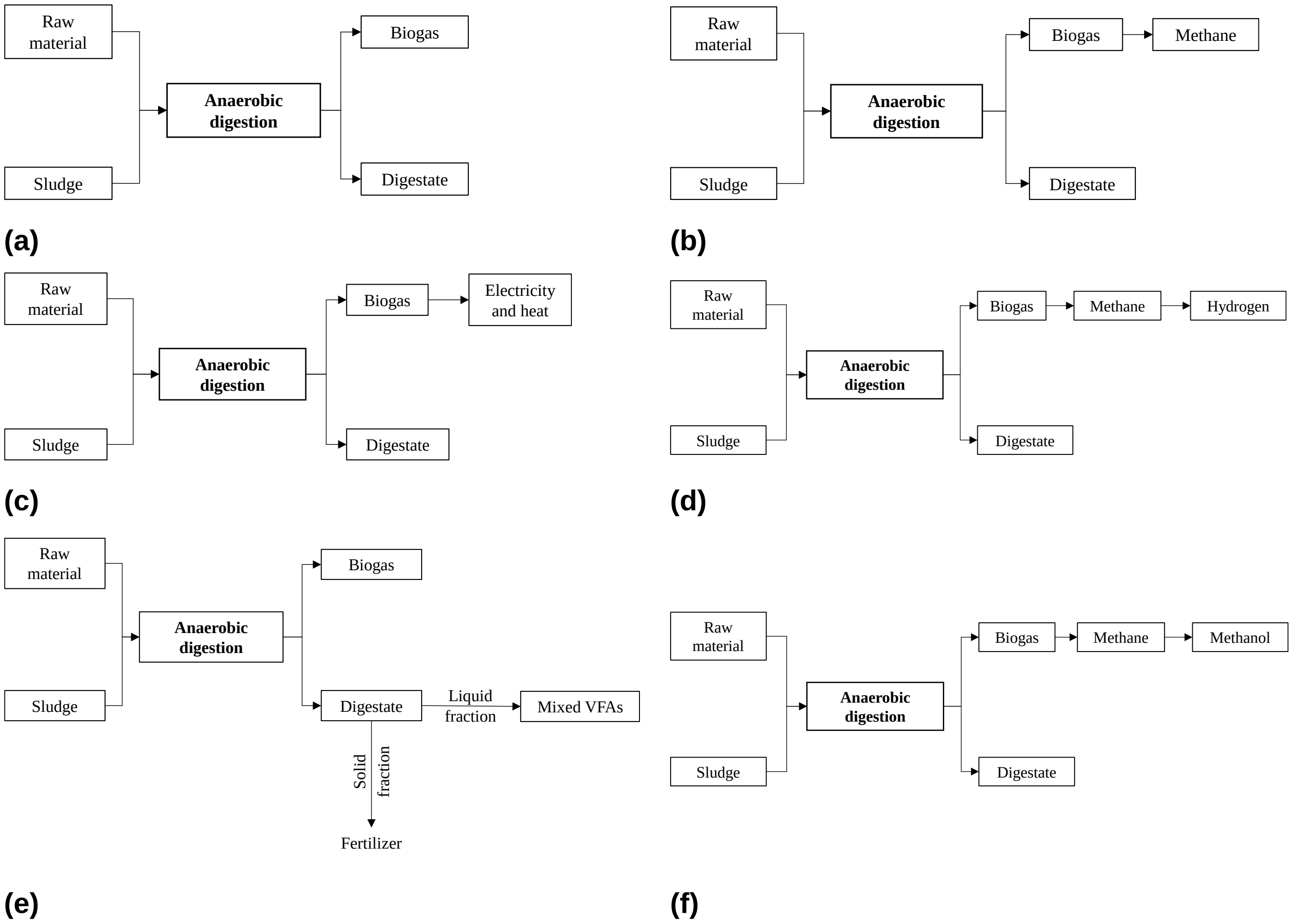
1.2.4.3.3. Nutrient removal

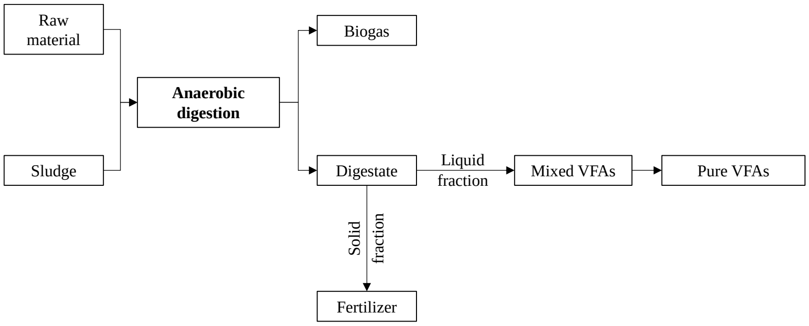
Due to the versatility of mixed VFAs produced from AD, other valorization alternatives have been proposed that are highly promising. For example, mixed VFAs can be used as an easily degradable and cost-effective carbon source for biological nutrient removal processes in wastewater treatment plants [162]. As reported by Shen et al. [163] mixed VFAs are only present in small amounts in wastewater, thus the addition of VFAs is required. Synthetic VFAs can be used as an additional carbon source, but represent additional costs, so as an economical solution, mixed VFAs can be produced on site through sludge AD and then introduced into the treatment steps in the process. Simon G [20] mentions that the production of sufficient mixed VFAs from sewage sludge and their use for water treatment is more cost-effective than the conventional chemical flocculation process. From a 50:50 acetic and propionic acid ratio, phosphorus removal was achieved.

1.3. Mixed volatile fatty acids and anaerobic digestion potential in biorefineries

The term anaerobic biorefinery is a promising concept, where the anaerobic digester acts as the backbone for the transformation of raw materials into various high-value products or intermediates. The AD process has several associated benefits (e.g., reduction of organic wastes, reduction of the environmental burden of the current disposal of these wastes, valorization of these wastes, among others) [164]. Conventional AD already applies the biorefinery concept due to obtaining energy in the form of biogas and high-value products (digestate). However, emerging processes involving all the fractions obtained in integral transformation routes still need to be valorized or proposed. When the operating conditions of the process are kept in the optimal range, the microbial consortium (depending on the digestion route) may contain non-degraded compounds of interest (digestate). In subsequent processes, the digestate may contain valuable compounds that can be utilized. For example, when lignocellulosic materials are used as substrate, the digestate may contain cellulosic fibers that can be transformed into sugars. These sugars can then be precursors to produce various products (bioenergy, organic acids, and biopolymers) [165]. **Figure 1.4** present the schematic of an anaerobic biorefinery for producing bioenergy and bio-based products.

There are several alternatives to use the digestion process as a valuable tool for generating a wide range of products, among them: (i) AD modified to favor other metabolic pathways (e.g., acidogenic stage, acetogenic stage). This allows for obtaining valuable compounds such as mixed VFAs [18]. These are economically more attractive than biogas. Separation and purification techniques can be performed to generate pure VFAs. They can also be used as substrates to be implemented in other processes (e.g., PHA production). (ii) generation of valuable products from conventionally generated AD products. The biogas generated can be implemented in reforming processes for H_2 production. H_2 has an energy potential 21 times higher than biogas. It can also be transformed into methanol, which facilitates its transport.





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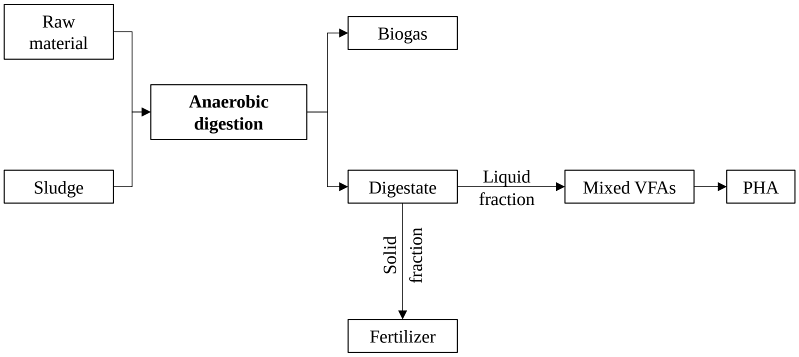

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Figure 1.4. Several schemes of biorefineries based on the AD process. (a) Conventional AD process, (b) biogas upgrading to biomethane, (c) Electricity and heat generation, (d) Methane reforming, (e) Recovery of mixed VFAs, (f) Methanol production, (g) Recovery of pure VFAs, (h) PHA production

2. Chapter 2: Methodology

2.1. Overview

The general methodology for developing the specific objectives proposed in this thesis is shown in **Figure 2.1.** The methodology started with the development of specific **objective 1**. This objective aimed to identify and typify the value chains of the selected raw materials (wastewater from starch production, organic kitchen food waste, and vinasses). In addition, to identify the bottlenecks in the chain. The results of specific objective 1 were used to identify the waste generated in the different links of the value chain. These results served as input data for the experimental process (biogas and mixed VFAs production (**objective 2**)) and for the simulation of biorefinery scenarios (**objective 3**). In addition, the results of specific **objective 1** and **objective 3** were used to perform the environmental assessment through the life cycle assessment proposed in specific **objective 4**. Finally, the indicators obtained in specific **objectives 3**, **4**, and **5** were normalized to perform a comprehensive analysis of the four dimensions of sustainability of the proposed biorefineries.

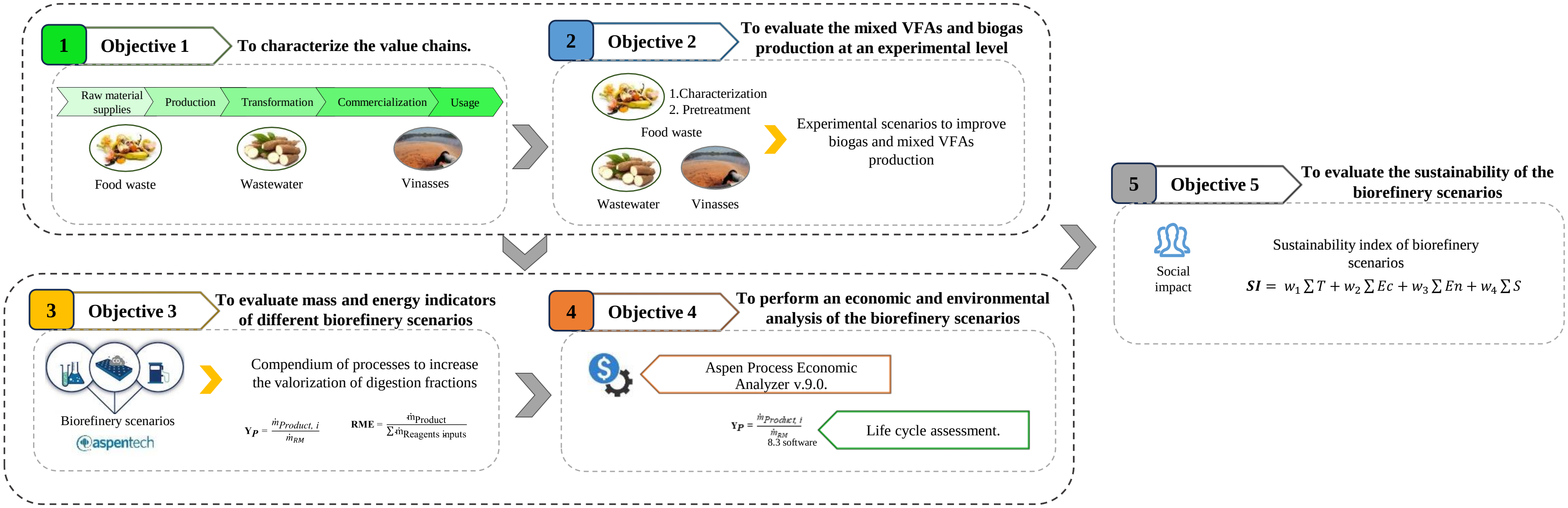


Figure 2.1. Methodology for the development of the specific objectives proposed in this thesis.

2.2. Functional analysis of the value chain

This objective aimed to characterize and analyze the VC of raw materials in a Colombian context. A functional analysis was conducted to determine the current state of the value chains (See **Figure 2.2**). The value chain analysis provided an understanding of the relevant impact pathways. It identified which chain stages, investments, and support could generate benefits and remove constraints and bottlenecks. The functional analysis covered three main areas [166]:

- The main characteristics of the value chain: identify key products, actors, functions, geographical location of activities and operations, as well as main flows.
- The main processes and technical practices: main technologies used; technical coefficients and productivity ratios; main known physical and technological constraints and risks.
- The organization and governance of value chains, in general and at all levels: organizations, institutions, coordination schemes, business environment and policy framework.

The first phase of this objective was to search for information reported in the open literature on the current state of the chains. For the cassava VC, this information involved aspects such as harvested and cultivated area, productivity, yield, typification of the chain, representative links and actors in the region, global flows for each actor of products and residues generated; in addition, the main agricultural practices and existing transformation processes were identified. The cassava VC's scope focuses on the Sucre, Colombia department. In this department, two types of cassava (sweet and agro-industrial) are grown by producers classified as smallholders. The cassava transformation is carried out in industries called rallanderias, characterized by their low technological level.

In the case of ethanol VC, the scope is focused on the Valle del Cauca department. However, since ethanol plants are complex facilities that produce only ethanol, sugar, and electricity, the characterization of this VC will be very general, and only the transformer link will be discussed in detail. They are characterized by a high technological level, where they perform a series of stages, from fermentation for ethanol production to distillation for ethanol recovery from the fermentation broth. Likewise, the food VC is a complex chain involving multiple value sub-chains of different foods. Therefore, the characterization of this VC will be very general, and only the link that generates OKFWs will be discussed in detail. In addition, these wastes present enough variability (context of the region) that it is necessary to delimit a model. The search for information involved identifying the actors that generate organic waste (e.g., food services, retail, households), current disposal of this waste, and the overall flows for each actor of the waste generated.

This information will be complemented with primary information obtained through field visits. These results made it possible to obtain a description of the current state of the chain and to establish the typology of the actor's present. In addition, the main bottlenecks or links that require greater investment to promote the sustainability of the VC were identified.

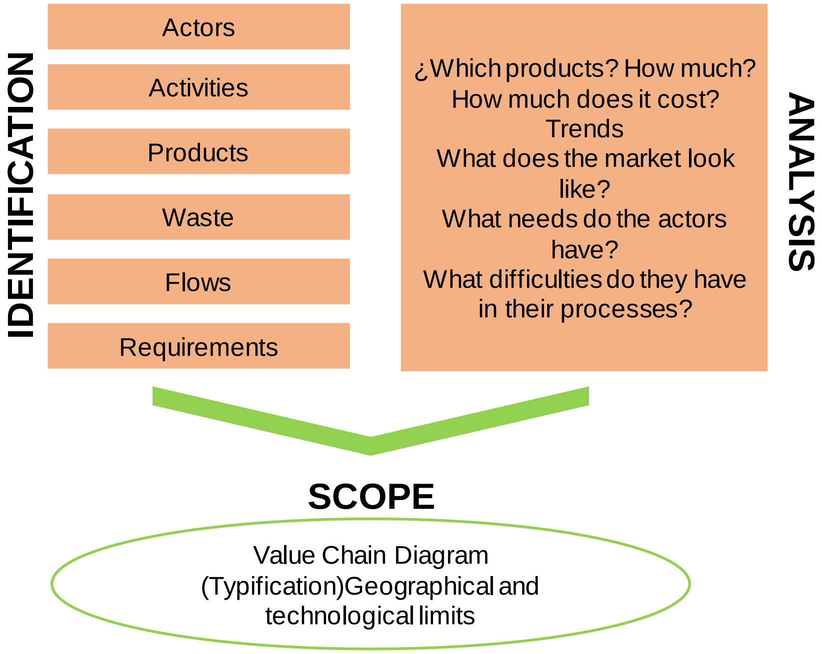


Figure 2.2. Representative aspects for the identification of value chains.

2.3. Experimental approach

The biogas and mixed VFAs production presented four stages. **Stage 1** consisted of the characterization of the raw material. The organic kitchen food waste (OKFW) was characterized by chemical, proximate, and solids analysis. Characterization of wastewater and vinasses was obtained at the sources of origin. The **stage 2** consisted of pretreatment of the OKFW by thermal pretreatment with hot liquid water. Then, **stage 3** involved biogas production with the three feedstocks and pretreated OKFW was considered. Finally, for the mixed VFAs production (**stage 4**), the same feedstocks as in biogas production and some techniques to increase the production of mixed VFAs were considered.

2.3.1. Raw material origin

2.3.1.1. Wastewater

Wastewater samples from starch production were obtained from cassava processing industries (rallanderias). The samples were obtained from the rallanderia located in the department of Sucre, Colombia (8.8114 °N, 72.7208 °W). Samples were collected and stored at 4 °C until further use. The physicochemical characterization of WW is shown in **Table 2.1**.

Table 2.1. Physicochemical characterization of wastewater.

Variable	Range of variation	Unit
pH	4.1–4.4	-
Total Alkalinity	0-10	mg $CaCO_3$ /L
Volatile fatty acids	12.6-31.8	mg /L
Chemical Oxygen Demand	3400-5400	mg/L
Biochemical Oxygen Demand	1876-2459	mg/L
Total Solids	2075-3500	mg/L
Total nitrogen	136-196.6	mg NTK/L
Total phosphorus	22.2-47.8	mg P /L
Sulfur	37.3-54.8	mg S/L
Nickel	<0.1	mg Ni/L
Zinc	0.7	mgZn/L
Sodium	16.5-18.2	mg Na /L
Calcium	21.8-25.8	mg Ca /L
Magnesium	12.4-24.4	mg Mg /L
Potassium	243-249	mg P /L

2.3.1.2. Vinasses

Vinasses samples were obtained from sugar cane processing mills (for ethanol generation). The samples were obtained from the mill Mayagüez, located in the department of Valle del Cauca (3.3977 °N, 76.3275°W). Samples were collected and stored at 4 °C until further use. The physicochemical characterization of WW is shown in **Table 2.2**.

Table 2.2. Physicochemical characterization of vinasses.

Variable	Value	Unit
pH	5.5	-
Volatile fatty acids	1200	mg /L
Total Organic carbon	27940	mg/L
Total nitrogen	654	mg NTK/L
Total phosphorus	74	mg P /L
Sulfates	4688	mg S/L
Total solids (TS)	33.10	g TS / 100 g vinasses
Volatile solids (VS)	23.38	g VS / 100 g vinasses

2.3.1.3. Organic kitchen food waste

An OKFW compositional model reported by Ortiz et al. was used [167]. The composition model considers a mathematical expression (see **Ec 2.1**) that involves seven groups of food (GF). Where, DP: Dairy products, FS: Fish and eggs, MP: Meat products, FV: Fruits and vegetables, OCP: Oily crops and pulses, RT: Roots and tubers and C: Cereals.

$$FW_{model} = \sum_{i=1}^{7} a_i GF_i = 0\%(DP) + 1\%(FS) + 2\%(MP) + 62\%(FV) + 2\%(OCP) + 25\%(RT) + 8\%(C) \quad \textbf{Ec.2.1}$$

The percentage distribution of the seven GF considered by the model was defined through reports from government entities [168] such as DANE and National Planning Department. From databases reported by government entities on consumption statistics, the most representative foods were defined according to the basic family basket from the department of Sucre. The residues of each GF considered in the OKFW model are presented in **Table 2.3.**

Table 2.3. Residues considered in each food group.

Food group	Food residue	Percent
Fish and eggs	Fishbone	15
	Eggs peel	85
Meat	Beef bone	40
	Chicken bone	45
	Meat waste	15

Cereals	Rice	100
Roots and tubers	Potato peel	20
	Cassava peel	35
	Yam peel	35
	Carrot peel	10
Fruits and vegetables	Tomato peel	22
	Onion peel	18
	Bean residues	11
	Pumpkin peel	1
	Lettuce residues	4
	Cabbage residues	3
	Tamarind residues	2
	Corozo residues	2
	Orange, lemon, and mandarin peel	10
	Banana peel	4
	Mango peel and seed	5
	Papaya residues	2
	Lulu residues	2
	Blackberry Pulp	4
	Passion fruit peel and Pulp	3
	Avocado peel and seed	2
	Watermelon peel	5

This model was performed manually in the laboratory from fresh waste. OKFW was liquefied and homogenized. The sample was dried at 40°C for 24 hours (Shimadzu MOC-120H moisture balance) and then ground with a blade mill (SR200 Gusseisen rotary mill, Redsch GmbH, Germany) to a particle diameter of 0.45 mm (40 mesh). Samples were collected and stored at 4 °C until further use.

2.3.2. Characterization

2.3.2.1. Organic kitchen food waste characterization

The extractives of the organic kitchen food waste were quantified using polar and non-polar solvents (i.e., ethanol-water). The extraction was carried out from a 250 mL soxhlet montage. Holocellulose content was performed to calculate hemicellulose content indirectly. Holocellulose represents the total fraction of biomass polysaccharides contributed by cellulose and hemicellulose. It was determined using the chlorination method from a sample free of extractives. The exhausted solid obtained in the holocellulose stage was used to calculate the cellulose content. Lignin was measured as Klason lignin (insoluble lignin) using sulfuric acid at 72% vol. Fat was extracted through percolation with n-hexane under reflux (Soxhlet) for 24 hours. Ash content was determined by total calcination from a heating ramp. Volatile matter was determined by exposing the sample to 950°C for seven minutes in a muffle. The fixed carbon content was measured as the difference between the volatile matter content and the mineralogical content of the sample (i.e., ash). Total solids content was determined by heating the sample at 105°C for six hours. Then, the volatile solids content was determined by exposing the exhausted sample to 550°C for two hours. The extractives were characterized in terms of TPC through the Singleton Folin-Ciocalteu colorimetric method. Antioxidant capacity was measured by α,α-Diphenyl-β-picrylhydrazyl radical (DPPH) inhibition. Finally, reducing sugars were determined considering the dinitro salicylic acid (DNS) methodology. The characterization was performed based on international standards and in triplicate as shown in **Table 2.4.**

Table 2.4. Characterization methods for the feedstock

Characterization	Method	Reference
Extractives	NREL/TP-510-42619	[169]
Fats	Solvent extraction	[170]
Hollocelulose	Chlorination	[171]
Lignin	NREL/TP-510-42618	[172]
Volatile matter	ASTM D7582-15	[173]
Total and volatile solids	ASTM E1756-08	[174]
TPC	Folin–Ciocalteu colorimetric method	[175]
CA	Method described by Marinova et al	[176]
Reducing sugars	DNS methodology	[177]

2.3.3. Pretreatment

2.3.3.1. OKFW pretreatment

The OKFW pretreatment involved the use of techniques focused on improving the accessibility of the raw material. Pretreatment was performed only for OKFW. A thermal pretreatment with liquid hot water (LHW) was carried out following the methodology proposed by Goh et al. Operating conditions of 85°C for 2 hours and a solid: liquid ratio of 1 to 10 were contemplated.

2.3.4. Biogas and mixed volatile fatty acids production

Biogas production was performed under different upstream scenarios to increase production yield. The process followed the methodology described in the standard method VDI 4630 [178]. An experimental setup was performed in 110 ml shots with a digestion volume of 90 ml. The inoculum was obtained from the anaerobic reactor located in a coffee processing plant (Buencafé Liofilizado Company) with a total solid (TS) and volatile solid (VS) of 6.41% wt and 5.78% wt respectively. A nutrients solutions reported by Angelidaki et al. [179] was added to increase the efficiency of the process as shown in **Table 2.5.** The pH was adjusted at 7 at the beginning of the process, and the temperature was constantly controlled (37 °C). Nitrogen was injected to ensure an oxygen-free medium. The tests were carried out in duplicate, and a volumetric displacement method was applied to determine the biogas quantity produced. The biogas composition was measured using the Gasboard - 3100P gas analyzer, reporting CH_4, CO_2, and H_2S composition. The measurements were performed every two days during the digestion time (30 days). In addition, samples of the digestion liquor were collected during digestion to determine the mixed VFAs generated. Samples were centrifuged, filtered, and stored at -4°C for analysis.

Table 2.5. Macro and micronutrients used to perform the AD process.

Macronutrients solution		**Micronutrients solution**	
Nutrient	**Concentration (g/L)**	**Nutrient**	**Concentration (g/L)**
NH_4Cl	100	$FeCl_2.4H_2O$	2
NaCL	10	$CoCl_2·6H_2O$	0.05

$MgCl_2.6H_2O$	10	$MnCl_2.4H_2O$	0.05
$CaCl_2.2H_2O$	5	$CuCl_2 \cdot 2H_2O$	0.038
$K_2HPO_4.3H_2O$	200	$ZnCl_2$	0.05
		H_3BO_3	0.05
		$(NH_4)6Mo_7O_{24}.4H_2O$	0.05
		$Na_2SeO_3 \cdot 5H_2O$	0.1
		$NiCl_2 \cdot 6H_2O$	0.092
		EDTA	0.5
		$AlCl_3$	0.05
		HCl 36%	0.5

Mixed VFAs production was performed considering different upstream scenarios to increase the production yield. The Modified AD conditions were considered as described by Iglesias R [180]. The pH was adjusted at 5-6 at the beginning of the process, and the temperature was constantly controlled (37°C). Nitrogen was injected to ensure an oxygen-free medium. The tests were carried out in duplicate. In addition, the nutrient solution described in **Table 2.5** was added to the digestion. The volume and composition of the gas generated was quantified as described above. In addition, samples of the digestion liquor were collected during digestion to determine the mixed VFAs generated. Samples were centrifuged, filtered, and stored at 4°C. The mixed VFAs concentration was quantified by chromatographic method [181]. The measurements were performed using a GC-2014 gas chromatograph (Shimadzu) equipped with a flame ionization detector (FID) and a Stabilwax capillary column (30 m 0.25 mm 0.25 lm). The carrier gas was nitrogen with a 40 ml/min flow rate and a split ratio of 30. The column had an initial temperature of 60 °C (holding time of 2 min), then increased at 10 °C/min to 140 °C and then further increased at 20 °C/min to 230 °C (holding time of 5 min). The injector and detector temperatures were 230 and 250 °C, respectively. The detector's volumetric flow ratio (ml/min) of hydrogen and air was set at 40:400. Concentrations were determined using standard curves obtained by injecting individual standard solutions of acetic, propionic, and butyric acid.

2.3.4.1. OKFW experimental scenarios

Regarding conventional AD, the **base case** involved fresh OKFW, i.e., dried and ground as shown in **Figure 2.3.** For scenario 1 (**Sc1**), pretreated OKFW was used. A thermal pretreatment (Liquid hot water-LHW) at 80°C for two hours and a solid: liquid ratio of 1:10 was considered. The literature has demonstrated that the hydrolysate obtained from the pretreatment contains dissolved sugars that can increase biogas production. Therefore, for scenario 2 (**Sc2**), the exhausted solids from the pretreatment and the hydrolysate were used. Following the proposed nomenclature, for modified AD, scenario 3 (**Sc3**) involved the use of fresh OKFW. For scenario 4 (**Sc4**), pretreated OKFW was used. The same pretreatment conditions as mentioned above were considered. For scenario 5 (**Sc5**), the exhausted solids from the pretreatment and hydrolysate were used. Inoculum pretreatment has been demonstrated to favor mixed VFA production. Methanogenic microorganisms are sensitive to temperature variations, resulting in their inactivation. On the contrary, acidogenic microorganisms are resistant [182]. Therefore, for scenarios 6 and 7 (**Sc6** and **Sc7**, respectively), thermal pretreatment of the inoculum was considered. The pretreatment involved exposing the inoculum to temperature conditions of 65°C for 30 minutes. Fresh OKFW was used for Sc6 and pretreated OKFW for Sc7.

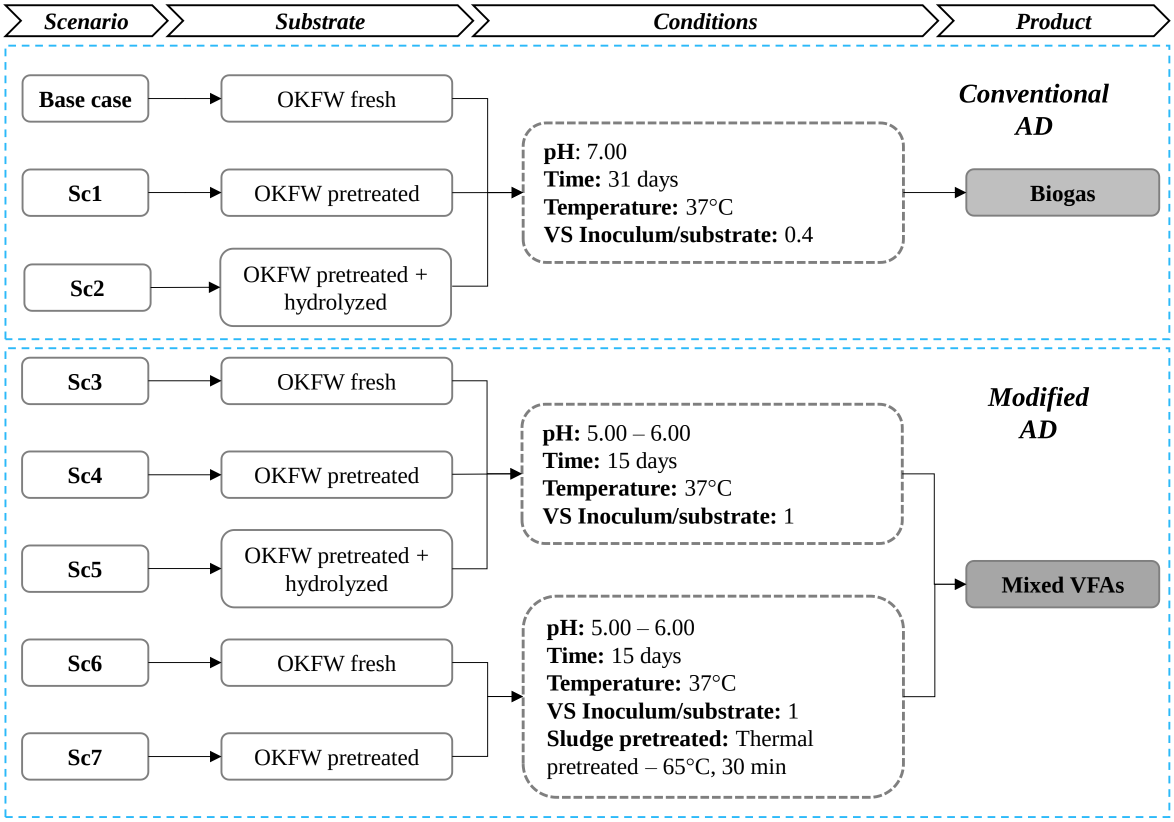


Figure 2.3. Experimental scenarios to promote biogas and mixed VFAs production through conventional AD and MAD using OKFW, respectively.

2.3.4.2. Vinasses experimental scenarios

In the **base case**, vinasse and the conventional route followed for biogas production were used as shown in **Figure 2.4**. Scenario 1 (**Sc1**) involved using vinasse for the mixed VFAs production. Finally, scenario 2 (**Sc2**) involved vinasse and thermal pretreatment of the inoculum. The pretreatment involved exposing the inoculum to a temperature of 65°C for 30 min.

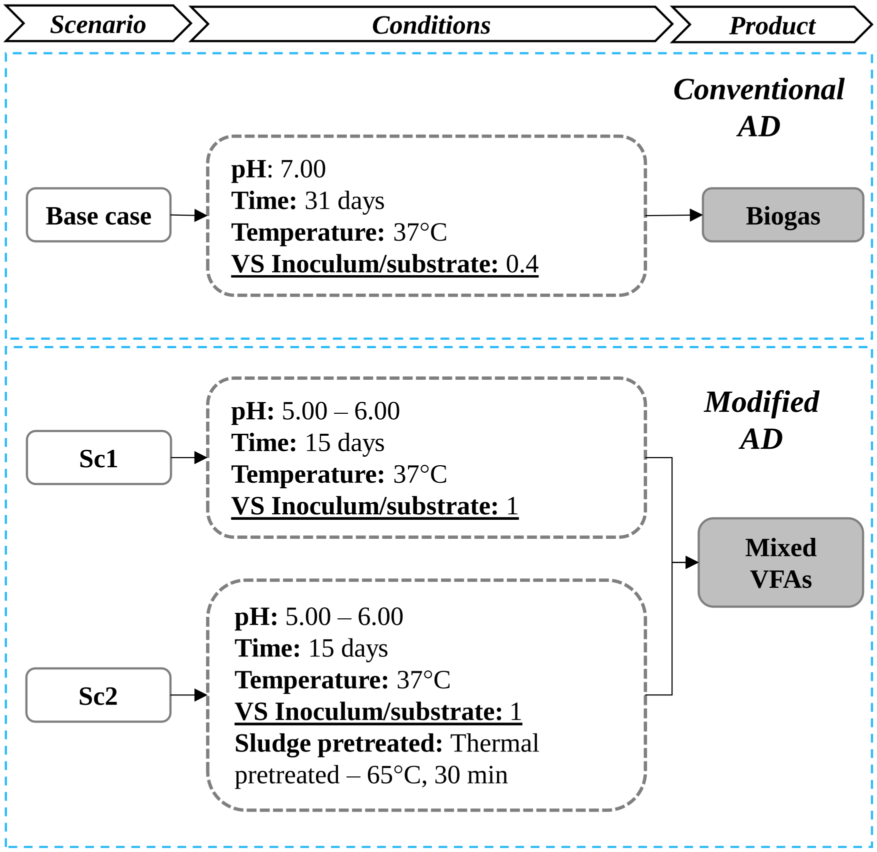


Figure 2.4. Experimental scenarios to promote biogas and mixed VFAs production through conventional AD and MAD using vinasses, respectively.

2.3.4.3. Wastewater experimental scenarios

In the **base case**, wastewater and the conventional route guided to biogas production were used as shown in **Figure 2.5**. In this scenario, a volumetric ratio of inoculum to substrate of 1:1 and 1:2 was used. Scenario 1 (**Sc1**) involved the use of wastewater for mixed VFAs production. Finally, scenario 2 (**Sc2**) involved wastewater and thermal pretreatment of the inoculum. The pretreatment was described above. Furthermore, the cassava starch extraction process was carried out in the laboratory to validate the experimental results, and the wastewater was collected. The mass balances provided by the producers were used.

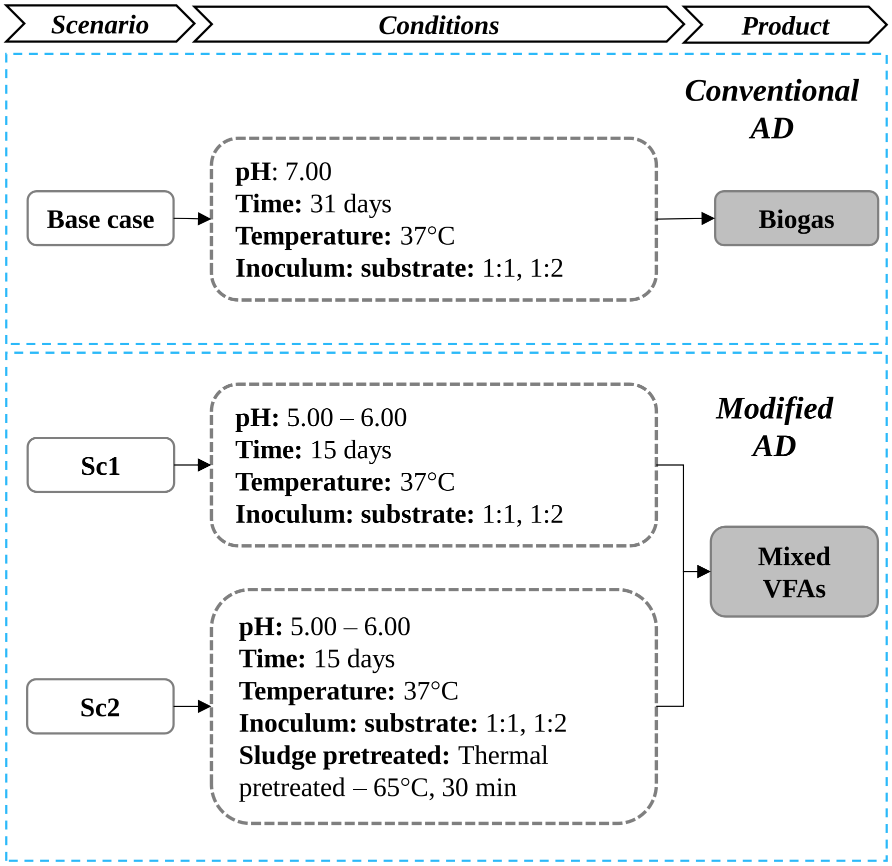


Figure 2.5. Experimental scenarios to promote biogas and mixed VFAs production through conventional AD and MAD using wastewater, respectively.

2.4. Sustainability analysis

The sustainability index of the biorefinery scenarios proposed by OKFW was carried out using the methodology proposed by Solarte-Toro et al [183]. This methodology consists of estimating the sustainability index (0-100%) considering the four dimensions of sustainability (i.e., technical, economic, environmental, and social). **Equation 2.2** shows the Sustainability Index (SI) reported by the author. There are different methodologies for weighting in sustainability analysis. The first is called equal weight factors, the second is Stakeholder values, and the third is Robust indicators [184]. However, the last two require a very high degree of expertise, which leads to subjectivity. Therefore, it is not a representative option for this analysis. The aim is to show the most important of the four dimensions of the sustainability index and to be able to identify possible bottlenecks and points for improvement. For this reason, the equal-weighted method is selected to assign the weights of each dimension and the weights of each indicator. Moreover, the dimension values were determined by estimating different metrics and an equal relative weighting, as shown in **Table 2.6.** The metrics were standardized, considering the best and worst scores for each indicator. The sustainability dimension scores were calculated as shown in **equations 2.3** to **2.6**. The metrics were standardized, considering the best and worst scores for each indicator as shown in **Equitation 2.7.**

$$\boldsymbol{SI} = w_1 \sum T + w_2 \sum Ec + w_3 \sum En + w_4 \sum S \qquad \textbf{Ec 2.2}$$

$$\boldsymbol{T} = w_a TI_1 + w_b TI_2 + \cdots + w_z TI_j \qquad \textbf{Ec 2.3}$$

$$\boldsymbol{Ec} = w_a EcI_1 + w_b EcI_2 + \cdots + w_z EcI_j \qquad \textbf{Ec 2.4}$$

$$\boldsymbol{En} = w_a EnI_1 + w_b EnI_2 + \cdots + w_z EnI_j \qquad \textbf{Ec 2.5}$$

$$\boldsymbol{S} = w_a SI_1 + w_b SI_2 + \cdots + w_z SI_j \qquad \textbf{Ec 2.6}$$

Where:

w: weighting factor for each dimension of sustainability,

w_j: weighting factor for the technical, economic, environmental, and social dimension.

T: Technical dimension,

Ec: Economic dimension,

En: Environmental dimension,

S: Social dimension

$$\boldsymbol{Normalized} = \frac{Estimated\ value - worst\ value}{Best\ value - worst\ value} \qquad \textbf{Ec 2.7}$$

Table 2.6. Best and worst cases used to normalize the indicators assessed.

Dimension	**Weighting factor**	**Indicator**	**Best case**	**Worst case**
Technical	0.25	Process mass intensity (PMI)	1.0	100.0
		Renewable material index (RMI)	1.0	0.0

Economic	0.25	Payback period (PBP)	1.0	20.0
		Turnover ratio (RR)	5.0	0.1
Environment	0.25	Carbon footprint (CF)	0.5	20.0
		Water depletion (WF)	1.0	20.0
Social	0.25	Minimum to living wage ratio (M/L)	1.0	0.5

2.4.1. Process simulation

The simulation was carried out using Aspen Plus v9.0 software (Aspen Technology Inc. USA). Through the Aspen software the model properties are predicted, especially for the separation of organic molecules and physicochemical processes (adsorption). Conventional AD was simulated using the non-random two-liquid activity (NRTL) model and the Peng-Robinson equation of state to describe the liquid and vapor phases, as described by Solarte et al [185]. The MAD was simulated using the non-random two-liquid thermodynamic/activity (NRTL-HOC) model. This thermodynamic model is suggested for processes involving carboxylic acids (as VFAs). The method uses the Hayden-O'Connell equation of state as the vapor phase model [151].

2.4.2. Biorefineries scenarios description

2.4.2.1. Scenarios for organic kitchen food waste biorefineries

The selected processing scale of the OKFW was 3 tons/h. The processing scale was selected based on the organic waste from the main landfill of the capital city (Sincelejo) of the Sucre department [168]. The biorefineries scenarios were designed based on the experimental results obtained in the first stage of this thesis. Likewise, a compendium of downstream processes was proposed to increase the fractions valorization obtained in the digestion process. The **base case** was performed using fresh OKFW. Scenario 1 (**Sc1**) used pretreated OKFW. Scenario 2 (**Sc2**) considered the recovery of unconsumed mixed VFAs and the use of fresh OKFW. For scenario 3 (**Sc3**), adding the hydrolysate to the AD (obtained in the pretreatment stage) and the pretreated OKFW was considered. Scenario 4 (**Sc4**) involved pretreated OKFW, the bioactive compound production from the hydrolysate, and the recovery of the unconsumed mixed VFAs. Scenarios 5 to 9 (**Sc5**-**Sc9**) involved modified AD for the mixed VFAs and liquid and solid digestate production. **Sc5** was performed using fresh OKFW. **Sc6** used pretreated OKFW. **Sc7** involved fresh OKFW and thermal pretreatment of the inoculum. **Sc8** considered using pretreated OKFW and the bioactive compound production from the hydrolysate. **Sc9** comprised fresh OKFW, thermal pretreatment of the inoculum, and polyhydroxybutyrate (PHB) production using the mixed VFAs as substrate. Detailed descriptions of the biorefinery scenarios are presented in **Annex A: Biorefinery scenarios description.** The areas considered for each scenario are shown in **Table 2.7.**

Table 2.7. Scenarios evaluated at simulation level for biorefineries based on OKFW.

Scenario	**Area**							
	Storage	**Conditioning**	**LHW pretreatment**	**AD**	**Mixed VFAs recovery**	**BC extraction**	**Inoculum pretreatment**	**PHB production**
	10	**20**	**30**	**40**	**50**	**60**	**70**	**80**
Conventional AD								
Base case	x	x		x				
1	x	x	x	x				
2	x	x		x	x			
3	x	x	x	x	x			
4	x	x	x	x	x	x		
Modified AD								
5	x	x		x	x			
6	x	x	x	x	x			
7	x	x		x	x		x	
8	x	x	x	x	x	x		
9	x	x		x	x		x	x

2.4.2.2. Scenarios for vinasses biorefineries

The processing scale selected for the vinasse was 129.4 kg/day based on the ethanol production from sugarcane in the Valle del Cauca department. The biorefinery scenarios were designed based on the experimental results. The vinasse is a by-product obtained from ethanol production. Evaporation processes with high energy consumption are necessary for its current valorization. For this reason, several scenarios were proposed to use vinasses in conventional and modified AD processes to generate biogas (and reduce process energy costs) and high-value products (mixed VFAs), respectively thought the biorefinery concept. The **base case** corresponds to the conventional process of ethanol production. **Scenario 1 (Sc1)** and **Scenario 2 (Sc2)** involves the integration of conventional AD in the **base case. Sc1** involved Biogas and digestate production. **Sc2** considered the recovery of unconsumed mixed VFAs from liquid digestate. **Scenario 3 (Sc3), Scenario 4 (Sc4),** and **Scenario 5 (Sc5)** involves the integration of modified AD in the **base case.** In **Sc3** the mixed VFAs were recovered from the liquid fraction of the digestate. **Sc4** considered the inoculum pretreatment stage to increase the mixed VFAs production. **Sc5** involved the polyhydroxybutyrate production using the mixed VFAs as substrate. Detailed descriptions of the biorefinery scenarios are presented in **Annex A: Biorefinery scenarios description.** The areas considered for each scenario are shown in **Table 2.8.**

Table 2.8. Scenarios evaluated at simulation level for biorefineries based on vinasses.

Scenario	**Area**					
	Ethanol production	**Storage**	**Inoculum pretreatment**	**AD**	**Mixed VFAs recovery**	**PHB production**
	10	**20**	**30**	40		50
Base case	x					
			Conventional AD			
1	x	x		x		
2	x	x			x	
			Modified AD			
3	x	x		x	x	
4	x	x	x	x	x	
5	x	x		x	x	x

2.4.2.3. Scenarios for wastewater biorefineries

The processing scale selected for the wastewater (WW) was 8.69 L/min considering the mass balances performed in previous studies of rallanderias with low technological levels. The biorefinery scenarios were designed based on the experimental results. WW is a residue obtained from the cassava starch production process. It is disposed of in water sources and has a high organic material content. For this reason, several scenarios were proposed using WW in conventional and modified AD processes to generate biogas and high-value products (mixed VFAs) through the biorefinery concept. The **base case** corresponds to the conventional process of cassava starch production. **Scenario 1 (Sc1)** and **Scenario 2 (Sc2)** involves the integration of conventional AD in the **base case. Sc1** involved biogas and digestate production. **Sc2** considered the recovery of unconsumed mixed VFAs from liquid digestate. **Scenario 3 (Sc3), Scenario 4 (Sc4),** and **Scenario 5 (Sc5)** involves the integration of modified AD in the **base case.** In **Sc3** the mixed VFAs were recovered from the liquid fraction of the digestate. **Sc4** considered the inoculum pretreatment stage to increase the mixed VFAs production. **Sc5** involved the polyhydroxybutyrate production using the mixed VFAs as substrate. Detailed descriptions of the biorefinery scenarios are presented in **Annex A: Biorefinery scenarios description.** The areas considered for each scenario are shown in **Table 2.9**.

Table 2.9. Scenarios evaluated at simulation level for biorefineries based on wastewater.

Scenario	**Area**					
	Starch production	**Storage**	**Inoculum pretreatment**	**AD**	**Mixed VFAs recovery**	**PHB production**
	10	**20**	**30**	40	50	60
Base case	x					
Conventional AD						
1	x	x		x		
2	x	x		x	x	
Modified AD						
3	x	x		x	x	
4	x	x	x	x	x	
5	x	x		x	x	x

2.4.3. Technical analysis

The biorefinery scenarios were analyzed based on the mass and energy requirements obtained from the simulation. The analysis was performed using a set of mass and energy indicators. Mass indicators were used to evaluate the degree of transformation of feedstocks into the desired products. In addition, these indicators were used as metrics to calculate the efficiency of each scenario based on reported in literature. The mass indicators were product yield (**Yp**) [186], process mass intensity (**PMI**), and renewability index (**RMI**) [187]. The indicators were calculated from equations **2.7, 2.8**, and **2.9.** Product yield is the ratio between the products obtained and the feedstock. The PMI quantifies the feedstock used to obtain one kilogram of product. Thus, this indicator can be used to analyze how to design less intensive and more productive processes. Finally, RMI allows for determining the renewability of a process from mass balance data.

$$Y_P = \frac{\dot{m}_{\text{Product, i}}}{\dot{m}_{OKFW}} \tag*{\textbf{Ec. 2.7}}$$

$$\text{PMI} = \frac{\sum \dot{m}_{\text{i inputs}}}{\sum \dot{m}_{\text{Products}}} \tag*{\textbf{Ec. 2.8}}$$

$$\text{RMI} = \frac{\sum_{i:1}^{N}\left(m_i^{in}\right)_{renewable}}{\sum_{i:1}^{N} m_i^{in}} \tag*{\textbf{Ec. 2.9}}$$

Energy indicators were used to evaluate the energy performance of the scenarios. For this analysis, the process utilities, the energy requirements in terms of heat and power of each equipment, and the heat content of each flow were used. Moreover, indicators related to overall energy efficiency ("η") and specific energy consumption (SEC) were considered. The indicators were calculated from equations **2.10** and **2.11.** SEC provides a quantitative description of the total energy consumed by the process to produce a product per unit mass of valuable product. "η" relates the output energy to the total energy consumption of the process. The self-generation index was determined for conventional AD biorefinery scenarios using vinasse and wastewater as feedstock (see **Ec 2.12**).

$$\eta = \frac{\dot{m}_{\text{Biogas}} * \text{LHV}_{\text{Biogas}}}{(\dot{m}_{OKFW} * \text{LHV}_{OKFW}) + \dot{Q} + \dot{W}} \tag*{\textbf{Ec 2.10}}$$

$$\text{SEC} = \frac{\dot{Q}_{Total} * \text{W}_{\text{Total}}}{\dot{m}_{OKFW}} \tag*{\textbf{Ec 2.11}}$$

$$\text{SGI} = \frac{(\dot{m}_{\text{Biogas}} * \text{LHV}_{\text{Biogas}}) * \eta_{\text{Conversion}}}{\dot{Q} + \dot{W}} \tag*{\textbf{Ec 2.12}}$$

2.4.4. Economic analysis

The economic analysis involved estimating the cost of capital (CapEx) using the Aspen Process Economic Analyzer. CapEx amortization was calculated using the straight-line amortization method. In addition, operating costs (OpEx) were estimated using the mass and energy balances obtained from the simulation. The OpEx relates the costs of raw materials, chemical reagents, labor, and utilities. The analysis was performed from a Colombian context with a project life of 20 years, as shown in **Table 2.10**. The economic feasibility was calculated from the estimation of economic metrics. The net present value (NPV), the payback time (PBP), and the turnover rate (TR) were calculated. The Detailed Economic Evaluation methodology reported by Rueda-Duran et al. [58] was applied to the feedstock scenarios. OKFW costs were established based on disposal and transportation costs in the Colombian context. The cost of transport was assumed as the price of the inoculum. The inoculum must be supplied during the first six months of plant operation in anaerobic digestion. During this time, fermentation stabilizes, and the inoculum is self-generated. For this reason, the cost of the inoculum is considered for the first six months.

Table 2.10. Economic parameters for biorefineries analysis.

Parameters in the Colombian context			
Parameter	**Value**	**Parameter**	**Value**
Tax rate	35%	Shifts	1 day/year
Interest rate	13%	CEPCI* 2022 [188]	815.98
Operating time	350 days/year	Working time	8 hours/day
Operators wage	232.15 USD/month	Supervisor wage	464.29 USD/month
Item	**Units**	**Value**	**Reference**
Utilities cost			
Cooling water	USD/m^3	0.042	[189]
Medium pressure steam	USD/ton	8.07	[190]
Low-pressure steam	USD/ton	7.89	[190]
Electricity	USD/kWh	0.055	[190]
Supplies cost			
OKFW	USD/ton	35.81	[191]
Vinasses	USD/kg	0.0460	
Sludge	USD/ton/km	0.0460	
Ethanol	USD/L	1.84	[192]

Water	USD/kg	0.33	[190]
Product price			
Solid digestate	USD/ton	7.36	[193]
Liquid digestate	USD/ton	4.16	[193]
Biogas	USD/m^3	0.79	[194]
Bioactive compounds	USD/kg	4	[195]
Mixed VFAs	USD/kg	3.8	[151]
PHB	USD/kg	3	[196]

2.4.5. Environmental analysis

The environmental assessment was developed according to the methodology proposed by the ISO 14040 standard, based on a life cycle assessment (LCA). SimaPro v9.1 software (PRé Sustainability, The Netherlands) and the Ecoinvent V.9 database were used. The analysis was performed quantitatively by estimating midpoint indicators (18 indicators). The most representative indicators were reported. The inventory of biorefinery scenarios were obtained from the mass and energy balances of the simulation. A cradle-to-gate approach were considered.

2.4.5.1. Environmental analysis for organic kitchen food waste biorefineries

Due to the complexity of the food VC of the Sucre department, the environmental impact only considers the link of the VC that generates the OKFW (consumers) and the proposed biorefinery scenarios. The objective of the LCA was to compare the environmental impact of the base case and the proposed biorefinery scenarios. For the OKFW generation stage, the acquisition of OKFW at the Oasis landfill, located in the capital of the Sucre department, was considered. An average distance from the landfill to the transformer unit (biorefineries) of 20 km was considered. The functional unit (FU) was 1 kg of OKFW processed.

2.4.5.2. Environmental analysis for vinasses biorefineries

The ethanol VC of the Valle del Cauca department is linked to the sugarcane agroindustry. Ethanol production is carried out in the sugar mills. Therefore, the environmental impact only considers the link between the VC generated by the vinasse and the proposed biorefinery scenarios. The objective of the LCA was to compare the environmental impact of the base case and the proposed biorefinery scenarios. The ethanol production unit at the sugar mill was considered for the vinasse generation stage. The FU was 1 kg of vinasse processed.

2.4.5.3. Environmental analysis for wastewater case

The environmental assessment of WW encompassed all the activities necessary for the production, processing, and commercialization of cassava in the Sucre department (see). The objective of the LCA was to determine the environmental impact of the cassava value chain in the Sucre department and to establish the bottlenecks in the chain. Also, to determine the environmental impact of the biorefinery scenarios. The objective involved the following specific objectives: (i) Identify the links in the chain with the greatest representation of the environmental impact of the value chain, (ii) To environmentally compare the biorefinery scenarios and establish their impact contribution to the value chain. Likewise, establish the stages of the process with the greatest environmental contribution. The FU was selected based on cassava productivity in the Sucre department. Therefore, this allows for comparing the different links in the value chain and the proposed biorefinery scenarios. In this analysis the environmental impact is assigned to 1 kg of WW. For the transformation link, inputs and utility consumptions were set according to the generation of 1 kg of WW. The geographical limits contemplated the value chain of sweet and agro-industrial cassava in the Sucre department. The cassava transformation is carried out directly in the region in sectors of cassava transformation to cassava starch called rallanderias. The information collected was obtained in the first and second quarter of 2023. This information was collected from primary information through field visits (interviews) and provided by regional experts (agro-industrial engineers). This information was complemented and supported by reports from secondary information sources. The system studied is the cassava value chain in the Sucre department, which includes four main links: (i) input suppliers, (ii) producers classified as small producers, and (iii) transformers. This study did not consider the distribution of cassava and cassava starch to the national market due to the wide national coverage. **Figure 2.6** shows the system's limits analyzed and the activities and processes involved and considered for this study.

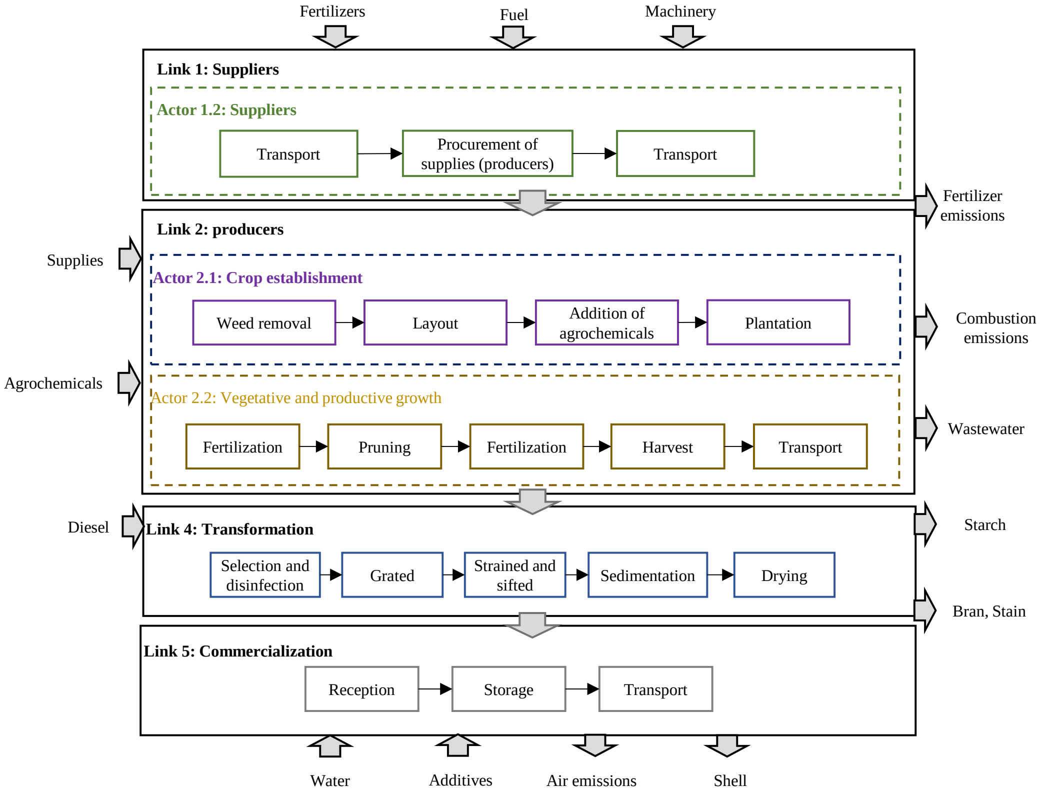


Figure 2.6. System boundaries analyzed from WW valorization.

The inventory was constructed for the value chain links typified in the functional analysis. Information for the first three links (i.e., producers, processors, and marketers) of the value chain was collected through primary information from field visits, meetings, and interviews with regional agricultural experts. The information includes inputs, agrochemicals, fertilizers, and outputs in the form of emissions to air, water, and soil. Direct and indirect nitrogen emissions to air and water (nitrous oxide-N_2O, nitrate-NO_3-, ammonia-NH_3) derived from the application of these fertilizers were determined from the standards defined by the Intergovernmental Panel on Climate Change (IPCC) [197]. Phosphorus emissions to the soil were determined by IPCC and SALCA-P [198]. In addition, solid residues (biomass) obtained from the leaves and stems of the plant are generated during harvesting and collection. Based on data provided by growers and local experts, for 1 hectare of crop, 5084 kg of leaves and 26474 kg of stems are generated. For the biorefinery scenarios, the inventory was based on mass and energy balances provided by the Aspen Plus software.

On the other hand, the considerations and limitations of this analysis are illustrated in **Table 2.11.** The activities in this link are related to crop establishment, vegetative and productive stages, harvesting, and collection, as shown in **Table 2.12.**

Table 2.11. LCI information, considerations, and limitations for the cassava VC.

Link	**Criteria**	**Commentary/Consideration/Limitation**
Input suppliers	Average distance between input suppliers and producer	Average distance 20 km. The distances were modeled with EURO 1 type engines (SimaPro). In addition, the cassava crop does not have previous stages of seedling growth in nurseries. Planting is done directly in the crop.
Producers	Crop establishment	The inputs and outputs considered in the producer link were adjusted according to the sweet cassava crop in the region. It is currently the most representative.
	Crop type	Monoculture is considered
	Seeding density	10000 plants per hectare
	Productivity	30.4 tons of cassava per hectare
	Laborers	The environmental impact associated with the transportation of day laborers was not considered.
	Transport to transformers	Average distance of 19 km. Distances were modeled with EURO 1 type engines (SimaPro).
Transformers	Raw Materials/Services	The inputs, outputs and energy consumed in the cassava starch process were taken from consolidated reports from qualified personnel in the region.

Market	Transport	The environmental impacts associated with the transportation of cassava and starch were not considered for the study due to the diversity of routes to be involved.
Biorefinery scenarios	Biorefineries	For the WW valorization, the mass and energy balances compiled from the simulation of the processes or scenarios evaluated using the Aspen plus v9 software were considered.

Table 2.12. Life cycle inventory for the first three links of the cassava value chain.

Link	Actor	Sub system	Activity	Input		
				Steam	**Flow**	**Unit**
Suppliers	**Suppliers**	**Suppliers**	Agrochemicals	Distance town center-crops	20	km
				Tons per kilometer	1.052	tkm
Producers	**Producers**	**Land conditioning**	Weed removal	Scythe (Oil)	0.56	L
				Scythe (gasoline)	7	L
			Layout	Manual		
			Drowning	Manual		
			Land preparation	Plow (ACPM)	20	L
				Aporcado (ACPM)	20	L
			Plantation	Manual (10000 plants per ha)		
		Growing - Vegetative and productive stage	Agrochemical addition	Trilla (Diuron 800g/L)	1	L
				Invetrina (Cypermethrin 200g/l)	2	L
				Water	600	L
			Weed control 1	Gramafin (Paraquat 200g/l)	2	L
			Fertilization	Triple 15 (NPK)	50	kg
				Total Nitrogen	7.5	kg
				Ammoniacal nitrogen	5.15	kg
				Nitric nitrogen	2.35	kg
				Assimilable phosphorus (P_2O_5)	7.5	kg

				Water soluble potassium (K_2O)	7.5	kg
			Seedling thickener addition	Seedling thickener	1	kg
				Total oxidizable organic carbon	0.04	kg
				Assimilable phosphorus (P_2O_5)	0.37	kg
				Water soluble potassium (K_2O)	0.5	kg
				Total sulfur (S)	0.094	kg
				Total sodium	0.01	kg
				Insoluble solids	0.021	kg
			Weed control 2	Scythe (Oil)	0.56	L
				Scythe (gasoline)	7	L
		Harvesting and gathering	Harvesting and gathering	Manual		
			Leaves and stems	Residual biomass (leaves)	5084	kg
				Residual biomass (stems)	26474	kg
		Productivity	Cassava	Cassava	30442	kg
Transformers	**Rallanderias**	**Transport**	Cassava transport to rallanderias	Crop-landing distance	19	km
				Tons per kilometer	304	tkm
				Cassava	1000	kg
		Washing	Dry cleaning	Electricity	1.9	kWh
				Shell (residue)	97.76	kg
			Wet cleaning	Electricity	1.91	kWh

				Wastewater	3907.33	m^3
				Wastewater (COD)	2620.8	mg/L
		Grated and strained	Grated and strained	Electricity	10.86	kWh
				Input water	7211.54	kg
				Afrecho (waste)	109.41	kg
		Filtering	Filtering	Electricity	2.51	kWh
				Input water	8604.07	kg
				Wastewater	3309.79	m^3
				Wastewater (COD)	2910.8	mg/L
		Sedimentation	Sedimentation	Electricity	1.28	kWh
				Stain (waste)	3.6	kg
				Wastewater	8998.8	m^3
				Wastewater (COD)	3419.2	mg/L
		Drying	Drying	Sun drying		

2.4.6. Social analysis

The social analysis involved the estimation of two indicators. The minimum living wage (M/L) was determined considering a Colombian context. A combined socioeconomic evaluation was conducted to assess the versatility and resilience of the proposed biorefineries scenarios by increasing the minimum/living wage (M/L) ratio. A sensitivity analysis was applied to analyze whether the biorefineries scenarios can provide a living wage to plant employees (i.e., M/L = 1) without a decrease of more than 20% in the cumulative net present value (NPV) at the end of the project. If the valorization scheme can achieve an M/L value of 1 without a sharp decrease in income, it is concluded that it has good social performance. However, if a minor change in the M/L ratio causes a sharp decrease in the economic viability of the process, the social performance is not good since the process cannot guarantee a better quality of life for the employees. On the other hand, the number of employments generated (operators and supervisors) in the biorefineries scenarios was determined. Based on the total number of working hours per year (8400 h/year). An additional person was added to the operators as a substitute is required. Three supervisors are also needed in each processing area. Subsequently, the number of operators was multiplied by the number of production lines in the biorefineries.

3. Chapter 3: Functional analysis of the value chain.

3.1. Overview

The Functional Analysis (FA) of the value chain (VC) performed in this thesis aimed to describe how the raw material is transformed from primary production on the farm to obtaining the different processed products reaching the final consumer. Likewise, the VC is understood as a system identifying and characterizing the chain's main direct and indirect actors. In addition, the FA allowed determining the current state of the VC and the main bottlenecks associated with the current waste generation and disposal. Thus, it was possible to determine the link in the chain that does not allow sustainable development and, therefore, to develop valorization alternatives, considering AD as a pillar for generating new products.

3.2. Food value chain

3.2.1. Food production in Colombia

Colombia has been positioning as a country with enormous productive potential and profiled as an agri-food pantry worldwide. According to the Food and Agriculture Organization of the United Nations (FAO), Colombia is the fourth country in Latin America regarding the availability of land suitable for agricultural production and the third regarding water availability and climatic diversity. Moreover, according to the Registro Único Empresarial y Social de Confecámaras, in 2022, there were 42940 companies engaged in food production. The geographical distribution of industrial establishments indicated that in 2020, the departments with the highest number of companies were Bogota (26.5%), Antioquia (22.8%), Valle del Cauca (15.3%), Atlántico (7.9%), Cundinamarca (6.9%) and Caldas (4.2%). According to the Annual Manufacturing Survey (EAM) of DANE, in 2016, there were 218 industrial establishments engaged in processing Processed Fruits and Vegetables, representing 2.6% of the total number of registered establishments. When classifying the industrial establishments whose main activity is the processing of Processed Fruits and Vegetables according to their business size, small companies were found to participate in 56.5%, while medium, micro, and large companies accounted for 20.3%, 18.8%, and 4.3%, respectively.

3.2.2. Food production in Sucre department

Food production in the Sucre department presents a great challenge for its development. According to the Annual Manufacturing Survey conducted by DANE in 2019, the department had 18 industrial establishments. 44% corresponded to other manufacturing industries and 56% to manufacturing other food products. However, agribusiness contributed 19% of the manufacturing sector's income. Furthermore, according to ProColombia, in 2020, only 5% of the department's agri-food exports were from the "bakery and milling products" subsector. Under the National Plan for Food and Nutritional Security (2012-2019) and according to the department's agricultural production, food was prioritized: rice, corn, mango, cassava, banana, cocoa, and derived products such as vegetable oil and beef. Also of special importance are yams, eggplant, chili peppers, avocado, and fruits such as papaya, mango, passion fruit, patilla, melon, and guava. The department is mainly supplied with agricultural products such as green plantains, potatoes, tomatoes, rice, onions, sugar, carrots, flour, oranges, lettuce, tree tomatoes, iodized salt, bananas, cucumbers, and yellow corn, mainly in the wholesale centers of Antioquia, Boyacá, Chocó, Córdoba, and Norte de Santander. According to the results of the ENSIN 2015, the degree of food insecurity in the Sucre department is high (73.9%), placing it above the national average (54.2%) and the highest in the Caribbean region. On the other hand, the existing food agroindustry in the department involves few small agricultural producers due to the low level of production technology, the limited transformation of products to add value, and insufficient associative processes to enhance cooperation among small producers and increase their negotiation power, productivity, and profitability.

3.2.3. Food value chain in Sucre department

Three main actors are considered in the primary phase: suppliers, producers (agricultural and livestock producers), and marketers. Producers are divided into (i) small producers and (ii) medium and large producers. Marketers include purchasing agents, rural and urban traders, and international fresh produce exporters as shown in **Figure 3.1**. The processing phase involves valorizing food (production chain) from local transformers and medium and large industries. The marketing phase includes the rural and urban markets. Finally, the consumption phase includes rural consumers, urban domestic consumers, restaurants, hotels, schools, institutions, and international consumers.

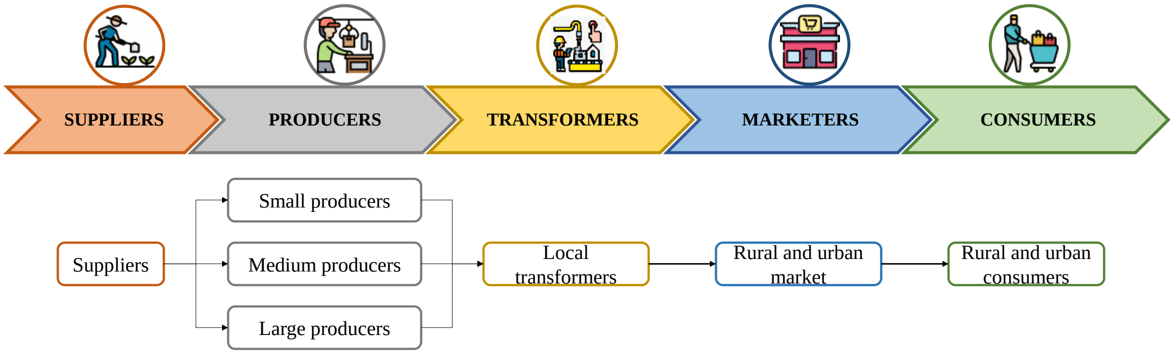


Figure 3.1. Food value chain in Sucre department.

The type of plantation guides the input suppliers and producers link. Generally, the link is described in five sub-stages: i) nursery, ii) soil preparation, iii) planting, iv) crop maintenance, and v) harvesting and post-harvesting. In the first stage, the planting design is given by the schematization of the land. The spacing between the crops is established since, depending on the crop type and each plant's growth, there must be an appropriate distribution. Likewise, the location of irrigation systems and drainage channels is identified to ensure adequate water absorption. The transformer link is guided to the type of transformation. It is generally described in three sub-stages: i) washing and disinfection, ii) transformation, and iii) packaging. Raw materials are received at the processing plants and undergo specific processes to obtain a product.

In Colombia, around 14974 Mton of waste is generated in the production and transformation links, including rice, banana, and banana chains. Most residues obtained in the producers' link are disposed of on crops as organic fertilizer. The transformer link generally sends the waste to incineration processes for energy generation. On the other hand, more than 2008 Mton and 1526 Mton are generated in the marketing and consumption links. Fruits and vegetables are the groups that most contribute. In addition, roots, tubers, and cereals are present in the composition according to the food consumption trend in Sucre. 53% of the waste generated in the last two links of the chain comes from households (e.g., organic kitchen food waste - OKFW). One person is estimated to generate 32 kg/year of OKFW. OKFW is generated daily and collected by companies that dispose of this waste in landfills. In 2020, Sucre reported 949,252 people. Thus, a mass flow of 21.64 tons/day is elucidated. In 2021, there was 100% coverage in collecting these wastes in the urban area of the municipality of Sincelejo. However, only 24% is covered in the rural area. This reflects a deficiency in waste management. OKFW are disposed of at the El Oasis landfill, operated by INTERASEO SA ESP and located within the municipality on the El Oasis farm, covering an area of 21 hectares. Only 2.8% of the department's organic waste is estimated to be valorized. Most of it is incorporated into processes to generate compost, energy, or vermiculture.

Based on the functional analysis of the food VC in the department of Sucre, the main bottlenecks in the chain were identified. The main strengths, weaknesses, opportunities, and threats (DOFA analysis) are consolidated in **Table 3.1.**

Table 3.1. DOFA analysis of food value chain.

Strengths	**Weaknesses**

• Crop diversity. • Presence of high and medium scale industries that promote the social development of the region. • Ideal climatic conditions to generate higher productivity. • Agroforestry production system with low use of agrochemicals.	• Producers are decentralized in the territory, preventing the generation of economies of scale. • Producers have a low technological level. • Small processors with low technological levels. • No linkage between actors (producers-transformers). • High amount of waste generated in the last two links of the chain
Opportunities	**Threats**
• National and international market.	• Deficient road infrastructure • Climate change and Depletion of water resources in the region. • Reduction of small producers

3.3. Cassava value chain

3.3.1. Cassava production in the word

In 2021, about 1 billion hectares of agricultural products were cultivated in the world, according to the Food and Agriculture Organization of the United Nations (FAO). The most representative crops were wheat, with 15.6% of the total area, followed by corn (14.5%), rice (12.15%) and soybean with 8.19% [199]. The cassava crop ranked 16th among the world's agricultural products. Cassava has been listed as a basic commodity in the food diet. Considered the cheapest source of starch used in more than 300 industrial products. Moreover, has increased its importance in global agriculture and is now a multi-purpose crop that responds to the priorities of developing countries, trends in the global economy, and the challenge of climate change [200]. In 2020, Nigeria was the leading producer of cassava in the world with 60,001,531 tons (19.8%), followed by the Dominican Republic with 41,014,256 tons (13.6%) and Thailand with 28,999,122 tons (9.6%). Only 10% of the area is dedicated to cassava cultivation in Latin America and the Caribbean.

3.3.2. Cassava production in Colombia

Colombia ranks 25th among Latin American cassava-producing countries. In 2018, the area planted was 210-250 hectares. Cassava production in the country is concentrated in the 5 departments of the Caribbean Region: Bolívar (30%), Córdoba (27%), Sucre (17%), Magdalena (11%), Atlántico (5%), Cesar (5%) and La Guajira (3.5%) [201]. The cassava crop is important for food security in Colombia. From 95% to 97% corresponds to cassava for fresh consumption (sweet cassava), and the remaining percentage represents cassava destined for value generation developed in the production areas (agro-industrial or native cassava). The value addition of agroindustrial cassava is destined for starch production processes (intermediate products for other industries), dried pieces, and flour production. In Colombia, there are three companies whose business model is based on the production of agro-industrial starch. One of them is the starch industry of Sucre. This is a large-scale industry offering native starch. 70% of the starch generated is destined for the food industry and the rest for other industries, such as brewing flavoring companies.

3.3.3. Cassava production in Sucre department

The cassava production and harvested area in the Sucre department for 2015-2021 is presented in **Figure 3.2**. A 34% decrease in production between 2020 and 2021 is evidenced. The Sucre municipalities with the highest productivity of sweet cassava are Ovejas, Los Palmitos, San Pedro, Sincelejo, and Betulia. Corozal, Sincelejo, Los Palmitos, San Pedro, and Sampues are the municipalities with the highest agroindustrial cassava productivity.

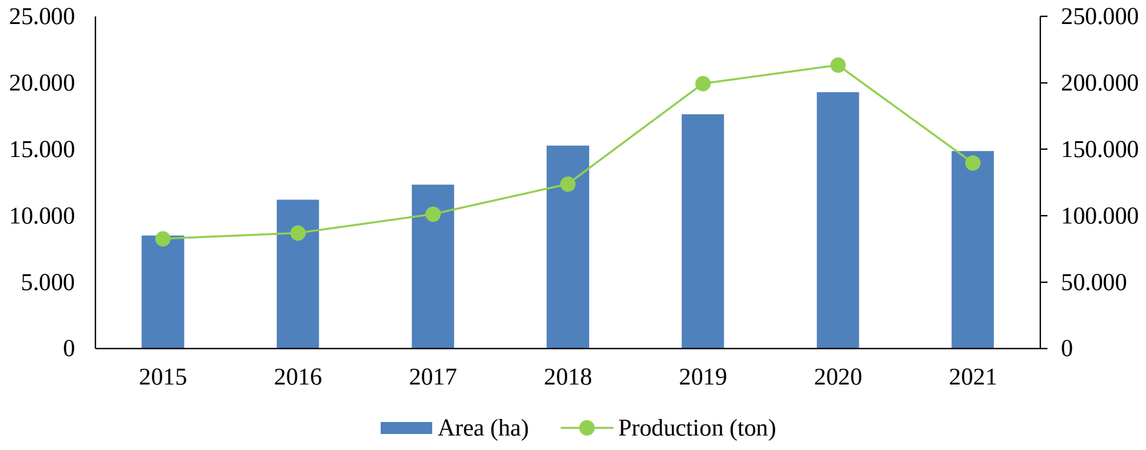


Figure 3.2. Harvested area and production of cassava in the Sucre Department.

3.3.4. Cassava value chain in Sucre department

The cassava VC in the Sucre department is characterized as a decentralized chain, where each link responds to independent interests and benefits. Two types of cassava are grown in the department: (i) industrial and (ii) sweet (M-Tai and Venezuelan varieties). The production of sweet cassava is destined for human consumption and involves producers, distributors or intermediaries, and final consumers. The product failing to satisfy the market's basic requirements is sent to the processing link. The cassava destined for agro-industry has the participation of producers, agro-industry (food and non-food), and finally, final consumers. In 2017, 81.3% of the total cultivated area was destined for sweet cassava, and the remaining was for industrial cassava. The general value chain of cassava in Sucre is shown in **Figure 3.3**, where the links and actors involved are visualized.

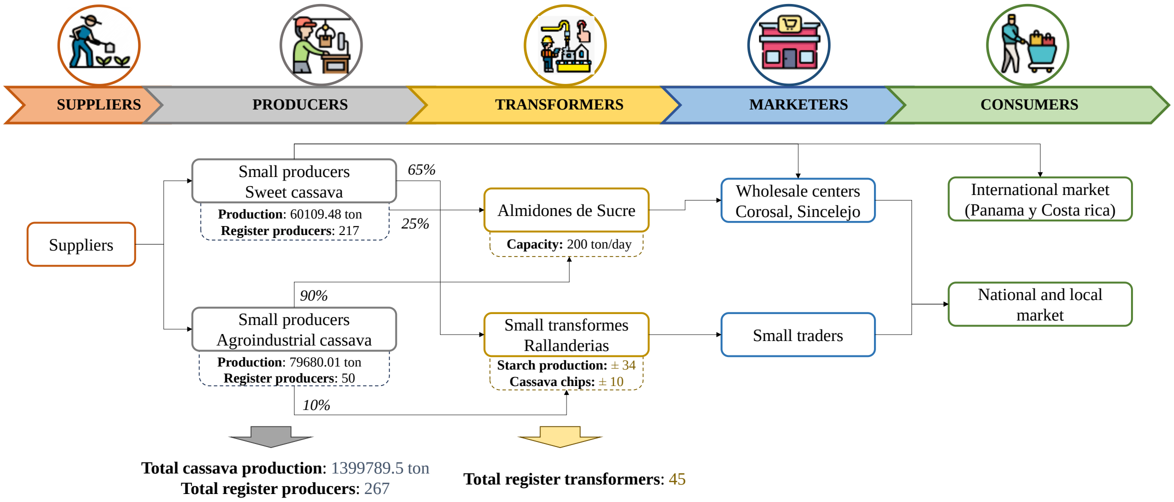


Figure 3.3. Cassava value chain in Sucre department.

The cassava VC scheme in the Sucre department consists of 5 links. The following typologies of actors were identified within each link: i. Suppliers: Suppliers of agricultural inputs and machinery; ii. Production: Small producers; iii. Transformation: Large and small local transformers; iv. Marketers: Starch traders and v. Consumer: Domestic consumers. The following is a detailed description of the actors.

3.3.4.1. Suppliers

Input suppliers only include one actor (input and equipment suppliers). These actors supply producers with the necessary materials (fertilizers, agrochemicals, and machinery) to perform their agricultural processes.

3.3.4.2. Producers

Only one actor is considered. The representative actor in this link is the producer of sweet cassava and agroindustrial cassava, typified as small producers, where the average area of cassava cultivation is less than 3 hectares. Producers generally have government-support research projects to improve production levels and benefit structures. Producers are members of various associations. According to the Colombian Federation of Cassava, there are 267 registered producers in the Sucre department. 43% are engaged in sweet cassava production, and 57% are engaged in agroindustrial cassava production. The region has a high informality rate, causing many producers to work independently without being linked to any association. In the Sucre department, the cassava crop is harvested by hand. Once the product is extracted from the soil, the cassava is packed in polypropylene bags and stored throughout the crop.

Producers were typified as small producers. The activities in this link are related to crop establishment, vegetative and productive stages, harvesting, and collection, as presented in Chapter 5. The environmental impact of manufacturing and transporting materials such as hand pumps and water pumps was not considered. The activities begin with removing weeds at the planting site using scythes with a gasoline consumption of 7 liters per hectare. The land is then conditioned by plowing and harrowing with a tillage tool to open furrows in the soil and remove the soil before planting. It has an ACPM consumption of 20 L per hectare. Tracing and hollowing is done manually. Planting is then done manually. It is estimated that in one hectare, there is a plantation of 10 thousand plants. After planting, the vegetative and productive stage of the crop begins. Agrochemicals such as herbicides and insecticides are added. First, threshing (diuron 800 g/L) is applied at a dose of 1 L per hectare. This is followed by invetrin (cypermethrin 200 g/L) at a dose of 2 L per hectare. Likewise, during this stage, two weed controls are performed with the addition of gramafin (paraquat 200 g/L) at a dose of 1 L per hectare. Seedling fertilization is carried out with triple 15 (NPK) additions, considering 50 kg per hectare coverage. Then, seedling thickener is added at 1 kg per hectare. Finally, harvesting is carried out. Between 12-24 months of the crop cycle is the optimal period for harvesting cassava when it is destined for the starch industry since this is when the maximum yield in roots is reached. Using fertilizers and agricultural crop inputs are sources of pollutant emissions to soil, air, and water. Nitrogen and phosphorus are among the compounds present in fertilizers to supply the nutritional needs of plants.

3.3.4.3. Transformers

The transformers focus their activities on producing intermediate products for the industry. Sweet cassava is destined to produce sweet starch. The starch is mainly destined for the food industry. Native starch (originating from agro-industrial cassava) is mainly destined for the bakery industry. Agroindustry plays a fundamental role in the cassava agro-food supply chain since the roots are used better, generating by-products that become fundamental raw materials for other industries. The Sucre department has companies transforming fresh roots, including Almidones de Sucre S.A.S., located at Km 4.5 Vía Sincelejo - Corozal. The company is dedicated to producing and marketing cassava starch and related products, with a fresh cassava reception capacity of 200 tons of cassava roots per day. There are also 10 small companies called rallanderias that produce native and sour starch in the municipalities of Sampues, Galeras and Sincelejo, Sincé, and La Unión. Ten agroindustrial companies are also engaged in producing dried cassava chips in the San Juan de Betulia, San Antonio de Palmito, Los Palmitos, Corozal, Ovejas, and San Pedro. This group of companies, including Almidones de Sucre, comprises processing companies (21 companies). A particularity of this practice in the Sucre department consists of processing in places exposed to the environment, allowing the deterioration of the quality of the product. The principle of the starch production process is based on the isolation of cassava components. **Figure 3.4** presents the main phases of the process. The process starts with the reception of the raw material. Then, the roots are cleaned, and the husk is removed. The root is taken to a grating process of releasing the starch from the root. In grating, the starch granules contained in the root cells are released. The efficiency of this operation is crucial to the yield of the process. Then, the straining and sieving process is carried out to separate the starch from the afrecho (by-product). Next, sedimentation is performed to separate the starch from the other less dense components, such as stain, fine fiber, and wasstewater. This process takes about three hours. At the end of this phase, a layer of compacted starch remains at the bottom. In the middle part, the stain remains, and the supernatant water is discarded.

The Secretary of Agriculture of Sucre has registered 27 rallanderias. Fifteen rallanderias operate regularly. In 2022, more rallanderías were recorded, especially during the harvest season (November to February), which operators and other intermediaries rented in Cauca. They generally process the roots in Sucre and Córdoba and transport the native starch to Cauca. Fermented starch is used in expanded products (such as doughnuts, cassava bread, or pandebono). Three new rallanderías were recently built (one in 2018 and two in 2022) in Sucre, with a processing capacity of 30 t/day of starch. This means they require about 150 t/day of fresh roots on average. One of the main constraints for the construction of these plants was the availability of water. Therefore, achieving high water flow rates can be challenging for the Sucre and Córdoba departments. Therefore, the environmental impact of starch production can be negative without proper water management. Considering the mass balance shown in **Figure 3.4**, wastewater (WW) represents about 67% of the total waste generated. On the other hand, the bran and stain are generally by-products destined for animal feed. WW is not valorized and is disposed of in water sources near the farm, causing environmental repercussions.

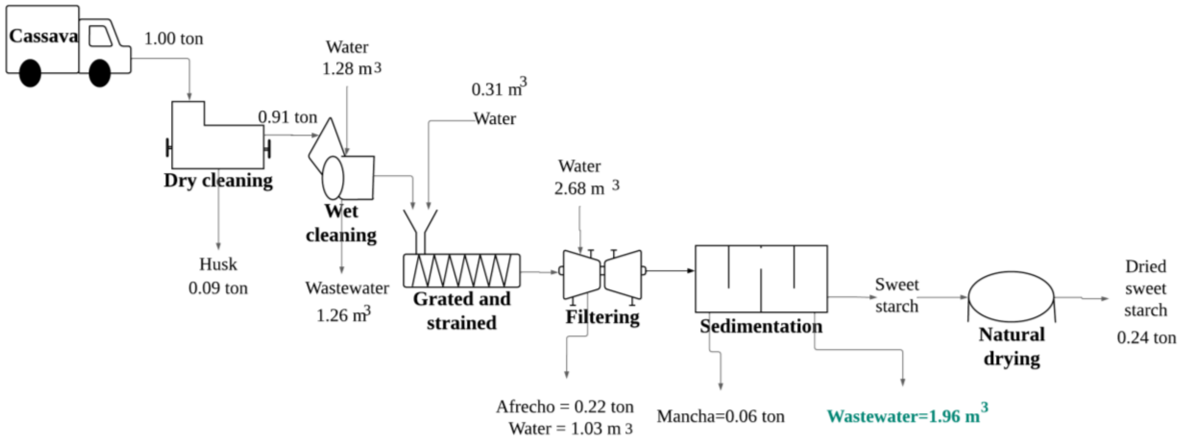


Figure 3.4. Stages of the cassava starch production process.

3.3.4.4. Marketers

This link includes those selling small and large quantities of fresh cassava or intermediate products. Local distributors such as market centers, stores, and small retailers are identified for this link in the cassava agroindustrial chain. In the Sucre department, there are two wholesale centers, one located in the Corozal municipality and the other in the city of Sincelejo. National distributors include market centers in other cities in the country and large chain stores, mainly in Montería, Cartagena, and Barranquilla.

3.3.4.5. Consumers

This link is made up of the consumers of the chain's products. They obtain the most important chain products: bakery, fresh cassava, and other by-products derived from cassava.

Based on the functional analysis of the cassava VC in the department of Sucre, the main bottlenecks in the chain were identified. The main strengths, weaknesses, opportunities, and threats (DOFA analysis) are consolidaed in **Table 3.2.**

Table 3.2. DOFA analysis of cassava value chain.

Strengths	**Weaknesses**
• Diverse crop materials: Different vegetable materials (sweet and agro-industrial) allow them to obtain products with market recognition.	• Producers are decentralized in the territory, preventing the generation of economies of scale.
• Easy access to inputs due to different projects in the region.	• Producers have a low technological level.
• Structured and consolidated market	• Small processors with low technological levels. This causes the flow of cassava to be destined for large industries.
• Ideal climatic conditions to generate higher productivity	• Inadecuade waste disposal in the transformer link (wastewater).
• Agroforestry production system with low use of agrochemicals.	• Transformer units in the open air. Generates productivity losses.
Opportunities	**Threats**
• National and international market: Cassava is a pillar crop for food consumption. The domestic market can consume the increased production.	• Deficient road infrastructure • Renting of external agents from processing plants • Depletion of water resources in the region.

3.4. Ethanol value chain

3.4.1. Ethanol production in the world

The production and consumption of liquid biofuels showed 4 % and 3 % increases in 2021 compared to 2020. Among the different types of liquid biofuels, bioethanol stands out as the one with the highest production, with a growing use of corn as raw material in the last 15 years. Moreover, there is an important production tradition derived from sugarcane, especially driven by Brazil. In the last decade (2012-2021), world production of liquid biofuels showed a cumulative growth of 41 %. The top five producers of liquid biofuels are the United States (42 %), Brazil (24 %), Indonesia (6 %), China (3 %) and Germany (3%). Worldwide, bioethanol production grew 23 % over the last decade (2012-2021). The five main bioethanol producers are the United States (54 %), Brazil (29 %), China (3 %), India (3 %) and Canada (2%).

3.4.2. Ethanol Production in Colombia

Colombia has a total installed ethanol production capacity of 17 million gallons/month. The ethanol produced in Colombia comes exclusively from sugar cane processing from the geographical Cauca River valley (Cauca, Valle del Cauca, and Risaralda). Ideal agroclimatic conditions in this region allow sugarcane to be harvested and milled year-round. The sugarcane agroindustrial sector is concentrated in over 50 municipalities in 5 departments (Valle del Cauca, Cauca, Risaralda, Caldas, and Quindío) along the Cauca River valley (see **Table 3.3**). The sugarcane agroindustrial sector is made up of (i) 4500 sugarcane growers, (ii) 15 sugarcane processing plants, (iii) 8 of the plants have attached distilleries for bioethanol production, (iv) all are energy co-generators, (v) 1 plant produces only ethanol, (vi) 4 support institutions for the sector (Cenicaña, Asocaña, Procaña and Tecnicaña), (vii) More than 50 specialized suppliers (transportation, packaging, agricultural services, among others). Valle del Cauca currently accounts for 73.53% of total national ethanol production.

Table 3.3. Installed capacity and ethanol production of sugar mills in the Cauca River valley.

Sugar mill	**Installed capacity (ton/day)**	**Ethanol production capacity (L/day)**
Riopaila	17600	400000
Incauca	17000	350000

Manuelita	11500	250000
Providencia	10000	300000
Mayaguez	10000	300000
Castilla	8000	
La Cabaña	5200	
Risaralda	5000	100000
Pichichi	4400	
Carmelita	2500	
San Carlos	2000	
María Luisa	800	
Total Valle del Cauca (ton/dia)	66800	1250000

3.4.3. Ethanol value chain in the department of Valle del Cauca

The ethanol value chain in the department of Valle del Cauca is characterized by considering the sugarcane agroindustry (sugar mills) as the central pillar. Ethanol production in Valle del Cauca is obtained from a by-product of the sugar industry. During the sugar production process, a by-product called molasses is obtained. Molasses contains a high content of organic matter suitable for ethanol production. The production of 1 ton of sugar generates 357.43 kg of molasses. The **Figure 3.5** shows the general ethanol value chain in the Valle del Cauca department, showing the links and actors involved.

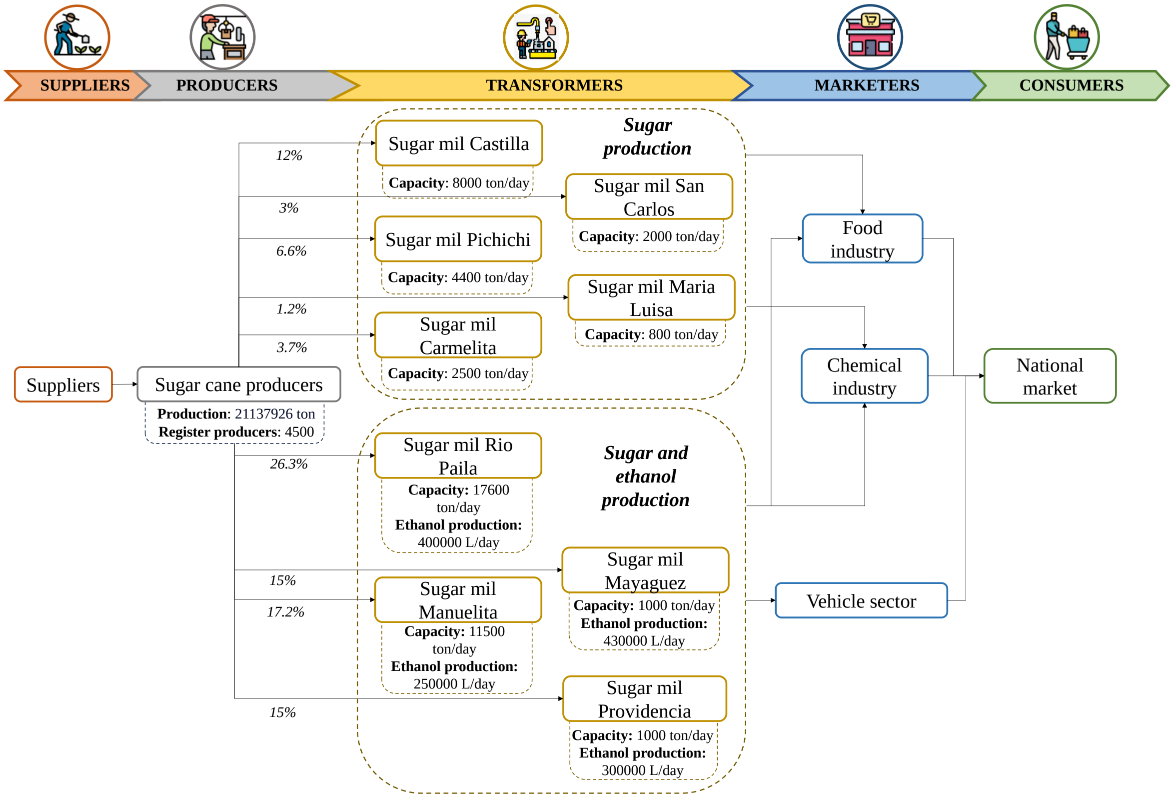


Figure 3.5. Ethanol value chain in Valle del Cauca department.

As mentioned above, the ethanol value chain in the Valle del Cauca department is linked to the sugarcane agroindustry. Sugarcane (Saccharum Officinarum) is a perennial crop that grows mainly in tropical and subtropical regions. In Colombia, the sugarcane cluster is an important contributor to the country's economy. In 2021, the sugarcane agroindustry accounted for 0.6% of the total national gross domestic product (GDP) and 3.7% of the national agricultural GDP. Sugarcane is located mainly in the geographic valley of the Cauca River. Primary sugarcane production is located in 30 municipalities in Valle del Cauca, 9 in Cauca, 5 in Caldas, 5 in Risaralda, and 1 in Quindío. In Valle del Cauca, 65.3% of the land is planted with sugarcane, and the area is approximately 60 hectares. Sugarcane is important since it is the raw material for the sugar industry, with an average per capita consumption of 20 kg of sugar per year and a production of 12.4 tons per hectare. In addition, sugarcane, and derivatives (honey, bagasse) can generate ethanol and energy, among others. There are currently 15 sugarcane processing plants for sugar production, nine in Valle del Cauca. The ethanol value chain scheme in the department of Valle del Cauca consists of 5 links. The following typologies of actors were identified within each link:

i. Suppliers: Suppliers of agricultural inputs and machinery; ii. Production: Major producers; iii. Transformation: Major local processors known as mills; iv. Marketers: Sugar and ethanol marketers; v. Consumers: National and international consumers. Since this paper focuses on the ethanol value chain, the following describes the main actors involved.

3.4.3.1. Suppliers

Input suppliers only include one actor ("Suppliers and equipment"). These actors supply producers with the necessary materials (fertilizers, agrochemicals, and machinery) for their agricultural processes. The agribusiness comprises over 50 suppliers specialized in inputs, machinery, and equipment for growers and mills.

3.4.3.2. Producers

Includes independent farmers (75% of the planted area) and sugar mills' crops. This link is classified as a large producer due to its productive capacity and the level of technology implemented. Colombia has the highest crop productivity worldwide. These levels have been achieved due to the innovation, research, and technological development of the association and the productive sector. In particular, the internationally recognized work of the Colombian Sugarcane Research Center (Cenicaña) in improving productivity, developing new varieties, and water management, among other aspects, has been a key factor in its success. Sugarcane cultivation generates agricultural residues such as the head and green leaves (8%), pods, and dry leaves (20%). These residues are generally disposed of in the field.

3.4.3.3. Transformers

Transformers focus their activities on the production of intermediate products for the industry. Sugarcane is destined for sugar production. Agroindustry plays a fundamental role in the chain by making better use of the stalks, generating by-products that become essential raw materials for other industries. 9 companies in the department process fresh stalks, including the Riopaila, Manuelita, Providencia, Mayaguez, Castilla, Pichichi, Carmelita, San Carlos, and María Luisa mills. These mills produce sugar with varying production capacities (see **Figure 3.5**). In addition, 4 of the mills mentioned (Riopaila, Manuelita, Providencia, and Mayaguez) produce ethanol from molasses. The Eder family owns the Manuelita sugar mill. The Hurtado-Holguin and Riopaila groups own Ingenio

Mayaguez, and the Caicedo Gonzalez family owns Central Catilla. These business groups and the governments have created an institutional framework that supports them throughout the agrofuels chain, represented by associations such as Asocaña (Colombian Sugarcane Growers' Association), Fedebiocombustibles (Colombian National Biofuels Federation), Procaña (Association of sugarcane producers and suppliers) and Procaña (Association of sugarcane producers and suppliers).

The principle of the ethanol production process is based on converting glucose monomers to ethanol. The **Figure 3.6** shows the main stages of the process. The process starts with molasses adequacy. Sucrose is not fermentable, but when hydrolyzed, the glucose-fructose complex is equivalent to two glucose molecules available for conversion to ethanol and carbon dioxide. Molasses is then fermented, generally with the microorganism Saccharomyces cerevisiae. The ethanol generated is then recovered and separated through a distillation and rectification tower. Each 1000 kg of molasses generates 246.43 kg of ethanol. On the other hand, vinasse is a by-product obtained from distillation. For 1 liter of ethanol, 14.12 liters of diluted vinasse are generated. Vinasse has been considered one of the most polluting materials in the ethanol sector. Even vinasse sometimes limited ethanol production capacity due to the difficulty and responsibility of the final disposal of this by-product. Due to the above, the model of alcohol production in Colombia through distilleries annexed to sugar industries, with recirculation of stillage and consumption of medium and low-pressure steam, with vinasse concentration at different values, was adopted. The result was environmentally positive as it reduced from 13 to 15 liters of vinasse produced per liter of ethanol to 4 to 2 liters per liter of ethanol. Therefore, two models for the final disposal of stillage were implemented in Colombia: (i) composting (low-solids vinasse) and (ii) the use of vinasse as a liquid fertilizer when mixed with urea (high-solids vinasse). Neither of the two models adopted turned out to be a perfect model since the time and areas required for the degradation of the organic matter contained in vinasse and compost made the areas grow significantly, requiring more human resources and machinery, among others. Concentrating the vinasse to 35 or 50 % of total solids w/w did not reduce the organic matter.

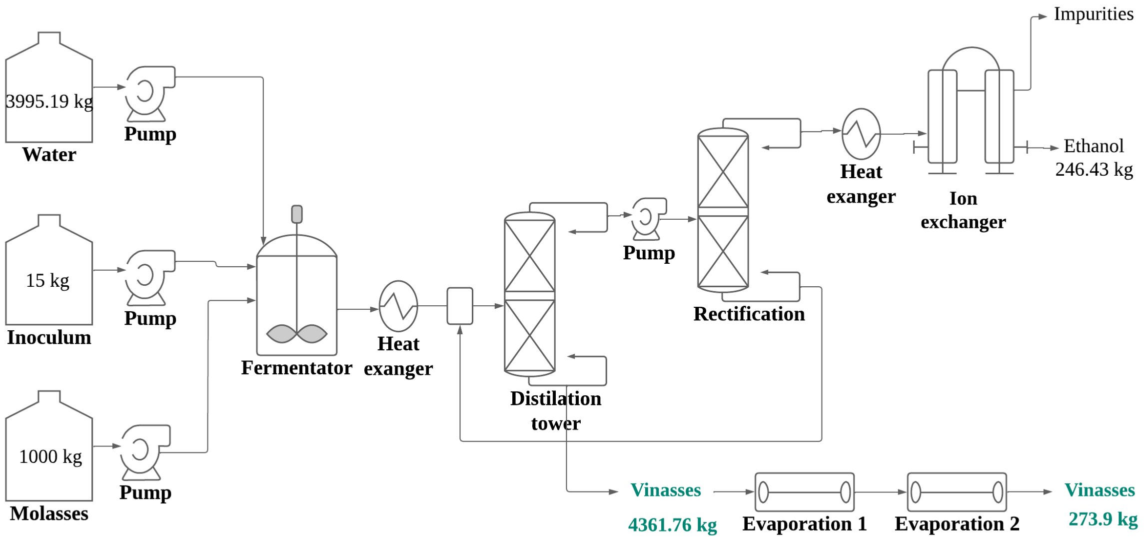


Figure 3.6. Stages of ethanol production process.

3.4.3.4. Marketers

Marketers are those responsible for the sale of sugar and ethanol. Wholesale distributors and marketers are identified in this link. Sugar mills in the department of Valle del Cauca send their products (i.e. sugar and ethanol) to departments such as Antioquia, Atlantico, Bolivar, Cundinamarca, Santander, Norte de Santander, Risaralda, Cesar and cities such as Medellin, Bogota, Barranquilla, Bucaramanga, Cartagena, Cucuta, Pereira, Cali, among others. In addition, exports have been made to other countries. The three companies that participated in exports from the Valle del Cauca department in 2021 were local Ambiocom SAS, Sucroal SA, and the Providencia sugar mill. The main destination countries were the United States, Panama, and Ecuador.

3.4.3.5. Consumers

Consumers are the consumers of the chain's products. They obtain the most important chain products: sugar and the automotive sector.

Based on the functional analysis of the ethanol VC in the Valle del Cauca department, the main bottlenecks in the chain were identified. The main strengths, weaknesses, opportunities, and threats (DOFA analysis) are consolidated in **Table 3.4.**

Table 3.4. DOFA analysis of ethanol value chain.

Strengths	**Weaknesses**
• High productive capacity of sugar cane. The crop characteristics allow harvesting throughout the year. • Easy access to inputs due to different projects in the region. • The production sector's innovation, research, and technological development is due to alliances with corporations such as Cenicaña. • Structured and consolidated market • Ideal climatic conditions to generate higher productivity	• According to secondary information reported in the literature, the sugarcane crop has the highest environmental impact (carbon footprint). • Current vinasse disposal can generate negative environmental impacts associated with eutrophication and soil acidity problems. • There is no consolidated treatment or valorization route for vinasse.
Opportunities	**Threats**
• National and international market: Sugarcane by-products (sugar, ethanol) are essential products that contribute to the country's economy.	• A greater supply of vinasses as fertilizers than the market demand

4. Chapter 4: Experimental results

4.1. Overview

This chapter presents the experimental results obtained from the characterization (chemical composition, proximate analysis, solids analysis) of OKFW. The WW and vinasse characterization were obtained from the supply source. Additionally, the results of the conventional AD and modified AD for biogas and mixed VFAs production are performed and discussed. The results of the compositional profile of the different VFAs (acetic, propionic, and butyric acid) generated in the process were performed and discussed. Then, the results were compared with data published in the literature.

4.2. Organic kitchen food waste

4.2.1. Characterization

The characterization (chemical, proximate, and solids) on a dry basis of the OKFW is presented in **Table 4.1.** OKFW obtained from specific point sources (restaurants) were obtained in two studies listed in the Table. The organic waste characterization is linked to the source and the socioeconomic context evaluated. For this reason, the organic waste composition model reported by Ortiz M et al. was implemented to develop this work. The model has been shown to adjust to the food consumption trends in a specific context and region. The model was based on consumption trends in the Colombian Caribbean region (department of Sucre). The extractives and fiber fractions (cellulose, hemicellulose, and lignin) were the most representative. These results are congruent due to the high content of tuber residues, specifically cassava and yam husks. These results are similar to those reported by Ortiz M et al. [167]. On the other hand, these results presented contrasting results with other authors, attributed to those mentioned above. The characterization of the raw material allows the definition of a suitable production or valorization process. This analysis provides essential information to propose a rational design. Based on the information provided in **Table 4.1Table 4.1**, specifically the proximate analysis results, the feedstock applicability in thermochemical processes (combustion, pyrolysis, gasification) can be elucidated from a conceptual perspective. Although the ratio of volatile matter to fixed carbon is low (6.96), showing the applicability in pyrolysis processes for bio-oil production [202], the high moisture content (>70%) hinders the use of this feedstock for thermochemical processes [203]. However, OKFWs have a high organic matter content, including carbohydrates, lipids, and proteins, resulting in high biodegradability [204]. Therefore, OKFWs are promising feedstocks to be valorized in biotechnological processes. This is confirmed by the high content of volatile solids (VS). The VS represents the amount of organic matter present in the feedstock [205].

Table 4.1. OKFW characterization generated from the compositional model and comparison with the literature.

Characterization	**This work**	**Ortiz M et al** [167]	**Rezwanul I et al** [206]	**Abdul-Sattar N et al** [207]

Country	Colombia	Colombia	Japan*	Saudi Arabia*
Organic waste composition	See **Table 2.3**	62% fruits and vegetables (20% tomato peel, 16% onion peel, 10% bean residue), 25% roots and tubers (60% potato peel, 30% cassava peel, 10% carrot peel)	Orange peel (50%) and equal proportions of potato peel, bp, egg peel, cow bones, chicken bones.	Rice (38.72%), meat waste (25.15), used oil (13.03%) and fruits and vegetables (2.16%).
Chemical characterization (%wt)				
Moisture	75.9 ± 0.76	83.15	7.26	38.4
Total extractives	34.55 ± 1.65	20.92 ±0.65	N.R	N.R
Water extractives	6.8 ± 1.52			
Ethanol extractives	27.75 ± 0.13			
Cellulose	31.18 ± 1.04	19.71 ±2.67	N.R	
Hemicellulose	4.70 ± 0.51	5.12 ±0.29	N.R	25.56
Lignin	16.65 ± 0.93	13.69 ±0.34	N.R	
Fats	7.96 ± 0.71	6.34 ±0.40	N.R	15.27
Ash	4.96 ± 0.08	3.23 ±0.03	N.R	3.21
Proximate analysis (%wt)				
Volatile matter	81.75 ± 0.95	N.R	63.85	N.R
Ash	6.51 ± 0.08	N.R	2.06	N.R
Fixed carbon	11.75 ± 0.88	N.R	26.83	N.R
Solid analysis (g/ 100 g OKFW)				
Total solids	90.54 ± 0.34	91.67 ± 0.29	N.R	N.R
Volatile solids	83.94 ± 0.37	80.54 ± 0.32	N.R	N.R

*Values on a dry basis, N.R No report

The characterization of the reducing sugars, total phenolic content (TPC), and antioxidant capacity (AC) contents of the extractives in water and ethanol and of the hydrolysate obtained from the LHW pretreatment are presented **Table 4.2**. The ethanol extractives presented higher reducing sugars and TPC content than the water extractives. These differences are mainly due to the polarity of the solvent used in the extraction. Compounds present in the extractives are more sensitive to polar compounds such as ethanol. These results are varied compared to those reported in the literature due to the compositional complex of the residues analyzed. For example, Castrica M et al. evaluated AC from agri-food residues. They determined that fruit and vegetable residues (a mixture of tomato peel, bell pepper, eggplant, lettuce, pear, and apple) presented a AC of 1.11 g eq Trolox per 100 grams of residues [208]. On the other hand, the TPC of banana peel and grape seed was 3.8 and 37.4 mg Gallic acid/ g residue, respectively [209]. The hydrolysate obtained from the thermal pretreatment (LHW) presented a reducing sugar content of 6.35 ± 0.28 g/L, TPC of 9.15 ± 0.01 mg Gallic acid/ g RM, and an AC of 0.282 ± 2.80 g eq Trolox/100 g RM. The reducing sugar content was similar to that reported by Tianyia F et al. [210]. In that work, hot water hydrolysis of food waste from a University in Beijing was performed. The content of reducing sugar was 6.96 g/L (T: 110°C, time 120 min, solid: liquid ratio of 1:10). However, the results are contrasted with other pretreatments reported. For example, Zhang C et al. [211] determined that the hydrolysate obtained from enzymatic hydrolysis of food waste was 125 g/L.

Table 4.2. Extractives and hydrolysate characterization of the LHW pretreatment.

Parameter	Ethanol extractives	Water extractives	Hydrolyzed
DNS (g/L)	4.28 ± 0.09	0.43 ± 0.03	6.35 ± 0.28
TPC (mg Gallic acid/ g RM)	6.20 ± 0.02	2.69 ± 0.02	9.15 ± 0.01
AC (g eq Trolox/100 g RM)	0.228 ± 0.043; 40.1% I	0.202 ± 0.092; 34% I	0.282 ± 0.080; 61% I

RM: Raw material; I: Inhibition

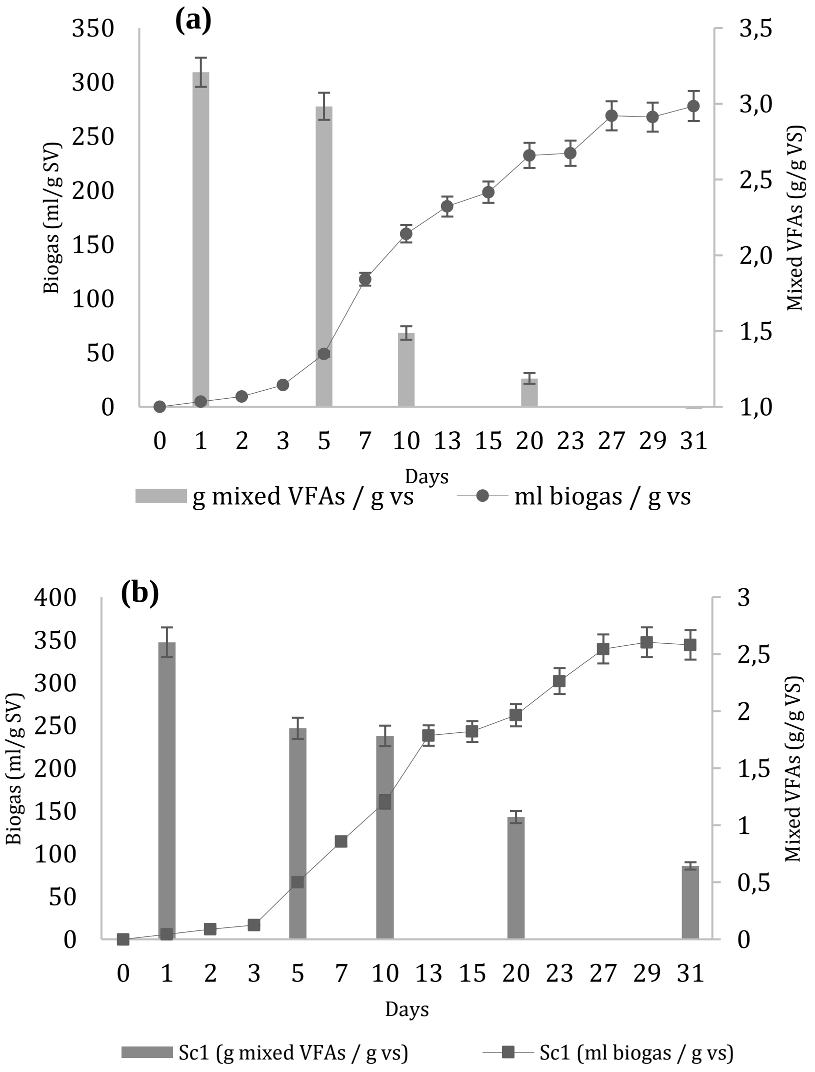
4.2.2. Biogas and mixed volatile fatty acids production

The biogas production yield from conventional AD is shown in **Figure 4.1.** The base case (**Figure 4.1 a**) generated a biogas production yield of 0.4321 Nm^3/kg SV and a CH_4 and CO_2 content of 59% and 41%, respectively.

From day nine onwards, the biogas production yield decreased, indicating substrate consumption. The biogas production was consistent with the production of mixed VFAs, which showed a decreasing trend along with the increasing biogas production. On day 1, the highest concentration of mixed VFAs (18.38 g/L) related to rapid hydrolysis and acidification occurred. At the end of digestion, the unconsumed mixed VFAs remaining in the liquor was 5.35 g/L. These results are similar to those reported in the literature. For example, Ortiz M et al. reported a biogas production yield of 0.42 Nm^3/kg SV OKFW [167]. Zhang C et al. performed biogas production from kitchen waste from Beijing University, obtaining yields of 0.62 Nm^3/kg SV with a methane content of 65% [212]. On the other hand, **Sc1**, (pretreated OKFW) generated a 25% increase in biogas production compared to the base case (0.5432 Nm^3/kg SV) as shown in **Figure 4.1 b**. The hydrolysis stage is similar in both cases. However, from day nine onwards, the biogas production of Sc1 increased. Therefore, the LHW pretreatment was effective in increasing biogas production. From day 21 of digestion, low volumes of biogas were recorded, indicating that most of the feedstock had been digested. Pretreatment achieved the lignocellulosic fractions solubilization to simpler fractions easily degradable by microorganisms in digestion [213]. The mixed VFAs production showed a similar (decreasing) trend to the base case. At the end of digestion, the unconsumed mixed VFAs was 6.48 g/L.

Recent studies have shown that thermal pretreatment implementing temperatures below 100°C can solubilize organic particles and improve biodegradability [214]. The content of lignocellulosic compounds is low for this type of biomass. Therefore, this type of pretreatment can satisfy the requirement to improve the biogas production yield without drastically impacting the cost associated with energy and reagent consumption [215]. Other authors have reported similar results. For example, Ma J et al. [216] reported an 11% increase in methane production by pretreating organic kitchen obtained from a company in Belgium (Majority components: carbohydrates derived from bread, cooked noodles, rice, proteins and fats from different types of meat and fish) and operating conditions of 120 °C for 30 min. Liao X et al. [217] reported an increase in biogas production of 24.4% when pretreatment was performed on activated sludge at 80 °C for 30 min. In addition, recent studies have shown that the hydrolysate obtained from pretreatment contains few inhibitors and can be used in fermentation processes [218]. Likewise, the hydrolysate characterization determined that the pretreatment removed phenolic compounds (see **Table 4.2**) that can be recovered.

Sc2 (added the hydrolysate and OKFW pretreated) (**Figure 4.1 c**) showed a reduction in hydrolysis time of over 80% was generated compared to base case. This is due to the presence of monomers more accessible to microorganisms. However, the presence of phenolic compounds in the medium inhibited the digestion process, reducing the biogas production (0.253 Nm^3/kg SV) compared to the base case. Biogas production was consistent with the mixed VFAs production. The mixed VFAs showed an increasing trend during the digestion time. The mixed VFAs formation was promoted by inhibiting methanogenic microorganisms, resulting in a final concentration of VFAs in the liquor of 27.25 g/L.



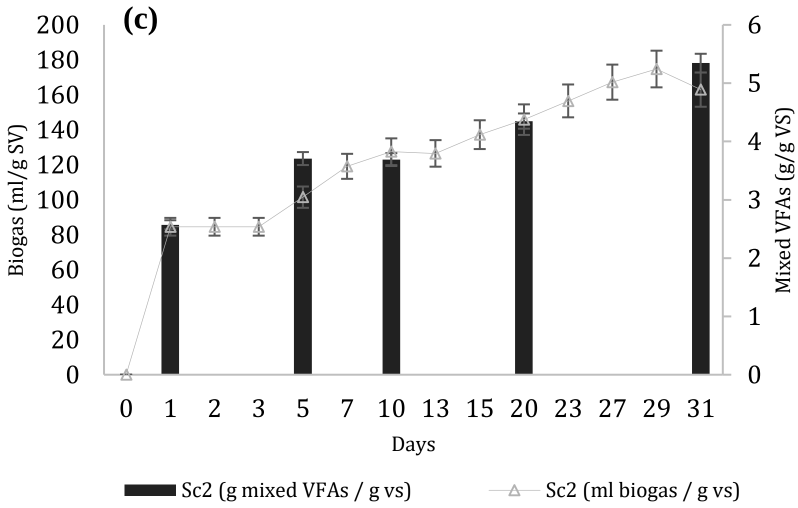
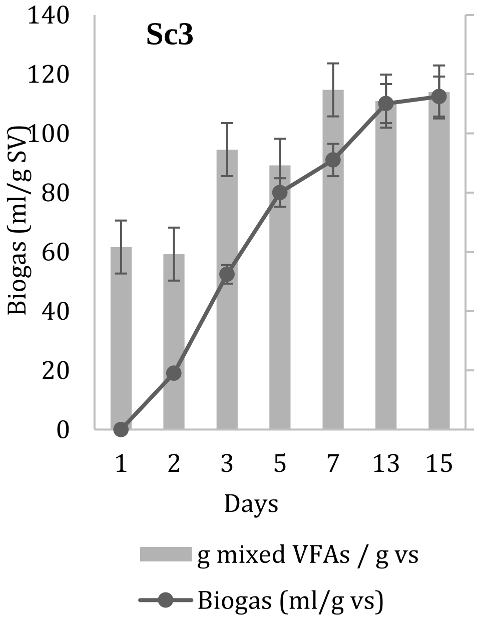
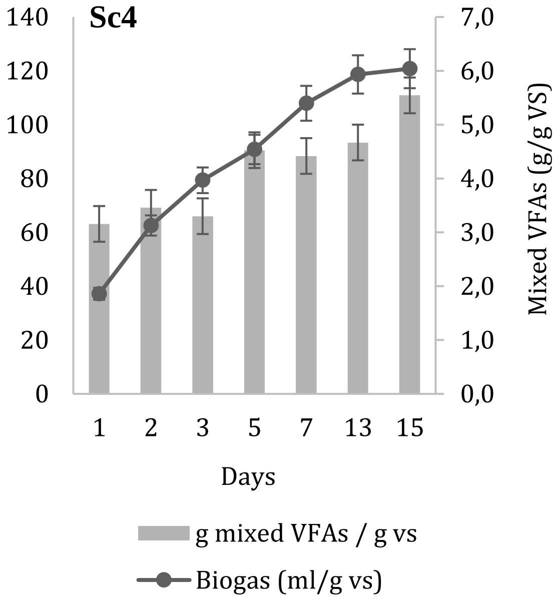
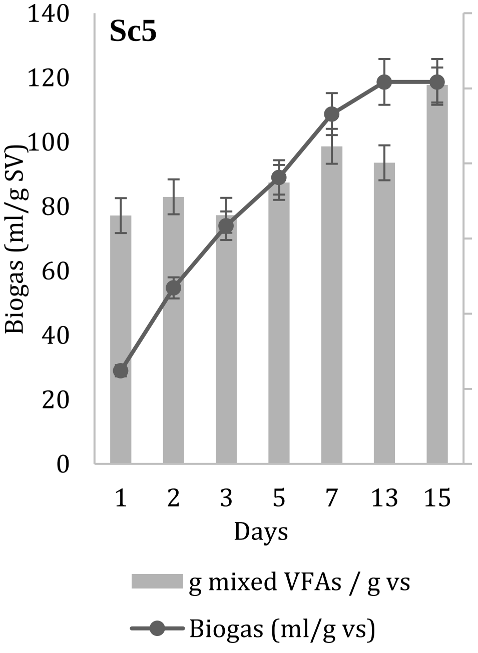


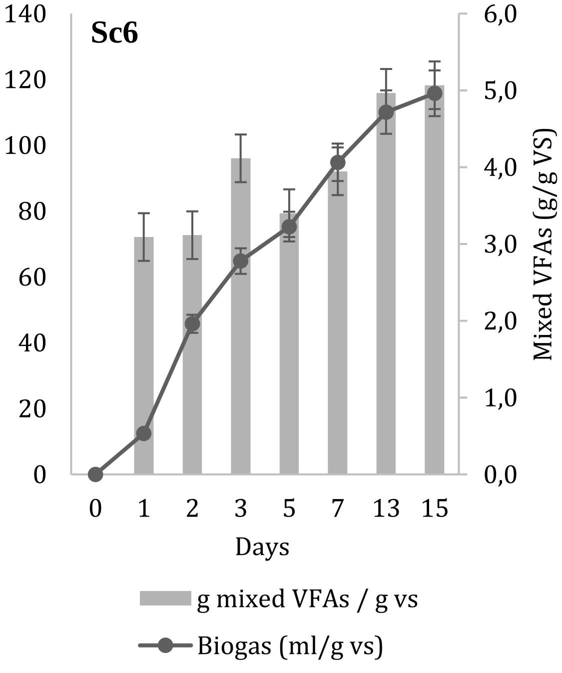
Figure 4.1. Biogas production yields from conventional AD and mixed concentrations of total VFAs. **(a)** Base case, **(b)** Sc1 and **(c)** Sc2.

The modified AD showed low biogas production during the digestion time for the evaluated scenarios as shown in **Figure 4.2**. The low biogas production is an indirect measure of the increasing mixed VFAs production. Likewise, the biogas composition (CH_4 and CO_2 content). The high CO_2 content indicates the VFAs production considering the stoichiometric equations of the stages of digestion. **Sc3** presented a biogas production of 0.188 Nm^3/kg SV and a CH_4 content of 14%. In **Sc4** (pretreated OKFW) the biogas production was similar. However, the CH_4 content was reduced by 14.3% compared to Sc3. Adding hydrolysate to the digestion (**Sc5**) decreased biogas production and methane content by 2.35% and 85.71%, respectively, compared to Sc3. On the other hand, the thermal pretreatment of the inoculum (**Sc6** and **Sc7**) (65°C, 30 min) generated a methane content of less than 1%.


Sc3
Biogas (ml/g SV)
140
120
100
80
60
40
20
0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
1
2
3
5
7
13
15
Days
g mixed VFAs / g vs
Biogas (ml/g vs)


Sc4
Mixed VFAs (g/g VS)
140
120
100
80
60
40
20
0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
1
2
3
5
7
13
15
Days
g mixed VFAs / g vs
Biogas (ml/g vs)


Sc5
Biogas (ml/g SV)
140
120
100
80
60
40
20
0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
1
2
3
5
7
13
15
Days
g mixed VFAs / g vs
Biogas (ml/g vs)


Sc6
Mixed VFAs (g/g VS)
140
120
100
80
60
40
20
0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
0
1
2
3
5
7
13
15
Days
g mixed VFAs / g vs
Biogas (ml/g vs)

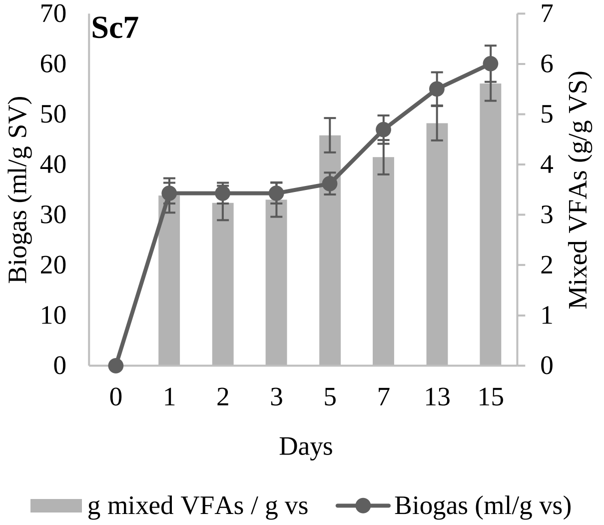


Figure 4.2. Biogas production yields from modified AD and mixed concentrations of total VFAs.

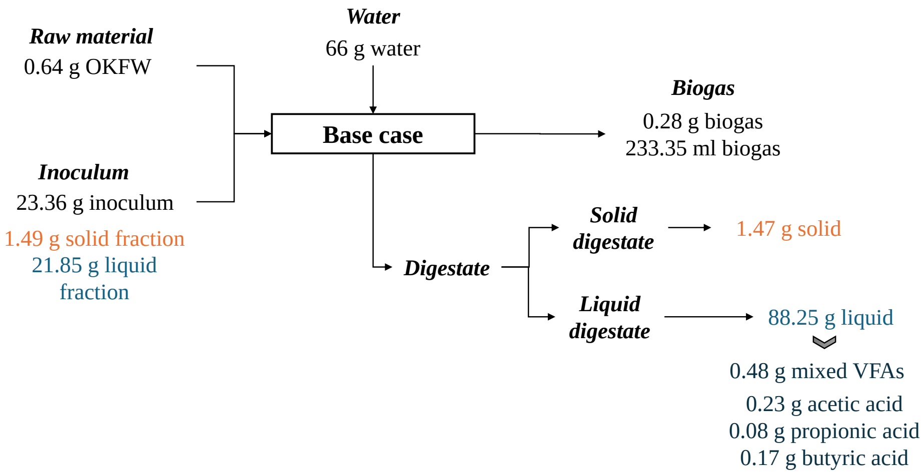
For all scenarios, a growing trend of mixed VFAs is shown. Using fresh OKFW (**Sc3**) generated a mixed VFAs yield of 18.75 g/L at the end of digestion. Using pretreated OKFW (**Sc4**) generated a production increase of 23.20% compared to Sc3. Adding hydrolysate to the digestion (**Sc5**) increased the mixed VFAs production by 53.29% compared to Sc3, as mentioned above, as shown in **Table 4.3.** The results of Sc5 are comparable with Sc2. This trend shows that although the operating conditions of the process promote biogas production (conventional AD), the addition of the hydrolysate obtained from the pretreatment generates an inhibition of methanogenic microorganisms. The mixed VFAs concentrations obtained for the OKFW of this work were in the range of the mixed VFAs yields obtained for similar food wastes in the literature (5–40 g/L) [75]. The variations in yields are attributed to the complex composition of the food waste and the operating conditions used [219].

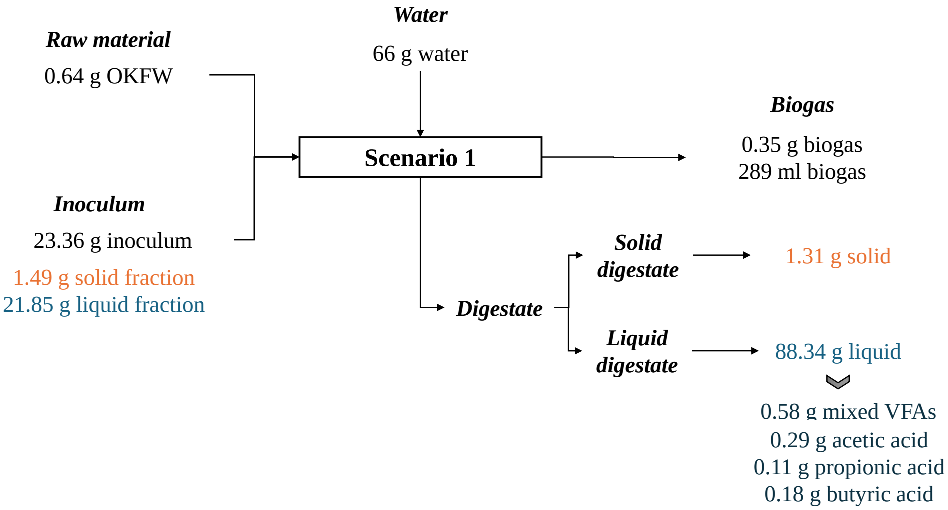
Table 4.3. Main results of the conventional and modified AD.

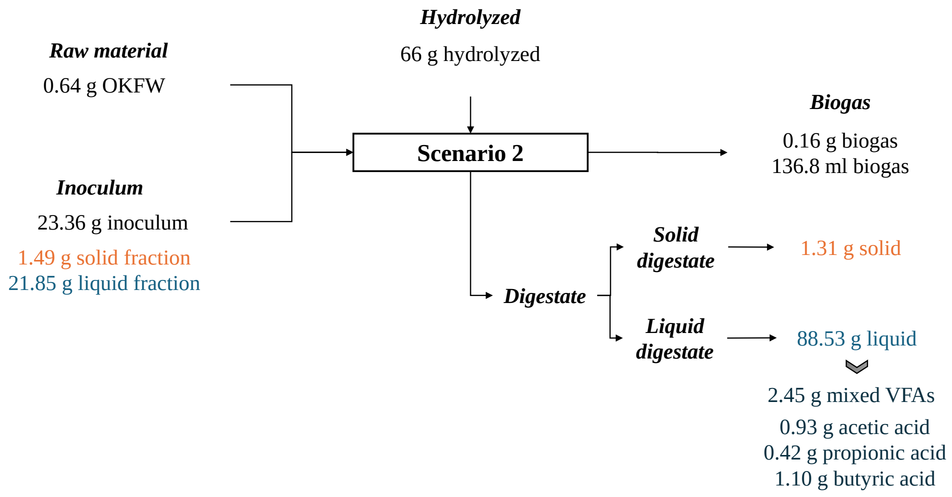
Scenarios	Scenario description	Biogas yield (ml/g VS)	%CH$_4$	%CO$_2$	Mixed VFAs yield (g/L)	Mixed VFAs composition (%vol)		
						Acetic	Propionic	Butyric
			Conventional AD					
Base case	OKFW fresh	432.13	59.00	41.00	5.35	48.11	17.51	34.38
Sc1	OKFW pretreated	535.19	65.00	35.00	6.48	50.12	18.19	31.68
Sc2	OKFW pretreated+ hydrolyzed	253.33	18.00	82.00	27.25	38.05	17.08	44.87
			Modified AD					
Sc3	OKFW fresh	121.51	14.00	86.00	18.75	45.11	15.62	39.27
Sc4	OKFW pretreated	120.79	11.00	89.00	25.11	48.40	13.19	38.41
Sc5	OKFW pretreated+ hydrolyzed	118.65	2.00	98.00	28.75	35.81	12.86	51.34
Sc6	OKFW fresh+ inoculum pretreated	115.79	<1	99.00	36.25	33.20	18.64	48.16
Sc7	OKFW pretreated + inoculum pretreated	60.04	<1	99.00	37.28	45.01	15.90	39.09

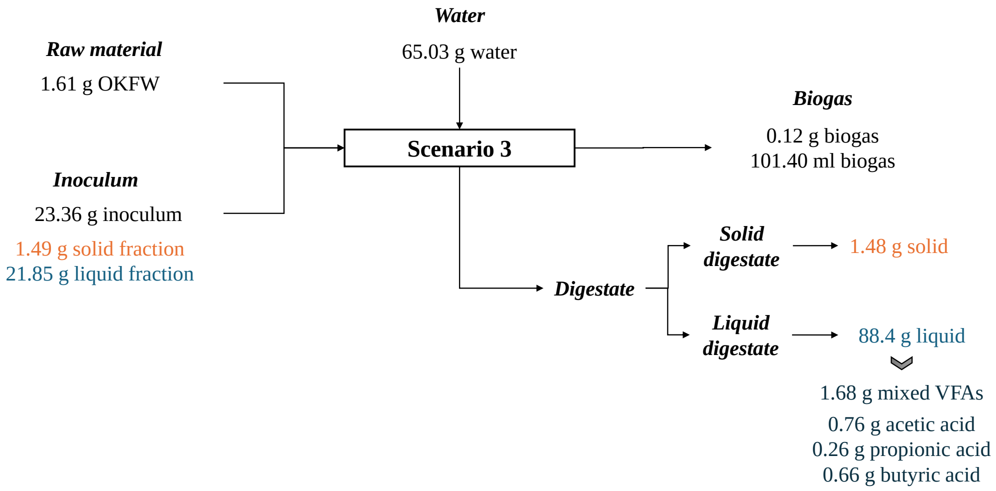
Using heat-treated inoculum (65°C, 30 min) demonstrated a considerable increase in the mixed VFAs production (**Sc6** and **Sc7**) as shown in the mass balances in **Figure 4.3**. The detailed mass balances for the proposed scenarios are shown in **Annex B: Detailed mass balances of experimental** . When inoculum pretreatment is performed, it is possible to use fresh or pretreated OKFW to obtain similar yields. Therefore, considering the economic aspects of the process, the raw material pretreatment step could be omitted. Heat treatment of the inoculum probably inhibited methanogenic microorganisms and allowed prolonged VFAs production. Several studies have reported similar results. Tampio et al. demonstrated that thermal treatment of the inoculum (94°C, 30 min) increased the production of mixed

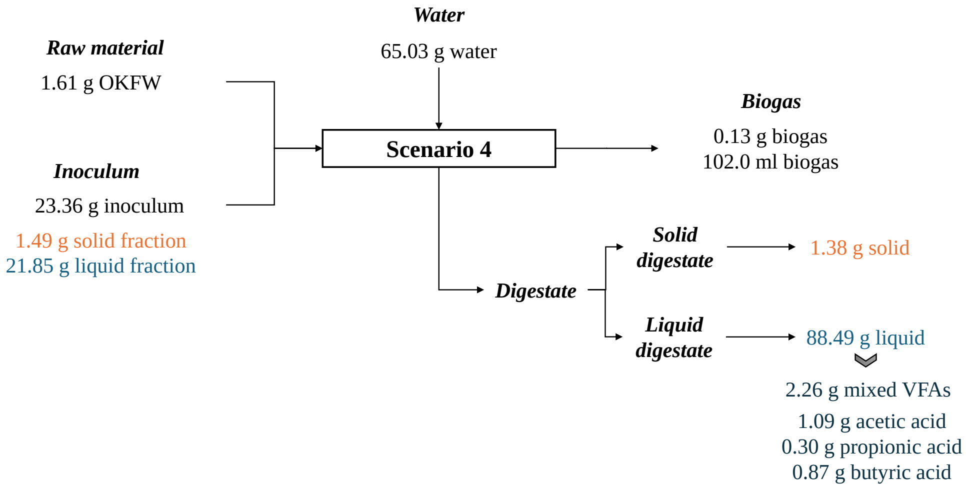
VFAs by more than 30% using food waste as raw material [220]. Blasco L et al. 2020 determined that digestions with inoculum subjected to different pretreatments, such as thermal or freezing-thawing, partially inactivated some archaea and bacteria, increasing VFAs production [221].

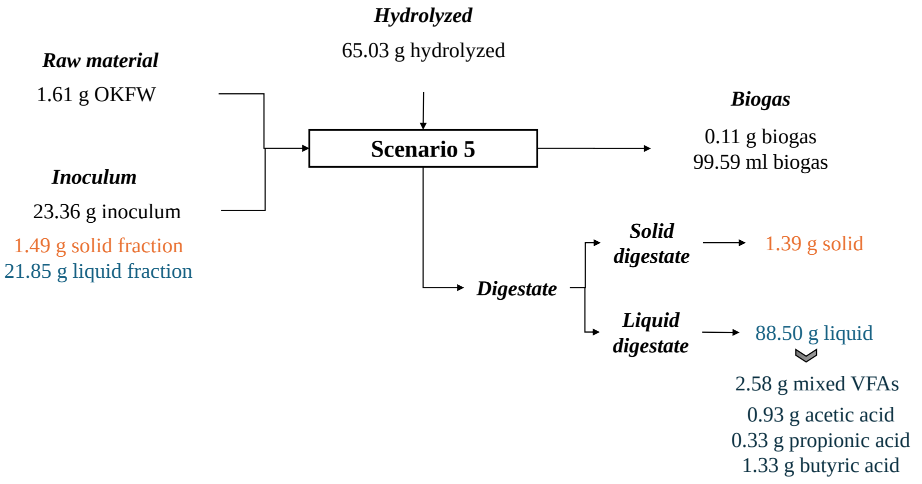


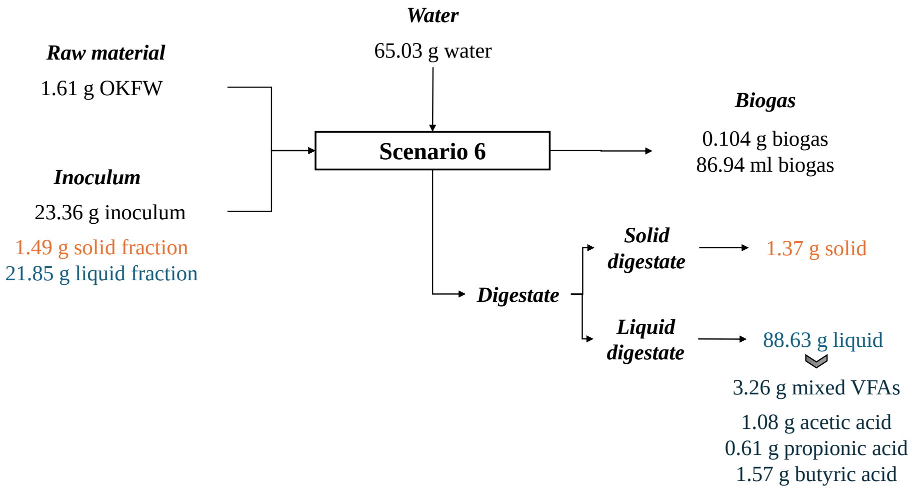



Hydrolyzed
66 g hydrolyzed
Raw material
0.64 g OKFW
Inoculum
23.36 g inoculum
1.49 g solid fraction
21.85 g liquid fraction
Scenario 2
Biogas
0.16 g biogas
136.8 ml biogas
Digestate
Solid digestate
1.31 g solid
Liquid digestate
88.53 g liquid
2.45 g mixed VFAs
0.93 g acetic acid
0.42 g propionic acid
1.10 g butyric acid


Water
65.03 g water
Raw material
1.61 g OKFW
Inoculum
23.36 g inoculum
1.49 g solid fraction
21.85 g liquid fraction
Scenario 3
Biogas
0.12 g biogas
101.40 ml biogas
Digestate
Solid digestate
1.48 g solid
Liquid digestate
88.4 g liquid
1.68 g mixed VFAs
0.76 g acetic acid
0.26 g propionic acid
0.66 g butyric acid


Water
65.03 g water
Raw material
1.61 g OKFW
Inoculum
23.36 g inoculum
1.49 g solid fraction
21.85 g liquid fraction
Scenario 4
Biogas
0.13 g biogas
102.0 ml biogas
Digestate
Solid digestate
1.38 g solid
Liquid digestate
88.49 g liquid
2.26 g mixed VFAs
1.09 g acetic acid
0.30 g propionic acid
0.87 g butyric acid


Hydrolyzed
65.03 g hydrolyzed
Raw material
1.61 g OKFW
Inoculum
23.36 g inoculum
1.49 g solid fraction
21.85 g liquid fraction
Scenario 5
Biogas
0.11 g biogas
99.59 ml biogas
Digestate
Solid digestate
1.39 g solid
Liquid digestate
88.50 g liquid
2.58 g mixed VFAs
0.93 g acetic acid
0.33 g propionic acid
1.33 g butyric acid


Water
65.03 g water
Raw material
1.61 g OKFW
Inoculum
23.36 g inoculum
1.49 g solid fraction
21.85 g liquid fraction
Scenario 6
Biogas
0.104 g biogas
86.94 ml biogas
Digestate
Solid digestate
1.37 g solid
Liquid digestate
88.63 g liquid
3.26 g mixed VFAs
1.08 g acetic acid
0.61 g propionic acid
1.57 g butyric acid

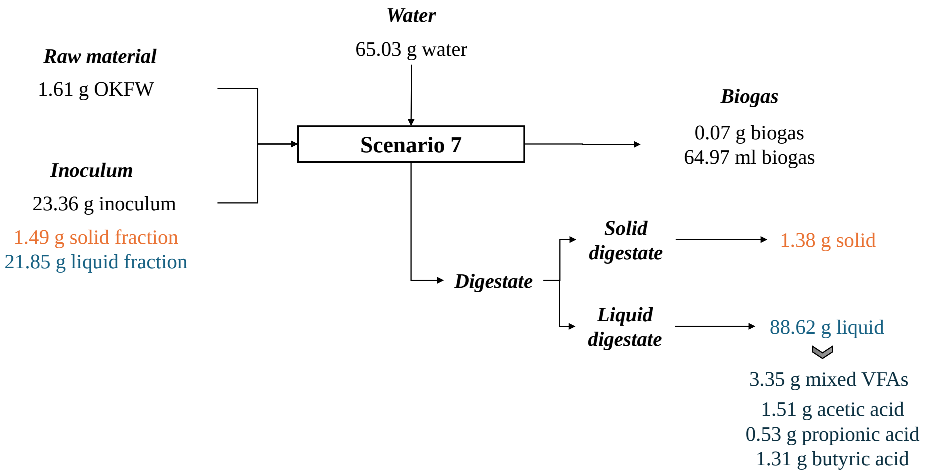
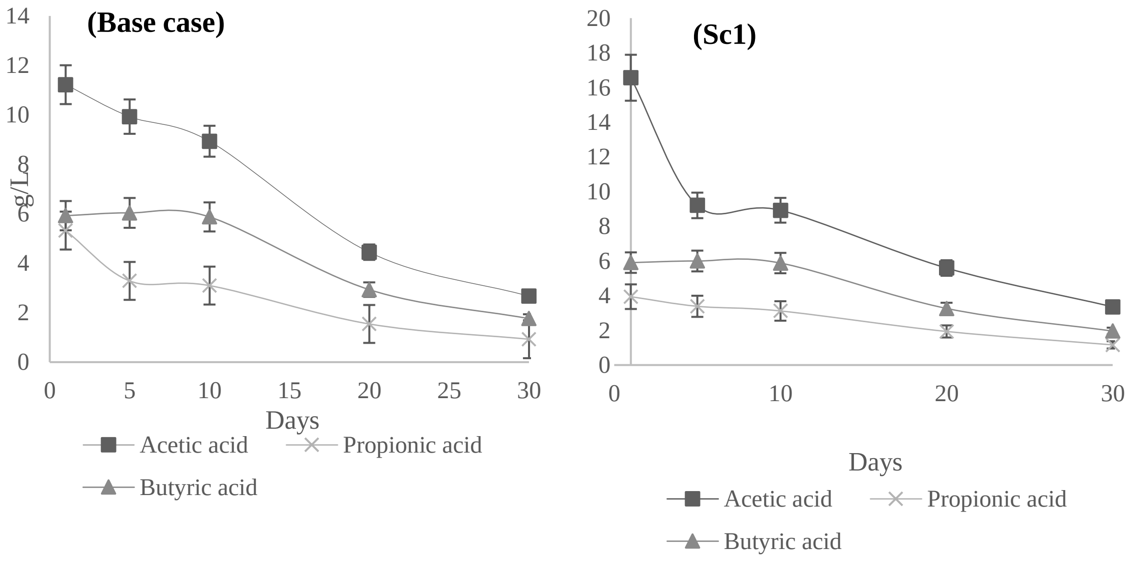

Water
65.03 g water
Raw material
1.61 g OKFW
Inoculum
23.36 g inoculum
1.49 g solid fraction
21.85 g liquid fraction
Scenario 7
Biogas
0.07 g biogas
64.97 ml biogas
Digestate
Solid digestate
1.38 g solid
Liquid digestate
88.62 g liquid
3.35 g mixed VFAs
1.51 g acetic acid
0.53 g propionic acid
1.31 g butyric acid

Figure 4.3. Mass balances of the experimental scenarios of conventional and modified AD using OKFW as raw material.

Regarding conventional AD, the VFAs profile showed a similar trend for the **base case** (fresh OKFW) and **Sc1** (pretreated OKFW) as shown in **Figure 4.4**. The mixed VFAs was mainly made up of acetic acid and had lower concentrations of butyric and propionic acid. The base case presented an initial content (day 1) of acetic acid of 59.70%, followed by 29.67% butyric acid and 10.63% propionic acid. Starting on day five, the concentration of acetic acid began to increase (from a minimum of 59.70% to 64.06% of the total). In comparison, propionic acid concentration decreased slightly (25.09% compared to day 1). The concentration of butyric acid showed increasing trends. **Sc1** (pretreated OKFW) showed an increase in the concentration of acetic acid at the beginning of digestion of 22.37% compared to the base case related to rapid hydrolysis and acidification. The acetic acid content decreased from the fifth day onwards (9.23% decrease compared to day 1). On the contrary, propionic, and butyric acid content began to increase. The addition of the hydrolysate (**Sc2**) increased the butyric acid content by 30.51% compared to the base case at the beginning of digestion.



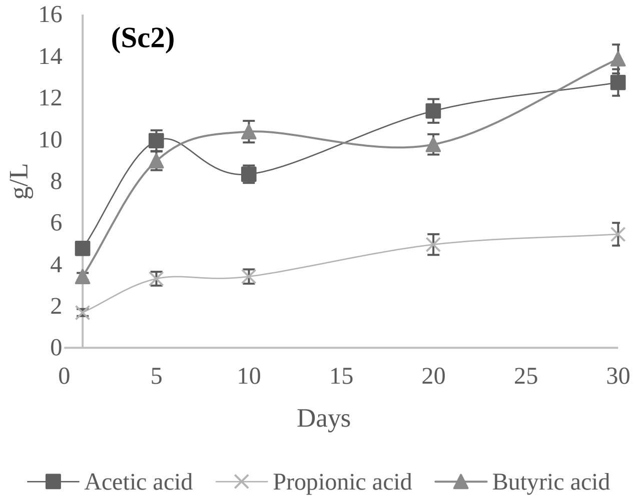
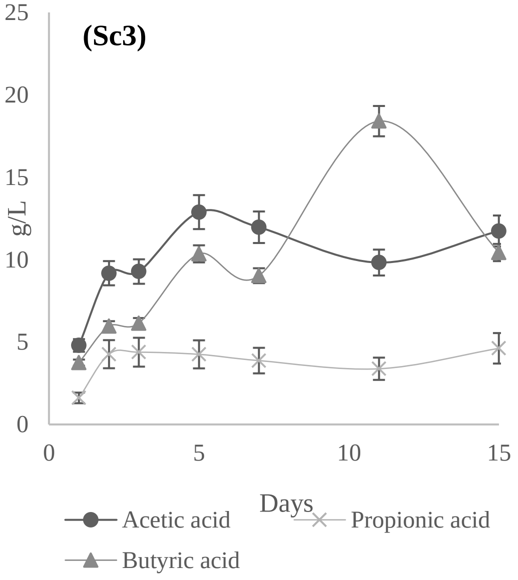
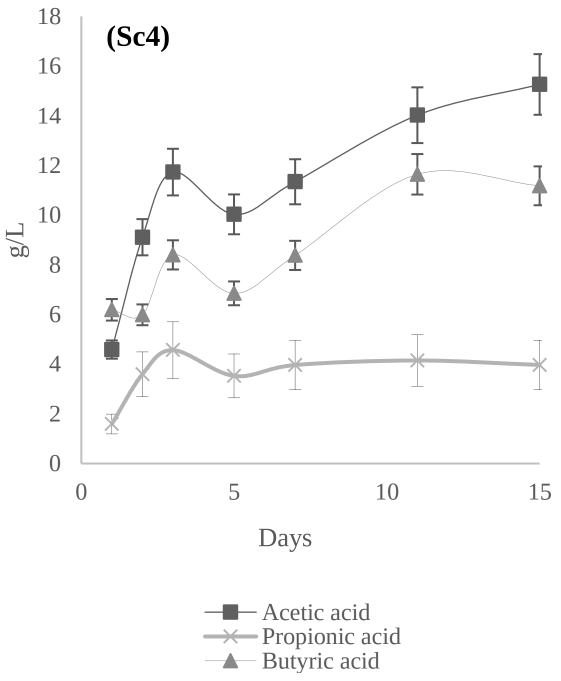
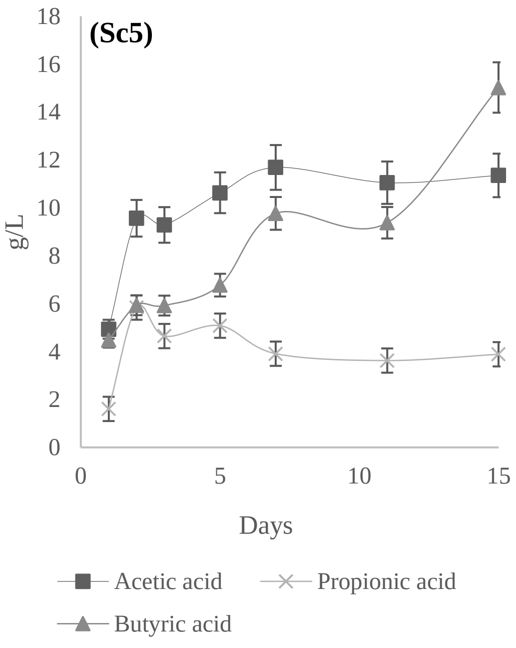


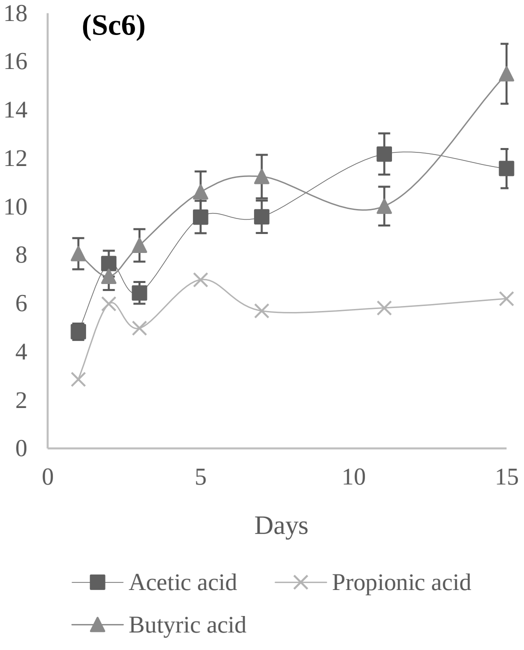
Figure 4.4. Profiles of mixed VFAs of conventional AD.

The modified AD (**Sc3, Sc4** and **Sc5**) presented the same trends as Sc2 (see **Figure 4.5**). At the end of digestion, **Sc3** (Fresh OKFW) presented similar acetic and butyric acid contents (42.10% and 40.53%, respectively). The pretreatment (**Sc4**) promoted the highest production of acetic acid (15% increase) compared to Sc3. **Sc5** (adding the hydrolysate and pretreated OKFW) did not differ from Sc2 in terms of yield. However, the composition was affected. Sc2 had a butyric acid content of 44% at the end of digestion. When compared to Sc5, there was an increase of 15%. On the other hand, pretreatment of the inoculum (65°C, 30 min) using fresh (**Sc6**) and pretreated (**Sc7**) OKFW generated similar yields but a different composition. The type of raw material used (i.e., fresh, or pretreated OKFW) determined the composition of the mixed VFAs. **Sc6** showed a higher butyric acid content (48.16% of the total), while Sc7 showed a higher acetic acid content (45.01%). Several studies with similar results have been reported. Tampio et al. 2014 demonstrated that heat treatment of the inoculum (94°C, 30 min) increased the production of mixed VFAs by more than 30% using food waste as raw material [221]. Blasco L et al. 2020 determined that digestions with inoculum subjected to different pretreatments, such as thermal or freeze-thaw, partially inactivated some archaea and bacteria, increasing VFAs production [220].


(Sc3)
g/L
25
20
15
10
5
0
0
5
10
15
Days
Acetic acid
Propionic acid
Butyric acid


(Sc4)
g/L
18
16
14
12
10
8
6
4
2
0
0
5
10
15
Days
Acetic acid
Propionic acid
Butyric acid


(Sc5)
g/L
18
16
14
12
10
8
6
4
2
0
0
5
10
15
Days
Acetic acid
Propionic acid
Butyric acid


(Sc6)
18
16
14
12
10
8
6
4
2
0
0
5
10
15
Days
Acetic acid
Propionic acid
Butyric acid

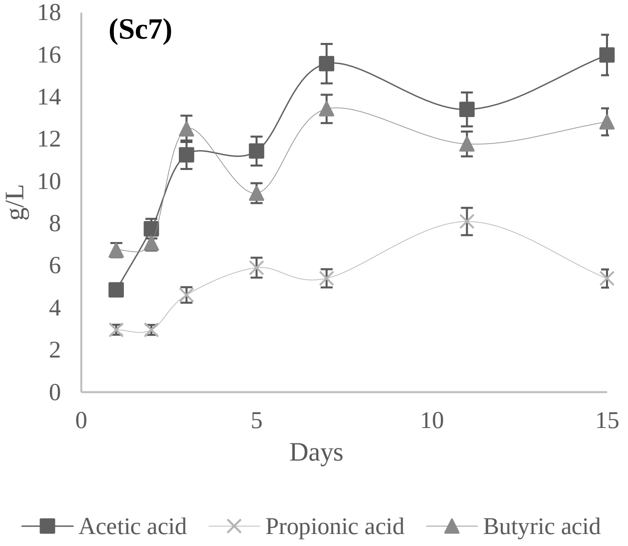
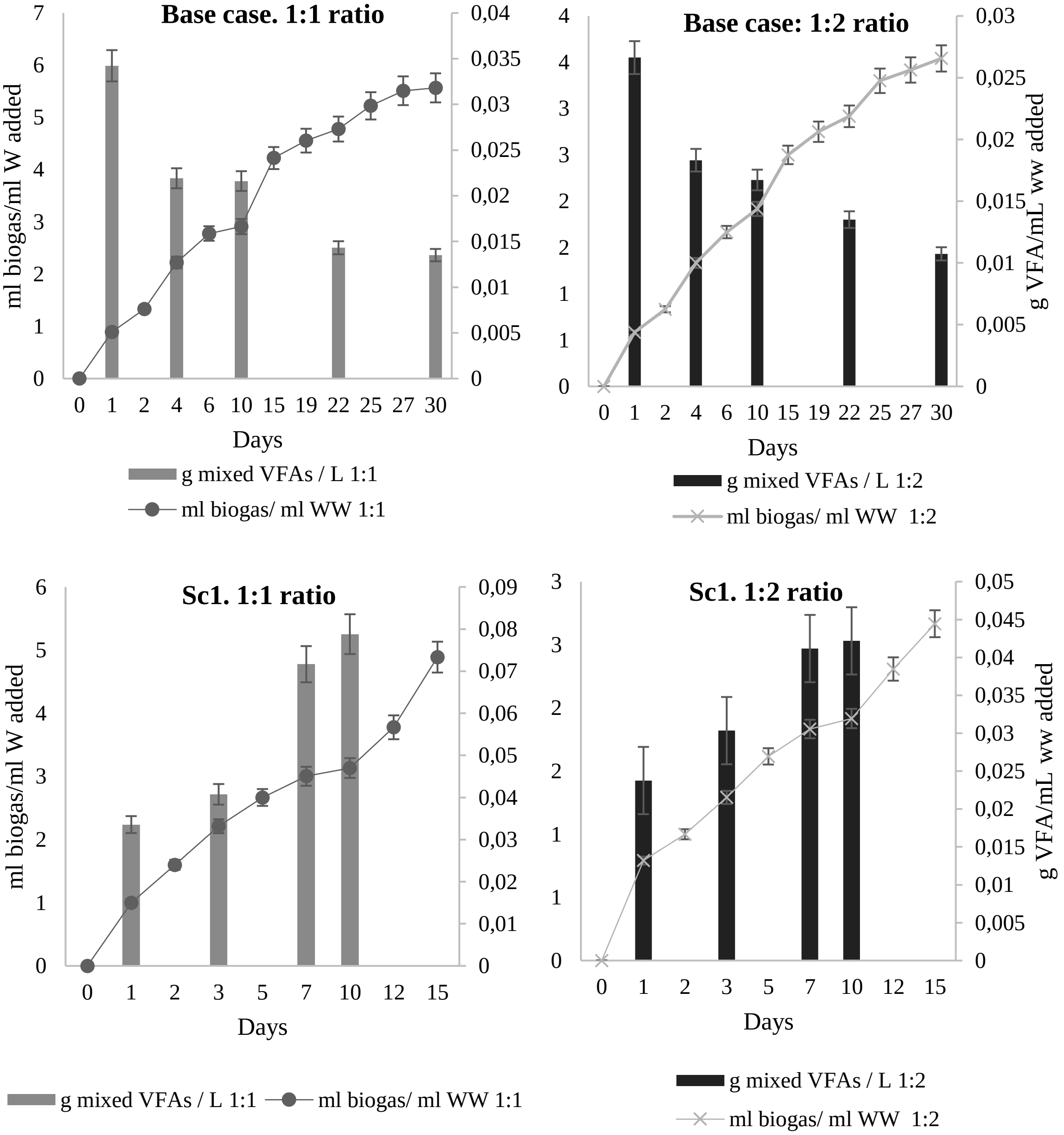


Figure 4.5. Profiles of mixed VFAs of modified AD.

The OKFWs evaluated in this work contain mainly carbohydrates and, to a lesser extent, fats, and proteins. Despite a high theoretical conversion efficiency of glucose (assuming it is the most abundant carbohydrate component) to acetic acid, this acid comprised only 40% and 60% of the VFAs produced in all samples analyzed, depending on the day, sampling, and scenario. These results are consistent with previous studies, which observed the prevalence of acetic and butyric acid in the AD of food waste [222]. The substrate's macromolecule composition (carbohydrates, proteins, lipids) affects the VFAs formation and is related to the degradation pathways of different molecules [223]. For example, high starch and glucose content have been linked to the formation of butyric acid. On the contrary, propionic acid is promoted by the high presence of proteins [224]. Another important factor that determines the mixed VFAs composition is the operating conditions of the process. It has been reported that decreasing pH at the beginning of digestion decreases propionic acid production and increases butyric acid production [225]. This is consistent with the results of this study.

4.3. Wastewater

The biogas production yield from conventional and modified AD during the digestion time is shown in **Figure 4.6.** The **base case** with a volumetric ratio of inoculum to substrate of 1:1 and 1:2 generated a biogas production yield of 5.57 ml/ ml WW with a CH_4, and CO_2 content of 75% and 32%, and 3.54 ml/ ml WW with a CH_4 and CO_2 content of 68.3% and 31.7% respectively. From day 15 onwards, the biogas production yield decreased for both ratios. This is indicative that the substrate had been consumed. Biogas production was consistent with the mixed VFAs production. The mixed VFAs production showed a decreasing trend as biogas was generated. At the end of digestion, a concentration of mixed VFAs of 13.05 mg / ml WW added was obtained for the 1:1 ratio and 10.7 g / ml WW added for the 1:2 ratio, as shown in **Figure 4.6**. WW is composed of organic matter (mainly starch), and its conversion to methane originates from complex metabolic interactions between digesting microorganisms [55]. Xueqin Lu et al. determined specific methanogenic activity (SMA) to characterize the methanogenic activity of granular sludge from a mesophilic anaerobic wastewater treatment plant digester. They determined that the starch-fed tests yielded an SMA of 0.18 L CH_4/ g VS. However, when glucose was used as a substrate, the SMA increased considerably to 0.256 L CH_4/ g VS [226]. This is indicative that the hydrolysis of starch to simpler monomers such as glucose is a limiting factor in biogas production yield. Periyasamy et al. analyzed biogas production from cassava processing wastewater. They determined that 65-70% was CH_4, and the remaining 20-25% was CO_2. The biogas yield was 3.39 ml per ml of WW [227]. There is a direct relationship between the biogas generated and the amount of WW fed. When using a 1:2 ratio, biogas production was reduced by 25% compared to a 1:1 ratio. These results are associated with the possible inhibition of digestion microorganisms. It has been reported that high substrate concentrations lead to inhibition of microorganisms [228].


Base case. 1:1 ratio
ml biogas/ml W added
0
1
2
3
4
5
6
7
0,04
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
0 1 2 4 6 10 15 19 22 25 27 30
Days
g mixed VFAs / L 1:1
ml biogas/ ml WW 1:1
Base case: 1:2 ratio
0
1
1
2
2
3
3
4
4
0,03
0,025
0,02
0,015
0,01
0,005
0
g VFA/mL ww added
0 1 2 4 6 10 15 19 22 25 27 30
Days
g mixed VFAs / L 1:2
ml biogas/ ml WW 1:2
Sc1. 1:1 ratio
ml biogas/ml W added
0
1
2
3
4
5
6
0,09
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
0 1 2 3 5 7 10 12 15
Days
g mixed VFAs / L 1:1
ml biogas/ ml WW 1:1
Sc1. 1:2 ratio
0
1
1
2
2
3
3
0,05
0,045
0,04
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
g VFA/mL ww added
0 1 2 3 5 7 10 12 15
Days
g mixed VFAs / L 1:2
ml biogas/ ml WW 1:2

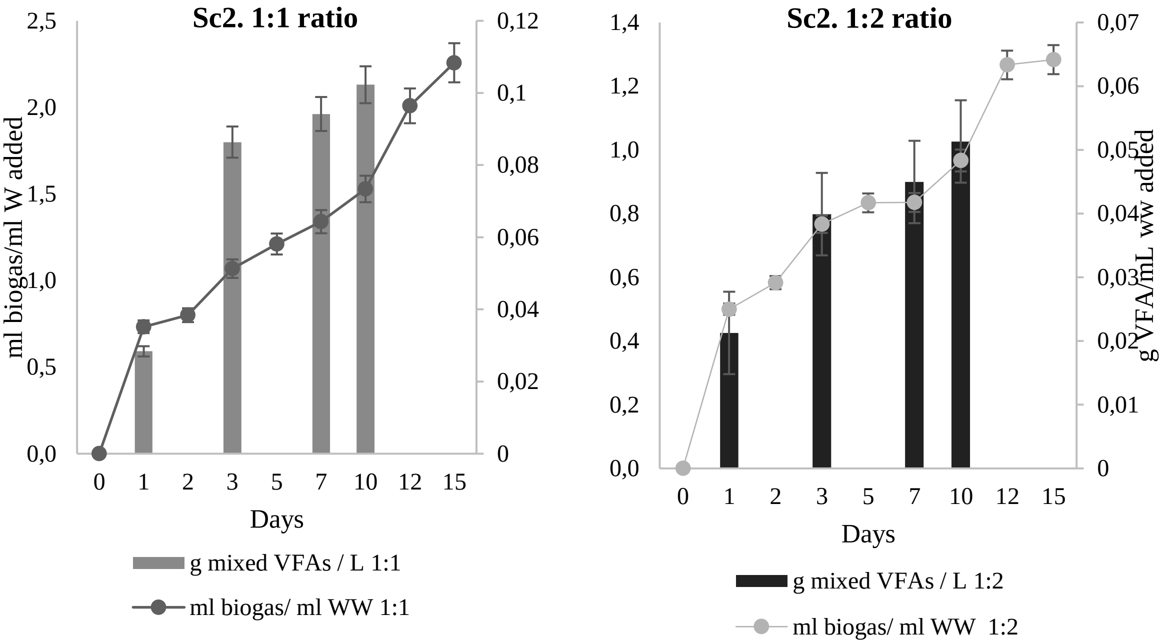


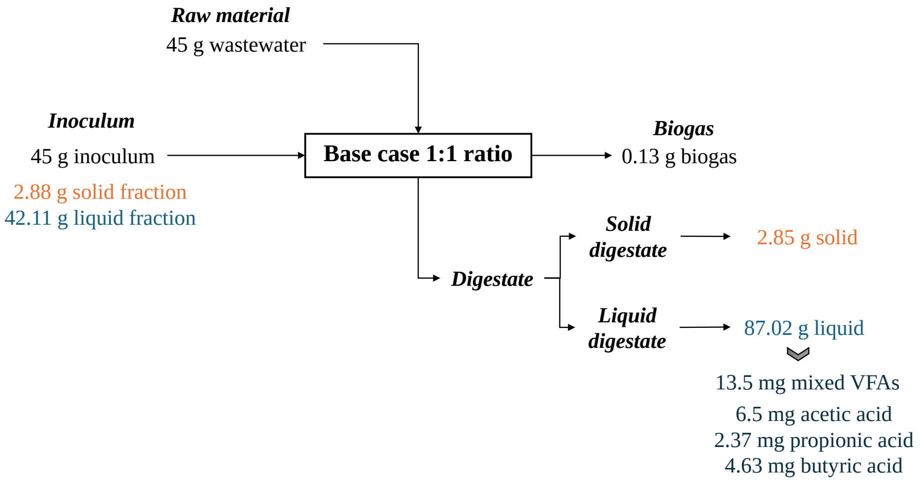
Figure 4.6. Biogas production yields from conventional and modified AD and mixed concentrations of total VFAs.

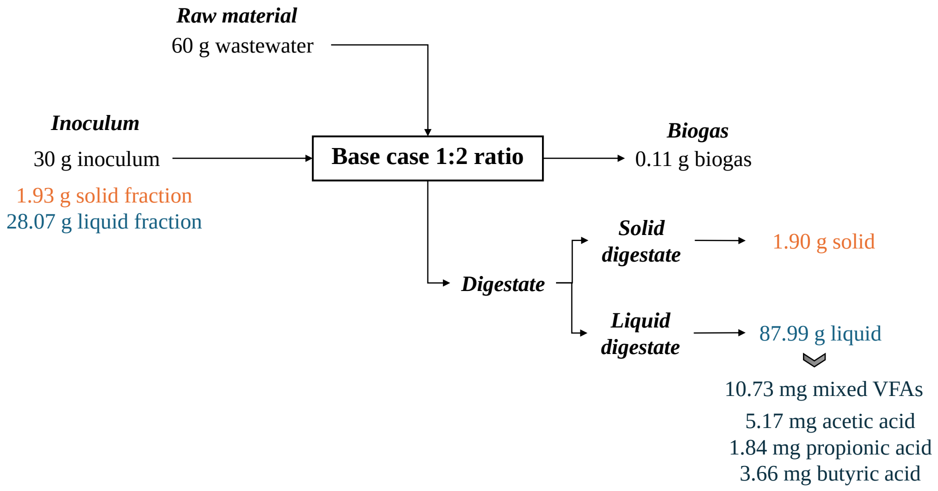
An increasing trend of mixed VFAs was observed for the modified AD scenarios. **Sc1** with an inoculum to substrate ratio of 1:1 generated a mixed VFAs production yield of 71.62 mg/L at the end of digestion, as shown in **Table 4.4.** 41.1 % reduction was generated when using a 1:2 ratio. This is attributed to those mentioned above as shown in the mass balance of the proposed scenarios in **Figure 4.7**. The detailed mass balances for the proposed scenarios are shown in **Annex B: Detailed mass balances of experimental .**The inoculum pretreatment (**Sc2**) generated the inhibition of the methanogenic microorganisms. Therefore, higher production yields of mixed VFAs (102.27 mg/L) were obtained with a 1:1 ratio. The same trend occurred when using a 1:2 ratio. The effect of inoculum pretreatment on methanogenic activity has been validated by several authors [229]. Wong et al. state that thermal pretreatment is the latest technology for treating mixed microflora when acidogenic bacteria are of interest [230]. Using the pretreated inoculum increased the mixed VFAs production after the third day compared to Sc1 (increase in production after the seventh day). The low mixed VFAs production was observed for both scenarios from the seventh day onwards. Likewise, there was an increase in biogas production. From day 12 onward, no traces of mixed VFAs were detected. Therefore, the methanogenic activity of the inoculum began to increase and generated the consumption of the VFAs.

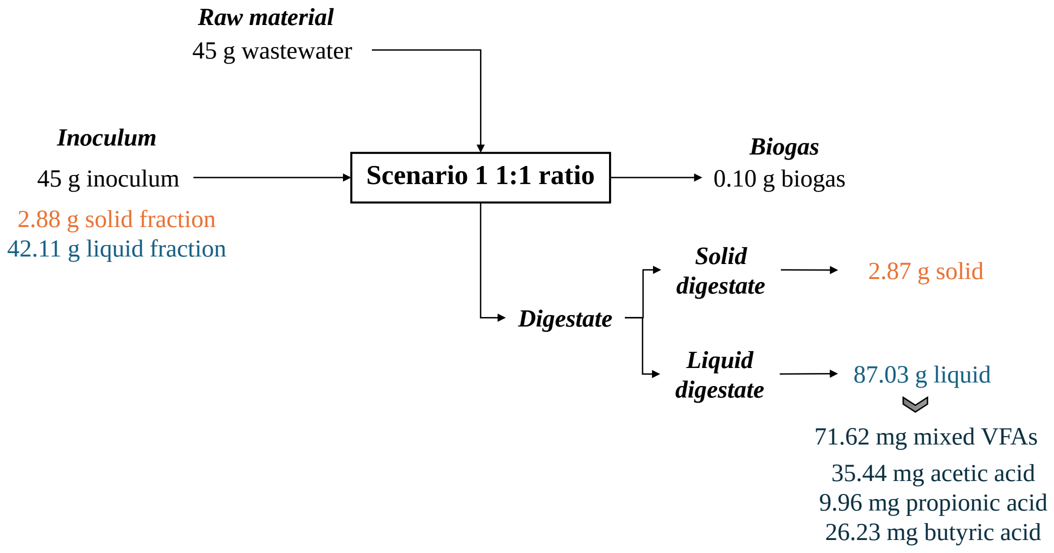
Table 4.4. Main results of the conventional and modified AD with WW as feedstock.

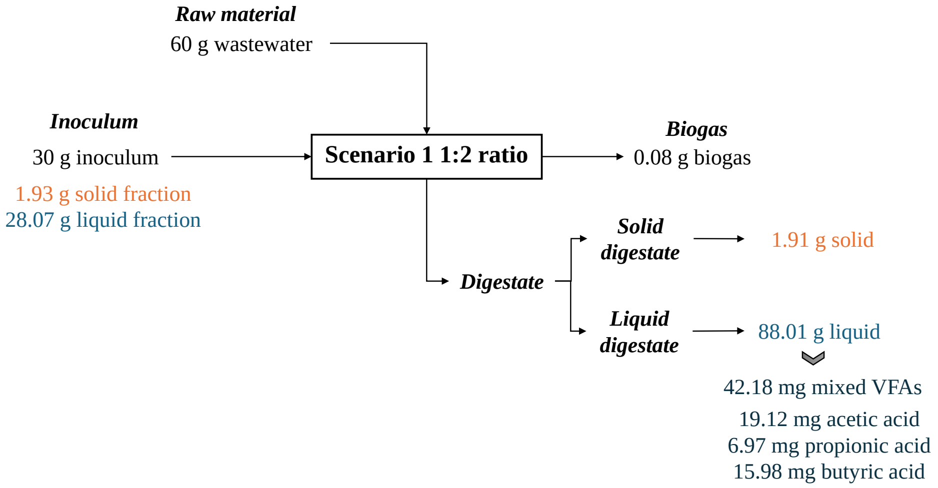
Scenarios	Scenario description	Inoculum to substrate ratio*	Biogas yield (ml/ ml WW added)	%CH$_4$	%CO$_2$	Mixed VFAs yield (mg/mL)	Mixed VFAs composition (%vol)		
							Acetic	Propionic	Butyric
Conventional AD									
Base case	WW	1:1	5.57	75.0	32.0	10.73	48.17	17.53	34.29
		1:2	3.54	68.3	31.7	13.50	48.15	17.17	34.14
Modified AD									
Sc1	WW	1:1	4.47	25.0	75.0	71.62	49.48	13.90	36.62
		1:2	2.67	18	82	42.18	45.34	16.53	37.89
Sc2	WW and inoculum pretreated	1:1	2.26	13.15	86.85	102.27	46.95	17.10	35.95
		1:2	1.28	11.3	88.7	51.32	44.95	17.84	37.21

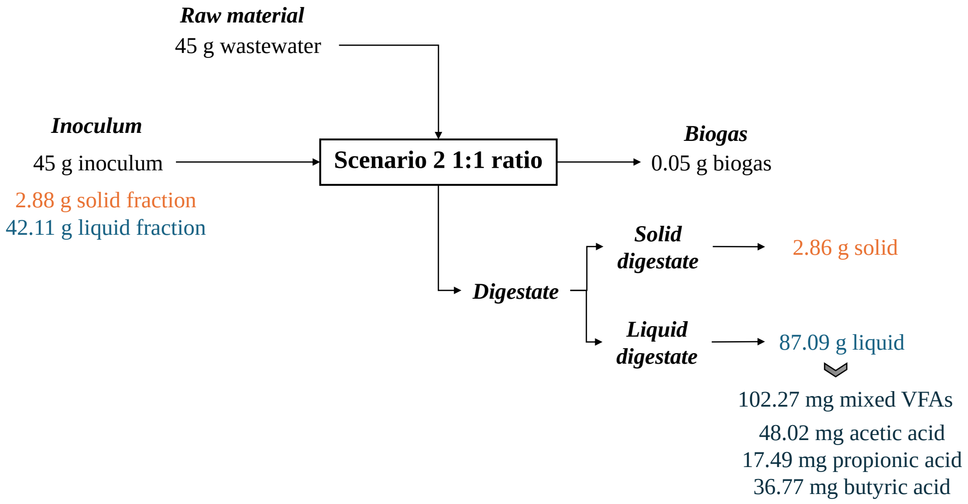
*Volumetric ratio


Raw material
45 g wastewater
Inoculum
45 g inoculum
2.88 g solid fraction
42.11 g liquid fraction
Base case 1:1 ratio
Biogas
0.13 g biogas
Digestate
Solid digestate
2.85 g solid
Liquid digestate
87.02 g liquid
13.5 mg mixed VFAs
6.5 mg acetic acid
2.37 mg propionic acid
4.63 mg butyric acid


Raw material
60 g wastewater
Inoculum
30 g inoculum
1.93 g solid fraction
28.07 g liquid fraction
Base case 1:2 ratio
Biogas
0.11 g biogas
Digestate
Solid digestate
1.90 g solid
Liquid digestate
87.99 g liquid
10.73 mg mixed VFAs
5.17 mg acetic acid
1.84 mg propionic acid
3.66 mg butyric acid


Raw material
45 g wastewater
Inoculum
45 g inoculum
2.88 g solid fraction
42.11 g liquid fraction
Scenario 1 1:1 ratio
Biogas
0.10 g biogas
Digestate
Solid digestate
2.87 g solid
Liquid digestate
87.03 g liquid
71.62 mg mixed VFAs
35.44 mg acetic acid
9.96 mg propionic acid
26.23 mg butyric acid


Raw material
60 g wastewater
Inoculum
30 g inoculum
1.93 g solid fraction
28.07 g liquid fraction
Scenario 1 1:2 ratio
Biogas
0.08 g biogas
Digestate
Solid digestate
1.91 g solid
Liquid digestate
88.01 g liquid
42.18 mg mixed VFAs
19.12 mg acetic acid
6.97 mg propionic acid
15.98 mg butyric acid


Raw material
45 g wastewater
Inoculum
45 g inoculum
2.88 g solid fraction
42.11 g liquid fraction
Scenario 2 1:1 ratio
Biogas
0.05 g biogas
Digestate
Solid digestate
2.86 g solid
Liquid digestate
87.09 g liquid
102.27 mg mixed VFAs
48.02 mg acetic acid
17.49 mg propionic acid
36.77 mg butyric acid

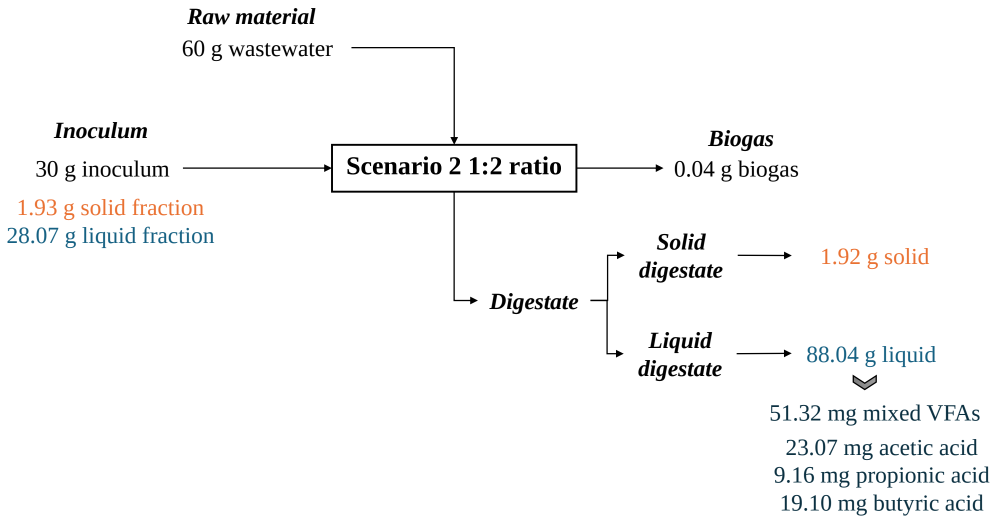
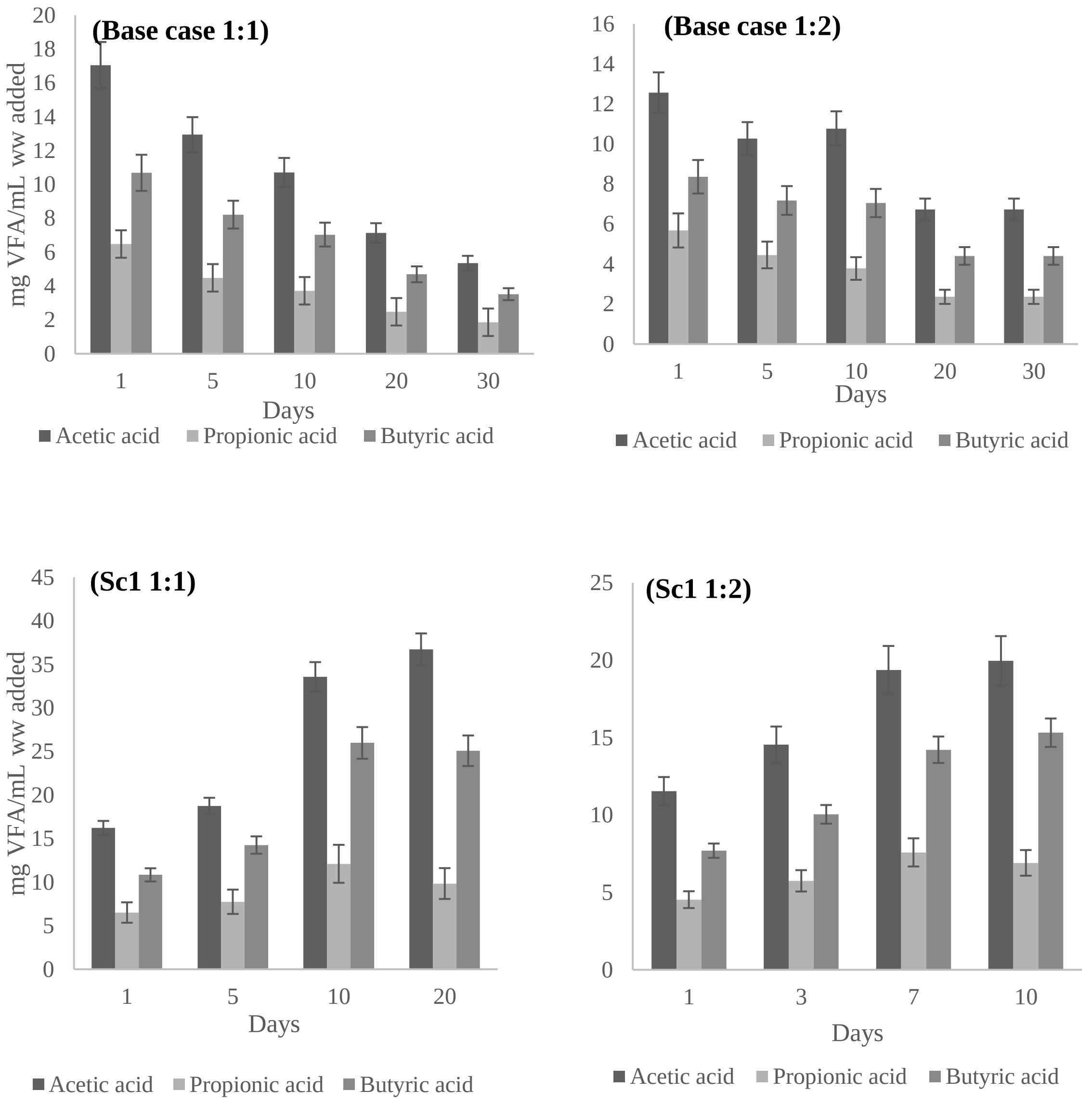


Figure 4.7. Mass balances of the experimental scenarios of conventional and modified AD using wastewater as raw material.

The profile of composition remained constant for conventional and modified AD. Acetic and butyric acid were the most representative acids, and propionic acid to a lesser extent as shown in **Figure 4.8**. These results are consistent with the literature. Starch and oligomers (amylase and amylopectin) promote acetic and butyric acid production [224]. Acetic acid remained in the 40-50% range throughout the digestion. Butyric acid was in the range of 32-38%, and propionic acid was in the range of 13-18%. Modified AD (pH decrease: 6) and inoculum pretreatment (70°C, 30 min) did not affect the mixed VFAs composition. Biswarup Sen et al. analyzed the hydrogen and mixed VFAs production from cassava starch production wastewater. Managed different pretreatments to the inoculum to increase the yield. Showed that acetic and butyric acid were the main soluble metabolic products in all experiments. They also determined propionic acid increased with chemical and acid pretreatments [231].


(Base case 1:1)
mg VFA/mL ww added
0
2
4
6
8
10
12
14
16
18
20
1
5
10
20
30
Days
Acetic acid
Propionic acid
Butyric acid
(Base case 1:2)
0
2
4
6
8
10
12
14
16
1
5
10
20
30
Days
Acetic acid
Propionic acid
Butyric acid
(Sc1 1:1)
mg VFA/mL ww added
0
5
10
15
20
25
30
35
40
45
1
5
10
20
Days
Acetic acid
Propionic acid
Butyric acid
(Sc1 1:2)
0
5
10
15
20
25
1
3
7
10
Days
Acetic acid
Propionic acid
Butyric acid

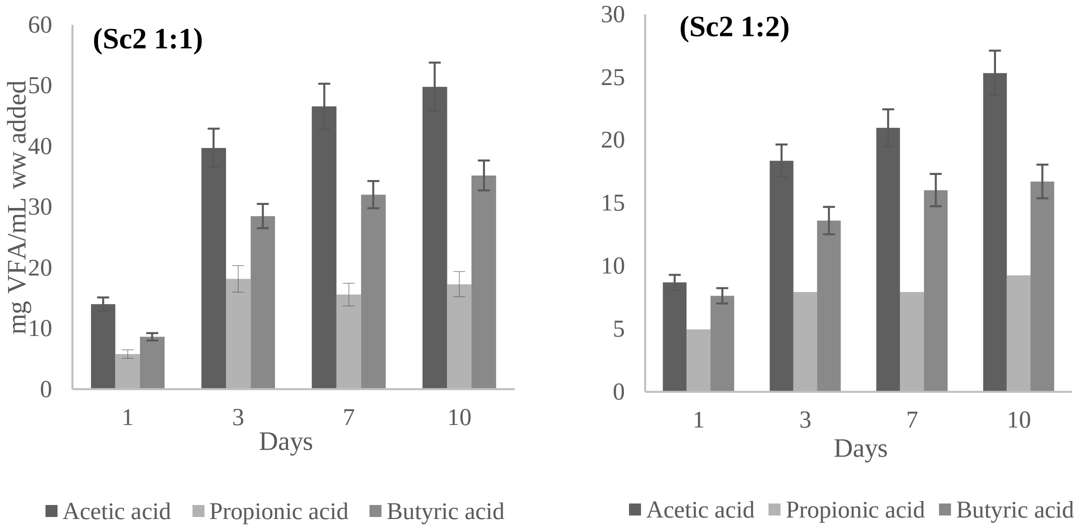


Figure 4.8. Profiles of mixed VFAs of conventional AD (Base case) and Modified AD (Sc1 and Sc2).

4.4. Vinasses

The biogas production yield from conventional and modified AD during the digestion time is shown in **Figure 4.9**. The **base case** generated a biogas production yield of 0.560 Nm^3/kg SV and a CH_4 and CO_2 content of 67.5% and 32.5%, respectively. From day 23 onwards, the biogas production yield decreased, indicating substrate consumption. Biogas production was consistent with mixed VFAs production, which showed a decreasing trend and increased biogas production. On day 1, the highest concentration of mixed VFAs (15.93 g/L) related to rapid hydrolysis and acidification occurred. At the end of the digestion, the unconsumed mixed VFAs that remained in the liquor was 4.46 g/L, as seen in **Figure 4.9**. The results obtained for the base case in this work showed differences from those reported in the literature. For example, Caillet H et al. reported biogas production yields of 0.284 Nm^3/kg SV for 40 days of digestion [232]. Cruz-Salomón A et al. determined a maximum biogas production of 0.264 Nm^3/kg SV and a CH_4 content of 60% using a UASB reactor and a time of 25 days [233]. On the other hand, Iltchenco et al. reported maximum performances of 0.613 Nm^3/kg SV [234]. The differences between the results obtained from this work and those reported in the literature are mainly due to the chemical characteristics of the vinasses and the operating conditions. It has been reported that vinasses may contain heavy metals and organic contaminants (phenols) that can affect digestion [235]. The modified AD showed low biogas production during the digestion time for the evaluated scenarios. The low biogas production is an indirect measure of the increasing of mixed VFAs production [236]. **Sc1** presented a biogas production of 0.228 Nm^3/kg SV and a CH_4 content of 9%. Sc3 presented a biogas production of 145.56 Nm^3/kg SV and a CH_4 content of 5%.

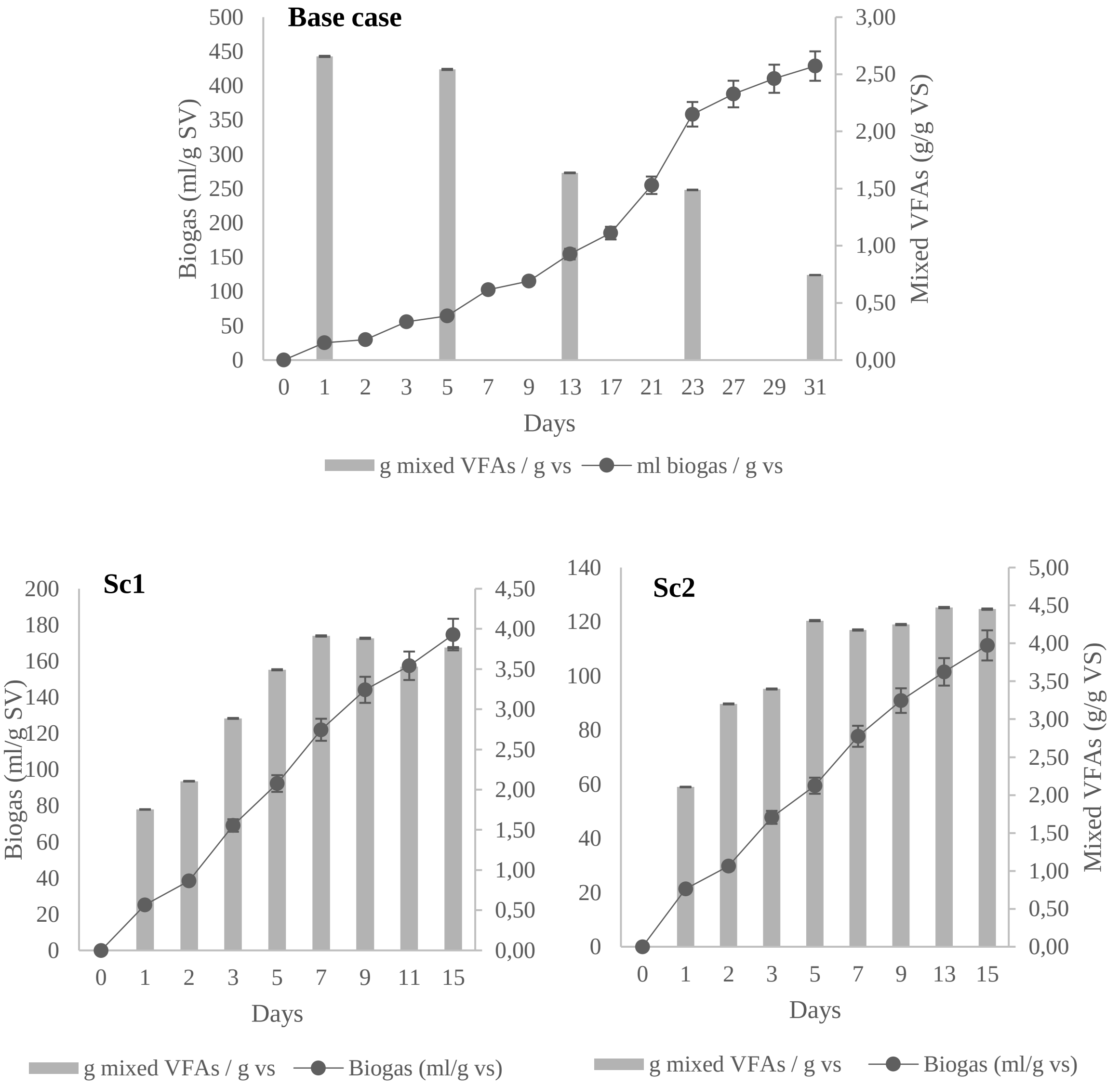


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Figure 4.9. Biogas production yields from conventional and modified AD and the total mixed VFAs concentration with vinasses as feedstock.

An increasing trend of mixed VFAs was observed for the modified AD scenarios. **Sc1** generated a mixed VFAs production yield of 22.59 g/L at the end of digestion, as shown in **Table 4.5.** The inoculum pretreatment (**Sc2**) generated the inhibition of methanogenic microorganisms, and consequently, the mixed VFAs production increased (30% compared to Sc1) as shown in the mass balance of the proposed scenarios in **Figure 4.10**. The detailed mass balances for the proposed scenarios are shown in **Annex B: Detailed mass balances of experimental .** Studies have shown that AD of liquid emissions (vinasse) from agro-industrial processes (ethanol production) could increase the amount of energy recoverable from organic waste [237]. The inoculum presents a very broad microbial consortium. When considering modified AD, metabolic pathways guided to producing mixed VFAs are promoted. Inoculum pretreatment before digestion is essential in inhibiting VFA-consuming populations [238]. For example, Rafieenia et al., 2018 performed AD to promote the hydrogen and mixed VFAs production. They analyzed four types of pretreatments (thermal shock, alkaline, aeration, and frying oil). They determined that thermal pretreatment (90°C for 30 min) increased the production of hydrogen and mixed VFAs by 50% [239].

Table 4.5. Main results of the conventional and modified AD with vinasses as feedstock.

Scenarios	Scenario description	Biogas yield (ml/g VS)	%CH_4	%CO_2	Mixed VFAs yield (g/L)	Mixed VFAs composition (%vol)		
						Acetic	Propionic	Butyric
Conventional AD								
Base case	Vinasses	432.13	59.00	41.00	4.46	58.20	14.11	27.69
Modified AD								
Sc1	Vinasses	187.78	9.00	91.00	22.59	32.18	18.55	49.27
Sc2	Vinasses and inoculum pretreated	145.56	5.00	95.00	26.70	30.7	19.1	50.3

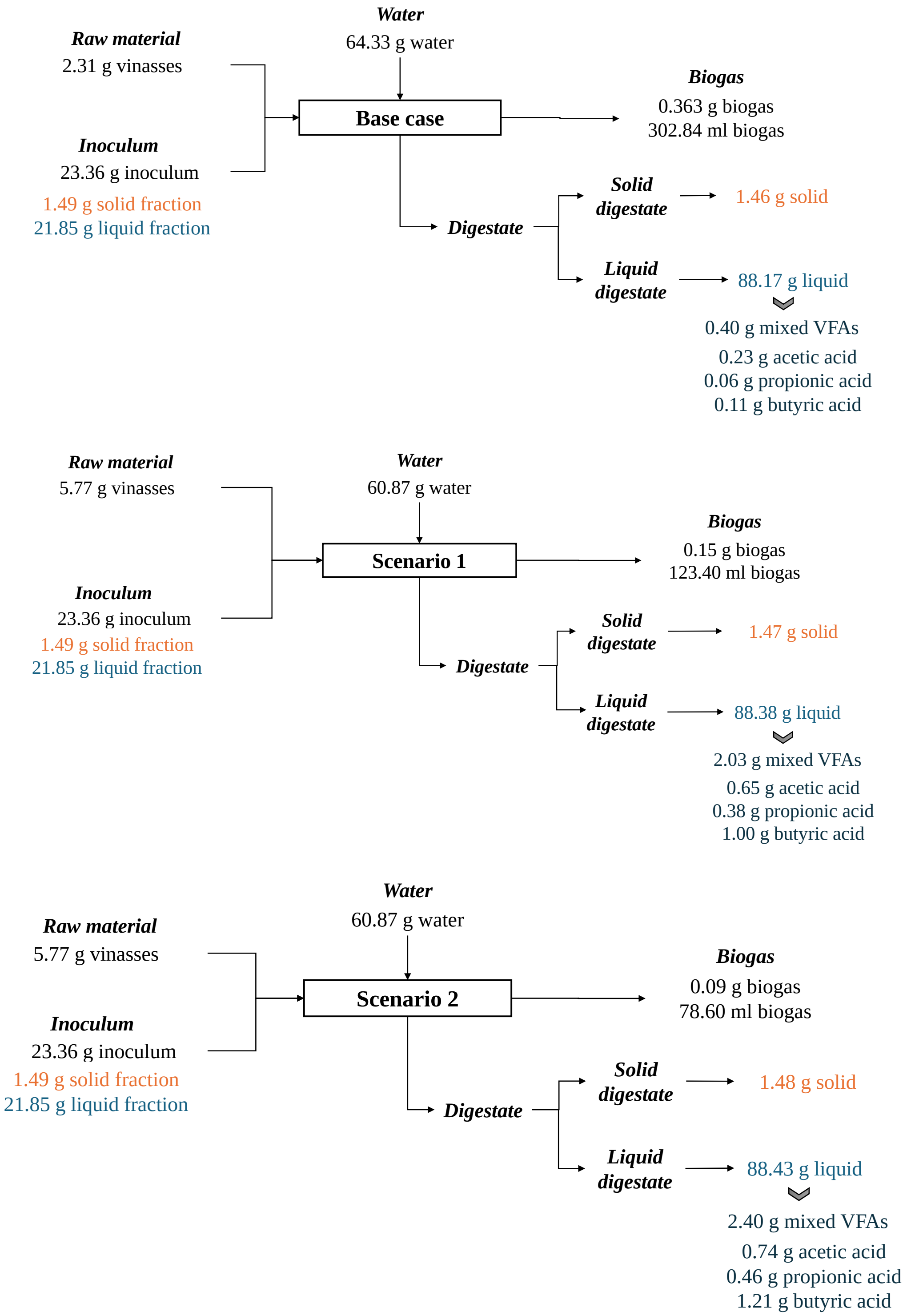
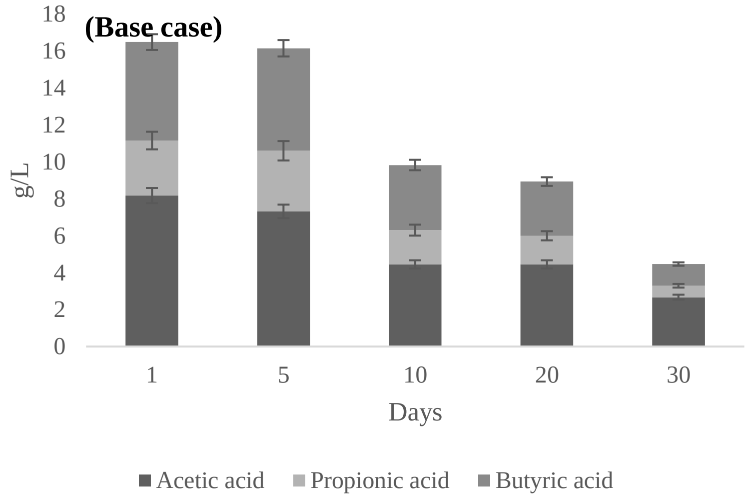


Figure 4.10. Mass balances of the experimental scenarios of conventional and modified AD using vinasses as raw material.

The composition profile of the mixed VFAs remained constant for conventional AD as shown in **Figure 4.11**. The base case presented a higher content of acetic acid (between 40% and 60%), followed by butyric acid (between 25% and 40%) and finally propionic acid (between 15% and 20%). On the other hand, considering the modified AD, for Sc1, the butyric acid content was predominant during the digestion time (between 45% and 50%). Thermal pretreatment of the inoculum generated an increase in the mixed VFAs production. In addition, similar profiles were obtained. The profile of the VFAs is linked to the composition of the raw material and the operating conditions of the digestion.



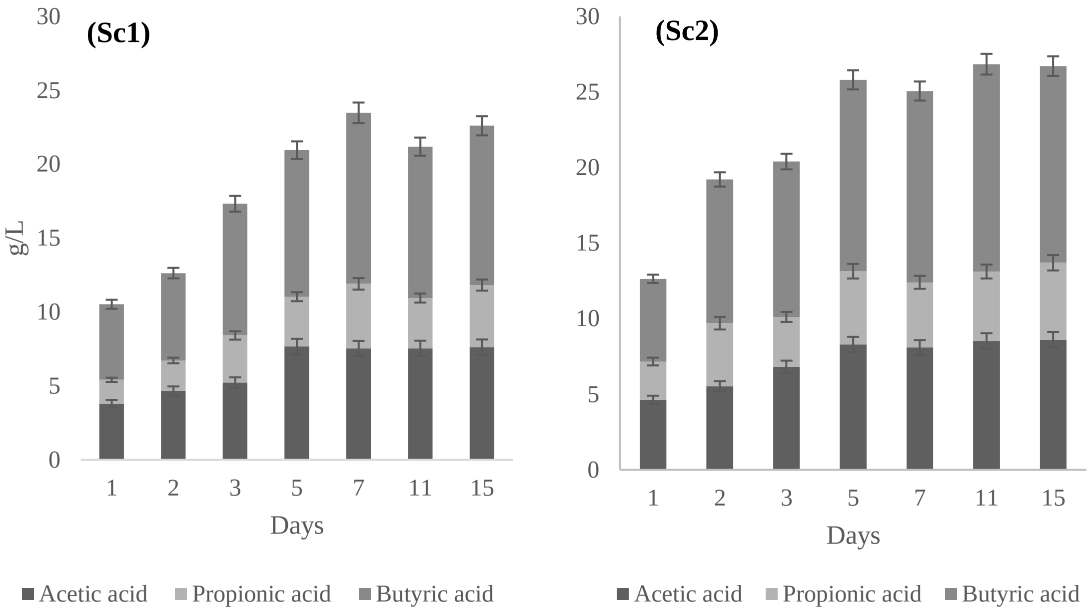


Figure 4.11. Profiles of mixed VFAs of conventional AD (Base case) and Modified AD (Sc1 and Sc2) with vinasses as feedstock.

Glucose monomers mainly constitute vinasse. Glucose monomers generally promote the acetic acid production [223]. Moreover, it has been reported that the pH of the digestion medium affects the mixed VFAs composition [240]. Magrini F et al. (2020) performed modified AD to promote the VFAs and hydrogen production using vinasse as feedstock. They handled different pH values. In addition, the influence of thermal pretreatment of the inoculum to increase yields was evaluated. Experiments conducted at pH 6 and the inoculum with T1 pretreatment (90°C, 30 min) showed that butyrate production was favored. In contrast, propionic acid predominance was observed under the same inoculum pretreatment conditions and at pH 5. Acetic acid production was favored at pH 7, and using inoculum without pretreatment [241]. Then, the results obtained in this work are consistent with those reported in the literature.

5. Chapter 5: Sustainability analysis of organic kitchen food waste biorefineries

5.1. Overview

The sustainability analysis of the OKFW biorefinery scenarios was performed considering the four dimensions of sustainability. First, a comparative analysis of the results of the scenarios was carried out, considering the indicators evaluated for each dimension. Then, the sustainability indices for each scenario were determined and compared to define the most viable configuration (i.e., the highest values of the sustainability impact).

5.2. Technical assessment

The technical analysis of OKFW biorefineries was carried out considering the experimental results. The PMI and RMI indicators were selected as indicators. The biogas and mixed VFAs production yields for each scenario were adjusted to the experimental data obtained in this thesis and are described in the **Annex B: Detailed mass balances of experimental .** The base case was the best scenario since it presented a PMI of 1,055 kg OKFW per kg product as shown in **Table 5.1.** This scenario has a lower consumption of raw materials. The index results show similarities to conventional AD. The raw material pretreatment (LHW) increased raw material consumption by 5.8% compared to the base case. The mixed VFAs recovery caused an increase of 23% associated with using the solvent (MTBE) in the liquid-liquid extraction. Moreover, the simultaneous recovery of mixed VFAs and bioactive compounds generated an increase in raw material consumption of 70% due to the use of ethanol. Regarding the modified AD, Sc5 and Sc7 obtained the lowest PMI (only raw materials for digestion and recovery of mixed VFAs are considered). On the contrary, Sc8 presented the highest PMI for the recovery of bioactive compounds. These results help to elucidate that the recovery process of bioactive compounds as a process step is not beneficial since a large amount of solvent is required to extract a small amount of bioactive compounds. The PMI values of the OKFW biorefineries in this work are low. These results represent a materially more efficient process. Likewise, these results are low compared to data reported in the literature for other processes.

Table 5.1. Technical and energy results of OKFW biorefineries.

Indicator	**Conventional AD**					**Modified AD**				
	Base case	**Sc1**	**Sc2**	**Sc3**	**Sc4**	**Sc5**	**Sc6**	**Sc7**	**Sc8**	**Sc9**
Technical indicators										
RMI	0.905	0.895	0.826	0.811	0.810	0.786	0.766	0.754	0.749	0.756
PMI	1.055	1.117	1.204	1.304	1.499	1.280	1.370	1.280	1.543	1.299
Energy indicators										
n	0.104	0.225	0.095	0.216	0.218	0.051	0.081	0.046	0.083	0.048

SEC	0.085	0.176	2.200	0.533	0.343	0.812	1.092	1.612	0.881	0.916

A comparative study among several biopharmaceutical companies showed that the PMI of biological processes ranges from 2,000 to 25,000 [242]. Similar studies, recovering organic wastes from AD, showed similar results. For example, M. Sofokleous et al. determined a PMI of 1.01 for the anaerobic digestion of green waste. Moreover, for bioethanol production after alkaline pretreatment combined with anaerobic digestion, the PMI was 1.15 [243]. On the other hand, higher RMI values mean a high flow of renewable feedstock in the process. The base case presented the best result. The scenarios involving mixed VFAs recovery presented the lowest indexes. This is congruent due to the use of solvents in the extraction. In addition, indicators related to overall energy efficiency ("η") and specific energy consumption (SEC) were considered. SEC quantitatively describes the total energy consumed by the process per unit mass of product. "η" relates the output energy to the total energy consumption of the process.

5.3. Economic assessment

The CapEx and OpEx for the proposed scenarios are presented in **Table 5.2**. In the conventional AD, the base case presented an OpEx of 20.23 mUSD and a CapEx of 6 mUSD. OKFW conditioning (drying and milling) accounted for 37% of the total CapEx, followed by AD. Sc2 (pretreated OKFW) represented an increase of 32.5% and 11.2% in CapEx and OpEx compared to the base case. Unconsumed mixed VFAs recovery (Sc3) when the hydrolysate was added to AD, generated, and increase in the CapEx by 1.4 times compared to the base case. In addition, the use of MTBE led to an increase in OpEx. (Sc4) involved pretreated OKFW, the bioactive compound production from the hydrolysate, and the recovery of the unconsumed mixed VFAs. The bioactive compound recovery represented an 206% increase in CapEx. The equipment maintenance and feedstock costs associated with disposal and transportation costs in the Colombian context contributed significantly to the OpEx for the conventional AD scenarios. Likewise, when the mixed VFAs recovery using liquid-liquid extraction technology was considered, an increase of 66% in the use of reagents was generated despite having a 90% recirculation. In modified AD, the

Sc6 (pretreated OKFW) increased the CapEx by 12.5% compared to Sc5. The addition of the inoculum pretreatment step (Sc7) represented 19% of the total CapEx compared to Sc5. On the other hand, adding the bioactive compound extraction step (Sc8) increased 22.8% of the total CapEx. The PHB production using mixed VFAs as substrate (Sc9) represented the highest share of the total CapEx for this scenario (32.7%).

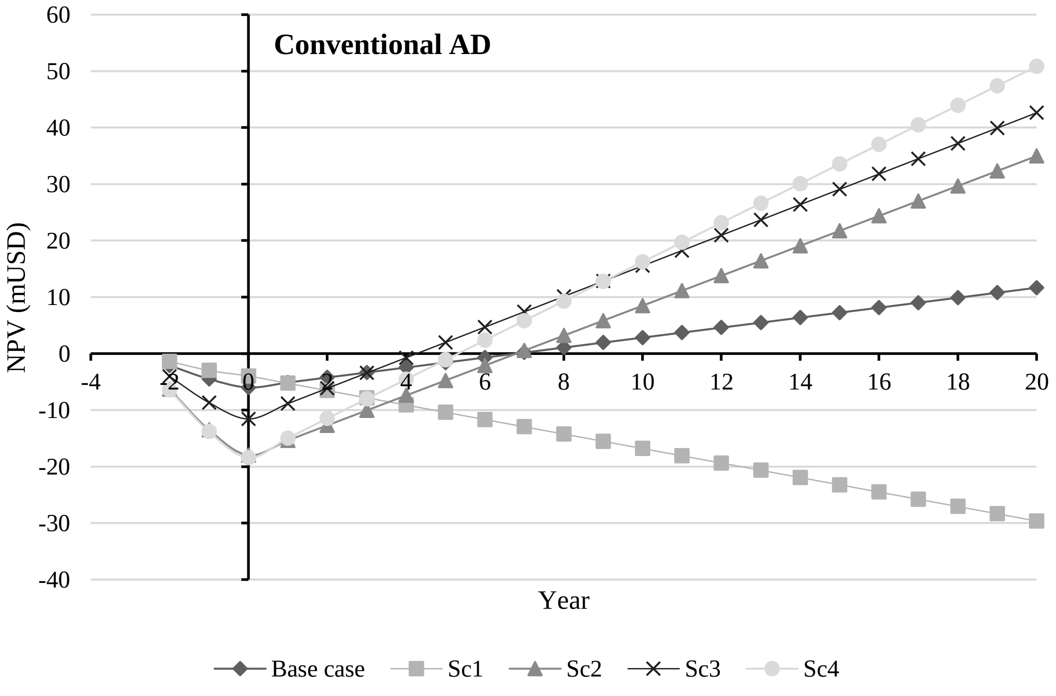
Table 5.2. Economic results of OKFW biorefineries scenarios.

Parameter	Conventional AD					Modified AD				
	Base case	**Sc1**	**Sc2**	**Sc3**	**Sc4**	**Sc5**	**Sc6**	**Sc7**	**Sc8**	**Sc9**
CapEx (mUSD)	6.00	7.96	15.38	14.47	18.42	15.45	17.31	18.32	18.90	20.42
OpEx (mUSD-year)	20.93	23.29	36.35	24.36	36.72	35.07	52.39	68.57	61.39	58.85
PBP (year)	6.90	Not apply	7.10	4.20	4.80	5.00	6.80	8.00	3.20	Not apply
NPV (mUSD)	11.66	-29.64	34.97	42.63	50.86	48.96	20.66	30.79	80.08	-7.17

In conventional AD, **Sc3** and **Sc4** presented the best economic viability. A payback period (PBP) and net present value (NPV) of 4.2 years and 42.64 mUSD for Sc3 and 4.8 years and 50.86 mUSD was achieved as shown in **Figure 5.1**. The economic viability is strongly linked to the economic allocation of the products. The liquid and solid fractions of the digestate were valued. In the base case, the economic allocation of liquid and solid digestate is higher than that of biogas, with values of 38.3% and 16%, respectively. In this sense, valorizing liquid and solid digestate is essential for the economic viability of OKFW AD. **Sc1** (Pretreated OKFW) generated an increase in biogas production. However, it was insufficient to amortize the costs (CapEx and OpEx of the process). **Sc2** (Unconsumed mixed VFAs recovery) generated that the process remained viable. However, the PBP was higher than the base case. On the contrary, adding the hydrolysate to the digestate (**Sc3**) promoted mixed VFA production, resulting in better economic results (PBP of 4.2 years).

Similar results were obtained for Sc4. **Sc4** involved not only the mixed VFAs recovery but also the bioactive compounds production.

In modified AD, **Sc5** (fresh OKFW) generated economic viability with a PBP of 5 years and an NPV of 48.96 mUSD. The use of pretreated OKFW (**Sc6**) proved to be economically viable. However, no reduction in PBP was possible to achieve compared to Sc5. The increase in the yield of mixed VFAs production failed to buffer the costs associated with pretreatment. Thermal pretreatment of the inoculum achieved the best results in technical terms. However, the costs associated with utilities (OpEx) and equipment did not lead to a lower PBP (**Sc7**) than Sc5. On the other hand, PHB is a high-value and high-selling price product in the market. However, the low production yield associated with additional operating costs resulted in economic infeasibility (**Sc9**). These results demonstrate that AD remains a promising alternative for OKFW valorization. It was also evidenced that implementing new processing lines in conventional AD through the biorefinery concept, such as the mixed VFAs recovery, generated better economic results.



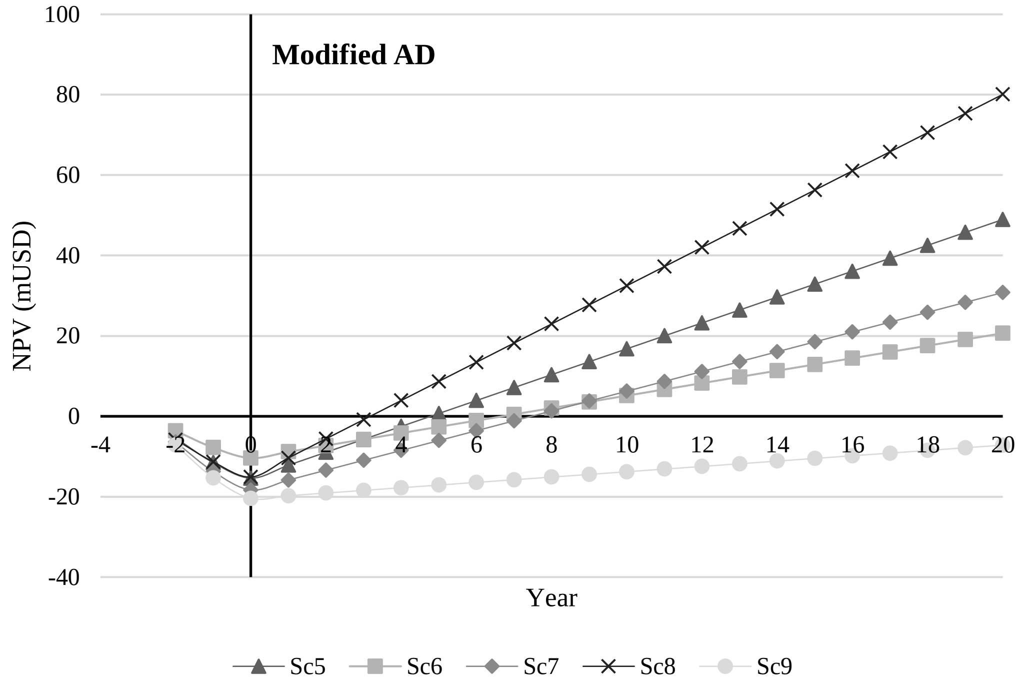


Figure 5.1. Net present value of OKFW biorefinery scenarios

5.4. Environmental assessment

The most representative impact categories were climate change, water depletion, fossil depletion, and human toxicity. The percentage of environmental contribution of the biorefinery scenarios is presented in **Figure 5.2**. The OKFW acquisition stage (i.e., transportation) for all scenarios and categories evaluated accounted for between 1 and 8% of the total impact. In conventional AD, for the **base case**, the climate change category was the most representative (70%). The base case had few processing units, where only steam was considered for energy requirements. The **Sc1** (pretreated OKFW) caused a 35% increase in the water depletion category compared to the base case. The unconsumed mixed VFAs recovery (**Sc2**) increased energy and reagent consumption (MTBE), reflected in a higher percentage contribution to the water depletion and human toxicity categories On the other hand, in the modified AD (**Sc5**), a 20% participation in the human toxicity category was observed due to the use of reagents.

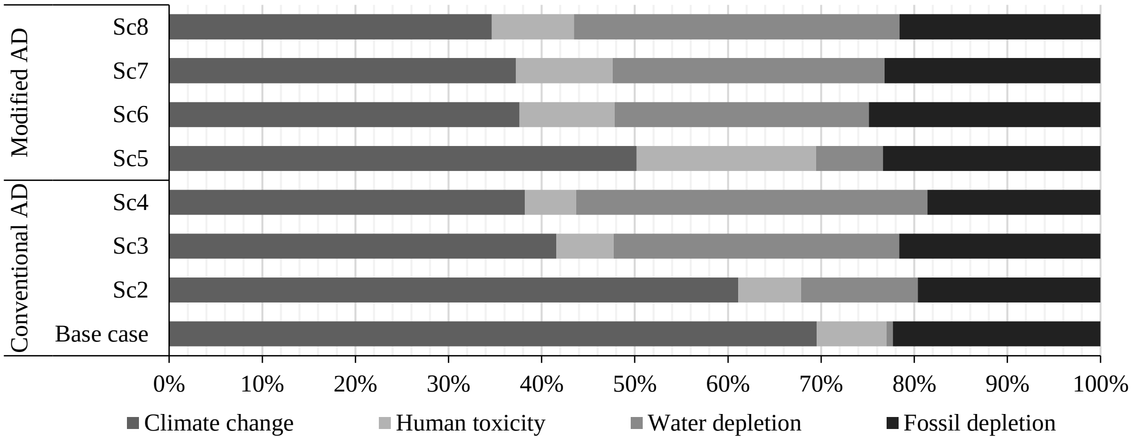
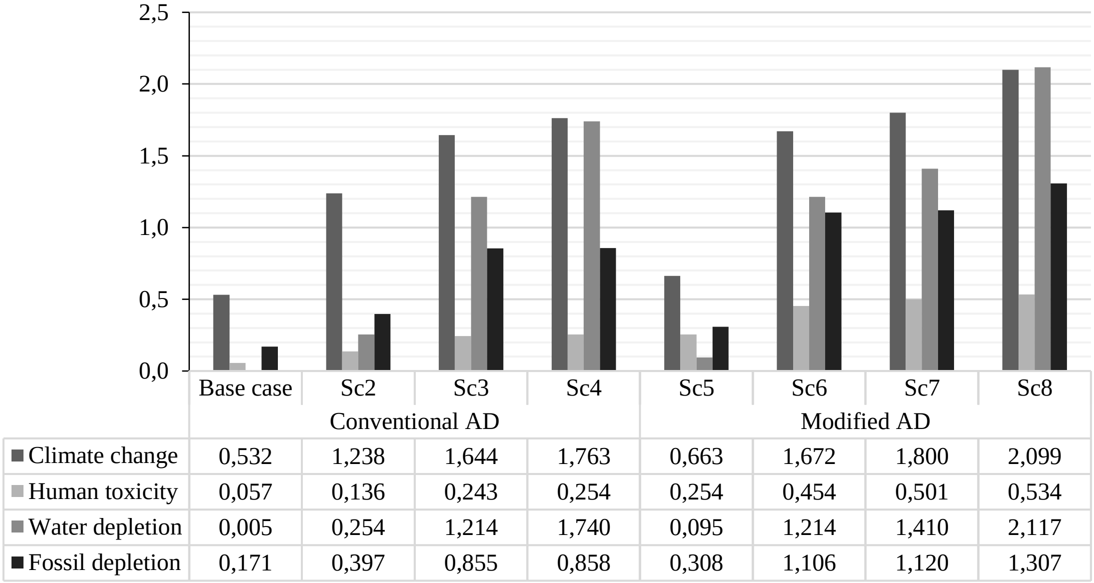


Figure 5.2. Percentage contribution of the most representative categories of the OKFW biorefinery scenarios

The **base case** presented the lowest values compared to the other scenarios. A carbon footprint (climate change) of 0.532 kg CO_2 per kg of OKFW was achieved as shown in **Figure 5.3.**. Sc2 (unconsumed mixed VFAs recovery) resulted in a 2.3-fold increase compared to the base case due to the additional use of service fluids and the use of reagents. The same trend is observed when bioactive compounds are extracted (**Sc4**). In the modified AD, the bottleneck, representing about 40-60% of the environmental impact, is the mixed VFAs recovery. Consistent with the additional process units associated with consuming service fluids and solvents (MTBE). The climate change values obtained in this research are in the range of some literature reports. For example, Yan Y et al. [244] report a carbon footprint of 0.3 kg CO_2ep/kg FW for a biorefinery contemplating oil extraction and electricity production through biogas combustion. On the other hand, Evangelisti S et al. [245] report values of 0.165 kg CO_2 per MJ generated from biogas in a UK facility.



	Conventional AD				Modified AD			
	Base case	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7	Sc8
■ Climate change	0,532	1,238	1,644	1,763	0,663	1,672	1,800	2,099
■ Human toxicity	0,057	0,136	0,243	0,254	0,254	0,454	0,501	0,534
■ Water depletion	0,005	0,254	1,214	1,740	0,095	1,214	1,410	2,117
■ Fossil depletion	0,171	0,397	0,855	0,858	0,308	1,106	1,120	1,307

Figure 5.3. Climate change (kg CO_2), human toxicity (kg 1.4-DB), water depletion (m3) and fossil depletion (kg oil eq) per kg of OKFW valorized of biorefinery scenarios.

5.5. Social assessment

The number of employees required to complete the work in a processing line of a plant is 5 operators, 1 substitute, and 1 supervisor per shift (i.e., 3 for working time). Therefore, the number of employees per processing line is nine (9) employees. The number of employees for the economically viable conventional and modified AD biorefinery scenarios is presented in **Figure 5.4**. **Sc4, Sc7** and, **Sc8** were the scenarios with the highest employment generation associated with the additional processing lines (extraction of bioactive compounds, inoculum pretreatment). On the contrary, the **base case** obtained the lowest employment generation since only the feedstock conditioning line and the AD are contemplated. The number of employees hired influences the M/L ratio. The **Base Case, Sc6**, and **Sc7** presented the lowest results. These results show a low resilience of the process. Any change in the process (employee wages) directly affects the cumulative NPV of the process. On the contrary, the remaining scenarios could hire more employees or increase existing employees' salaries without affecting the process's economic dimension.

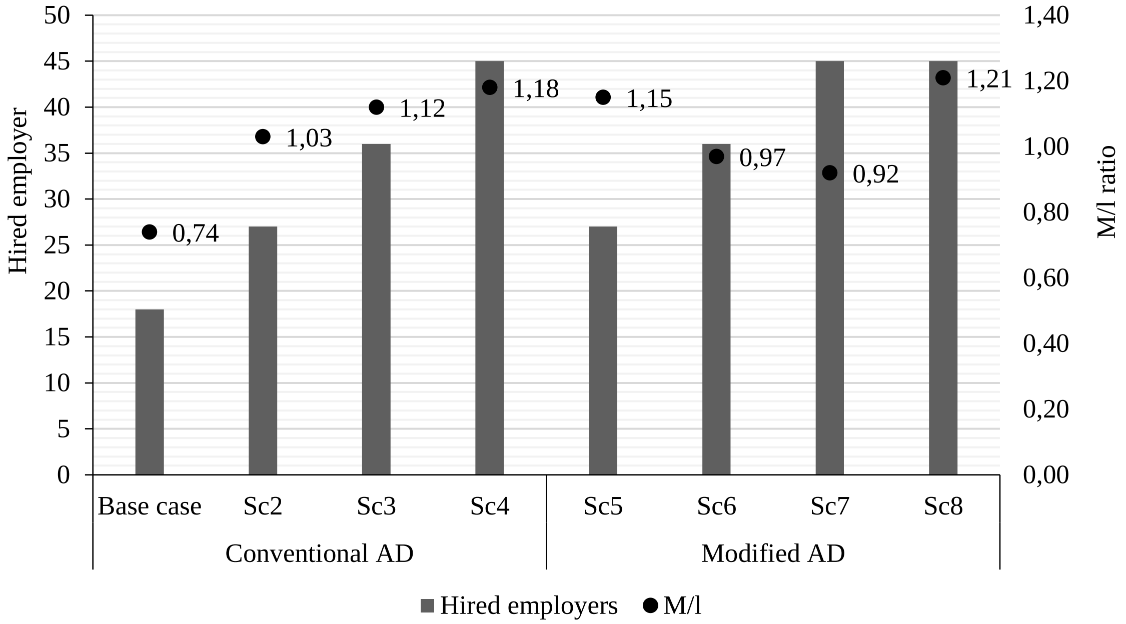


Figure 5.4. Employment generated and M/L ratio for the OKFW biorefinery scenarios.

5.6. Sustainability index

The results of the sustainability index for each scenario are shown in **Figure 5.5**. **Sc4** (from the conventional AD), **Sc5**, and **Sc8** (from the modified AD) presented the highest sustainability indices (>90%) with values of 91.23%, 91.17%, and 92.15%, respectively. These results are attributed to high economic and social results. The main difference is related to the technical and environmental results. The **base case** presented the lowest index of conventional AD. Although this scenario obtained the best results in the environmental dimension (45.63%), the lowest score was obtained in the economic dimension (9.68%). Considering the modified AD, **Sc6** (pretreated OKFW) and **Sc7** (inoculum pretreatment) presented the lowest rates (79.80% and 76.46% respectively). The economic dimension mainly influences these results. These results allow a preliminary glimpse that the AD (conventional or modified) of OKFW integrated into the biorefinery concept allows for obtaining energy while obtaining value-added products such as volatile fatty acids liquid, and solid fertilizers.

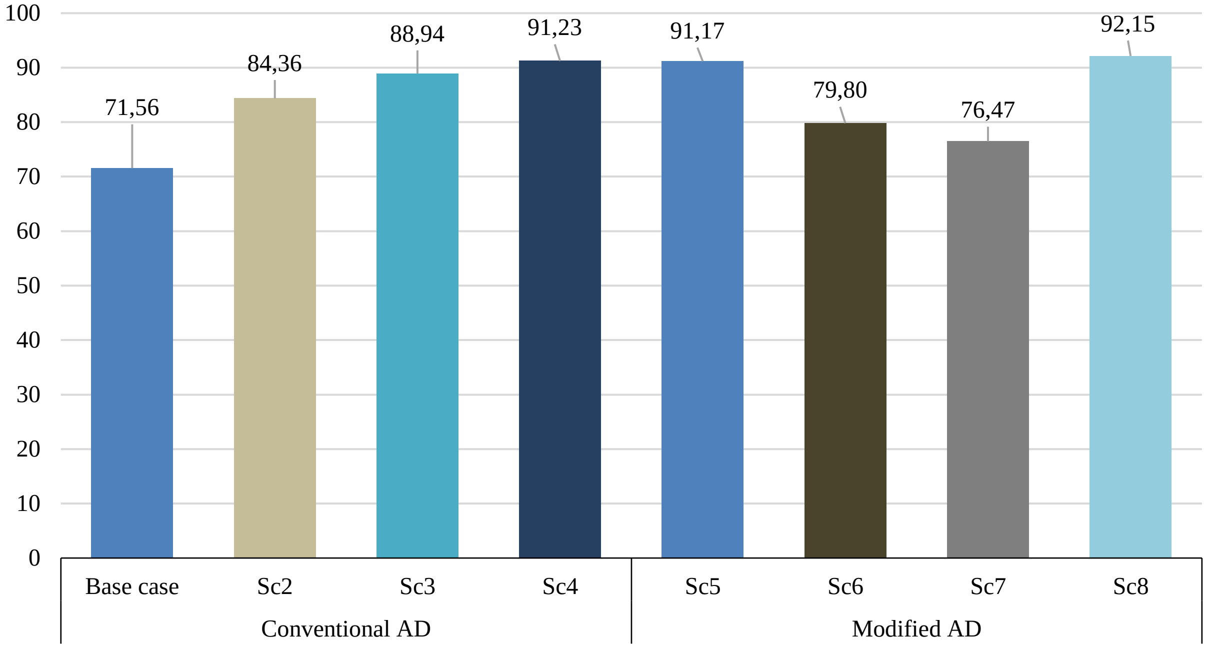


Figure 5.5. Sustainability index for the OKFW biorefinery scenarios.

6. Chapter 6: Sustainability analysis of wastewater from cassava starch production biorefineries

6.1. Overview

The sustainability analysis of the WW biorefinery scenarios was performed considering the four dimensions of sustainability. First, a comparative analysis of the results of the scenarios was carried out, considering the indicators evaluated for each dimension. Then, the sustainability indices for each scenario were determined and compared to define the most viable configuration (i.e., the highest values of the sustainability impact).

6.2. Technical assessment

The technical analysis of WW biorefineries was carried out considering the experimental results. The PMI and RMI indicators were selected as indicators. The biogas and mixed VFAs production yields for each scenario were adjusted to the experimental data obtained in this thesis and are described in the **Annex B: Detailed mass balances of experimental .** The base case, i.e., the cassava starch production process in the rallanderia, presented a PMI of 3.45 kg of raw material per kg of product as shown in **Table 6.1.** This scenario has a higher consumption of raw materials (mainly water). The integration of conventional or modified AD (Sci, Sc2, Sc3, Sc4, and Sc5) using WW as raw material achieved a reduction of this indicator ranging from 30% (Sc5) to 44% (Sc1). This is due to the valorization of a fraction of the base case and the generation of new products (biogas, mixed VFAs, liquid and solid fertilizer). The mixed VFAs recovery (Sc2) generated an increase of 15% compared to Sc1. The modified AD showed the same trends. These results help to elucidate that the mixed VFAs recovery process from liquid-liquid extraction technology as a process step is not beneficial since a large amount of solvent is required to extract a small amount of product.

Table 6.1. Technical and energy results of WW biorefineries

Indicator	**Base case**	**Conventional AD**		**Modified AD**		
		Sc1	**Sc2**	**Sc3**	**Sc4**	**Sc5**
			Technical indicators			
RMI (%w/w)	0.809	0.716	0.671	0.644	0.625	0.644
PMI (kg raw material/kg product)	3.456	1.908	2.206	2.213	2.147	2.394
			Energy indicators			
n		0.948	0.823	0.371	0.131	0.404
SEC (MJ/kg WW)	0.01847	0.0303	0.0646	0.1130	0.1301	0.1007
SGI		12.651	4.258	0.413	0.105	0.475

Higher RMI values mean a high flow of renewable raw materials in the process. The base case presented the best result. The scenarios involving mixed VFAs recovery presented the lowest rates. This is congruent due to the use of solvents in the extraction. In addition, indicators related to overall energy efficiency ("η") and specific energy consumption (SEC) were considered. The SEC quantitatively describes the total energy consumed by the process per unit of product mass. The addition of new process lines to the base case generated an increase in the energy consumed in the process. For example, Sc1 increased by 64.2% concerning the base case. Sc4 (pretreatment of the inoculum in the modified AD) increased seven (7) times, and Sc5 (PHB production) increased five (5) times. These results help to elucidate that integrating conventional and modified AD into existing processing lines, such as cassava starch production (Base Case), is not beneficial in terms of energy because a large amount of energy is required to generate new products. However, when considering the SGI (i.e., self-generation index), the biogas generated in Sc1 supplies 12.65% of the process energy while generating other products (liquid and solid fertilizer). On the other hand, the mixed VFAs recovery was not degraded by the microorganisms (Sc2), decreasing the amount of energy supplied in the process by 75% due to the new processing line. The biogas generated in the modified AD has a very low CH_4 content. Therefore, the energy supplied to the process is lower.

6.3. Economic assessment

The economic evaluation of the proposed WW biorefinery scenarios involved key aspects such as capital costs (CapEx and operating costs (i.e., feedstock and utilities - OpEx), among others. The **base case** (i.e., the cassava starch production) presented an OpEx of 3.38 mUSD and a CapEx of 2.21 mUSD as shown in **Table 6.2.** The CAD integration guided to the energy generation (in the form of biogas), liquid and solid fertilizer (**Sc1**) increased the CapEx and OpEx by 1.5 times and 0.28 time, respectively. Adding the mixed VFAs recovery not consumed stage (**Sc2**) generated an increase in OpEx and CapEx of 0.38 times and 1.57 times, respectively compared to Sc1. The increase is due to the solvent (MTBE) and service fluids (steam and cooling water) consumption for the OpEx and facilities for the CapEx. Considering the integration of MAD (**Sc3-Sc5**), the OpEx and CapEx increased considerably. The mixed VFAs production yield is higher for these scenarios, generating higher input usage for the mixed VFAs recovery stage. The inoculum pretreatment (**Sc4**) increased OpEx and CapEx by 51.2% and 168%, respectively compared to **Sc3**. PHB production using mixed VFAs as substrate (**Sc5**) increased by 75% and 187%, respectively compared to Sc3.

Table 6.2. Economic results of the WW biorefinery scenarios

Parameter		PBP (year)	NPV (mUSD)	CapEx (mUSD)	OpEx (mUSD-Year)
Base case		12	5.39	2.21	3.38
Conventional AD	Sc1	15	2.78	5.53	4.35
	Sc2	10.8	7.61	5.69	4.67
Modified AD	Sc3	8.8	12.04	5.75	4.91
	Sc4	9.6	9.93	5.94	5.11
	Sc5	N.A	N.A	6.35	5.92

N.A Not apply.

The **base case** presented economic viability resulting in a payback period (PBP) and net present value (NPV) of 12 years and 5.39 mUSD, respectively as shown in **Figure 6.1.** The high sales price of the products and by-products generated in the rallanderias (cassava starch and afrecho, respectively), associated with the low technological level represented by the low capital costs, allows the economic viability of the process. Integrating CAD into the base case (**Sc1**) generates an energy recovery of 12.65% and new products (liquid and solid fertilizer). However, the low yields combined with increased capital and operating costs increase the PBP by 25% as shown in **Figure 6.2**. The mixed VFAs recovery stage not consumed by the microorganisms (**Sc2**) in the CAD caused continued viability of the process, although low yield of mixed VFAs was achieved. Moreover, a reduction of 10% in PBP was achieved. These results showed that integrating the recovery of non-degraded mixed VFA is welcome while reducing the PBP and increasing the net present value. Considering the integration of MAD (**Sc3**) in the base case (see **Figure 6.3**), a reduction in PBP of 26.6% and an increase in NPV of 123% were achieved. The MAD generated the inhibition of biogas production and increased the mixed VFAs production. With MAD using WW for the mixed VFAs production, lower yields are obtained than other feedstocks. However, the high selling price allowed for amortizing the costs associated with utilities, reagents (MTBE), and equipment costs. The pre-treatment of the inoculum (**Sc4**) led to better technical results. In addition, economic feasibility is obtained. However, it is not enough to be economically competitive with Sc3. On the other hand, although PHB is a high-value product with a high selling price in the market, the low production yield associated with the additional operating costs of the process resulted in an economically unviable process. These results allow us to elucidate that the integration of MAD in WW valorization rallanderias is welcome.

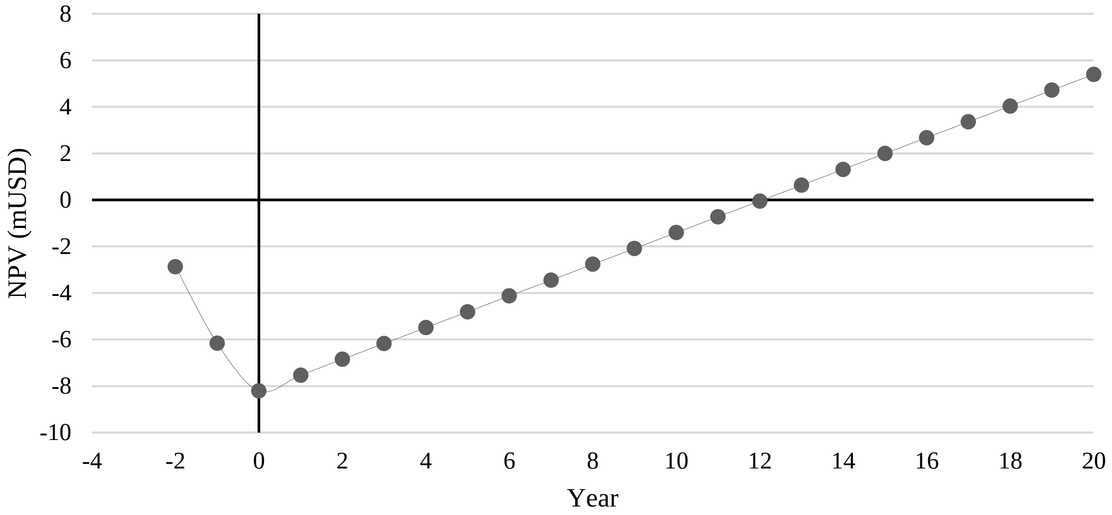


Figure 6.1. Net present value of base case (cassava starch production)

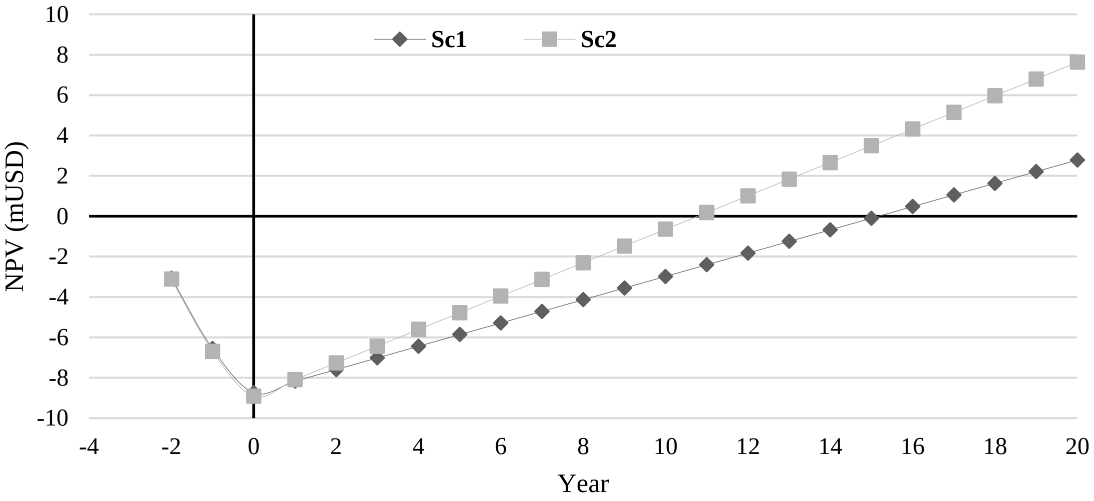


Figure 6.2. Net present value of Conventional AD integrates in base case (cassava starch production)

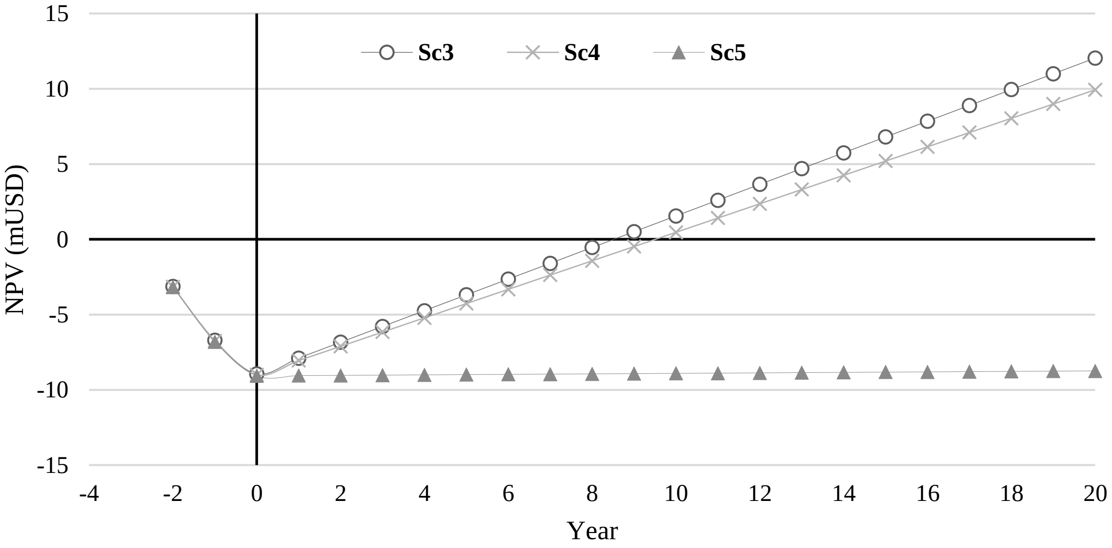
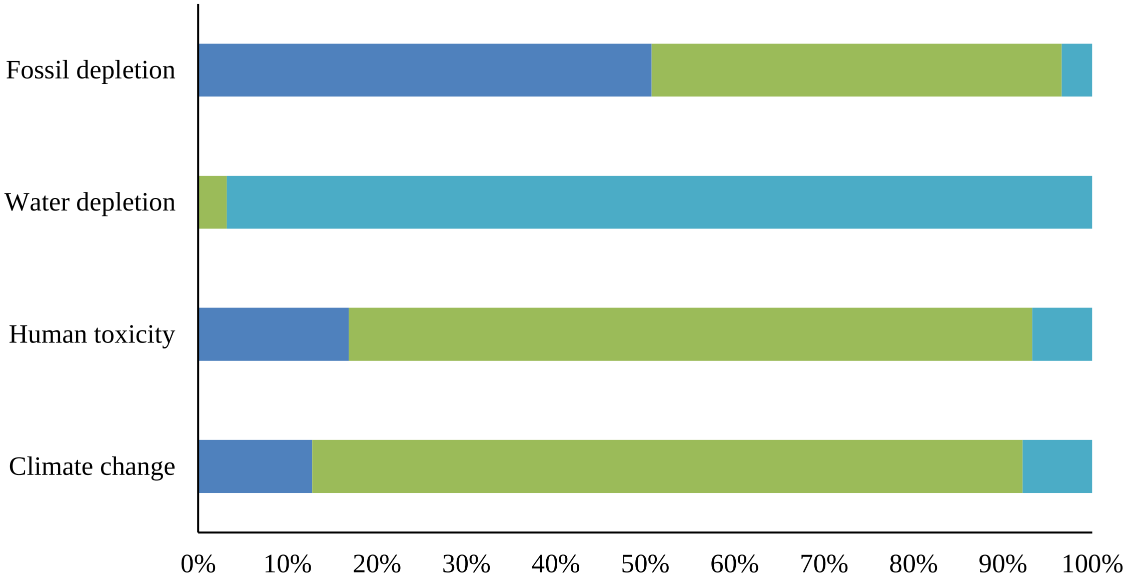


Figure 6.3. Net present value of modified AD integrates in base case (cassava starch production)

6.4. Environmental assessment

The results presented below are shown by the percentage participation of the activities performed by each link in the cassava VC for different representative relative impact categories. As mentioned above, the cassava value chain in the Department of Sucre includes the participation of input suppliers, producers, and processors. The most representative impact categories for the chain were climate change, human toxicity, water depletion, and fossil depletion. The producers contributed 79.4% and 76.5% for the first two categories mentioned, respectively (see **Figure 6.4**). On the other hand, the transformer link represented the greatest contribution to the water depletion category (96.7%), and the input suppliers link was the greatest contributor to the fossil depletion category (50.72%), followed by the producer link (45.87%).



	Climate change	Human toxicity	Water depletion	Fossil depletion
■ Suppliers	12,77%	16,85%	0,00%	50,72%
■ Producers	79,44%	76,45%	3,22%	45,87%
■ Transformers	7,80%	6,70%	96,78%	3,42%

Figure 6.4. Percentage contribution of the first three links of the cassava value chain in different impact categories

The environmental contribution of the activities related to the producer link described in **Table 2.12** is shown in **Figure 6.5.** The crop's disposal of organic residue, i.e., leaves and stems, is the most representative activity in the climate change and human toxicity impact categories. The leaves and stems that remain after the harvesting and collection process are generally left in the field and considered an organic crop fertilizer. This waste is currently not valorized. Generally, greenhouse gas (GHG) emissions associated with the climate change category are lower per unit area in organic agriculture [246], for example, when organic residues are disposed of in the crop but higher per unit of product.

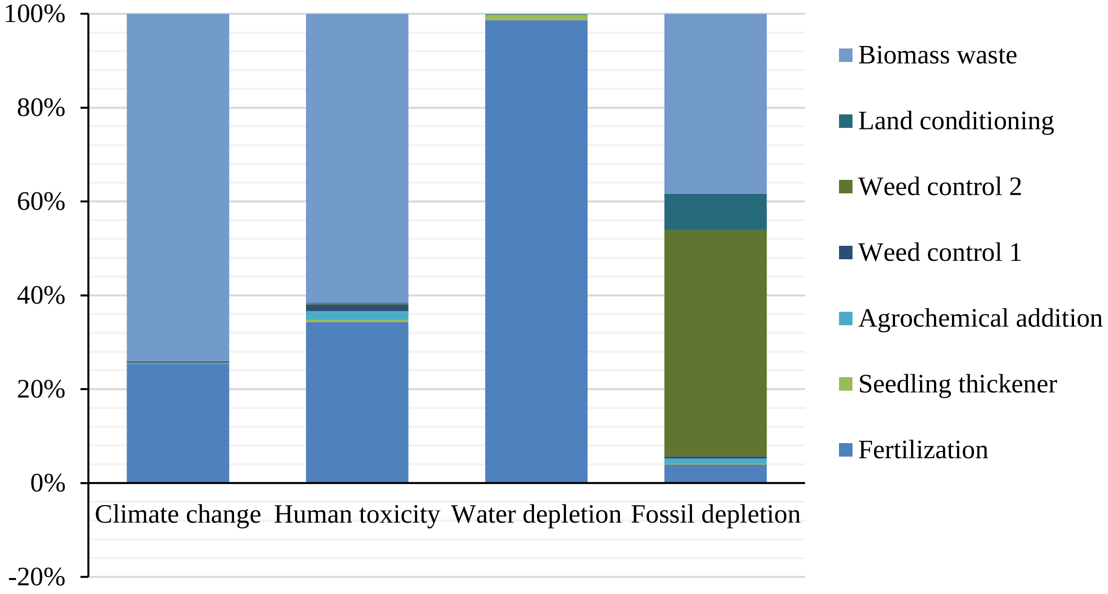


Figure 6.5. Percentage contribution of the activities related to the producer link.

GHG emissions from the addition of synthetic fertilizers and agrochemicals contribute to global warming. The use of these types of agrochemicals (triple 15, invetrin, threshing) generates the nitrous oxide (N_2O) formation occurring during nitrification and denitrification processes affecting the ecosystem and human health [247]. Agrochemicals leach into the surrounding soil and water bodies and enter the chain, leading to bioaccumulation and environmental damage [248]. However, the contribution of fertilizers to 98% of the water depletion category is also reflected. The use of organic fertilizers implies a greenhouse gas reduction rate compared to chemical fertilizers. For example, Kitamura R et al. [249] report that a reduction of about 25% is achieved by using manure and slurry as fertilizers. Similar results have been reported by Havukainen et al. [250] and Yuttitham [251].

The environmental contribution of the activities related to the transformer link described in **Table 2.12** is shown in **Figure 6.6**. The transformation process is characterized by a low technological level, i.e., low-capacity equipment and limited use of additional inputs. The energy required in the different stages of the process is obtained from electricity supplied by the national power grid (hydroelectric plants); therefore, this factor does not significantly influence the environmental impact.

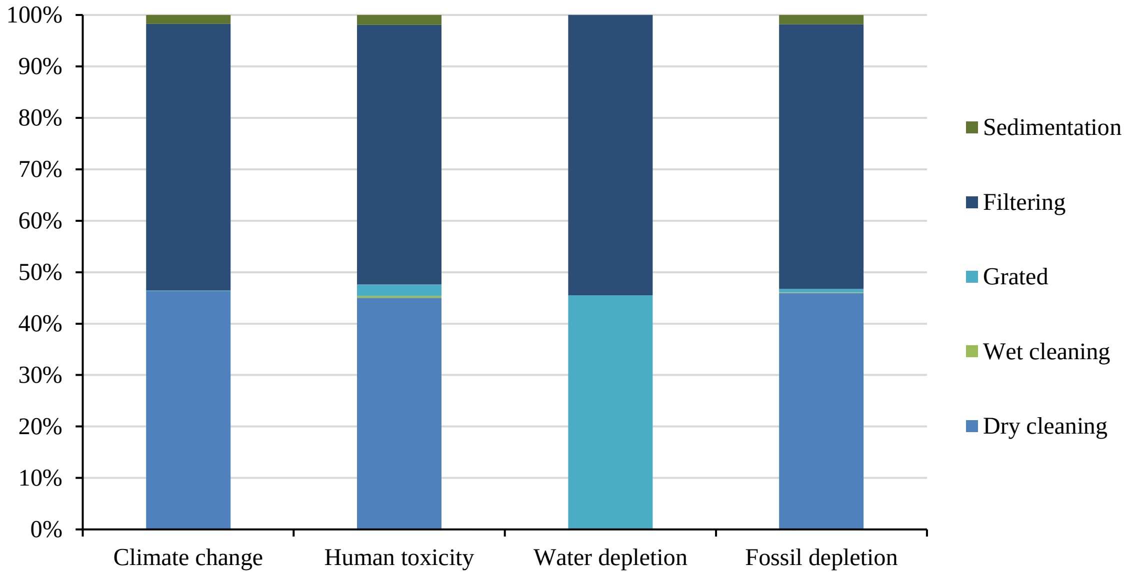


Figure 6.6. Percentage contribution of the activities related to the transformer link.

The raw material in traditional industries does not generally use automated processes; most are produced during the pressing of the peeled roots. The characteristic of this type of process is that a smaller amount of effluent is obtained. However, it is more concentrated. The untreated effluent is discharged close to the producing region, polluting the soil and rivers. In addition, the high content of organic compounds present in the effluent generates a considerable drop in the oxygen levels of the rivers [252]. The stages contributing most to the environmental impact (in the climate change, human toxicity, and fossil depletion categories) were the dry drying and filtration stages. The cassava husk is generated and disposed of in the field in the drying stage. The filtering stage generates the afrecho (cassava bran). The afrecho is a by-product destined for animal feed. 80% of the waste generated in the processing link corresponds to wastewater. Wastewater has a low pH (4-5) and a high organic matter content. They generally have a chemical oxygen demand between 6000 and 10000 mg/L and a total solids content of 150-180 mg/L [253]. These characteristics make WW one of the main contributors to the water depletion category [254]. They are generated in three of the four stages of the process.

Considering the above, climate change and water depletion were the most influential categories regarding environmental impact. The contribution of kg of CO_2 eq and water depletion expressed in m^3 per functional unit of each link in the VC is shown in **Figure 6.7**. The cassava value chain generated 2.63 kg of CO_2 eq per functional unit. The producer link accounts for 74%. The transformer link is the least representative. The cassava value chain generates 85.14 m^3 of water per functional unit. The transformer link accounts for 96%. The supplier's link is the least representative.

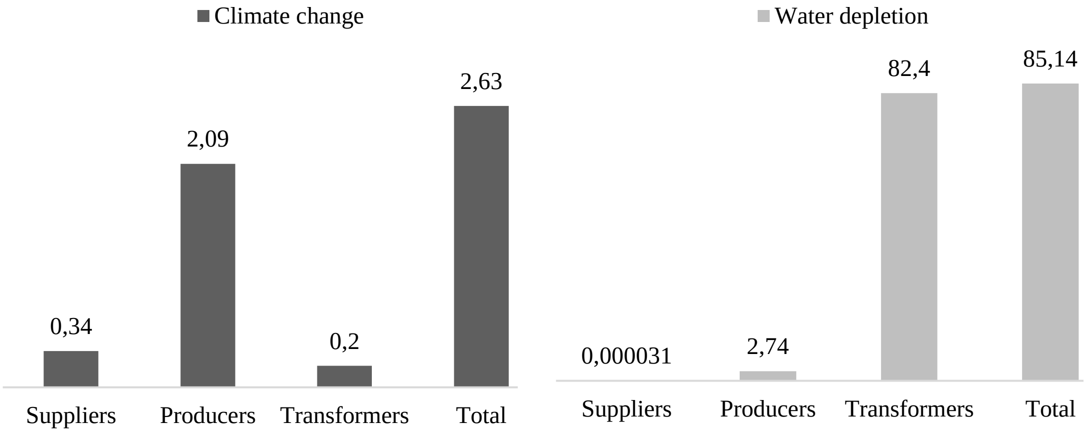


Figure 6.7. Carbon footprint (kg CO_2) and water footprint (m^3) per FU of the cassava value chain in the department of Sucre for each link.

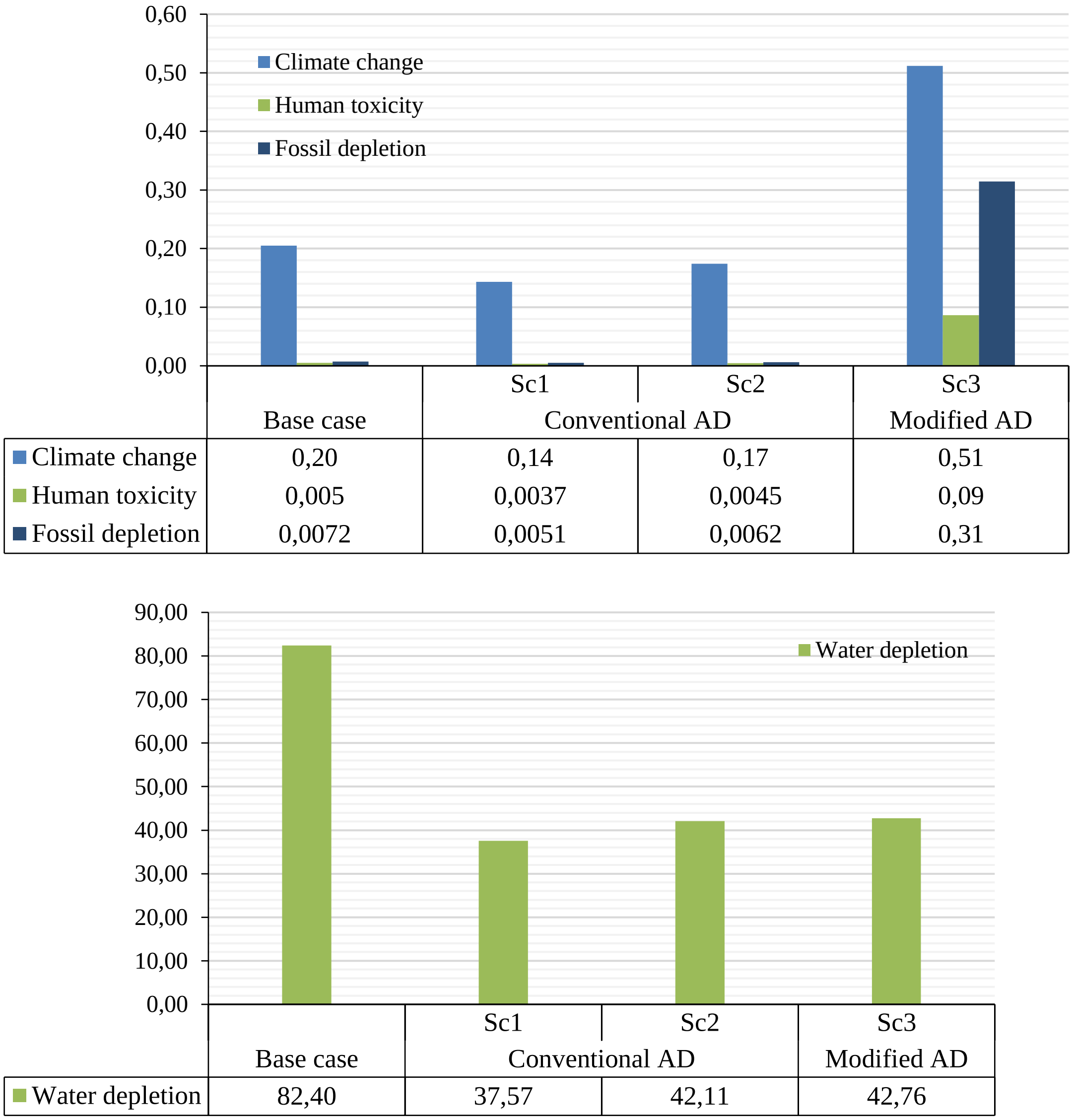
Based on the life cycle assessment, the cassava value chain's environmental impact in the Sucre department was identified. In this sense, the cumulative impacts of each actor and link in the cassava chain (i.e., suppliers, producers, processors) are mainly influenced by the activities in the production and processing links. The most representative categories were climate change and water depletion. The activities with the greatest environmental impact in these links were: (bottlenecks - hotspots)

- The addition of fertilizers and agrochemicals to seedlings in the producer's link.
- The use of water in the transformation process of cassava
- The current disposal of waste generated in the transformer link.

Considering the above, WW obtained from the transformer link is not valorized and is disposed of in water sources, generating a high environmental impact. Therefore, the biorefinery scenarios were proposed to be integrated into this link for WW valorization. The environmental analysis was performed only for those scenarios that represented similar economic feasibility to the base case (i.e., Sc1, Sc2, Sc3). The most representative impact categories were climate change, water depletion, fossil depletion, and human toxicity.

The base case, i.e., the cassava starch generation unit, was discussed previously. All biorefinery scenarios have additional processing units where steam is involved to satisfy the energy requirements. Moreover, conventional AD allowed energy recovery of the process by 12.65% and 4.26% for **Sc1** and **Sc2**, respectively. This generated a reduction in the carbon footprint in the above scenarios. Sc1 generated a 30% reduction in the carbon footprint (0.14 kg CO_2 per functional unit). Adding the unconsumed mixed VFAs recovery generated a 14.8% reduction (0.14 kg CO_2 per functional unit) as shown in **Figure 6.8.** This shows that conventional AD, even recovering the unconsumed mixed VFAs, allowed the reduction of the environmental impact of the evaluated categories by energy recovery. Considering the modified AD, **Sc3** (use of WW guided to the mixed VFAs, liquid and solid fertilizer production) increased carbon footprint by 1.49 times compared to the base case due to the additional use of solvent and service fluids. In Sc3, there was a low energy recovery; therefore, it was not enough to reduce the environmental impact.

The carbon footprint of base case results was comparable with the literature. Thierry T et al. reported that a low-scale starch processing industry has a carbon footprint of 0.15 kg CO_2 per UF. The main factor generating these results was energy use [255]. Richard K et al. report that the starch production process in Africa produces 1.8 kg CO_2 eq per functional unit [256].



	Base case	Sc1 Conventional AD	Sc2 Conventional AD	Sc3 Modified AD
🟦 Climate change	0,20	0,14	0,17	0,51
🟩 Human toxicity	0,005	0,0037	0,0045	0,09
■ Fossil depletion	0,0072	0,0051	0,0062	0,31

	Base case	Sc1 Conventional AD	Sc2 Conventional AD	Sc3 Modified AD
🟩 Water depletion	82,40	37,57	42,11	42,76

Figure 6.8. Climate change (kg CO_2), human toxicity (kg 1.4-DB), water depletion (m^3) and fossil depletion (kg oil eq) per kg of WW valorized of biorefinery scenarios.

Integrating new processing lines through the conventional or modified AD generates a maximum increase of 1.49 times the base case in the climate change category (0.51 kg CO_2 eq per UF). However, these results are low compared to other technological processes (with higher technification and production levels). For example, rice production in China generates 2.5 kg CO_2 eq per UF [257]. On the other hand, the valorization of wastewater for generating new products resulted in a considerable reduction in the biorefinery scenarios. Sc1, Sc2, and Sc3 generated a decrease in water depletion of 54.4%, 48.8%, and 48.1%, respectively.

6.5. Social assessment

The social analysis was developed considering the employment generated in the biorefinery scenarios and the relationship between minimum wage and living wage. The living wage represents the wage for a person to have a decent life. The employment generated was estimated based on the Colombian context. For one year of work, 91 days off (associated with vacations, holidays, and family days) were considered. The number of employees required to fulfill the work in a plant processing line is 5 operators, 1 substitute, and 1 supervisor per shift (i.e., 3 for working time). Therefore, the number of employees per processing line is nine (9) employees. The number of employees for the economically acceptable conventional and modified AD biorefinery scenarios is presented in **Figure 6.9**. The base case presented a total of 36 jobs. Adding the new processing lines generated a maximum increase of 50% (Sc4) and a minimum increase of 25% (Sc1). Sc2 and Sc3 presented the same number of jobs generated because they comprise the same processing units under different conditions.

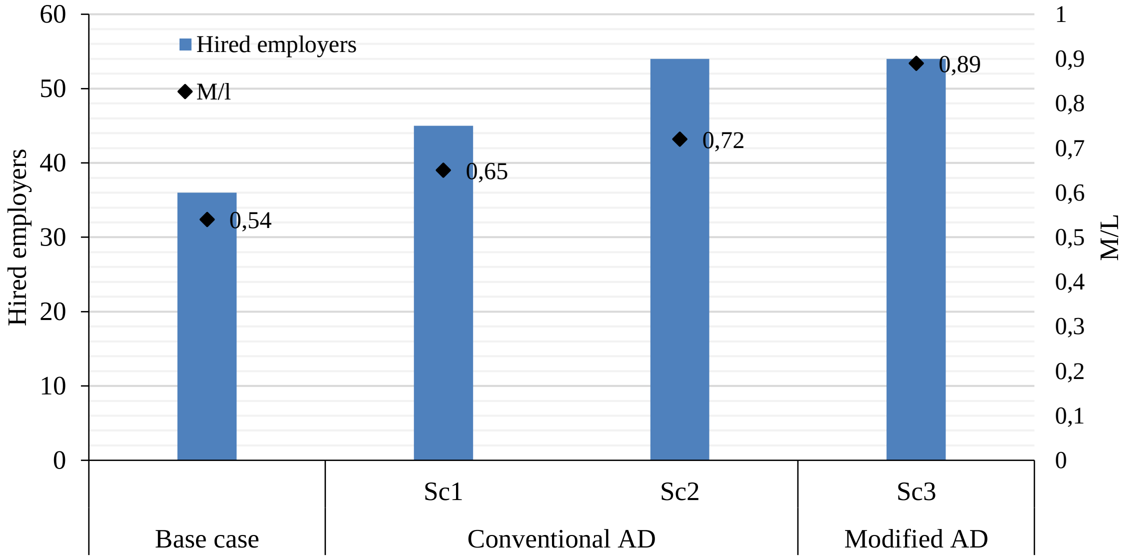


Figure 6.9. Employment generated and M/L ratio for the WW biorefinery scenarios.

The number of employees hired influences the M/L ratio. M/L was determined based on the minimum wage variation until a 30% decrease in the accumulated NPV was obtained (without drastically affecting the economic profits of the biorefinery). Similar to the number of employees generated, the M/L ratio was determined only for the economically attractive scenarios. The ideal value for the M/L ratio is equal to 1. The Sc3 obtained the best results. In this sense, these biorefinery can hire more employees or increase existing employees' salaries without affecting the process's economic dimension. Therefore, these scenarios presented a high social impact.

6.6. Sustainability index

The sustainability index was determined by considering equal weights for each dimension (technical, economic, environmental, and social). The results of the sustainability index for each scenario are shown in **Figure 6.10**. **Sc1** (use of WW guided to conventional AD) was the lowest sustainable scenario, as the sustainability index was 51.25. This scenario obtained the lowest sustainability index due to the low technical (17.43%), economic (3.87%) and social (5%) dimensions. The base case presented similar results. The base case presented an economically feasible. However, the current disposal of WW generated in the process directly affects the sustainability index. The recovery of unconsumed mixed VFAs in the conventional AD (**Sc2**) and the modified AD route guided to increase the mixed VFAs production (**Sc3**) obtained similar results. Sc2 obtained an index of 66.99, and Sc3 an index of 70.40. The main differences were observed in the economic and environmental dimensions. Sc3 requires more service fluids and inputs (MTBE) to extract and recover the mixed VFAs, increasing the carbon footprint. Likewise, the PBP of Sc3 is 60% higher than Sc2. Therefore, modified AD biorefinery guided to promote mixed VFAs production integrated into ethanol production is more sustainable than conventional AD using vinasse as feedstock.

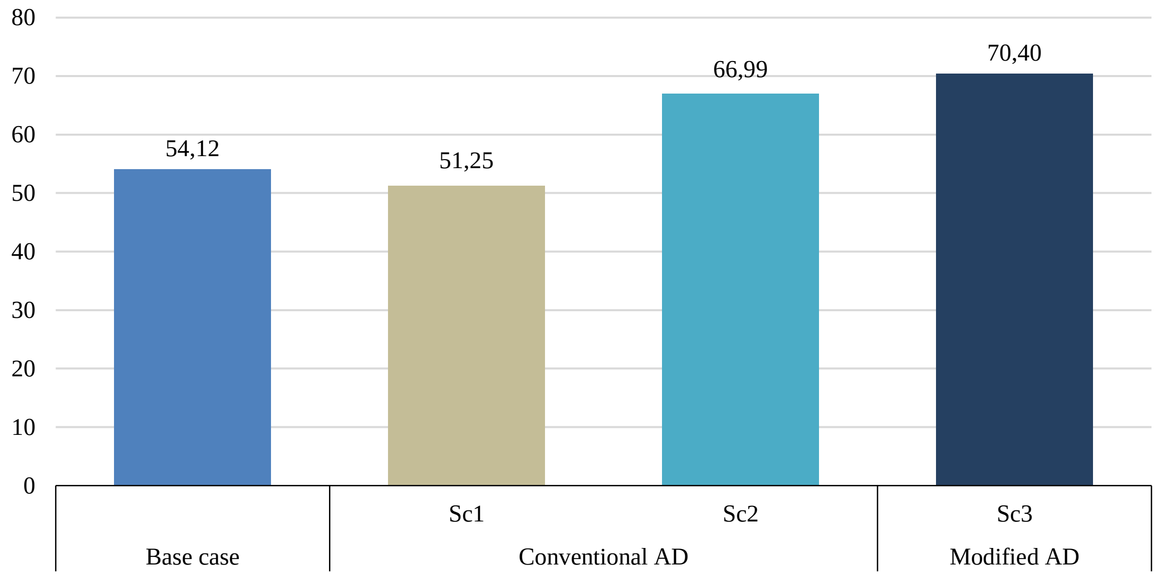


Figure 6.10. Sustainability index for the WW biorefinery scenarios.

7. Chapter 7: Sustainability analysis of vinasses from ethanol production biorefineries

7.1. Overview

The sustainability analysis of the vinasses biorefinery scenarios was performed considering the four dimensions of sustainability. First, a comparative analysis of the results of the scenarios was carried out, considering the indicators evaluated for each dimension. Then, the sustainability indices for each scenario were determined and compared to define the most viable configuration (i.e., the highest values of the sustainability impact).

7.2. Technical assessment

The technical analysis of vinasses biorefineries was carried out considering the experimental results. The PMI and RMI indicators were selected as indicators. The biogas and mixed VFAs production yields for each scenario were adjusted to the experimental data obtained in this thesis and are described in the **Annex B: Detailed mass balances of experimental .** The base case, i.e., the ethanol production process, presented a PMI of 20.526 kg of raw material per kg of product as shown in **Table 7.1.** This scenario has a higher consumption of raw materials (water, H_2SO_4 and, NaOH). The integration of conventional or modified AD (Sc1, Sc2, Sc3, Sc4, and Sc5) using vinasses as raw material achieved a reduction of this indicator over 90%. This is due to the valorization of a fraction of the base case and the generation of new products (biogas, mixed VFAs, liquid and solid fertilizer). The mixed VFAs recovery (Sc2) generated an increase of 10.7% compared to Sc1. The modified AD showed the same trends. These results help to elucidate that the mixed VFAs recovery process from liquid-liquid extraction technology as a process step is not beneficial since a large amount of solvent is required to extract a small amount of product.

Table 7.1. Technical and energy results of vinasses biorefineries

Scenario		**Technical indicators**		**Energy indicators**		
		RMI (%w/w)	**PMI (kg raw material/kg product)**	**n**	**SEC (MJ/kg Vinasses)**	**SGI**
Base case		0.79	20.526		0.252	
Conventional AD	Sc1	0.977	1.102	0.92	0.303	8.1
	Sc2	0.96	1.22	0.920	0.323	7.07
Modified AD	Sc3	0.921	1.31	0.76	0.334	2.25
	Sc4	0.917	1.32	0.66	0.359	1.33
	Sc5	0.9	1.42	0.71	0.34	1.74

Higher RMI values mean a high flow of renewable raw materials in the process. The base case presented the low result. The scenarios involving mixed VFAs recovery presented the lowest rates. This is congruent due to the use of solvents in the extraction. In addition, indicators related to overall energy efficiency ("η") and specific energy consumption (SEC) were considered. The SEC quantitatively describes the total energy consumed by the process per unit of product mass. The base case generated a SEG of 0.252 MJ/ kg vinasses. One liter of ethanol generates 14.12 L of vinasse as a by-product. The evaporation unit achieves a reduction of this ratio by 1.15. Moreover, the evaporation unit represents 24.7% of the total energy consumed of the process.

The addition of new process lines (AD biorefineries) to the base case generated an increase in the energy consumed in the process. For example, Sc1 increased by 20.2% concerning the base case. Sc4 (pretreatment of the inoculum in the modified AD) increased 1.42 times, and Sc5 (PHB production) increased 1.34 times. These results help to elucidate that integrating conventional and modified AD into existing processing lines, such as ethanol production (Base Case), is not beneficial in terms of energy because a large amount of energy is required to generate new products. However, when considering the SGI (i.e., self-generation index), the biogas generated in Sc1 supplies 8.10% of the process energy while generating other products (liquid and solid fertilizer). On the other hand, the mixed VFAs recovery was not degraded by the microorganisms (Sc2), decreasing the amount of energy supplied in the process by 12.65% due to the new processing line. The biogas generated in the modified AD has a very low CH_4 content (see **Table 4.5**). Therefore, the energy supplied to the process is lower.

7.3. Economic assessment

The economic asessment of the vinasses biorefinery scenarios involved key aspects such as capital costs (CapEx and operating costs (i.e., feedstock and utilities - OpEx), among others. The economic results for the proposed scenarios are presented in **Table 7.2**. The base case (i.e., the ethanol production process) presented an OpEx of 12.27 mUSD and a CapEx of 3.73 mUSD.

Table 7.2. Economic results of the vinasses biorefinery scenarios

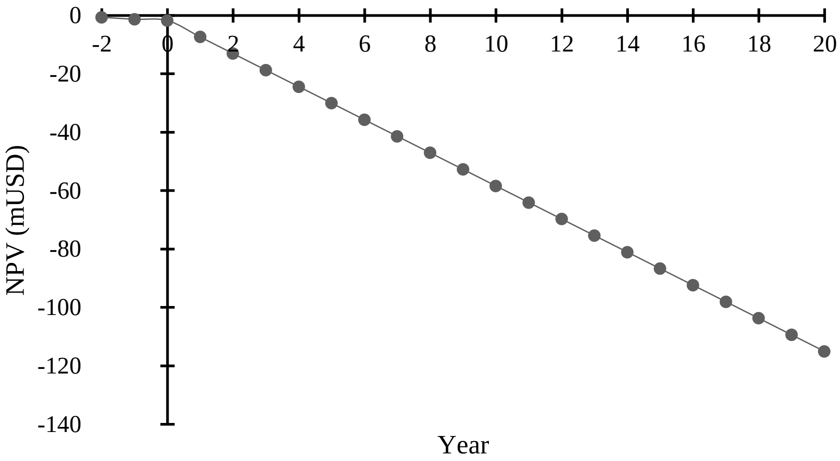
Scenario description	**Ethanol production**	**Energy recovery from biogas. Liquid and solid digestate**	**Recovery of unconsumed mixed VFAs**	**Recovery of mixed VFAs**	**Thermal pretreatment of inoculum**	**PHB production using mixed VFAs as substrates**
Parameter	**Base case**	**Conventional AD**		**Modified AD**		
		Sc1	**Sc2**	**Sc3**	**Sc4**	**Sc5**
PBP (year)	N.A	18.00	9.80	6.80	7.10	N.A
NPV (mUSD)	N.A	0.16	3.29	6.02	8.28	N.A
CapEx (mUSD)	3.73	3.85	4.15	4.88	5.24	5.97
OpEx (mUSD-Year)	12.27	12.41	15.03	15.83	16.14	16.26

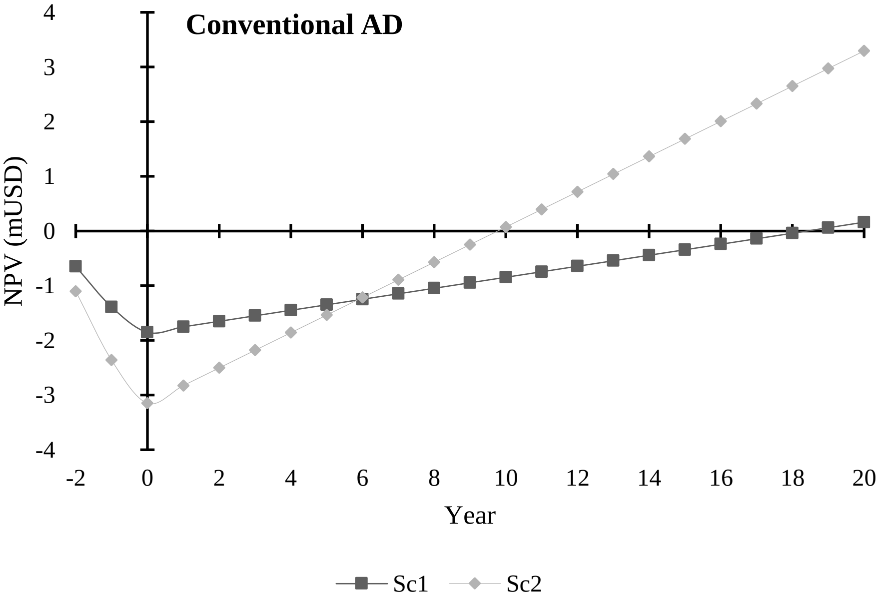
N.A: not apply.

In the base case, the operating parameters that most influenced the OpEx were reagents, equipment maintenance, insurance, and taxes (34.43%, 25.28%, and 15.55%, respectively). conventional AD integration (**Sc1**), increased the utilities increase 6.5 times the base case due to the additional service fluids (steam). On the contrary, in **Sc2** (recovery of the mixed VFAs), the contribution of the service fluids to the OpEx increases up to 20 times the base case.

The conventional AD integration guided to the energy generation (**Sc1**), liquid and solid fertilizer increased the OpEx and CapEx by 1.14% and 3.2%, respectively compared to the base case. **Sc2** (unconsumed mixed VFAs recovery) generated an increase in OpEx and CapEx of 21.09% and 7.79%, respectively compared to Sc1. The increase is due to the solvent (MTBE) and service fluids (steam and cooling water) consumption for the OpEx and facilities for the CapEx. In modified AD (**Sc3-Sc5**), the OpEx and CapEx increased considerably. The mixed VFAs production yield is higher for these scenarios, generating higher input usage for the mixed VFAs recovery stage. The inoculum pretreatment (**Sc4**) increased OpEx and CapEx by 1.9% and 7.3%, respectively compared to Sc3. PHB production using mixed VFAs as substrate (**Sc5**) increased by 2.7% and 22.33%, respectively compared to Sc3.

The base case presented economic unviability as shown in **Figure 7.1**. The ethanol production unit is considered an independent unit. These results were obtained mainly due to the large amount of energy used to produce ethanol, resulting in high utility costs. These results are similar to those reported in the literature. Parascanu M et al. [258] determined that ethanol production from molasses and agave was economically unfeasible due to the high energy costs of the process. Comparing these results with the conventional production process in the Colombian context, i.e., integrated with sugar mills, the energy recovery by burning bagasse (in the sugar production unit) managed to amortize the costs of the ethanol production unit. The biorefinery scenarios presented economic viability. When conventional AD is integrated with biogas and liquid and solid fertilizer generation (**Sc1**), the PBP and de NPV were 18 years and 0.16 mUSD respectively. The mixed VFAs recovery (**Sc2**) caused continued viability of the process, although low yield of mixed VFAs was achieved. Moreover, a reduction of 54.4% in PBP was obtained as shown in . These results showed that integrating the recovery of unconsumed VFAs is welcome while reducing the PBP and increasing the net present value. The integration of modified AD (**Sc3**) in the base case, caused a reduction in PBP of 62.2% and an increase in NPV by 37 times compared to Sc1. The modified AD generated the inhibition of biogas production and increased the mixed VFAs production. The high selling price allowed for amortizing the costs associated with utilities, reagents (MTBE), and equipment costs. The pre-treatment of the inoculum (**Sc4**) led to better technical results. In addition, economic feasibility is obtained. However, it is not enough to be economically competitive with Sc3. Furthermore, although PHB is a value product with a high selling price in the market, the low production yield associated with the additional operational costs in the process resulted in an economically unviable process.





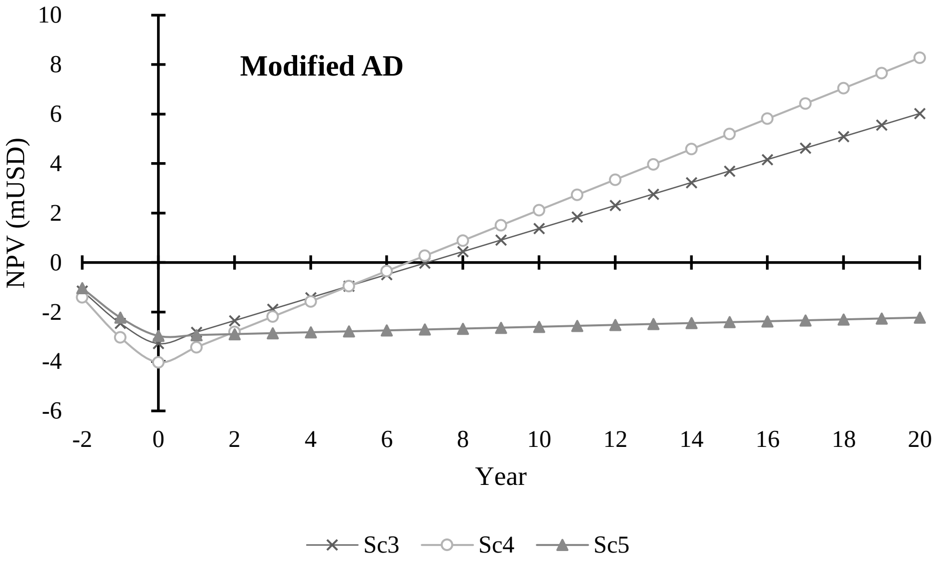


Figure 7.1. Net present value of vinasses biorefinery scenarios.

7.4. Environmental assessment

The environmental analysis was performed only for the most economically attractive scenarios (Sc1, Sc2, Sc3, and Sc4). In addition, the environmental analysis was performed for the base case to analyze the environmental performance when integrating the digestion biorefinery. The most representative impact categories were climate change, water depletion, fossil depletion, and human toxicity. The percentage of environmental contribution of the biorefinery scenarios and the base case is presented in **Figure 7.2.** Ethanol production in sugar mills is highly technological. The stages considered for ethanol production were (i) molasses conditioning, (ii) fermentation, (ii) distillation and rectification, and (iii) evaporation (to concentrate the vinasses obtained from distillation). Reagents such as H_2SO_4 and NaOH are used in the raw material conditioning process. H_2SO_4 is corrosive. Therefore, any living being in contact with H_2SO_4 can have health repercussions. In addition, oxygen in the water can be reduced, affecting aquatic life [259]. This process stage affects the human toxicity and water depletion categories by 26.07 % and 11.45 %, respectively. Only energy consumption (steam and cooling water) is considered in the fermentation, distillation, and evaporation stages. Steam is generated by boilers that consume fuel. The result is climate change and the fossil fuel depletion. The reaction stage represents 31.63% of the total energy of the process, the distillation stage 43.67%, and the evaporation stage 24.7%. In this sense, distillation and reaction were the most influential stages in the previously mentioned categories. These results are similar to the literature. For example, Parascanu M et al. [258] performed an environmental analysis of ethanol production from molasses. They considered three main stages: (i) Sugar cane cultivation, (ii) Sugar extraction, and (iii) Ethanol production (molasses). They determined that the most influential stages of environmental impact (climate change) were sugar cane cultivation and sugar extraction. They also determined that the energy consumption of the fermentation and distillation process (in the ethanol production stage) drastically influenced the environmental impact of ethanol production.

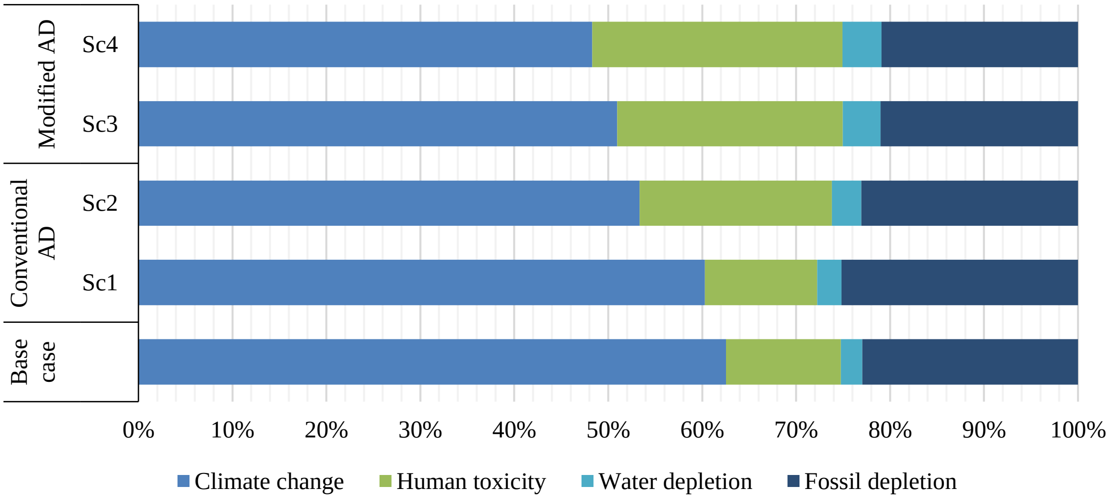
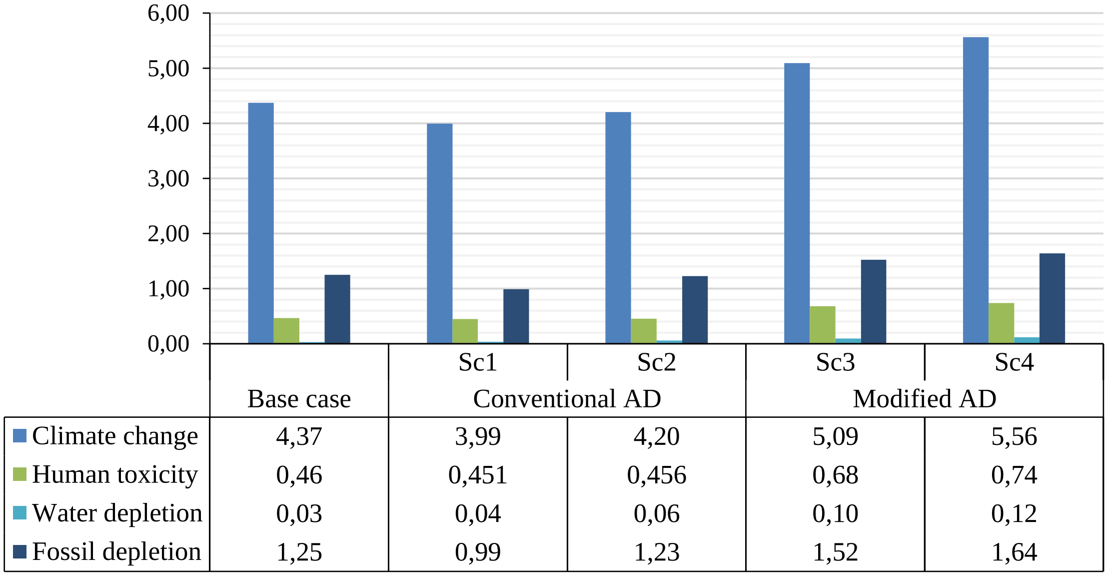


Figure 7.2. Percentage contribution of the most representative categories of the vinasses biorefinery scenarios

Integrating the biorefinery scenarios to the ethanol VC (Sc1, Sc2, Sc3, and Sc4), the evaporation stage of the base case is omitted. Conventional AD allowed energy recovery of 8.1% and 7.07% for Sc1 and Sc2, respectively. This allowed the reduction of the environmental impact of the categories mentioned above, as shown in the **Figure 7.3**.

The base case presented the lowest values compared to the other scenarios. The base case obtained a carbon footprint (climate change) of 3.99 kg of CO_2 per kg of vinasses. By including additional stages to the process, such as conventional AD for energy recovery (in the form of biogas) and liquid and solid fertilizer (**Sc1**), 8.10 % of the total energy of the process was recovered. Therefore, there was a reduction of 8.5% in the climate change category compared to the base case. By including the stage of unconsumed mixed VFAs recovery (**Sc2**) through liquid-liquid extraction generated a reduction of 3.7% in the climate change category compared to the base case. In the modified AD (**Sc3** and **Sc4**), the bottleneck, accounting for over 28-57% of the environmental impact, is the mixed VFAs recovery. This is consistent with the additional process units that consume service fluids and solvents (MTBE). This shows that conventional AD, even recovering the unconsumed mixed VFAs, allowed the reduction of the environmental impact of the evaluated categories by energy recovery.



		Sc1	Sc2	Sc3	Sc4
	Base case	Conventional AD		Modified AD	
■ Climate change	4,37	3,99	4,20	5,09	5,56
■ Human toxicity	0,46	0,451	0,456	0,68	0,74
■ Water depletion	0,03	0,04	0,06	0,10	0,12
■ Fossil depletion	1,25	0,99	1,23	1,52	1,64

Figure 7.3. Climate change (kg CO_2), human toxicity (kg 1.4-DB), water depletion (m^3) and fossil depletion (kg oil eq) per kg of vinasse valorized of biorefinery scenarios.

7.5. Social assessment

The social analysis was developed considering the employment generated in the biorefinery scenarios and the relationship between minimum wage and living wage. The living wage represents the wage for a person to have a decent life. The employment generated was estimated based on the Colombian context. For one year of work, 91 days off (associated with vacations, holidays, and family days) were considered. The number of employees required to fulfill the work in a plant processing line is 5 operators, 1 substitute, and 1 supervisor per shift (i.e., 3 for working time). Therefore, the number of employees per processing line is nine (9) employees. The number of employees for the economically acceptable conventional and modified AD biorefinery scenarios is presented in **Figure 7.4**. The addition of the new processing lines in the base case generated a maximum of 63 employees (Sc4) and a minimum of 45. Sc2 and Sc3 presented the same number of jobs generated because they comprise the same processing units under different conditions.

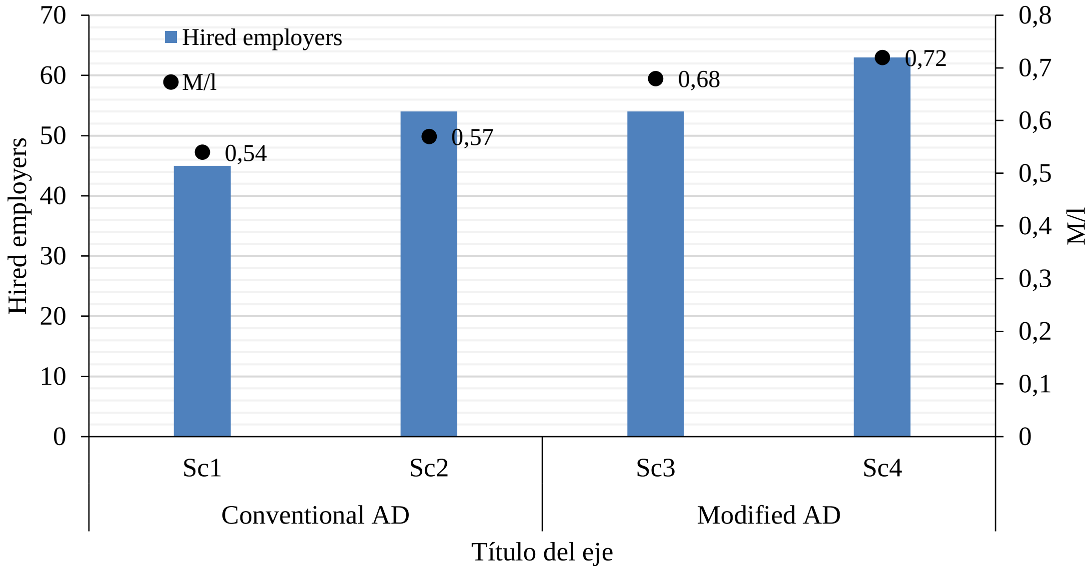


Figure 7.4. Employment generated and M/L ratio for the vinasses biorefinery scenarios.

The number of employees hired influences the M/L ratio. M/L was determined based on the minimum wage variation until a 30% decrease in the accumulated NPV was obtained (without drastically affecting the economic profits of the biorefinery). Similar to the number of employees generated, the M/L ratio was determined only for the economically attractive scenarios. The ideal value for the M/L ratio is equal to 1. The Sc3 and, Sc4 obtained the best results. In this sense, these biorefineries can hire more employees or increase existing employees' salaries without affecting the process's economic dimension. Therefore, these scenarios presented a high social impact.

7.6. Sustainability assessment

The sustainability index was determined by considering equal weights for each dimension (technical, economic, environmental, and social). The results of the sustainability index for each scenario are shown in **Figure 7.5**. The Sc4 (modified AD) scenario was the most sustainable, as the sustainability index was 67.82. This scenario obtained the highest sustainability index due to the high economic (17.97%) and social (28%) results. Sc1 (conventional AD) presented the lowest result. In this scenario, the environmental dimension obtained the highest result. However, the low technical and economic results did not allow to obtain a higher index. Sc2 obtained similar results, the difference being in the economic dimension (lower PBP). Therefore, modified AD biorefinery guided to promote mixed VFAs production integrated into ethanol production is more sustainable than conventional AD using vinasse as feedstock.

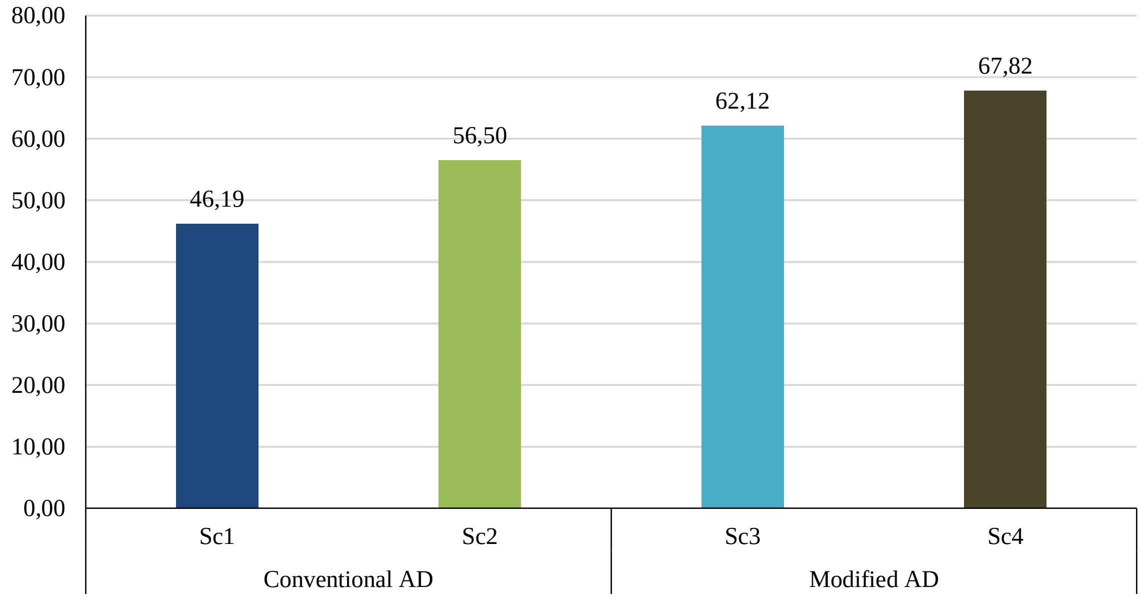


Figure 7.5. Sustainability index for the vinasses biorefinery scenarios.

8. Conclusions and recommendations

Anaerobic digestion (AD) is a simple and widely used technology for organic waste degradation and renewable energy generation. It was demonstrated that organic wastes from different links of value chains (such as organic kitchen food waste and wastewater from cassava starch production and vinasses) could be valorized and integrated into existing processes (the last two feedstocks) for energy generation or mixed VFAs.

The feedstock composition was a key factor affecting the biogas production yield in the conventional AD. Likewise, the composition and operating conditions affect the compositional profile of the mixed volatile fatty acids (VFAs) generated in the modified AD. A high glucose and starch content promotes acetic acid production. Slight acidity (pH=6) promotes the butyric acid production.

The functional analysis of the value chains (VCs) of the organic wastes analyzed in this thesis allowed the identification of bottlenecks in the chains. Based on this, the integration of AD was proposed as a pillar for waste valorization through the biorefinery concept. AD can be oriented to energy or high-value products by conventional and modified AD, respectively. The feedstock influenced the best sustainability indices for conventional or modified AD analyzed (organic kitchen food waste, wastewater from rallanderias, or vinasses). For example, considering organic kitchen food waste, conventional AD resulted in better sustainability indices. Adding new process lines (mixed VFAs recovery, PHB production) could be preliminary considered a good option, however it is increased operating and capital costs. The same results were obtained when considering the AD integration into an established VC (cassava VC) valorizing rallanderias wastewater. Modified AD can be comparable to conventional AD when the scale of the process is increased. By integrating the AD into the ethanol VC, the modified AD presented better sustainability indexes.

This thesis proved that anaerobic digestion to produce biogas will always be an alternative for any feedstock where the composition or even the scales of production are very diverse (with a very wide range). Anaerobic digestion has always been seen as the only or best alternative for waste that cannot be easily standardized. However, this thesis demonstrates, in detail, that additional assessments of the metabolic pathways of anaerobic digestion are always welcome. In the case of the mixed VFAs, all these modifications allowed for obtaining an excellent economic performance in a preliminary way, making the anaerobic digestion processes even more viable. Likewise, the concept of biorefinery can be fully involved in the development of processes based on anaerobic digestion because biogas, volatile fatty acids, and digestate can be produced at the same time to achieve better performance and sustainability of these proposed biorefineries. The three raw materials investigated in this thesis are very different. In this thesis, non-standard raw materials such as organic kitchen food waste presents very high logistical constraints that cause it to have variations not only in composition but also in scale. It also was approached raw materials from the agro-industrial sector, such as wastewater from cassava starch production (low scale) and the particular case of ethanol vinasses (large scale). Despite being so dissimilar, these three cases were investigated in this thesis, and it was demonstrated that anaerobic digestion is still one of the best alternatives, even with modifications. It was possible to establish that the economic yields and overall sustainability could be even higher, especially when the mixed VFAs production is also considered.

Annex A: Biorefinery scenarios description

A1: Organic kitchen food waste biorefinery scenarios description

The integral biorefinery scenarios of all the fractions generated during the conventional and modified AD process is shown in **Figure A1. 1.** The first area (**Area 10**) represented the storage area for the raw materials and inputs required for the process. **Area 20** involved the raw material conditioning stages. The raw material was dried and ground to a 1-2 mm particle size. Then, for the scenarios considering pretreatment, an LHW pretreatment was performed at 80°C, atmospheric pressure, and a solid: liquid ratio of 1:10 (**Area 30**). In addition, adding the hydrolysate from the pretreatment to the digestion stage was contemplated for some scenarios. **Area 40** involved the AD process. The temperature was 37°C at atmospheric pressure, and a substrate SV: inoculum ratio of 0.4 was managed for conventional AD. The temperature was 37°C at atmospheric pressure, and a substrate: inoculum SV ratio 1 was used for modified AD. The liquid and solid fractions of the digestate were separated by a centrifuge. **Area 50** included the recovery process of the mixed VFAs from the liquid fraction of the digestate. The liquor was sent to extraction column. The VFAs recovery unit consists of an extraction column, rectification, and water-stripping column. Methyl tert- butyl ether (MTBE) was selected as extractive agent because it has lower boiling point and enthalpy of vaporization. The feed mixture has a greater density than the solvent and is fed in at the top end of the extraction tower. Inside the tower it streams towards the bottom and in the process gives off VFAs to the extraction agent. The extraction ability of MTBE increases in the order Butyric acid > Propionic acid > Acetic acid. The flow rate of MTBE is manipulated to have an acetic acid concentration of 0.0025 at the bottom of extraction column. The aqueous phase (Raffinate) in the extraction tower is saturated with MTBE. Therefore, it is recovered in a downstream stripping tower. High pressure steam at 9.5 atm is used to strip MTBE from water. The water at the bottom of striping column is sent to wastewater treatment unit. The extract stream from extraction column is sent to rectification column to separate VFAs from MTBE. The extraction agent accumulates at the top end of the rectification tower and the VFAs at the bottom of the tower resulting in VFAs concentrations of 100 %. MTBE recovered from top of the stripping column and rectification column are condensed and sent to a decanter. The organic phase containing mostly MTBE is recycled to extraction column [151]. **Area 60** involved the extraction of bioactive compounds from the hydrolysate by solvent extraction. The extraction was performed using 60% vol ethanol at a solid: liquid ratio of 1:20 and at 50°C. The stream rich in bioactive compounds are finally conducted to an ethanol separation step that is reintegrated into the system. **Area 70** considered the inoculum pretreatment stage. The inoculum was exposed to heat treatment at 65°C and atmospheric pressure [220]. **Area 80** involved the polyhydroxybutyrate (PHB) production using the mixed VFAs recovered from Area 40 as substrate. The consumption yields of acetoacetic, propionic, and butyric acids were considered according to those reported by Yamane T [260].

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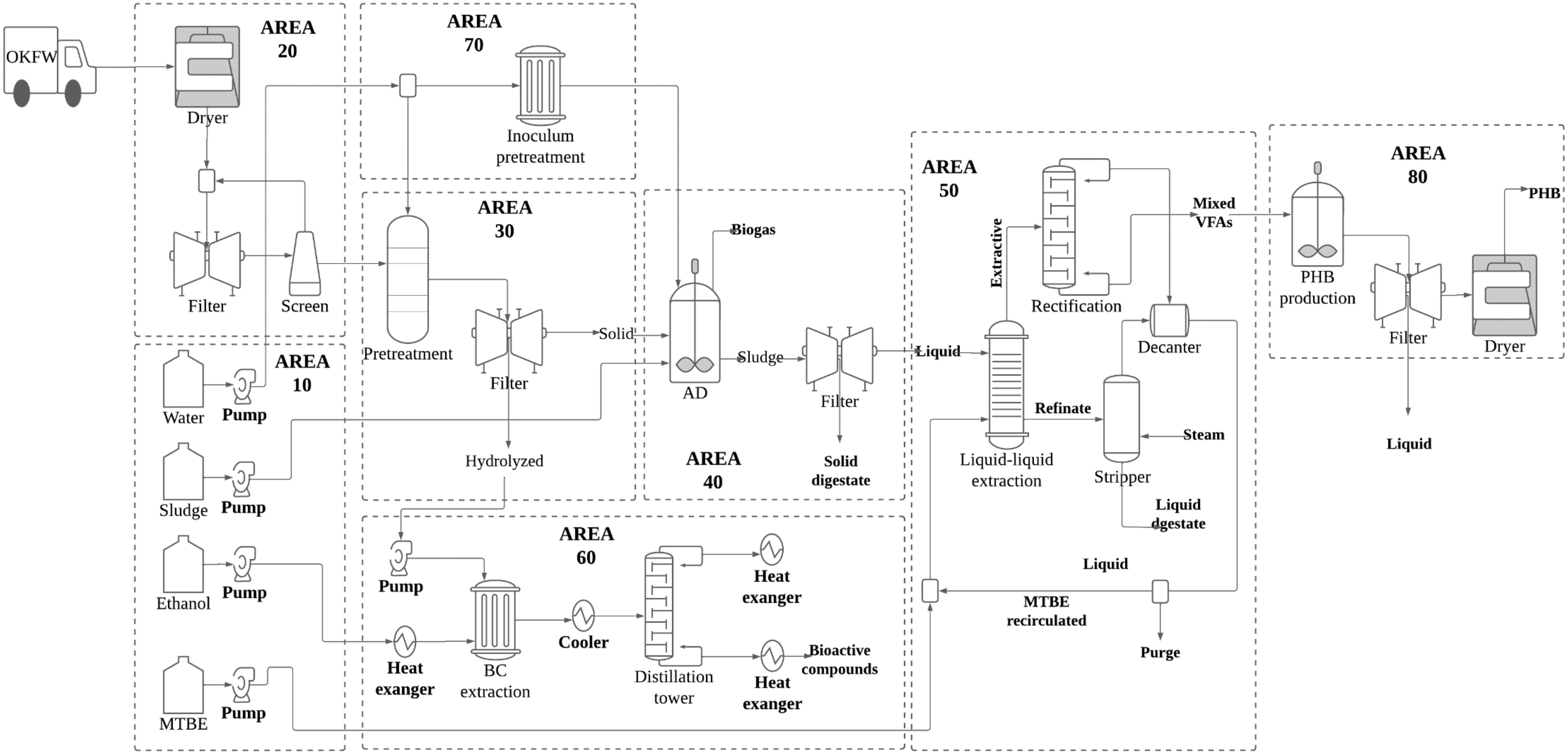

Figure A1. 1. Process diagram of the units of the proposed scenarios in conventional and modified AD.

A2: Vinasses biorefinery scenarios description

The integration of the ethanol production process with AD biorefinery using vinasse as substrate is presented in **Figure A2. 1. Area 10** corresponds to the conventional ethanol production process in sugar mills. This process includes stages such as fermentation with the microorganism *Saccharomyces cerevisiae*, distillation, and rectification to recover ethanol. Then, the vinasse is directed to evaporation sequences to remove the water. **Area 20** represented the storage area for the raw materials and inputs required for the process. **Area 30** considered the inoculum pretreatment stage. The inoculum was exposed to heat treatment at 65°C and atmospheric pressure [220]. **Area 40** refers to using diluted vinasse in the conventional or modified AD. The temperature was 37°C at atmospheric pressure, and a substrate SV: inoculum ratio of 0.4 for conventional AD and 1 for MAD was used. Considering conventional AD, the biogas generated is recirculated as energy to the process. For the modified AD, the mixed VFAs are recovered as products. The liquid and solid fractions of the digestate were separated using a centrifuge. **Area 50** included the recovery process of the mixed VFAs from the liquid fraction of the digestate. The methodology described in previous section was used. Finally, **Area 60** involved the PHB production using the mixed VFAs recovered from Area 40 as substrate. The consumption yields of acetoacetic, propionic, and butyric acids were considered according to those reported by Yamane T [260].

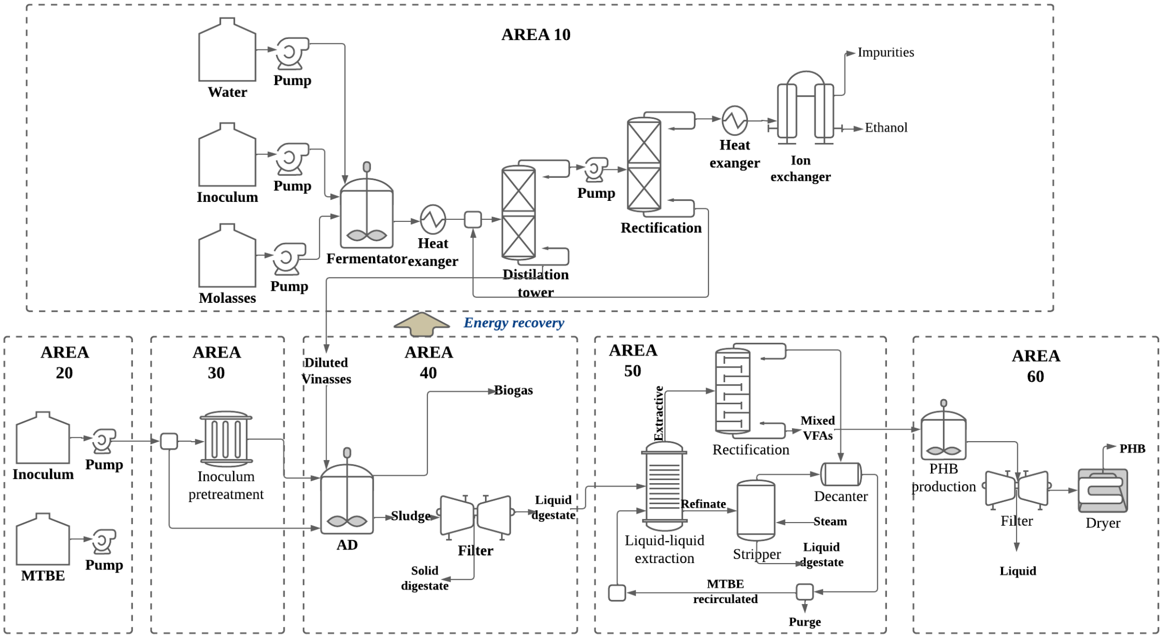

Figure A2. 1. Integration of vinasses biorefinery scenarios to the ethanol production process.

A3: Wastewater biorefinery scenarios description

The integration of cassava starch production process with AD biorefinery using WW as substrate is presented in **Figure A3. 1.** The base case corresponds to the conventional process of a rallanderia (i.e., starch generation from cassava). The biorefinery scenarios considered integrating conventional or modified AD and generating new products in the base case. **Area 10** corresponds to the conventional cassava starch production process in rallanderias. The process starts with the raw material reception. Then, the roots are cleaned. The first stage corresponds to the roots washing and is divided into two sub-stages. The first corresponds to a dry washing to eliminate the cassava peel. Then, a wet washing is carried out to eliminate any other impurities. Afterward, the cassava is subjected to grating to remove the internal fibers and obtain the slurry. At this stage, water is added to facilitate removal, and a residue called "afrecho" is generated. Then, the slurry is filtered to eliminate the afrecho and other impurities. The filtered slurry is taken to sedimentation tanks. The starch is sedimented at the bottom, and other compounds, such as fine fiber and residual water, are separated. Finally, starch from the sweet cassava, i.e., sweet starch, is sent directly to natural drying (in the sun). On the other hand, sour starch from agro-industrial cassava is subjected to fermentation processes and stored. **Area 20** represented the storage area for the raw materials and inputs required for the process. **Area 30** considered the inoculum pretreatment stage. The inoculum was exposed to heat treatment at 65°C and atmospheric pressure [220]. **Area 40** refers to using diluted vinasse in the conventional or modified AD. The temperature was 37°C at atmospheric pressure, and a substrate SV: inoculum ratio of 0.4 for conventional AD and 1 for MAD was used. Considering conventional AD, the biogas generated is recirculated as energy to the process. For the modified AD, the mixed VFAs are recovered as products. The liquid and solid fractions of the digestate were separated using a centrifuge. **Area 50** included the recovery process of the mixed VFAs from the liquid fraction of the digestate. The methodology described in previous section was used. Finally, Area 60 involved the PHB production using the mixed VFAs recovered from Area 40 as substrate. The consumption yields of acetoacetic, propionic, and butyric acids were considered according to those reported by Yamane T [260]. The units considered for each scenario are shown in **Table 2.9**.

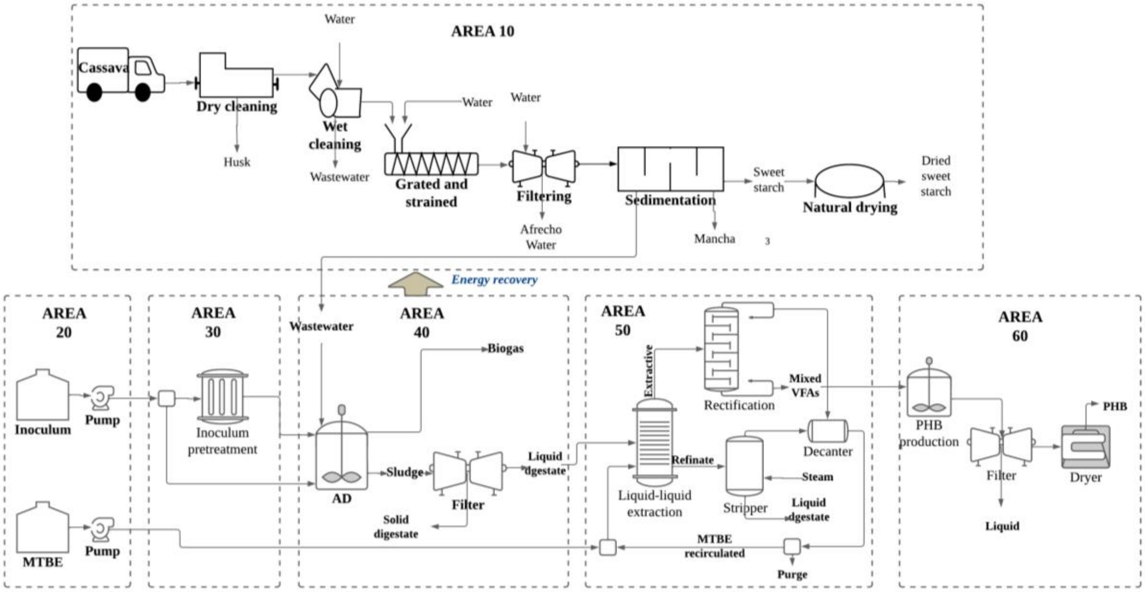

Figure A3. 1. Integration of wastewater biorefinery scenarios to the starch production process.

Annex B: Detailed mass balances of experimental scenarios.

B1: Detailed mass balances of the experimental scenarios of conventional and modified AD using OKFW as raw material.

Scenarios	Scenario description	Input					Output									
		OKFW (g)	Inoculum			Water / Hydrolyzed (g)	Biogas			Digestate			Mixed VFAs in liquid fraction			
			Total (g)	Solid fraction (g)	Liquid fraction (g)		Biogas (g)	CH_4 (g)	CO_2 (g)	Total (g)	Solid fraction (g)	Liquid fraction (g)	Total (g)	Acetic	Propionic	Butyric
Conventional AD																
Base case	OKFW fresh	0.64	23.36	1.49	21.85	66	0.28	0.17	0.11	89.72	1.47	88.25	0.48	0.23	0.08	0.17
Sc1	OKFW pretreated	0.64	23.36	1.49	21.85	66	0.35	0.23	0.12	89.65	1.31	88.34	0.58	0.29	0.11	0.18
Sc2	OKFW pretreated+ hydrolyzed	0.64	23.36	1.49	21.85	66	0.16	0.03	0.13	89.84	1.35	88.53	2.45	0.93	0.42	1.1

Modified AD																
Sc3	OKFW fresh	1.61	23.36	1.49	21.85	65.03	0.12	0.02	0.10	89.88	1.48	88.4	1.68	0.76	0.26	0.66
Sc4	OKFW pretreated	1.61	23.36	1.49	21.85	65.03	0.12	0.013	0.109	89.87	1.38	88.49	2.26	1.09	0.3	0.87
Sc5	OKFW pretreated+ hydrolyzed	1.61	23.36	1.49	21.85	65.03	0.12	0.002	0.117	89.89	1.39	88.5	2.58	0.93	0.33	1.33
Sc6	OKFW fresh+ inoculum pretreated	1.61	23.36	1.49	21.85	65.03	0.10	0.001	0.103	90	1.37	88.63	3.26	1.08	0.61	1.57
Sc7	OKFW pretreated + inoculum pretreated	1.61	23.36	1.49	21.85	65.03	0.08	0.001	0.077	90	1.38	88.62	3.35	1.51	0.53	1.31

B2: Detailed mass balances of the experimental scenarios of conventional and modified AD using vinasses as raw material.

Scenarios	Scenario description	Input					Output									
		Vinasses (g)	Inoculum			Water (g)	Biogas			Digestate			Mixed VFAs in liquid fraction			
			Total (g)	Solid fraction (g)	Liquid fraction (g)		Biogas (g)	CH_4 (g)	CO_2 (g)	Total (g)	Solid fraction (g)	Liquid fraction (g)	Total (g)	Acetic	Propionic	Butyric
Conventional AD																
Base case	Vinasses	2.31	23.36	1.49	21.85	64.33	0.36	0.25	0.12	89.63	1.46	88.17	0.4	0.23	0.06	0.11
Modified AD																
Sc1	Vinasses	5.77	23.36	1.49	21.85	60.87	0.15	0.01	0.13	89.85	1.47	88.38	2.03	0.65	0.38	1
Sc2	Vinasses and inoculum pretreated	5.77	23.36	1.49	21.85	60.87	0.09	0.005	0.09	89.91	1.48	88.43	2.4	0.74	0.46	1.21

B3: Detailed mass balances of the experimental scenarios of conventional and modified AD using wastewater as raw material.

Scenarios	Scenario description	Input				Output									
		Wastewater (g)	Inoculum			Biogas			Digestate			Mixed VFAs in liquid fraction			
			Total (g)	Solid fraction (g)	Liquid fraction (g)	Biogas (g)	CH_4 (g)	CO_2 (g)	Total (g)	Solid fraction (g)	Liquid fraction (g)	Total (mg)	Acetic (mg)	Propionic (mg)	Butyric (mg)
Conventional AD															
Base case	Inoculum to substrate ratio 1:1	45	45	2.88	42.12	0.13	0.09	0.04	89.87	2.85	87.02	13.5	6.50	2.37	4.63
	Inoculum to substrate ratio 1:2	60	30	1.93	28.07	0.11	0.07	0.03	89.89	1.9	87.99	10.73	5.17	1.84	3.66
Modified AD															
Sc1	Inoculum to substrate ratio 1:1	45	45	2.88	42.12	0.10	0.03	0.08	89.9	2.87	87.03	71.62	35.44	9.96	26.23
	Inoculum to substrate ratio 1:2	60	30	1.93	28.07	0.08	0.01	0.07	89.92	1.91	88.01	42.18	19.12	6.97	15.98

Sc2	Inoculum to substrate ratio 1:1	45	45	2.88	42.12	0.05	0.01	0.04	89.95	2.86	87.09	102.27	48.02	17.49	36.77
	Inoculum to substrate ratio 1:2 and inoculum pretreated	60	30	1.93	28.07	0.04	0.004	0.03	89.96	1.92	88.04	51.32	23.07	9.16	19.10

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