Evaluation of the biobutanol production from two agroindustrial wastes generated in the coffee growing region: plantain peel and milk whey

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A mis padres, a David: Mi vida, mi gran apoyo

"No basta saber, se debe también aplicar. No es suficiente querer, se debe también hacer"

Johann Wolfgang von Goethe

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Evaluación de la producción de biobutanol a partir de dos residuos agroindustriales generados en la región cafetera: Cáscara de plátano y lactosuero

Resumen

La necesidad de reemplazar los combustibles fósiles ha llevado a la búsqueda de nuevas alternativas que permitan reducir la contaminación generada. El butanol es un biocombustible que presenta grandes ventajas sobre otros más estudiados como el etanol. Adicionalmente permite el planteamiento de una plataforma bajo el concepto de biorrefinería para la obtención de productos de valor agregado. Por otro lado, la disposición inadecuada de la mayoría de residuos agroindustriales e industriales genera gran contaminación, por lo que se busca darles un uso adecuado. Es por esto que esta tesis presenta la evaluación experimental de la producción de butanol a partir de cáscara de plátano y suero de leche, usando la cepa *Clostridium acetobutylicum* ATCC 824, así como la evaluación técnica, económica y ambiental de una plataforma química para la obtención de acetato de butilo, acrilato de butilo y propionato de butilo a partir del butanol obtenido en las fermentaciones. Los resultados demuestran que es posible la obtención de butanol a partir de ambos residuos, sin embargo, el suero de leche es un proceso que se debe realizar a gran escala, ya que no presenta los mismos rendimientos de azucares que la cascara de plátano, obteniendo así menores flujos de butanol y por ende de los productos de valor agregado, haciendo inviable económicamente el proceso.

Palabras clave: Butanol, cáscara de plátano, lactosuero, biorrefinería

Abstract

The need to replace fossil fuels has led to the search for new alternatives to reduce the pollution generated. Butanol is a biofuel that has great advantages over others more studied like ethanol. Additionally, it allows the approach of a platform under the concept of biorefinery to obtain added-value products. On the other hand, the inadequate disposal of the majority of agro-industrial and industrial waste generates great contamination. For that reason, it is necessary to find an adequate use for them. That is why this thesis presents the experimental evaluation of the production of butanol from plantain peel and milk whey, using the strain *Clostridium acetobutylicum* ATCC 824, as well as the technical, economic and environmental evaluation of a chemical platform for obtaining butyl acetate, butyl acrylate and butyl propionate from the butanol obtained in the fermentations. The results show that it is possible to obtain butanol from both residues. However, the milk whey is a raw material for the process that must be carried out only at high scale, since it does not present the same yields of sugar as the plantain peel, obtaining lower butanol flows and, therefore, value-added products, making the process economically unviable.

Keywords: Butanol, plantain peel, milk whey, biorefinery

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Daniela Parra-Ramírez, Christian D. Botero-Gutiérrez, German Aroca, Julián Quintero, Carlos A. Cardona. “Analysis of the improvement of ABE fermentation through the production of butyl acetate”. *Chemical Engineering Science*. Status: Under review

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Daniela Parra-Ramírez, Alfredo Martínez, Germán Aroca, Julián A. Quintero, Carlos A. Cardona. Biobutanol as a platform for future biorefineries. Biorefineries: Concepts, Advancements and Research. Nova Science Publishers.

Participation of this Thesis in Research Projects

ERANet-LAC: Latin America, Caribbean and European Union. Project: Development of modular small-scale integrated biorefineries to produce an optimal range of bioproducts

from a variety of rural, agricultural and agroindustrial residues/wastes with a minimum consumption of fossil energy (SMIBIO).

Internship

During this thesis a research internship was carried out in the Metabolic Engineering Laboratory of the Biotechnology Institute of the National Autonomous University of Mexico.

Introduction

The fuels will always be a primary need for humans, as several of their daily needs require it, like starting the car or turning on the heating, in addition to the large amounts required daily in industries. A worrying fact is that the world oil production, the main source for the generation of fuels, approaches its peak and the world is now finding a new barrel of oil for every four that consumes [1]. For this reason, the production of biofuels is a great alternative to replace fossil fuels. Alcohol fuels can replace gasoline spark ignition engines, while biodiesel, green diesel and dimethyl ether (DME) are suitable for use in compression ignition engines [2]. Several technologies are being developed to produce biofuels such as sugar substrate fermentation, conversion of ethanol mixed hydrocarbon, cellulose hydrolysis, biobutanol by fermentation, gasification of various biological materials, etc.

Butanol is a four carbon straight-chained alcohol. It is an important chemical precursor for paints, polymers and plastics, mainly used as a solvent, chemical intermediate, and extractant in cosmetics and pharmaceutical industries and also for the production of butyl acrylate and methacrylate [3]. Butanol can be produced by petrochemical route from ethanol dimerization [4] or in the propylene synthesis [5] and by the biological route through ABE fermentation [6].

The butanol has been generating interest in researchers as a good candidate for an alternative biofuel. Ethanol has been presented as biofuel and is perhaps the most studied. However, the butanol has many advantages over ethanol. Butanol is less corrosive; the Reid vapor pressure of n-butanol is 7.5 times lower than that of ethanol, thus making it less evaporative/explosive [7]. The biggest problem is its high volatility, for which it evaporates easily and generates losses for the consumer. Butanol has higher energy content, is less volatile and is less hygroscopic [2], [8]. In conclusion, butanol reaches 95% of the energy than the same volume of gasoline, while ethanol reaches no more than 75%. It can be mixed with conventional gasoline, without having to adjust the car engines, in higher proportion than the ethanol, tolerates better water contamination, is less corrosive and has a lower vapor pressure than ethanol.

In this work, an experimental ABE fermentation based on agroindustrial wastes is done. The raw materials were plantain peel and milk whey, which are selected due to the great amounts generated in the coffee growing region. A modeling of a bioreactor was done based in the experimental kinetic curve. Simulation procedures were developed using Aspen Plus to evaluate technically, economically and environmentally (using additionally an environmental tool) a biorefinery to obtain different added-value products with the butanol as chemical platform.

Thesis hypothesis

The plantain peel and the milk whey from the coffee growing region are raw materials that allow obtaining biobutanol with production costs similar to those of other raw materials like sugars and starch.

Thesis objectives

General objective

To design and analyze from a technical, economic and environmental point of view the production of biobutanol from agroindustrial residues of the coffee growing region: plantain peel and milk whey.

Specific objectives

- To characterize physical-chemically the plantain peel and the milk whey.
- To evaluate experimentally the production and separation of biobutanol from plantain peel and milk whey.
- To model a bioreactor for the production of biobutanol from plantain peel and milk whey.
- To evaluate technically, economically and environmentally the process of obtaining ABE.
- To evaluate the biobutanol obtained as a platform for obtaining other added-value products.

1. Biobutanol production from agroindustrial residues

The use of agro-industrial wastes to obtain value-added products has been studied extensively. Most of these residues are of lignocellulosic nature and through different processes it allows the obtaining of sugars such as glucose and xylose that can be used in the production of ethanol, xylitol, and butanol, among others [9]. However, there are other types of residues from which fermentable sugars can be obtained, such as whey, mucilage, and stillage [10]–[12]. Almost all of these products obtained have a great added value for their importance in the industry (e.g. lactic acid). Products as ethanol and butanol are of great importance because they represent some of the best alternatives to fossil fuels, allowing to reduce the environmental contamination [7]. But these last products are not only good biofuels, they are promising compounds because of the large quantity of products that can be obtained from them [13], [14]. In the case of the butanol, products as resins, lacquers and paintings can be obtained. The actual industrial distribution of butanol into different products is shown in **Figure 1-1**. These compounds with higher added value allow the consideration of butanol as a great platform for many uses more than a gasoline additive for which very little would be required [13].

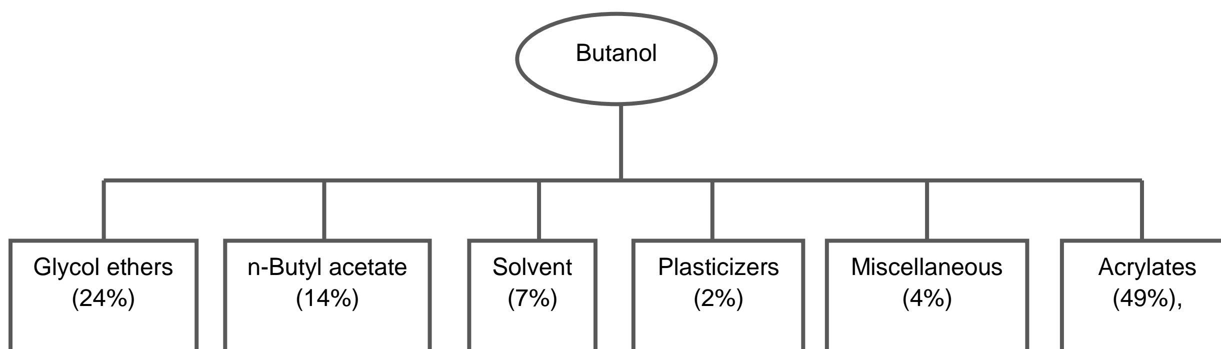


Figure 1-1. Main products obtained from butanol

1.1 Biobutanol in the world

Production of butanol began in 1916 using the method of ABE fermentation. Now butanol is produced starting from petroleum via hydrolysis of haloalkanes or hydration of alkenes. The butanol produced from biomass is denominated biobutanol despite it has the same characteristics as the butanol from petroleum [15].

Today fossil fuels occupy 80% of the energy consumed worldwide [16]. Sources of production of these fuels are depleted over time. Consumption also generates environmental problems, related to greenhouse gases, melting glaciers, destruction of ecosystems, etc. An increasing energy demand leads to an increase in crude oil prices that affects directly the global economic activity [17]. All these factors contribute to the need to find a better alternative as renewable sources, generating sustainability and primarily to reduce the impact generated in the environment. Some biofuels produced from biomass are ethanol, methanol, biodiesel, Fischer-Tropsch diesel, hydrogen and methane [18]. Environmental Energy Inc. now is one of a few companies in USA that works on the creation of experimental installation for producing biobutanol. First, the company intended to produce biobutanol for the solvents industry, but further it will produce and sale it as alternative fuel. The company demonstrates that the production of butanol by fermentation is more economic compared with petrochemicals methods [19]. Increasing industrialization has led to a large increase in demand for fuel, most of which are petroleum products [20].

To have knowledge about the butanol market in Colombia,

Figure 1-2 shows the values of exportations and importations in the last years. The country has a large number of imports and exports in recent years but by 2015 both values decreased, this may mean that the butanol produced in Colombia is being used in the processes that is required. Increasing the production of butanol can minimize the imports and exports to improve the country economy. The country of origin with more importations is United States (96%), then Brazil (3%) and Germany (1%) [21]. The exportations goes to Venezuela (65%), Ecuador (26%) and domestic consumption in the Barranquilla Free Zone (9%) [22]

For this reason, it is necessary to develop platforms to be implemented in Colombia for the production and use of butanol. Butanol, offers the possibility of producing a promissory

biofuel by adding one part of butanol to gasoline and would provide greater economic and social stability to the country by the creation of new jobs and the possibility of trading products of high added value.

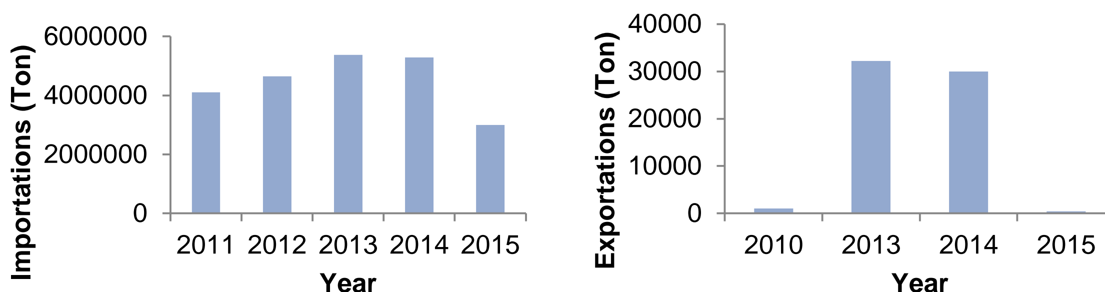


Figure 1-2. Butanol importations and exportations in Colombia [21], [22]

1.2 Paths to obtain butanol

Butanol is an alcohol that can be obtained from different routes. The main one is the biological route, due to the possibility of using residues that made the process more environmentally friendly. However, it is possible to synthesize the butanol from other chemicals as ethanol.

1.2.1 Petrochemical route

The butanol can be produced from the petrochemical route based on hydroformylation of propylene followed by an hydrogenation to obtain butanol [5]. Other alternative to obtain butanol is the dimerization of ethanol over solid-based catalysts [23], [24]. The costs of synthetic production of butanol are directly related to the propylene market and are very sensitive to oil prices [3].

1.2.2 Biological route – ABE fermentation

Biobutanol has been produced traditionally through the anaerobic fermentation of sugar substrates using various species of solventogenic clostridia [25]. This process is referred often to as the ABE fermentation, due to its main chemical products: acetone, butanol and ethanol. The ABE fermentation is a proven industrial process that uses solventogenic

clostridia to convert sugars or starches into solvents [3]. Fermentation using *Clostridium sp.* is limited by some factors such as substrate inhibition, generating low butanol production, low cell density and great cost in the substrate. So researchers have proposed some solutions to these problems as the development of a microbial strain with higher yields [3], *in situ* product recovery [26] and the application of different strategies to increase cell density [27]. The main advantage of the biological route is the use of agro-industrial wastes and is much friendlier to the environment than the petrochemical way, which generates a lot of pollution to both, water (with liquid chemicals) and the air (with the release of toxic gases resulting from the use of fossil fuels) and possible waste production. In addition, the reagents used in the petrochemical route are usually flammable and explosive generating greater insecurity for operators and industries.

1.3 Technologies for biobutanol production

There are different types of processes to produce butanol by fermentation. Some have developed designs to overcome production problems, reduce costs and increase productivity. The main processes are continuous, batch and fed-batch. **Table 1-1** shows some of the processes for butanol production with their conditions.

Table 1-1. Processes of butanol production

Batch Process		
Microorganism	**Process description**	**Ref.**
C. saccharoperbutyl-acetonicum N1-4 (ATCC13564)	Initial pH of 5.8, temperature 30°C, agitation speed 100 rpm. Produces 0.9 g/L of butanol and 2.09 g/L of ABE	[28]

Fed-batch Process		
Microorganism	**Process description**	**Ref.**
C. pasteurianum NRRL B-598	Fed-batch cultivations were initiated in batch mode, 37 °C, without pH control, stirring 200 rpm, under anaerobic conditions. After 14 h, when the glucose concentration had decreased to 5 g/L, pulse-feeding was commenced at a frequency of approx. 4 h. Produces 8.3 g/L of butanol and 12.3 g/L of ABE	[29]

Continuous Process		
Microorganism	**Process description**	**Ref.**
C. pasteurianum NRRL B-598	Agitation speed 200 rpm and 37 °C, anaerobic atmosphere and the pH was adjusted to 6.6–7.0. Produces 10.4 g/L of butanol.	[30]

In situ recovery (Adsorption) + Fed-batch Process		
Microorganism	**Process description**	**Ref.**
C. acetobutylicum ATCC 824	Temperature of 37 °C, agitation 300 rpm, anaerobic conditions, pH 5.5. Produces 27.2 g/L	[31]

1.3.1 Batch and Feed Batch processes

Batch fermentation efficiently achieves very high titers and product yields, the main problems are the low productivities for the substrate and product inhibition, and it is also limited to a single amount of substrate. Fed-batch fermentation helps reducing substrate inhibition and slightly increases system productivity due to the addition of substrate and the medium dilution [29].

1.3.2 Continuous process

Continuous fermentation offers more advantages as the fermenter volume is used efficiently, the proportion of productive to non-productive period increases, product inhibition is removed and the amount of inoculum per product unit is decreased. However, lower titers product, greater demands on the sterility of the large volumes of process and waste water can be the major disadvantages [29].

1.3.3 Recovery *in situ*

Butanol production with microorganisms generally has a problem with the end-product inhibition caused by the accumulation of toxic products. Therefore, to avoid the toxicity, it is necessary to dilute the fermentation conditions of the conventional processes, which means inefficient processes of separation and purification decreasing the viability [31]. To solve these problems, *in situ* product recovery techniques have been developed.

Pervaporation is one of these, where the butanol diffuses across a membrane into a stream of gas [32]. Reverse osmosis membrane, solid adsorbents, extractive fermentation [33], adsorption and gas stripping are other techniques to recovery butanol *in situ* [34].

1.4 Technologies for biobutanol separation

The separation is a very important part of the butanol process, due to the complexity of the composed broth, mainly butanol, ethanol, acetone and water. Different separation schemes are presented. Some of these are based on *in-situ* recovery of the product as explained above, to reduce product inhibition.

1.4.1 Distillation

Recovering butanol by distillation has a high cost. This is due for the butanol low concentration in the fermentation broth caused by the product inhibition. In addition, more energy is required because the boiling point of butanol is higher than that for water [35]. **Figure 1-3** shows the general scheme of the process. The fermentation reactor is followed by a distillation train, which is required for recovery of the other products obtained in the fermentation.

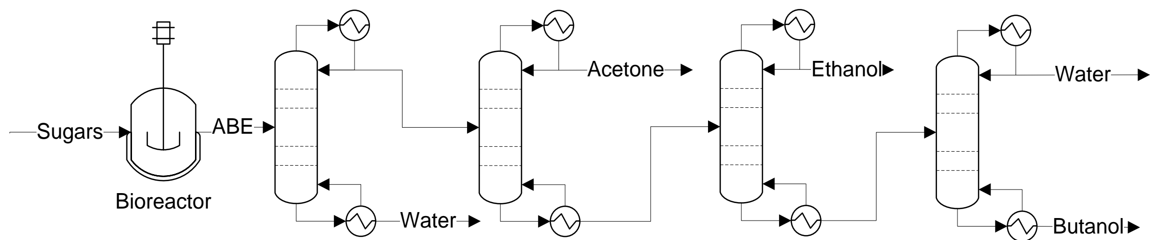


Figure 1-3. Butanol production process with product recovery by simple distillation.

1.4.2 Liquid – Liquid extraction

The separation by liquid – liquid extraction is based on the differences of the components distribution to be separated in two liquid phases with a solvent. It depends on the mass transfer of the component to be extracted. When the distillation and the crystallization cannot be used or is very costly, this technique is a good option [36]. **Figure 1-4** shows the general scheme of this system. While the fermentation broth is continuously removed, the

compounds of interest are extracted. The organic phase contains the product of interest and a part of the aqueous phase can be returned to the process.

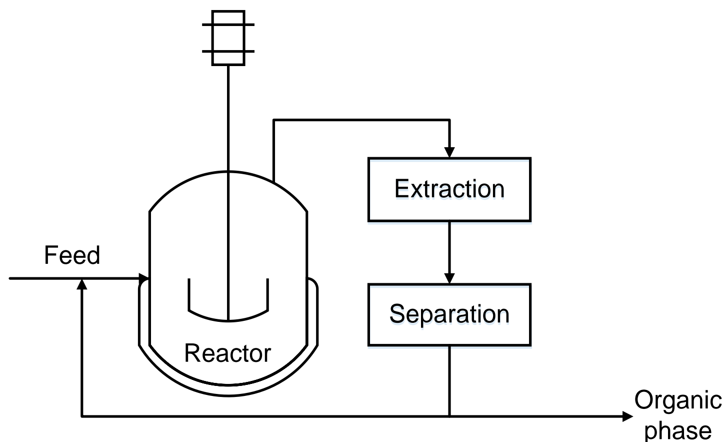


Figure 1-4. Butanol production process with product recovery by liquid-liquid extraction

1.4.3 Extractive distillation

Products recovery *in situ* by an extractive technology cannot be sustainable on a large scale for many reasons, such as low mass transfer in the solvent phase and the formation of emulsions, among others [37]. Therefore, it is proposed a continuous removal of the products in an extraction column [38]. In this case, the microorganisms must be retained by immobilization or by ultrafiltration. The major advantages of this system are: high rates of mass transfer, use of powerful but toxic solvents and temperature can be optimal [38]. **Figure 1-5** shows the towers train required for the extractive distillation. In the first extraction tower, the fermentation products are separated. In the second recovering of the solvent is made and then it is recycled to the first tower. The final distillation is for recovering the butanol by bottoms.

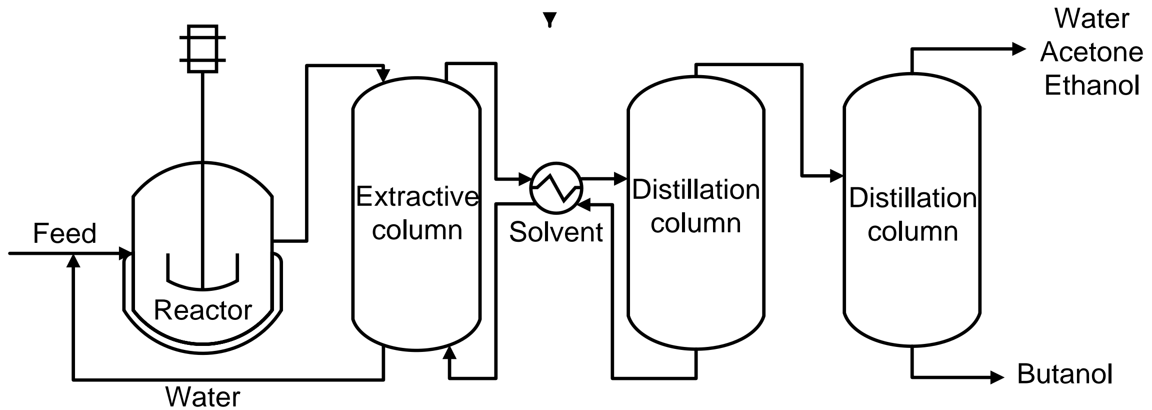


Figure 1-5. Butanol production process with product recovery by extractive distillation

1.4.4 Liquid – Liquid extraction and membrane solvent extraction

In the membrane extraction with solvent system, the products are separated from the broth by membranes. Dispersion-free extraction is possible, leading to an easy operation of the equipment. This separation process improves emulsion formation problems mentioned above. The silicone rubber membranes are used to separate butanol from water [39]. The process of recovering butanol by liquid-liquid extraction and membranes is shown in the **Figure 1-6**.

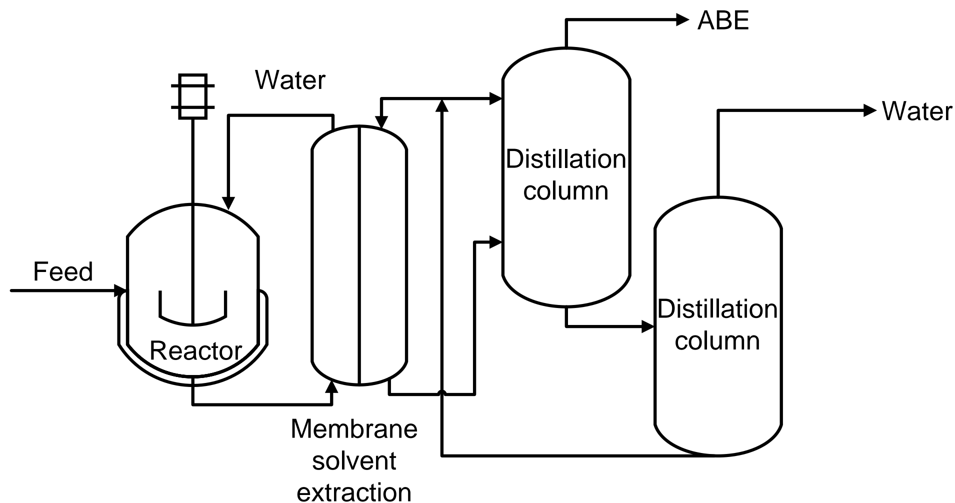


Figure 1-6. Butanol production process with product recovery by liquid-liquid extraction and membrane solvent extraction

1.4.5 Gas stripping

Gas stripping is a technique that involves bubbling gas (nitrogen or fermentation gases). This gas captures solvents and then are condensed, gas can be recirculated to the fermenter. This technique is one of the most economical [35], [40]. This process is represented in **Figure 1-7**, where the condenser and the recycle gas is shown.

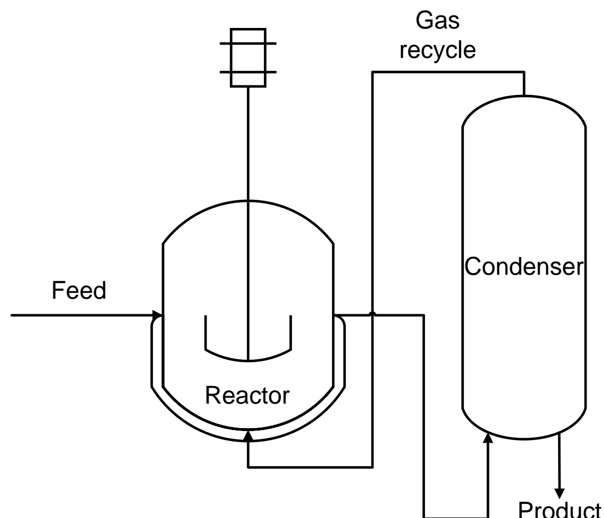


Figure 1-7. Butanol production process with product recovery by gas stripping

1.4.6 Pervaporation

Pervaporation is a technique that uses membranes to separate the butanol. The membrane has a selectivity, which allows the diffusion of the butanol through it. The butanol is removed and condensed because it gets out of the membrane as vapor [41]. Pervaporation is considered one of the best techniques for butanol recovery, as it is not dangerous to the microorganism and is less expensive than the distillation. The most used membrane materials in the ABE fermentation are silicone and propylene [42]. **Figure 1-8** shows the flow sheet of butanol production process coupled with pervaporation, in this case the fermentation broth enter to the pervaporation tower, the product passes the membrane and the broth is recycled to the bioreactor.

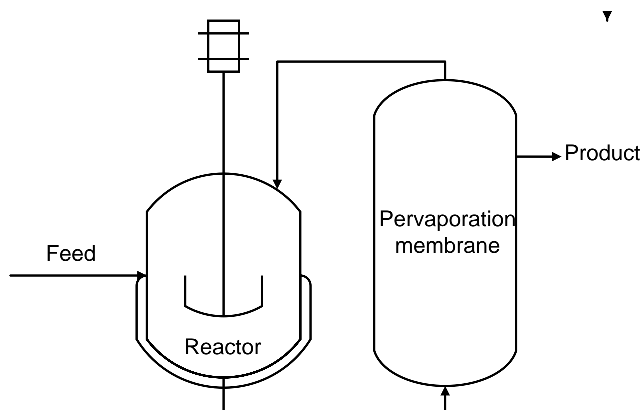


Figure 1-8. Flow sheet of butanol production process with product recovery by pervaporation

1.5 Substrates for biobutanol production

1.5.1 Sugars and starch

The ABE fermentation is carried out using carbon substrates. The *Clostridium sp.* has strong amylase activities with which can consume starchy substrates without needing an hydrolysis [43]. One of the highest concentration of butanol using sugars was reported using sugar cane juice and it was 28 g/L of butanol with *Clostridium acetobutylicum* JB200 [44]. For the starch, the butanol has been produced from waste peanuts obtaining 21.7 g/L of ABE [45] and from corn starch obtaining 18.4 g/L of ABE [46]. These substrates offer great advantages because of their high yields and the minimum requirements of a pretreatment. However, they are highly competitive with the food industry because of their nutritional potential, so it is necessary to find other alternatives with less industrial competition.

1.5.2 Lignocellulosic materials

The use of lignocellulosic materials for the production of butanol by fermentation gives an important added value to this product, because it is not only a renewable fuel, in addition it can be produced from wastes. Lignocellulosic materials have a composition of 40-50% cellulose, 25-30% hemicellulose and 15-20% lignin [47].

The lignocellulosic material does not have competition with the food industry, but it requires a pretreatment to obtain sugars that can be digested by the microorganism. The pretreatment breaks the lignocellulosic matrix and frees the simple sugars, but in this process, some inhibitory compounds are generated affecting the microorganism. This compounds include furfural, hydroxymethyl furfural (HMF), and acetic, ferulic, glucuronic, and r-coumaric acids, among others [48]. One of the most important lignocellulosic materials are agricultural wastes, among which are: wheat straw, barley straw, pea straw, corn stover, corn fiber, grass hay, switchgrass, and sugarcane bagasse [48]–[50].

1.5.3 Industrial wastes

In the industry, there are many residues that can be fermented due to their characteristics. In the case of the butanol, residues as glycerol, microalgae wastes, syngas and milk whey, among others, have been studied.

Glycerol is a substrate recently studied as carbon source for the production of butanol, as it appears as a major byproduct of the biodiesel industry. However, the crude glycerol has a large amount of impurities which reduce its value. With the large production of biodiesel, the production of this glycerol has increased. Development of glycerol-based butanol production processes can add significant value to the biodiesel industry [51]. The most commonly microorganism used is *Clostridium pasteurianum* [52].

The waste from microalgae harvesting or processing is a good raw material for fermentation for several reasons. Although not all species of algae have the same carbohydrate composition, many species used industrially as *Chlorella vulgaris* can accumulate a significant portion of their dry weight in easily digestible glucans like starch, with the presence of small quantities of other sugars as xylose, mannose and galactose [53]. Recently, pretreated algal biomasses such as, *Nannochloropsis sp., Arthrospira platensis,* and wastewater algae have been tested as substrates for butanol production. This alternative substrate is very important because biomass does not compete with food supply, does not require large cultivation land and obtains higher yield per area compared with plants [43]. However, butanol production by clostridia was not sufficiently demonstrated and the productivity of butanol is low [43].

Syngas can be used as substrate to produce butanol. The advantages of this substrate are that it is avoided the recalcitrance of biomass and it can be utilized without the complex pretreatment and some clostridia can produce butanol from syngas. Nevertheless, the mass transfer from gas to liquid is poor and the productivity of butanol is very low [54].

1.6 Microorganisms

Currently there is a large number of microorganisms capable of producing butanol through ABE fermentation. **Table 1-2** shows each of them with their respective strains, fermentation times, substrates and production of butanol or ABE. The most used microorganisms are *C. pasteurianum* and *C. acetobutylicum.*

Table 1-2. Microorganism, substrate and butanol production.

Microorganism	Strain	Fermentation Time	Substrate	Butanol or ABE	Ref.
Clostridium acetobutylicum	YM1	20 h	Glucose (50 g/L)	17 g/L of butanol and 21.71 g/L of ABE	[55]
	NRRL B-591	72 h	Autohydrolysis pretreatment, enzymatic hydrolysis of softwood pine	104.5 g ABE/kg pine	[47]
	NRRL B-591	72 h	Autohydrolysis pretreatment, enzymatic hydrolysis of hardwood elm	43.4 g ABE/kg elm	[47]
	P262	75 h	Sago starch	16 g/L of butanol 18 g/L of ABE	[56]
	ATCC 824	120 h	10% domestic organic waste	3 g/L of butanol and 3.3 g/L of ABE	[57]
Clostridium pasteurianum	DSM 525	38 h	Pure glycerol	10.4 g/L of butanol	[30]
	NRRL B-598	60 h	Glucose	8.3 g/L of butanol and 12.3 g/L of ABE	[29]
	ATCC 6013	240 h	Glycerol (21.5 g/l)	7.8 g/L of butanol and 18.3 g/L of ABE	[58]
Clostridium beijerinckii	TISTR 1461	36 h	Diluted sweet sorghum juice	15.46 g/L of butanol	[59]
	P260	70 h	Barley straw hydrolysate sugars	47.20 g/L ABE	[60]
	P260	70 h	Corn stove hydrolysate sugars	50.14 g/L ABE	[60]
	ATCC 55025	72 h	Wheat bran hydrolysis (Reducing sugars 53.1 g/L)	8.8 g/L of butanol and 11.8 g/l of ABE	[61]
Clostridium saccharobutylicum	DSM 13864	36 h	*C. vulgaris* (ionic liquid extracted algae) pretreated 1% sulfuric acid	4.99 g/L of butanol 9.06 g/L of ABE	[53]
C. saccharoperbutyl-acetonicum	N1–4	96 h	10% acid/base pretreated algae	2.3 g/L of butanol 2.8 g/L of ABE	[62]
C. carboxidivorans	P7T	96	Carbon monoxide	0.002 g/L of butanol and 0.009 g/L of ABE	[54]

Through genetic engineering, some microorganisms have been modified to solve some problems like inhibition and obtain higher concentrations of butanol. Some examples are *Escherichia coli* [63], *Synechococcus elongatus* (cyanobacterium) to produce butanol from carbon dioxide [64], *Saccharomyces cerevisiae* [65] and *Clostridium acetobutylicum*, which despites produces naturally butanol has also been modified to increase its yields [66].

The microorganisms presented in **Table 1-2** depending on the strain have different accessibility for use in laboratories and industries. The availability of some strains is presented in **Table 1-3.**

Table 1-3. Strain accessibility

Microorganism	Accessibility strain		
	Commercial	**Agreement**	**No accessible**
Clostridium acetobutylicum	ATCC 824	NRRL B-591 ATCC 824	YM1 P262
Clostridium pasteurianum	DSM 525 ATCC 6013	NRRL B-598	
Clostridium beijerinckii	ATCC 55025	TISTR 1461	P260
Clostridium saccharobutylicum	DSM 13864	NRRL B-634	
C. saccharoperbutylacetonicum	ATCC 27021		N1–4
C. carboxidivorans	ATCC BAA-624		P7C

Clostridium species perform fermentation in two phases: acidogenic and solventogenic. In the first phase, which is the acidogenic, the productions routes of acids are activated and the main products are acetate, butyrate, hydrogen and carbon dioxide [3]. Occasionally there may be lactate formation depending of the conditions [15], [25]. This phase usually occurs during the exponential phase [3mirar]. In the second phase, the solvents acetone, ethanol and butanol are produced using the acetate and butyrate excreted in the first phase [15]. This last stage is very linked to the sporulation of the microorganism [3].

During fermentation, hexoses and pentoses are catabolized through their respective routes to produce pyruvate. This is converted into acetyl-coenzyme A, which can be converted into acetate, ethanol or acetoacetyl-coenzyme A to obtain acetone or butyryl-coenzyme A for the production of butyrate and butanol [67]. The obtaining of the products depends on the enzymes that act on the secondary metabolites. **Figure 1-9** shows a brief diagram of the biphasic route for the production of butanol.

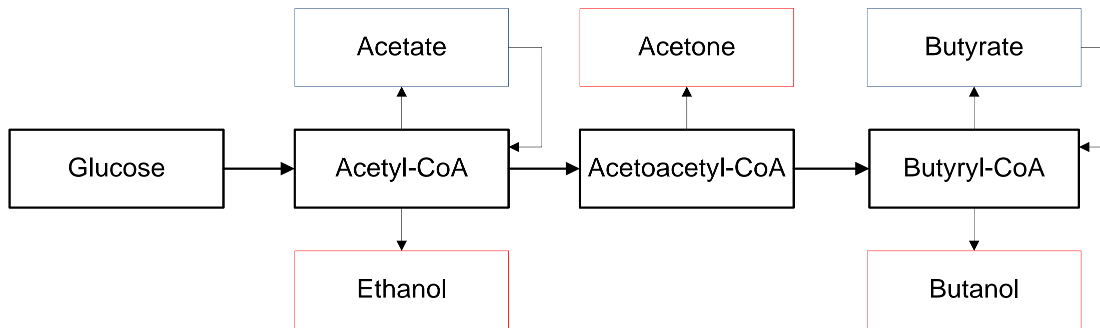


Figure 1-9. Two phases route of butanol fermentation (Adapted from Li et. al [67])

It is important to note that one of the main problems in this fermentation is the inhibition of solvents. The metabolism of clostridium is affected when there is a concentration equal to or greater than 20 g/L of solvents [3]. Resulting in low productivity.

1.7 Final remarks

Biofuels are the new alternative to fossil fuels. Butanol is a compound very studied as a biofuel. It is traditionally produced by ABE fermentation. There are many technologies for their development developed to solve the problems of inhibition by product. The microorganisms used for these fermentations are *Clostridium sp*. Which can consume glucose, starch, among other types of carbohydrates. Due to this, different studies have been developed for the production of butanol from industrial and agronomic waste.

2. Raw materials in Colombia

Colombia is a country with a great variety of crops and different industries. Being a tropical country has different climates that are appropriate to different types of crops. Colombia is divided into different regions, including the coffee region, which as its name says is the main coffee producer. However, there are other types of fruit crops that generate large residues such as orange, plantain, banana and tangerine. On the other hand there are food industries that generate waste that can be studied to give them an added value, including the dairy industry, base for gelatin, food (sweets, juices), among others.. Plantain Peel and Milk Whey are two of the most generated residues in the growing coffee region. Both raw materials have high availability and low cost. The plantain peel is a lignocellulosic residue and the milk whey is an industrial waste. Below is presented a brief description of each raw material.

2.1 Plantain peel

The plantain is one the most important crops in Colombia. It is produced by the whole country. The plain region is where there is more production, followed by the south-central region and the coffee growing region. According to statistics, it is one of the largest permanent crops in the country. Four departments comprise the coffee growing region: Antioquia, Caldas, Risaralda and Quindío. The Ministry of Agriculture in Colombia reports the production of plantain by departments and towns [68], **Figure 2-1** shows the location of the coffee growing region in Colombia and the distribution of the plantain crops in each department.

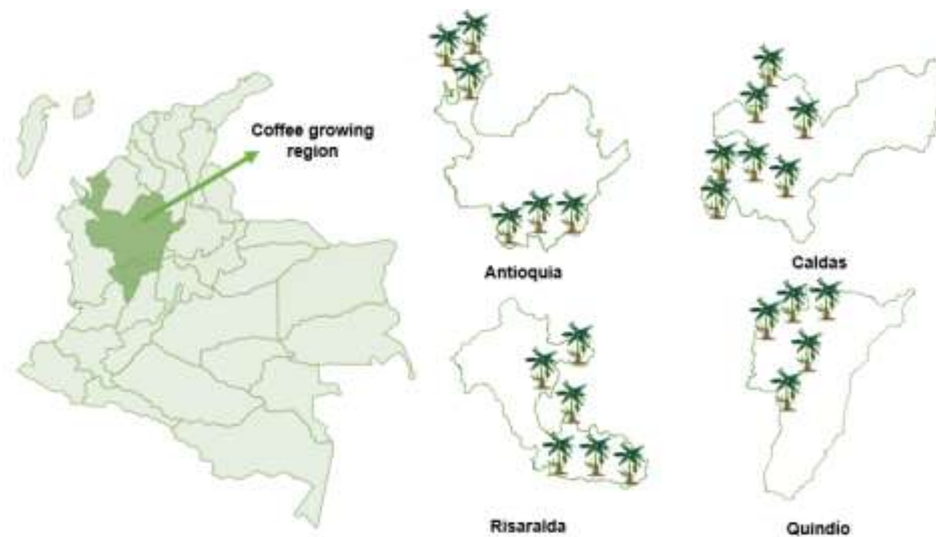


Figure 2-1. Coffee growing region location and plantain crops distribution by department.

The highest concentration of plantain crops is found in the central area of the coffee growing region, except for crops in the north of Antioquia. This department is the one of greater surface and therefore has greater amount of cultivated area as can be seen in **Figure 2-2**.

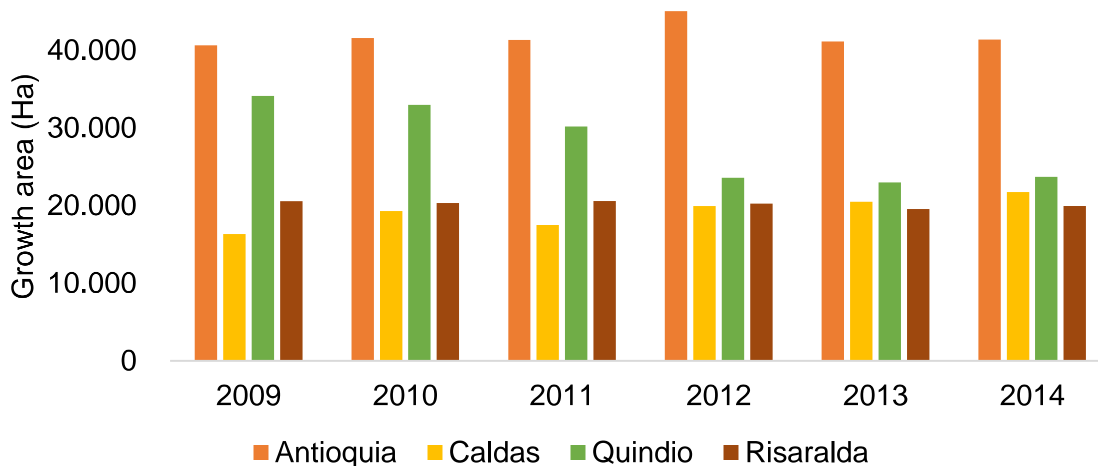


Figure 2-2. Plantain crops area by department in the coffee growing region. (Data adapted from [68])

In the last years, the cultivated areas of plantain in the coffee growing region have remained almost constant. In the department of Quindio, there has been a slight decrease in the cultivated areas. However, if the surface area of this department is compared with the

others, much of this area is destined for plantain plantations. In fact, these crops represent around 50% of permanent crops in the department. From the four studied departments, Antioquia is the largest producer, followed by Caldas, Quindío and Risaralda. **Figure 2-3** shows the percentage of production of each of these for 2014.

Although Antioquia is the largest producer of plantain in the coffee growing region, its production is not very far from the departments of Caldas and Antioquia. This means that despite being the largest department its percentage of cultivated area of plantains is not great. For this reason, plantain represents in this department only 16.21% of permanent crops. Caldas is the area with the highest crop yield with 11.71 tons of plantain per hectare of cultivated area, followed by Quindío, Risaralda and Antioquia with 10.24, 8.64 and 6.94, respectively [68].

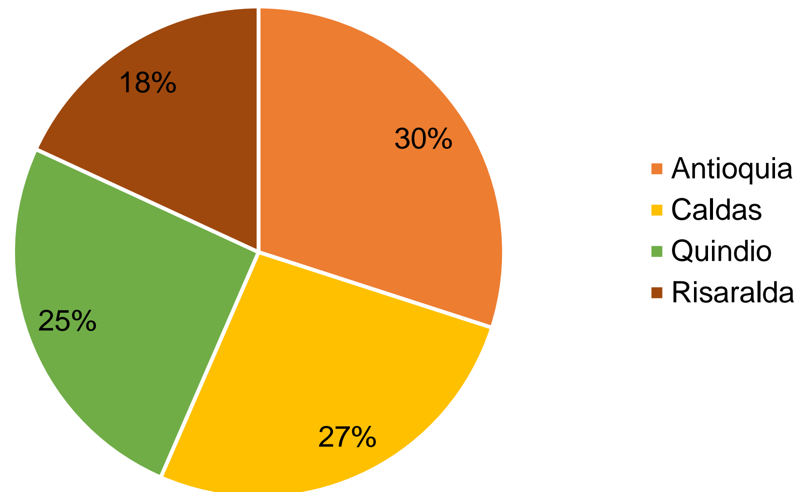


Figure 2-3. Plantain production by department in the coffee growing region in 2014. (Data adapted from [68])

The increase in the use of plantains in the food industry has led to accumulation of the generated waste. The plantain plant is composed of two parts, an edible part that corresponds to 20% of the total plant and a non-edible that corresponds to 80%. [69]. The plantain peel represents 40% of the total weight of the fresh fruit (edible part) [70]. **Figure 2-4** shows the parts of a plantain plant.

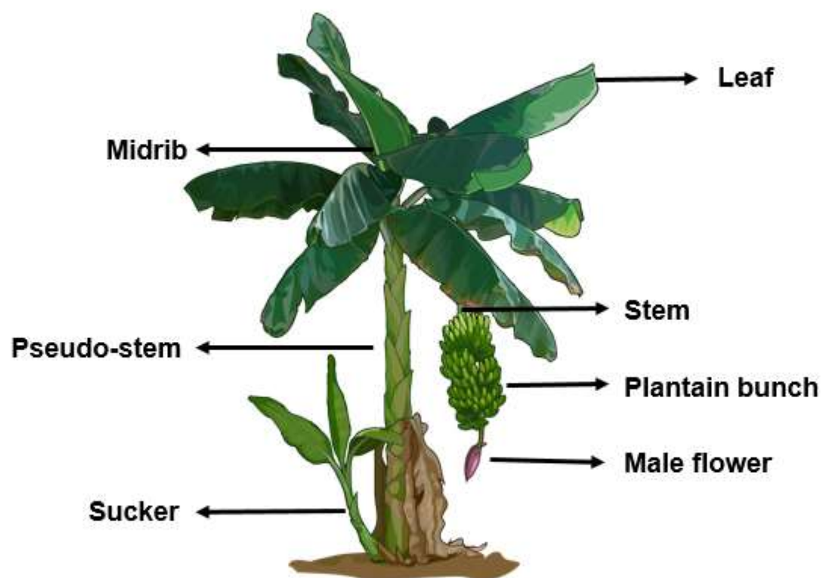


Figure 2-4. Plantain plant parts

As reported by the Ministry of Commerce, Industry and Tourism of Colombia in 2015 [71], about 143 tons of plantains were produced in the country. Taking into account the previous relationship, there were 57.2 tons of plantain peel of which 80% is left in the fields without using them. The plantain peel has a high lignin content but the hemicellulose concentration is low [72]. Currently, different researches are carried out to valorize this agroindustrial residue. Some of these are biogas by anaerobic digestion [73], bioethanol by fermentation [74], and antioxidant compound extraction [75], among others.

2.2 Milk Whey

Milk whey is the main residue of the dairy industry and is generated in the process of obtaining cheese. Milk production and processing is focused in each department on the coffee growing region. **Figure 2-5** shows the distribution of the main milk processing companies. In Antioquia companies such as Colanta, Alpina, Alqueria and Parmalat are located in Medellin. In Caldas, La central lechera de Manizales (Celema) is located in Manizales and La Dorada, the company Alpina is located in Chinchina and Normandy in Manizales. The company Parmalat is located in Pereira, Risaralda and in the department of Quindio, Colanta is located in Armenia [76].

Figure 2-5. Main milk processing companies' location in the coffee growing region

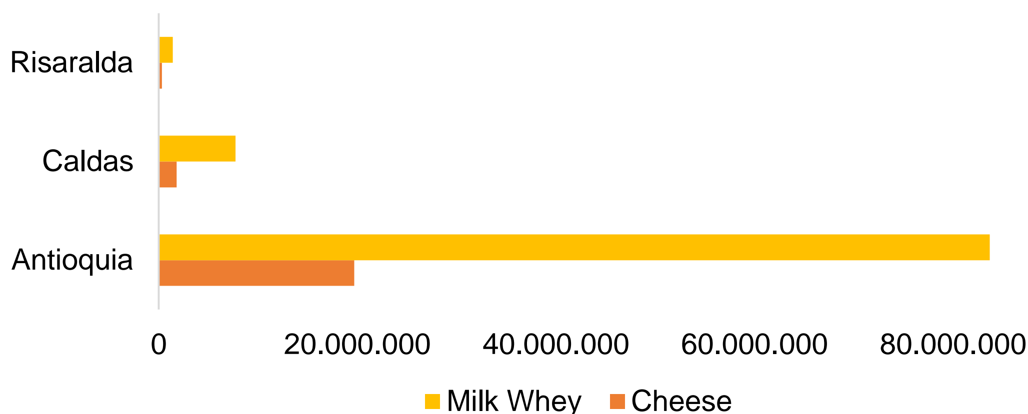


Figure 2-6. Milk Whey and Cheese production in the coffee growing region. (Data adapted from [68])

The Ministry of Agriculture and Rural Development does not report the production of cheeses for the department of Quindío, which is why it is not found in the information presented in **Figure 2-6** for the production of cheeses and whey in the departments of the coffee growing region for 2015. The production of milk whey was calculated taking into account the ratio of 8.5 liters of milk whey per two kilograms of produced cheese [77].

Milk whey is a residue from the dairy industry, accounting for around 90% of the total milk used for the production of cheeses. The whey retains about 55% of the total components

of the milk, some of them are lactose, soluble proteins, lipids and mineral salts [78], therefore the whey is highly contaminating. The Federación Nacional de Ganaderos (Fedegan) reported a production of 6.391 million liters of milk in 2016, from which about 18% were used for cheese production. With these statistics, 1.03 million liters of milk whey were generated in Colombia. The whey has been studied to give an added value. However, for its large quantities, in most industries a percentage is used and the rest is discarded. In the literature it is possible to find different studies where the milk whey has been used to produce butanol [12], biogas [79], lactic acid [80], among others. However, it is not used efficiently [81].

2.3 Final remarks

Raw materials are one of the most important parameters in the design of a process, the availability and quantities generated from these determine the scale and productivity of the process. Colombia is divided into different regions and due to its location has great variety in agricultural products. One of these regions is the coffee region where the largest amount of coffee is found, however there are other types of waste that are not given so much attention and you are looking for a better disposition. Some of the agroindustrial waste available in this region is the citrus trees, peels of the same, pseudostem and banana peel, among others. Industrially there are residues such as milk whey, the spent coffee ground, among others. For this reason, it is proposed to use plantain peel and the milk whey, teo different raw materials, for the evaluation of the production of biobutanol and thus be able to give added value to this agroindustrial residue.

3. Materials and methods

In this chapter, the experimental procedure developed in this thesis is presented. The experimental description includes the characterization of the raw materials, the specific pretreatments and the laboratory-scale fermentation. Subsequently, in the simulation procedure, a proposed platform is described. Finally, the guidelines in order to analyze the processes from the economic, energy and environmental point of view are presented.

3.1 Plantain Peel characterization

The plantain peel characterization comprises the determination of the cellulose, hemicellulose, lignin, extractives, ash and starch content.

3.1.1 Sample preparation

The plantain peel was collected from La Violeta, Manizales, Caldas. Before that, it should be cut in particle size of 2 cm, dried and milled. The raw material was dried in an oven at 50 °C until a moisture content < 10%. The moisture content was measured in a Shimadzu moisture balance MOC - 120H. Subsequently, samples were milled and sieved using a gyratory mill and sieve 40 mesh (0.4 mm).

3.1.2 Extractives

Extractives are the non-structural material of biomass such as inorganic material, fatty acids, fatty alcohols, phenols, structural sugars, and waxes, among others, that are soluble in water and ethanol. The determination was developed following the National Renewable Energy Laboratories (NREL/TP-510-42619) [82].

The extraction bags should be weighted without sample. 10 g of sample was weight and added into the bags. Then the bags were placed in a Soxhlet extraction unit. Subsequently, 250 ml of distilled water were added to a 500 ml round bottom flask with boiling chips, the heating rates were adjusted to provide a minimum of 4-5 siphon cycles per hour. After 24 hour the bags with sample were dried at 50 °C during 24 hours. The dried bags were transferred to a desiccator for one hour and then were weighted. The samples with water extraction were introduced in the Soxhlet extraction unit to start the same procedure with 280 ml of ethanol. The extractives percentage was calculated according to the equation 3.1:

$$\text{Extractives (\%)}=\frac{W_f}{W_i}\times 100 \quad (3.1)$$

Where $\mathbf{W_F}$ is the final biomass weight (g) after the extraction and $\mathbf{W_i}$ is the initial dry biomass weight (g) before the extraction.

3.1.3 Ash

The Ash content in the sample was measured based on the National Renewable Energy Laboratories (NREL/TP-510-42622) [83]. The ash is the remaining material after combustion at 575 ± 25 °C, this material is mainly mineral salts and other inorganic matter in the fiber.

First, 0.5 g of dry matter were weighted in a crucible and were placed in a furnace with the following temperature ramp program: Ramp from room temperature to 105°C. Hold at 105 °C for 12 minutes. Ramp to 250 °C at 10 °C per minute. Hold at 250 °C for 30 minutes. Ramp to 575 °C at 20°C per minute. Hold at 575 °C for 180 minutes. Allow temperature to drop to 105 °C. Hold at 105 °C. Finally, the crucible was removed and was placed into a desiccator until cooled to room temperature, and then the weight was taken. The ash percentage was calculated according to the equation 3.2:

$$\text{Ash (\%)}=\frac{W_f}{W_i}\times 100 \quad (3.2)$$

Where $\mathbf{W_F}$ is the final biomass weight (g) after the combustion and $\mathbf{W_i}$ is the initial dry biomass weight (g) before the combustion.

3.1.4 Holocellulose

Holocellulose is defined as the water insoluble carbohydrates fraction composed of cellulose and hemicellulose. The holocellulose was determined based on the chlorination method reported by the ASTM Standards (D-1104). The sample must be extractives-free, 2.5 g of sample were added to a 250 ml Erlenmeyer flask, 80 ml of distilled water, 0.5 ml of acetic acid and 1 g of sodium chlorite were added. An Erlenmeyer flask of 100 ml was put inverted in the neck of the reaction flask to capture the generated gases. The Erlenmeyer flask was placed in a water bath at 70 °C. During 6 hours, 1 g of sodium chlorite and 0.5 ml of acetic acid were added each 1 hour. After the six hours, the mixture was left at 70 °C for 24 hours. Then, the holocellulose was filtered on a pre-weighed filter paper using a Buchner funnel washing with 20 ml of acetone and hot water until the yellow color and the odor was removed. The filter paper with the holocellulose was dried at 50 °C for 24 hours. The sample was placed in a desiccator and weighed. The holocellulose percentage was calculated according to the equation 3.3:

$$\text{Holocellulose (\%)} = \frac{W_f}{W_i} \times 100 \qquad (3.3)$$

Where $\mathbf{W_F}$ is the final biomass weight (g) and $\mathbf{W_i}$ is the initial dry biomass weight (g).

3.1.5 Lignin

The lignin content was determined based on the acid-insoluble lignin in wood and pulp method (TAPPI-T222 os-74). The sample should be extractives and moisture free. In an Erlenmeyer, 200 mg of extractives-free samples were weighed. Then, 2 ml of sulfuric acid 2 % (v/v) were added. The mixture was kept at 30 °C for one hour. Subsequently, 56 ml of distilled water were added and the flask was placed in the autoclave at 121 °C and 15 psi for one hour. The lignin was filtered using a pre-weighed filter paper and washing with distilled water until remove the acid. The sample was dried at 30 °C during 24 hours, then was transfered to a desiccator for an hour and weighed. The lignin percentage was calculated according to the equation 3.4:

$$\text{Lignin (\%)}=\frac{W_f}{W_i}\times 100 \qquad (3.4)$$

Where $\mathbf{W_F}$ is the final biomass weight (g) and $\mathbf{W_i}$ is the initial dry biomass weight (g).

3.1.6 Cellulose

The preparation of cellulose is a procedure that continues after the holocellulose determination procedure in pursuit of the ultimately pure form of fiber [84]. From the obtained holocellulose, 2 g were weighed in a beaker flask covered with a watch glass. From a solution of NaOH 17.5 % (w/v), 10 ml were measured and added to the beaker. At 5-minute intervals, 5 ml of NaOH 17.5 % (w/v) were added during 45 minutes and then the mixture was kept at 20 °C for 30 minutes. After that, 33 ml of distilled water were added and the mixture was kept at 20 °C for 60 minutes. The cellulose was filtered using a pre-weighed filter paper and washing with distilled water, and 15 ml of acetic acid 10 % (v/v). Finally, the filter paper with the cellulose was dried at 50 °C for 24 hours and weighed. The cellulose percentage was calculated according to the equation 3.5:

$$\text{Cellulose (\%)}=\frac{W_f}{W_i}\times 100 \qquad (3.5)$$

Where $\mathbf{W_F}$ is the final biomass weight (g) and $\mathbf{W_i}$ is the initial dry biomass weight (g).

3.1.7 Starch

The content of starch was measured using the iodine method [85]. For this method, it was necessary an iodine-potassium iodide solution prepared with 20 g of potassium iodide dissolved in 20 ml of distilled water, 2 g of iodine and mixed in a 1-liter volumetric flask. To prepare the patron curve 0.1 g of starch was dissolved in 10 ml of perchloric acid 52% (w/w). After 10 minutes, the volume was completed at 100 ml with distilled water. In four volumetric flasks of 50 ml with 2.5 ml of perchloric acid 52% (w/w), 1 , 1.5, 2 and 2.5 ml of starch solutions were added and the volume was completed with distilled water. For each point, 10 ml was taken and 0.5 ml of iodine-potassium iodide solution were added. The mixture was stirred and kept in darkness. After 10 minutes, the absorbance was measured

at 600 nm in a spectrophotometer. The sample was treated in the same way that the patron curve.

3.2 Milk whey characterization

The milk whey characterization comprises the determination of the titratable acidity, ash, fat, protein and lactose.

3.2.1 Moisture

The moisture content was determined based on the official methods of analysis – moisture content (AOAC-1999) [86]. A crucible was dried at 105 °C for 3 hours, after that it was transferred to a desiccator and weighed. In the dry crucible, 3 g of sample were measured. Then, the sample was placed in an oven at 105 °C during 3 hours and the crucible at room temperature was weighed. Finally, the crucible was placed again in the oven at 105 °C for one hour and weighed, until constant weigh. The moisture content was calculated according to the equation 3.6:

$$\text{Moisture (\%)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (3.6)$$

Where $\mathbf{W_F}$ is the final dry weight (g) and $\mathbf{W_i}$ is the initial weight (g).

3.2.2 Ash

The ash in the milk whey was measured following the official methods of analysis – Ash of milk (AOAC 945.46). In a crucible 5 g of sampled were weighed. Then, the sample was placed in a furnace at 525°C for 3 hours. Subsequently, the crucible with the ash was transferred to a desiccator and weighed. The ash percentage was calculated according to the equation 3.7:

$$\text{Ash (\%)} = \frac{W_f}{W_i} \times 100 \qquad (3.7)$$

Where $\mathbf{W_F}$ is the final weight (g) and $\mathbf{W_i}$ is the initial weight (g).

3.2.3 Titratable acidity

The titratable acidity was measured based on the Standard IS. 166-1973 of the bureau of Indian standards [87]. In a 250 ml Erlenmeyer, 10 g of sample were measured and 10 ml of distilled water were added. 1 ml of phenolphthalein is added and the sample was titrated with NaOH 0.1 N. The titratable acidity was calculated according to the equation 3.8:

$$\text{Titratable acidity as lactic acid}=\frac{9AN}{W} \tag{3.8}$$

Where **A** is the required volume of NAOH, **N** is the normality of NaOH and **W** is the sample weight.

3.2.4 Protein

The protein content was determined using the Biuret method. For the preparation of the Biuret reagent, the following amounts are required per 100 ml:

- Sodium hydroxide - 3 g
- Sodium potassium tartrate - 0.6 g
- Copper sulphate - 0.15 g

The 3 g of NaOH were diluted in 100 ml of distilled water, once dissolved the sodium potassium tartrate was added and finally the copper sulphate. The solution was stirred for approximately one hour until all the reagents were completely dissolved. It was stored in amber bottles to protect it from light.

For the preparation of the calibration curve, bovine serum albumin was used as the standard reagent. For this, a stock solution of 2 g/L was prepared. From this solution, the dilutions were made with protein concentrations of 0, 0.4, 0.8, 1.2, 1.6 and 2 g / L. In a test tube, 300 µl of each solution and 1 ml of Biuret reagent were added, each was stirred for 30 seconds and allowed to stand for 10 minutes before recording the absorbance at 540 nm using a UV / Visible Spectrophotometer 6405 (Jenway). Each measurement was done in triplicate.

To generate the standard curve, the recorded absorbance values for each of the solutions with the known concentration of albumin were plotted. A linear regression of the data was made and the equation of the generated line with the correlation between the studied variables were obtained.

For each deproteinization test, 1 ml of the initial and final sample were taken. In a test tube 300 µl were taken from each sample and 1 ml of Biuret reagent was added, allowed to stand for 10 minutes and then the absorbance was recorded at 540 nm. With the obtained values, the amount of protein present in each sample was calculated.

3.3 Pretreatments

3.3.1 Acid pretreatment

The acid breaks the lignocellulosic matrix to decompose the cellulose and hemicellulose into sugars that can be fermented by a microorganism. The main advantages of acid hydrolysis are that the acid penetrates easily the lignin and the rate of this process is greater than the enzymatic one. However, one problem is that the glucose is rapidly degraded in the acid process. The acid pretreatment process employs usually sulphuric acid and hydrochloric acid at concentrations of 1–10% using a moderate temperature (in the range of 100–150 °C) [88]. This process generates toxics compounds, so that, the biomass has to be detoxified [89]. For the plantain peel, 20 g of dry sample were weighed in a flask and 200 ml of sulfuric acid 2% (w/w) were added. The flask was closed and placed in an autoclave at 121 °C, 15 psi for 1 hour. To continue with the enzymatic hydrolysis, the mixture obtained after the acid pretreatment was neutralized with potassium hydroxide (KOH).

3.3.2 Autohydrolysis

The autohydrolysis is simple, environmentally friendly and cost effective. In the process, hemicellulose oligomers are recovered. This pretreatment allows selective hydrolysis of hemicellulose at high temperature using water as the only reagent, resulting in a "solid pre-treated" and an extract "auto-hydrolysate" [47]. It contains sugars and oligomers with minor

inhibiting composition. Therefore, the auto-hydrolysis can be used before and enzymatic hydrolysis process as a strong hydrolysis of the hemicellulose portion of lignocelluloses [47]. For the plantain peel, 20 g of dry sample were weighed in a flask and 200 ml of distilled water were added. The flask was closed and placed in an autoclave at 130 °C and 15 psi for 1 hour.

3.3.3 Enzymatic hydrolysis

Enzymatic hydrolysis of lignocellulosic materials is slow and therefore difficult to develop economically, for this reason it is usually performed after an acid hydrolysis. Enzymatic hydrolysis is a heterogeneous reaction so affected by the structural characteristics of the substrate, such as crystallinity, lignin content and surface area [90]. The enzymatic hydrolysis procedure was done based on the NREL/TP-510-42629 method [91]. For a total volume of 10 ml, the sample equivalent to 0.1 g of cellulose is weighed into an Erlenmeyer flask. Subsequently, 5 ml of 0.1 M citrate buffer, pH 4.8 and 100 µl of a solution of sodium azide were added. The required amount of distilled water required to complete the volume at 10 ml after addition of the enzyme was calculated, assuming a specific biomass weight of 1 g/ml. The mixture was heated at 50 °C. When the temperature is reached, the necessary volumes of enzymes were added (Cellulase complex - NS22086 (Novozymes), β-glucosidase). The Erlenmeyer was closed and maintained at 50 °C and 150 rpm for 24 hours. Samples were taken at intervals of time to be analyzed.

3.3.4 Deproteinization with heat treatment

To use milk whey as a substrate in the fermentation it is necessary remove the protein to avoid precipitation or agglomeration that can affect the growth of the microorganism. The conventional pretreatment consist in deproteinize the milk whey with heat treatment at 90 °C for 30 min, at this temperature most of the whey proteins is denature and precipitate [12]. Remove the precipitate by centrifugation at 5000 rpm and 10 °C for 30 minutes. Sterilize the supernatant in an autoclave at 121 °C for 20 minutes.

3.3.5 Deproteinization with ultrafiltration

The ultrafiltration process fractionated the milk whey into a protein concentrate and a deproteinized whey permeate fraction [92]. To carry out this pretreatment, the milk whey is autoclaved at 115 ° C for 15 minutes, then filtered with filter paper and passed through an ultrafiltration cellulose membrane. Then, the pH is adjusted to 7 and centrifuged at 6000 rpm for 10 minutes.

3.3.6 Lactose hydrolysis

For the hydrolysis of lactose, a procedure very similar to that reported for lignocellulosic materials was used based on the conditions reported by Jurado et. al [93]. The chosen conditions were 40 ° C, 150 rpm, pH 4.5 for 6 hours. The enzyme used was a commercial lactase with an activity of 9000 FCC. Enzyme was added to each assay at a concentration of 80 FCC / g lactose. Samples were taken and analyzed.

3.4 ABE fermentation

For the development of the fermentation, it is necessary to take into account different parameters such as propagation of the strain and the preparation of the inoculum, the procedures for these stages are presented as follows.

3.4.1 Microorganism propagation

For the propagation of the microorganism, it is necessary to perform a heat shock to the bottle that contains it, in a bath at 80 ° C for 10 minutes, after which it is cooled in ice water. The microorganism must be propagated in P2 culture medium, the components and their respective concentrations are shown in **Table 3-1.** Composition of P2 culture medium

Table 3-1. Composition of P2 culture medium

Compound	Concentration [g/L]
Potassium phosphate	0,5
Dibasic potassium phosphate	0,5

Magnesium sulfate	0,2
Manganese sulfate	0,01
Sodium chloride	0,01
Ammonium acetate	2,2
Ferrous sulfate	0,01
P-aminobenzoic acid	0,001
Thiamin	0,001
Biotin	0,00001
Yeast extract	1
Cysteine	0,5
Glucose	60

The reagents were diluted in distilled water to the indicated concentration. 1% of the total prepared volume of a resazurin solution at 0.025% (w/v) was added as an anaerobic indicator. This solution was prepared by diluting the required amount of reagent in boiled water cooled with nitrogen, after dilution, it was again bubbled with nitrogen in the bottle in which it would be stored. The prepared medium was heated until boiling, removed rapidly and cooled in ice with nitrogen bubbling. Once the solution was cooled, the cysteine was added (not made before as high temperature begins to react with the dissolved oxygen and the reducing power would be lost before autoclaving). The pH of the medium was adjusted to 6.8 with NaOH 3N. The medium was distributed in working bottles. These bottles should be sealed with a plug impermeable to nitrogen and oxygen. The bottles were autoclaved at 121 °C and 15 psi for 20 minutes. The preparation of the vitamins was done separately and are added only when the inoculation is to be performed. Each bottle should be inoculated with 10% of the total volume. The inoculum is taken from the bottle to which the heat shock was performed. Incubate at 37 °C for 96 h without shaking, store at room temperature (20 °C).

3.4.2 Inoculum preparation

The inoculum must be prepared following the same procedure for the propagation of the microorganism. Incubate at 37 °C for 48 h at 150 rpm.

3.4.3 Hydrolysate fermentation

Before carrying out the experiments with the hydrolysates, a fermentation was made with glucose as a substrate to know the behavior of the microorganism. All hydrolysates were prepared under asepsis conditions to avoid contamination by other microorganisms. First, the pH is adjusted to six with a 6N KOH solution. Subsequently all components mentioned in **Table 3-1** were added except the glucose. Then continued with the same protocol of the microorganism propagation without autoclaving. After the bottles were sealed with oxygen and nitrogen impermeable plugs, they were inoculated with 20% of the total volume, incubated at 37 ° C and 120 rpm for 100 hours. After having carried out the experiments in the vials, fermentations were made in a 1.5 L bioreactor (Biotron), with nitrogen bubbling during the first 24 h, preparing the medium in the same way as for the vials, the agitation was 90 rpm.

3.4.4 Preliminary experiments

In order to know and evaluate the management of the strain and its products yields, a preliminary experiment was carried out from plantain peel with autohydrolysis as the only pretreatment. This experiment was carried out in the metabolic engineering laboratory of the biotechnology institute of the Universidad Autónoma de México, in Cuernavaca, Mexico. In this laboratory, it had all the necessary materials for the development of an anaerobic fermentation and management of microorganisms, as well as personnel experienced in the treatment and cultivation of this type of strains. As the purpose of this experiment was to know the capacity of the microorganism for the production of solvents, only the production of acetone, ethanol and butanol was measured during fermentation by gas chromatography, and the glucose consumption by HPLC.

3.4.5 Experimental separation

In order to know some separation data of the mixture obtained after the fermentation, a basic scheme of separation was proposed. At the end of each fermentation, the culture medium was stored in a refrigerator at 4 ° C until testing. For the test the medium was transferred to a flat bottomed balloon, boiling beads were added and connected to a

condenser. Taking into account the azeotropes, the boiling points and the present volume of each component, the mixture was kept at a controlled temperature until the calculated volume of the pure component or the respective azeotrope was collected. Finally, each of the samples obtained was measured. This procedure was carried out for the three raw materials.

3.4.6 Sample analysis

3.4.6.1 Sugars

The sugars content was determined using the DNS method. For the preparation of the DNS reagent, the following amounts are required per 100 ml:

- Sodium hydroxide – 1.6 g
- Sodium potassium tartrate - 30 g
- 3,5-dinitrosalisilic acid – 1 g

The 1.6 g of sodium hydroxide were diluted in 10 ml of distilled water. In other recipient the 30 g of tartrate sodium potassium were dissolved in 30 ml of water. Both solutions were mixed and the DNS was dissolved in it with heat. Finally, the volume was completed until 100 ml and the solution was stored in in an amber bottle to protect it from light.

For the sugars determination, two different calibration curves were made. One with glucose as patron for the plantain peel fermentation and other with lactose for the milk whey fermentation. For each reagent, a stock solution of 10 g/L was prepared. From this solution, the dilutions were made with the respective patron concentrations of 0, 1, 2, 3, 4 and 5 g / L. In a test tube, 500 µl of each solution and 500 µl of DNS reagent were added, each was stirred for 30 seconds and put into a bath at 90 °C for 5 minutes, then was allowed in cold water for 10 minutes. After this time 5 ml of distilled water were added and the absorbance at 540 nm was recorded using a UV / Visible Spectrophotometer 6405 (Jenway). Each measurement was done in triplicate.

To generate the standard curve, the recorded absorbance values for each of the solutions with the known concentration of the respective sugar were plotted. A linear regression of

the data was made and the equation of the generated line with the correlation between the studied variables were obtained.

For each sample, 1 ml of the initial and final sample were taken and centrifugated in a centrifuge Z 300 K (Hermle Labortechnik GmbH) at 10000 rpm during 10 minutes. The supernatant was taken and stored for the sugar analysis. In a test tube 500 µl were taken from each sample and 500 µl of DNS reagent were added. Each was stirred for 30 seconds and put into a bath at 90 °C for 5 minutes, then was allowed in cold water for 10 minutes. After this time, 5 ml of distilled water were added and the absorbance was recorded at 540 nm. With the values obtained, the amount of sugar present in each sample was calculated.

3.4.6.2 Biomass

The determination of biomass was done by the dry weight method. For this, Eppendorf tubes were marked and dried in an oven at 110 ° C for 24 h and then cooled. Subsequently the weight of each tube was recorded. In each tube was placed 1 ml of sample and centrifuged at 10000 rpm for 10 minutes, for this a centrifuge Z 300 K (Hermle Labortechnik GmbH) was used. The supernatant was removed and stored for further analysis. The precipitate was dried in an oven at 110 ° C for 24 hours and the weight recorded until it was constant. For each test 1 mL of medium without inoculum was taken as a blank to subtract the weight of the solids present in the culture broth.

3.4.6.3 Fermentation products

The determination of solvents of interest in the fermentative runs was performed by gas chromatography. For the measurement, the supernatants obtained in the biomass determination test were used. For each sample, the corresponding dilution was performed and filtered with Millipore filters.

Measurements were made using a GC-2014 (Shimadzu) gas chromatograph equipped with a flame ionization detector (FID) and a capillary column Stabilwax.

The operating conditions of the gas chromatograph were:

- Injection volume: 10 µl
- Injector temperature: 220 °C
- Detector temperature: 270 °C

- Gas carrier: Nitrogen
- Gas flow in the column: 0.5 ml/min
- Running time: 21.75 min
- Column temperature: 65 °C for 8 minutes, increase at 20°C/min until 140 °C and keep for 10 minutes.

For the determination of each product, first, the retention time was determined and then a calibration curve was made with the concentrations shown in **Table 3-2**. The reactants used were butanol, acetone, ethanol. The calibration curve and sample analysis were performed using the software GC Solutions.

Table 3-2. Standards concentration for gas chromatography

Curve point	Butanol (g/L)	Acetone (g/L)	Ethanol (g/L)
0	0	0	0
1	2	1	1
2	5	2	2
3	7	3	3
4	10	4	4

The determination of acids of interest in the fermentative runs was performed by High Performance Liquid Cromatography (HPLC). For the measurement, the supernatants obtained in the biomass determination test were used. For each sample, the corresponding dilution was performed and filtered with Millipore filters.

Measurements were made using a HPLC chromatograph LC2010 (Shimadzu) with a C18 column.

The operating conditions of the chromatograph were:

- Injection volume: 10 µl
- Mobile phase: Acetonitrile, 20mM NaH_2PO_4 in HPLC water adjusted to pH 2.2 with phosphoric acid.

- Flow: 0.5 ml/min
- Running time: 18 min

Table 3-3. Standards concentration for HPLC

Curve point	Acetic acid (ppm)	Butyric acid (ppm)	Lactic acid (ppm)
0	0	0	0
1	100	100	100
2	300	300	300
3	500	500	500
4	800	800	800
5	1000	1000	1000

3.5 Bioreactor design

3.5.1 Specific growth rate, yield and productivity

These parameters are preliminarily calculated considering the model proposed by Jacques Monod without taking into account any inhibition in the organism. The specific growth rate (μ) of the microorganism was calculated using the equation 3.9.

$$\mu=\frac{\ln(x_1/x_0)}{t_1-t_0} \qquad (3.9)$$

Where $\mathbf{x_1}$ is the cell concentration at the time of fermentation 1, $\mathbf{x_0}$ is the cell concentration at the time fermentation 0, $\mathbf{t_1}$ is the fermentation time 1 and $\mathbf{t_0}$ is the fermentation time 0.

The products yield ($Y_{p/s}$) was calculated using the equation (3.10).

$$Y_{p/s}=\frac{\text{grams of generated product}}{\text{grams of consumed substrate}} \qquad (3.9)$$

The productivity (P) was calculated using the equation (3.11).

$$P=\frac{\text{grams of generated product}}{\text{fermentation volume (L)} \times \text{fermentation time (h)}} \tag{3.11}$$

3.5.2 Bioreactor type

The bioreactor chosen to obtain butanol by fermentation ABE is a CSTR (continuous stirred-tank reactor) bioreactor type for different reasons. The main one is that it is the most used in the industry for these purposes. In addition, it is the one that most resembles to the experiments since it is a tank in which the reactant mass is continuously agitated, which allows considering a complete mixture. That allows assuming that all properties are uniform at any point in the reactor. Equation (3.12) is the design equation of a complete mixing reactor.

$$\frac{V}{F_{A0}}=\frac{X_A}{-r_A} \tag{3.12}$$

Where **V** is the reactor volume, **F_{A0}** is the molar flow of the limiting reagent, **X_A** the conversion of A and **r_A** the reaction rate of A.

The design of a CSTR reactor requires to know the reactor volume and the operating time, taking into account the time periods necessary for the cleaning of the equipment. It is also necessary to carry out the sizing of the tank and the agitators. As well as the required power and heat exchangers needed to maintain the reactor temperature.

3.5.3 Sizing

To perform the sizing the correlation present in the **Table 3-4** was used.

Table 3-4. Correlations for bioreactor sizing (Taken from McCabe et. al [94])

$\frac{D_a}{D_t}=\frac{1}{3}$	$\frac{J}{D_t}=\frac{1}{12}$	$\frac{W}{D_t}=\frac{1}{5}$

$\frac{H}{D_t}=1$	$\frac{E}{D_a}=1$	$\frac{L}{D_a}=\frac{1}{4}$

Each variable present in the **Table 3-4** is represented in the **Figure 3-1**..

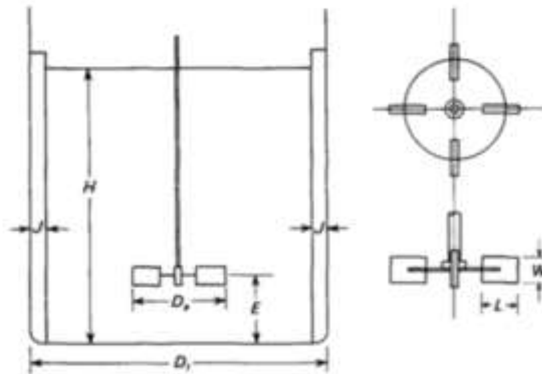


Figure 3-1. Dimension of stirred tank (Taken from McCabe et. al [94])

3.5.4 Power required by the stirring system

To calculate the power required by the stirring system, it is necessary calculate the Reynolds number (Re) with the equation (3.13).

$$Re=\frac{\rho N D_a^2}{\mu} \tag{3.13}$$

Where **ρ** is the density, **N** is the agitation speed, $\mathbf{D_a}$ is the agitator diameter and **μ** is the viscosity.

To know the required power, it is necessary choose one of the agitator types in the **Figure 3-2**, and then read the power number (N_p) in the same figure.

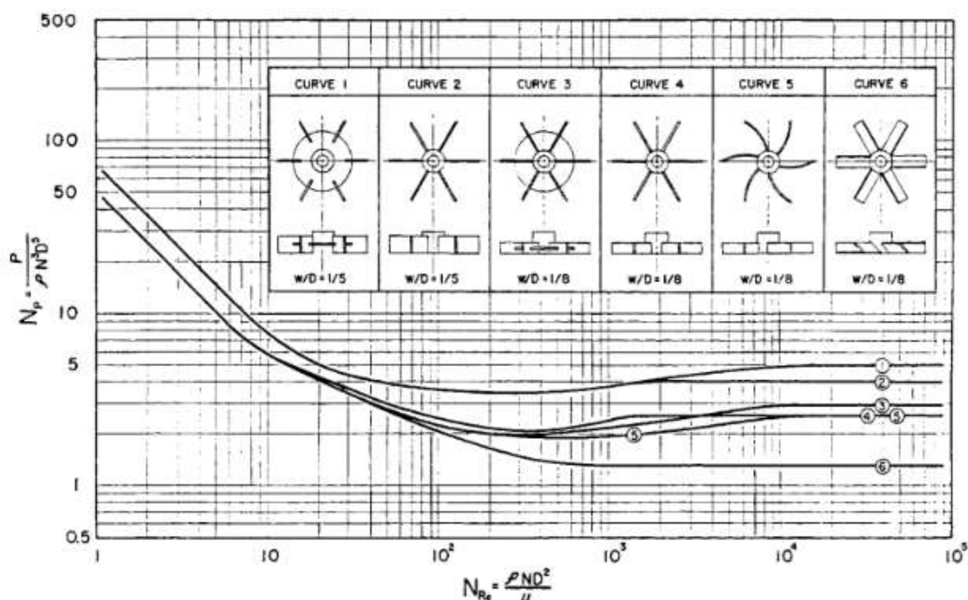


Figure 3-2. Power number vs Reynolds number (Taken from McCabe et. al [94])

After that, the power required by the stirring system can be calculated with the equation (3.14).

$$P=\frac{\rho N^3 D_a^5}{N_p} \quad (3.14)$$

3.6 Proposed platform simulation procedure

In this section, the proposed biorefinery with butanol is presented as a platform for obtaining three added value products. These products are butyl acetate, butyl propionate and butyraldehyde. The recuperated ethanol is considered without purification. In the plantain peel case, the solid wastes (lignin) are evaluated in a cogeneration process.

3.6.1 Thermodynamic-topological analysis of mixtures

Thermodynamic-topological analysis is based on the classic works of Zharov [95] where the relationship and similarity between mixture liquid-vapor equilibrium and the behavior of the residue curves in an open evaporation process is analyzed [96] (It consists in a simple evaporation stage without any reflux). The main problem when it has a variety of

components in a mixture, which it wishes to separate, it is the tendency of these components to form azeotropes that make separation difficult, increasing operating costs [96]. For this reason, it is important before to propose the separation scheme of a multicomponent mixture, carry out an analysis of its composition, as well as to know its behavior for choosing the best separation scheme. Thermodynamic-topological analysis can be applied in conventional distillation by describing the phase equilibrium in a multicomponent system through curves of constant boiling or trajectories of the distillation process [97]. The methodology presented for the development of the topological analysis was carried out as reported by Dussan et. al [98] and Pisarenko et. al [99].

1. **Azeotropes identification**: By proposing the phases equilibrium and building the binary and ternary systems present in the mixture, the presence of these points is determined where the vapor and liquid phase have the same composition.

2. **Topological characterization of the sample:** For this step the diagram is proposed with all the components present in the sample and the residue curves are plotted. These curves represent the evolution of the mixture composition through time in a simple distillation. When the residual curves divide the plane of the mixture into two or more sections, border lines are formed which will be the separation limit (separatrixes) in this region.

3. **Separation in regime ∞/∞:** A column that operates at regime ∞/∞, it is a hypothetical distillation column which operates at total reflux, and infinite number of stages. This premise implies that it is a distillation column that is working at its maximum efficiency, that is to say, an idealized distillation column, it is not operable in practice, but serves to obtain a preliminary and qualitative view of the behavior of a real distillation column. In addition, it allows to know the initial estimates to perform the separation system in Aspen Plus. For this, the feed composition is located and a line called balance line is drawn through this point connecting distilled and bottoms compositions. There are two types of balance lines that represent the direct and indirect separation in a distillation column. The direct separation line connects the feed point with the lowest boiling vertex, and the indirect separation line connects the feed point with the highest boiling vertex in a distillation region.

After knowing the methodology for the topological analysis, the general scheme of the biorefinery that will be evaluated is proposed. The process has different stages: The pretreatment step for obtaining fermentable sugars. The fermentation and separation step. The production of added-value products and the co-generation stage for the plantain peel. The **Figure 3-3** show the general scheme of the biorefinery.

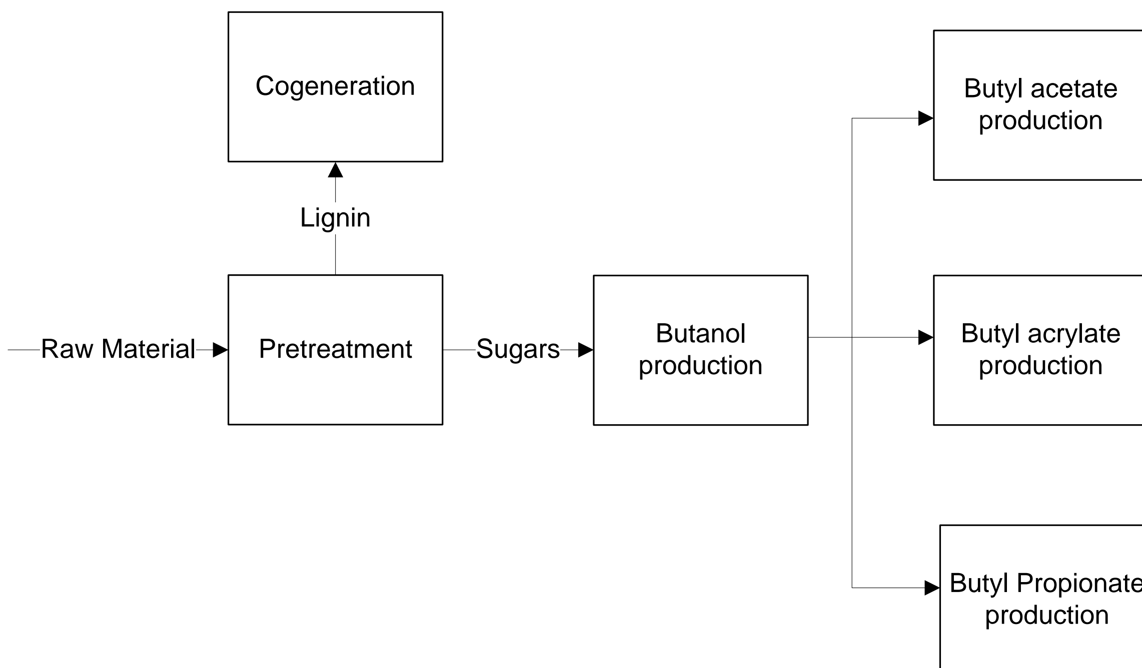


Figure 3-3. General scheme of the biorefinery

3.6.2 Butanol production

The sugar-rich stream obtained in the pretreatment step is entered into a bioreactor where it is mixed with a stream containing primarily water and ammonia as nutrients for the microorganism *Clostridium acetobutylicum*. The fermentation temperature is 37 ° C and the obtained yields in the experimental procedure were used. **Figure 3-4** shows the diagram process for butanol production.

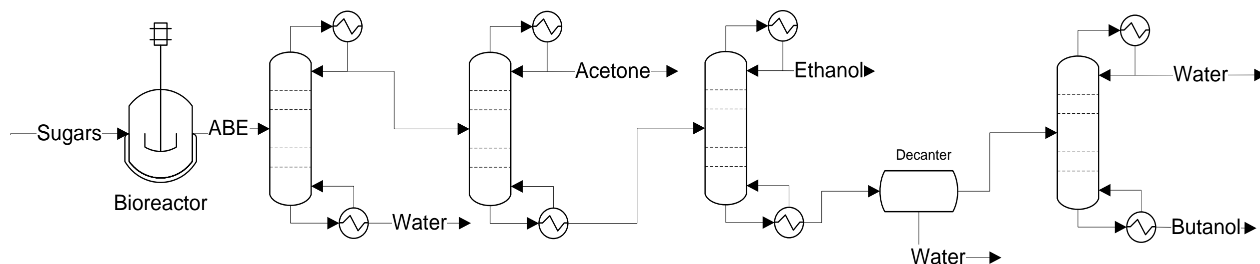
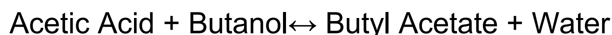


Figure 3-4. Butanol production process

After the fermentation, the produced gases are purged. The stream is entered to a train of distillation towers for obtaining products. Given the boiling points, acetone is recovered in the first distillation tower. A more complex mixture of alcohols and water is entered into a decanter to create aqueous and organic phases. This final phase is entered into a distillation tower to recover butanol and ethanol-water mixture. Finally, the butanol is purified into a third tower. This obtained butanol can be used as biofuel or bulk chemical to produce other chemicals. From the above, it is important to note that at this stage it is intended that the process of obtaining butanol have high efficiency and productivity to achieve an increase in the production of added-value products and have a profitable process.

3.6.3 Butyl acetate production

At industry level, butyl acetate is produced through esterification reaction, to which is added butanol and acetic acid as reagents [100]. In the last years different alternatives to carry out the esterification process have been investigated with the objective to increase the conversion in this process. The flowsheet of butyl acetate production is shown in **Figure 3-5**, while the reaction is described as follows:



Acetic Acid + Butanol↔ Butyl Acetate + Water

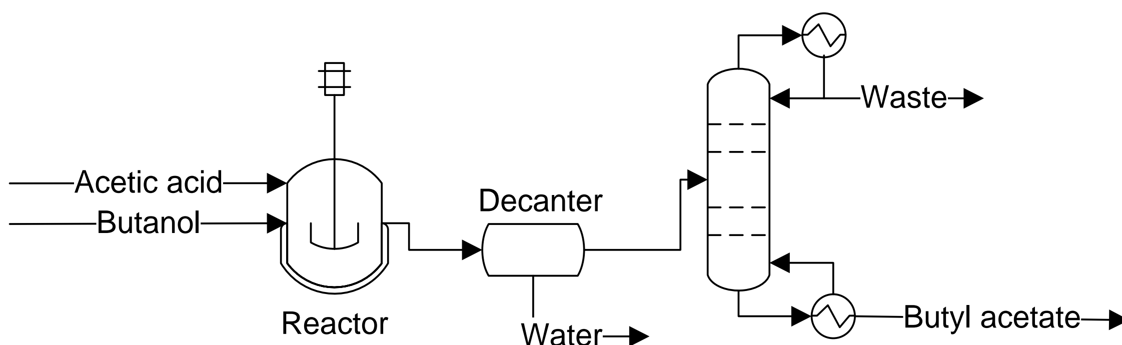
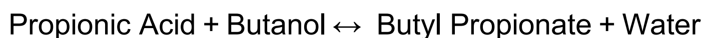


Figure 3-5. Butyl acetate production process

The 34% of the butanol obtained in the ABE fermentation is fed with the acetic acid, with a molar ratio of 1:1.2. Then the outlet stream is carried out to a decanter to retire water and then to a distillation tower to separate the butyl acetate. This process is carried out at atmospheric pressure. The kinetic model for the production of butyl acetate is reported by Cardona et. al [101].

3.6.4 Butyl propionate production

Chemical products such as esters are of great interest in the chemical industry as these are used in the synthesis of high added value products such as fragrances, flavors, solvents and plasticizers [102]. The butyl propionate is an ester with low volatility, product of esterification of propionic acid with butanol and is used as cleaning solvent. This ester can be replacing cleaning solvents more volatile such as ethyl acetate. This advantage has caused that this product has gained importance in the chemical market currently [103]. The esterification reaction to produce butyl propionate is presented as follows:



Propionic Acid + Butanol ↔ Butyl Propionate + Water

The process flow diagram to obtain this product is shown in **Figure 3-6**.

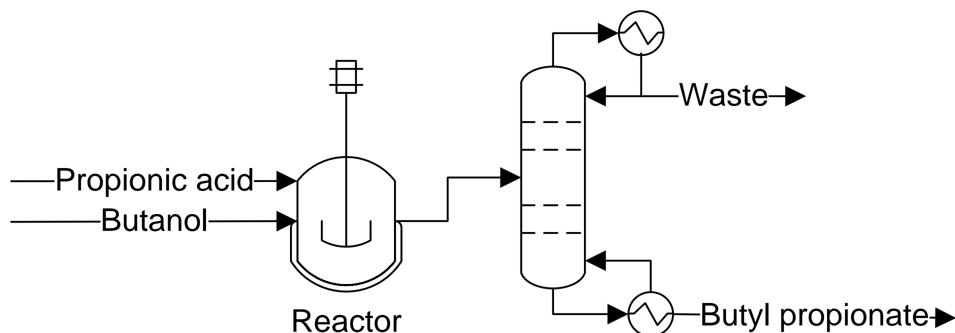


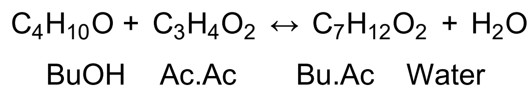
Figure 3-6. Butyl propionate production process.

The 33 % of the butanol obtained in the fermentation is fed with the propionic acid to the reactor where the esterification reaction is carried out. The liquid from the reaction stage is send to a distillation tower. Finally, the butyl propionate is purified by the bottoms of the tower and the waste is removing by head. The kinetic model for the production of butyl propionate was reported by Lee et. al [104].

3.6.5 Butyl acrylate production

Acrylates are one of the main components of many coating resins, so their secondary reactions have been studied, finding that polymerization rates are more significant as measured by laser pulsed polymerization [105], [106]. One of the most used acrylates is the butyl acrylate, which is produced by the esterification of butanol and acrylic acid. This compound is used in the industry as a precursor of vinyl, adhesives, textiles and polymers [107].

The butyl acrylate is produced by esterification of acrylic acid and butanol according to the following reaction:



$$\underset{\text{BuOH}}{C_4H_{10}O} + \underset{\text{Ac.Ac}}{C_3H_4O_2} \leftrightarrow \underset{\text{Bu.Ac}}{C_7H_{12}O_2} + \underset{\text{Water}}{H_2O}$$

The process flow diagram to obtain this product is shown in **Figure 3-7**.

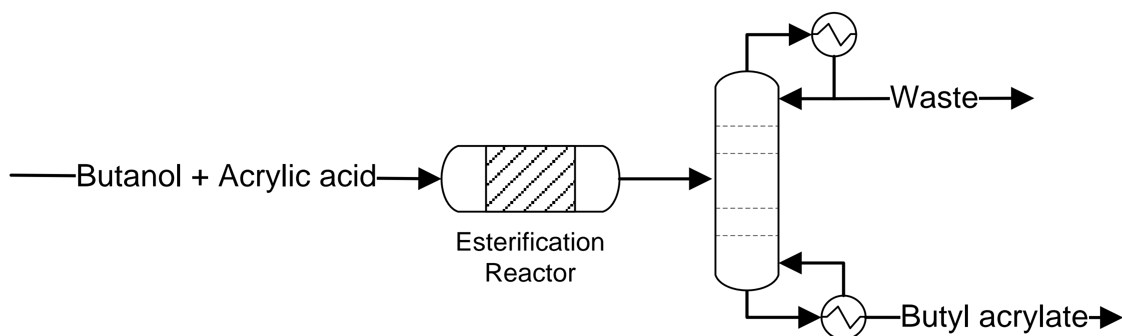


Figure 3-7. Butyl acrylate production process.

The reactants are fed to a reactor at 120 °C. The reactor output stream is fed to a distillation tower, which was designed taking into account that the butyl acrylate is the heaviest component, and water the lighter component of the mixture. With these data it was obtained that for a distillation tower with a total condenser operating at 1 bar, 16 stages are necessary by entering the feed stream in stage 8. The kinetic model for the production of butyl propionate was reported by Schwarzer et. al [108].

3.6.6 Cogeneration system

For the plantain peel process, it is possible including a cogeneration system in the biorefinery. Cogeneration can be defined as a thermodynamically efficient way of using energy with the ability to complete or partially cover the plant requirements [109]. There are a number of companies employing biomass cogeneration in its processes; some of them are ethanol plants and paper mills. If enough electricity can be produced, it is possible in some cases to satisfy the needs of communities near the site, increasing the economic and social viability of the process [109]. This process includes a step of gasification to produce syngas and use it into a gas turbine to produce electricity. The biomass gasification process consists in the conversion of a solid organic compound in a gas phase called “syngas” that has a high heating power and can be used as a chemical platform or an energy vector for heat of power generation [110]. Temperature ranges of gasification process vary between 875-1275 K [111]. In this process, lignin and compressed air enters into the gasifier. The

reactions that occur in this process are pyrolysis, combustion and reduction to obtain a hydrogen, methane, carbon monoxide and carbon dioxide mixture. This is centrifuged to separate solid particles as ashes. Finally, this then enters into a turbine to produce power.

3.7 Analysis of the proposed platform

3.7.1 Economic assessment

The estimation of total production costs was calculated using the Aspen Process Economic Analyzer (Aspen Technology Inc., USA) commercial software. These costs are based on material and energy balances from simulation. This software package calculates the capital costs, operating costs and all costs related to raw materials, utilities, equipment and some administrative aspects. The production costs are estimated in dollars for ten years of service life plant considering a linear depreciation method for calculating the capital depreciation. It is based on Colombian conditions as an income tax of 33% and an interest rate of 16.02%. With these values, an analysis of the influence of the percentage of variation of the sales cost with the NPV is made. For the economic analysis the prices used for the products were [112]: acetone 1.82 USD/kg, butyl acetate 2 USD/kg, butyl acrylate 1.88 USD/kg, butyl propionate 2.3 USD/kg, ethanol 0.86 USD/kg (However, since the ethanol obtained has a purity of 70%, a value of 0.43 USD/kg is used). The operator and supervisor labor costs were 2.14 USD/h and 4.29 USD/h, respectively, considering the Colombian context [113]. For the raw materials the cost used were 1 USD/ton for the plantain peel and 3 USD/ton for the milk whey. These costs of the raw materials were calculated taking into account the transport, the whey has higher cost since being a liquid waste requires specialized transport.

3.7.2 Environmental assessment

Environmental impact is measured using the Waste Reduction Algorithm WAR GUI, developed by the National Risk Management Research laboratory of the U.S. Environmental Protection Agency (EPA). This method calculates de potential environmental impact (PEI) based on the impact of input and output flow rates from the process. The program shows the results in two ways. The first is to show the impact generated by the streams of output of the process. While the second shows the general impact generated, which is calculated with the difference between the impact of the output

currents and the input currents. When the value is positive it means that the output currents generate more environmental impact than the input ones. This tool uses the mass balance and energy requirements generated in Aspen Plus. The WAR GUI software measures eight categories. These are human toxicity potential by ingestion (HTPI), human toxicity potential by skin exposure and inhalation (HTPE), terrestrial toxicity potential (TTP), aquatic toxicity potential (ATP), global warning potential (GWP), ozone depletion potential (ODP), photochemical oxidation potential (PCOP) and acidification potential (AP). Finally, the sum of all impacts results into the final impact per kg of products [114].

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4. Discussion and results

This chapter presents the results obtained during the development of this thesis work. Initially, the physicochemical characterizations of each raw material is presented. Subsequently, the results obtained during the pretreatments made to the raw materials are analyzed and are selected those that present the best results to carry out the fermentations, from which the yields obtained for each of the products are presented. Finally, with these yields, the simulations and the analysis are carried out to know the economic and environmental viability of each one of the proposed platforms.

4.1 Raw material characterization

This section presents the characterization of the raw materials used in this work. These results allowed to know the viability of each these raw materials for obtaining fermentable sugars. The results obtained were compared with some reported in the literature taking into account that the differences found may be due to the origin of the raw material or the method used for the characterization.

4.1.1 Plantain peel

The plantain peel was characterized following the parameters presented above. The results obtained are shown in the **Table 4-1**. As can be seen in this table, it was found that the plantain peel has a high content of extractives, this is due to the large amount of soluble sugars it has. The values of cellulose and hemicellulose are similar and allow knowing the possibility to obtain sugars from this waste through pretreatment processes. In this table it is found also other characterizations for this residue reported in the literature.

Table 4-1. Plantain peel characterization.

	This work (%)	Literature (%)	
Moisture	86	89.10[a]	57.8 0± 2.00[c]
Extractives*	50.60 ± 1.03	29.83[b]	NR
Cellulose*	12.60 ± 1.51	12.17[b]	13.17 ± 1.50[c]
Hemicellulose*	11.80 ± 2.01	10.19[b]	4.61 ± 7.60[c]
Lignin*	17.40 ± 1.41	14[a]	17.10 ± 0.10[c]
Ash*	7.60 ± 0.22	9.81[b]	12.70 ± 1.00[c]

[a] Monsalve et al. [115], [b] Oberoi et al. [116], [c] Agama et al.[75] * Dry basis, NR. Non-reported

The results obtained in this work do not differ significantly from the values reported in the literature. In the case of extractives, soluble sugars not determined in this work can be the cause of the difference in the value of this parameter. As reported by Agama et al. [75], the plantain peel has a high content of antioxidant components such as polyphenols and tannins with high antioxidant capacity that can be extracted to add value to the residue. As previously mentioned, the content of cellulose and hemicellulose allows obtaining fermentable sugars, in the literature some works have been reported where the sugars obtained from this residue have been used for the production of different products by fermentation such as ethanol and biogas [117], [118]. The lignin content of this material is not very high compared to other lignocellulosic materials. However, it is considered that it can be used in a cogeneration process, where the remaining solids of the pretreatments, which are mainly lignin, are used for the production of steam and electricity. Additionally, the content of starch in the plantain was determined obtaining a result of 39.17% ± 0.87. This value is very important because some microorganisms consume this carbohydrate as a substrate. It can also be hydrolyzed with enzymes such as α-amylase to obtain simple sugars that can be consumed by most microorganisms. In the literature, Agama et al. [75] report a starch content in the plantain peel of 39.29%. Happi et al. [119] report a content of 35.40%, both values are very close to the obtained.

4.1.2 Milk whey

The milk whey is a liquid waste, so it must be characterized with different methods to those used for lignocellulosic materials. **Table 4-2** presents the results obtained for the physicochemical characterization of the milk whey, as well as data reported in the literature for this residue.

Table 4-2. Milk whey characterization

	This work (%)	Literature (%)	
Moisture	94.32 ± 0.01	93.1[a]	94[b]
Protein	1.34 ± 0.20	0.9[a]	NR
Lactose	3.61 ± 0.45	4.9[a]	4.05[b]
Fat	0.19	0.3[a]	0.3[b]
pH	4.02	NR	4.8[b]
Titratable acidity	1.16%	NR	NR
Density	1.03 g/mL	NR	NR

[a] Durán [120], [b] Antonelli et al.[121], NR: Non reported

The milk whey used in this work is characterized as an acid whey due to its pH. Although the protein content is not very high, can generate floccules and precipitates that trap the microorganism, affecting the growth of this, so it is necessary to remove it before any fermentation. Compared with the literature data, the whey used for the development of this work has a lower content of fats and lactose, and a higher protein content. These differences can be given by the methods used for the characterization or by the process from which the whey is obtained, such as the type of cheese from which it comes. Lactose is the main sugar in milk whey. However, most microorganisms are not able to metabolize it. For that reason, it is necessary perform a lactose hydrolysis to obtain simpler sugars. At the experimental level, the whey has a large number of studies to obtain added-value products such as biogas, lactic acid, butanol, and ethanol, among others. [12], [80], [92], [121], [122].

4.2 Pretreatments

In this section, the results obtained for the pretreatments evaluated for each of the raw materials are presented. In the case of the plantain peel, acid pretreatment and autohydrolysis are evaluated. After knowing which one presents the best results, the

enzymatic hydrolysis is carried out. For the milk whey, are evaluated a thermal pretreatment and other one with ultrafiltration to remove the protein.

4.2.1 Acid pretreatment

For the acid pretreatment with sulfuric acid 2%, a sample was taken at the end of the process. This sample was analyzed by HPLC to know the composition of the obtained solution. Another characteristic of this pretreatment is the production of toxic compounds such as hydroxymethylfurfural and furfural, which can be proved to be generated during the process . Another characteristic of this pretreatment is the production of toxic compounds such as hydroxymethylfurfural and furfural.

Table 4-3 shows the components analyzed and their composition in the hydrolyzate. The glucose composition obtained can be considered high. However, the concentration of xylose is considered quite low, since commonly, the pretreatment with acid is used to treat the fraction of hemicellulose and obtain a xylose-rich stream. Nevertheless, due to the low content of hemicellulose in the plantain peel, low yields of xylose are obtained. Another characteristic of this pretreatment is the production of toxic compounds such as hydroxymethylfurfural and furfural.

Table 4-3. Compounds in plantain peel liquid fraction after the acid pretreatment

	Cellobiose	Glucose	Xylose	HMF	Furfural
Conc. (g/L)	0	10.29 ± 0.19	0.93 ± 0.01	0.19 ± 0	0.07 ± 0.02

The yield of glucose was 0.10 g/g of plantain peel and that of xylose of 0.0093 g/g of plantain peel. García [123] performed the glucose and xylose measurement of different lignocellulosic materials, for the *Pinus patula* (Cellulose: 44.78%, Hemicellulose: 23.75%) found a yield of 0.049 and 0.034 grams of glucose and xylose per gram of raw material, respectively. On the other hand, for coffee cut stems (Cellulose: 40.39%, Hemicellulose: 34.01%) found a yield of 0.023 and 0.107 grams of glucose and xylose per gram of raw material, respectively. These results show that, regardless of the type of lignocellulosic material, sugar yields will always depend on their composition. Materials with higher hemicellulose content will have higher xylose yields.

4.2.2 Autohydrolysis

The pretreatment with autohydrolysis has the same purpose of the acid pretreatment, to break the hemicellulose chain to leave the cellulose of the matrix more accessible for a later enzymatic hydrolysis. This process aims to be friendlier to the environment because it uses only water as a reactant. However, due to the high temperatures it is possible the production of inhibitory compounds [124]. **Table 4-4** shows the results for this pretreatment. Unlike acid pretreatment, cellobiose is obtained and there is no production of furfural. In addition, the glucose yield is higher (0.18 g/g of plantain peel). On the other hand, the concentration of xylose is almost half of that obtained with acid, which shows that the acid pretreatment is better to obtain xylose-rich streams.

Table 4-4. Compounds in plantain peel liquid fraction after the autohydrolysis

	Cellobiose	**Glucose**	**Xylose**	**HMF**	**Furfural**
Conc. (g/L)	0.65 ± 0.04	18.24 ± 0.16	0.50 ± 0.01	0.19 ± 0	0

In the literature, there are different studies about the use of autohydrolysis as pretreatment of lignocellulosic materials. Parajó et al. [125] reports a production of less than 5 g/100g of raw material of glucose and xylose for rice and barley husks, eucalyptus wood and corncobs. For this case, the glucose yield was 18.24 g/100 g of peel, the difference with other lignocellulosic materials reported in the literature is mainly due to the amount of soluble sugars present in the plantain peel. [72], which are released by putting the material in contact with hot water. Finally, it can be concluded that this pretreatment has no formation of furfural as an inhibitory component, there is formation of cellobiose and additionally the only reagent is water, autohydrolysis is chosen as the base pretreatment for the process with plantain peel.

4.2.3 Enzymatic hydrolysis

Initially to make the evaluation of the enzymatic hydrolysis, the pre-treatment of autohydrolysis to the plantain peel was performed and the solid fraction was separated from the liquid one. Using the solid fraction, enzymatic hydrolysis was performed, after 24 hours of hydrolysis a glucose concentration of 3.04 g/L ± 0.01 was obtained. Due to the low concentration of glucose, the separation of the solid fraction from the liquid is not

recommended. For this reason the enzymatic hydrolysis was carried out again without the separation of these

After the autohydrolysis process, the necessary amounts to the buffer of sodium citrate and citric acid were added, the sodium azide was too added, the pH was adjusted and the enzyme was incorporated. The same procedure proposed was followed during enzymatic hydrolysis. The **Figure 4-1** shows the development of the enzymatic hydrolysis of the plantain peel after the autohydrolysis and without separation of the solid fraction of the liquid fraction.

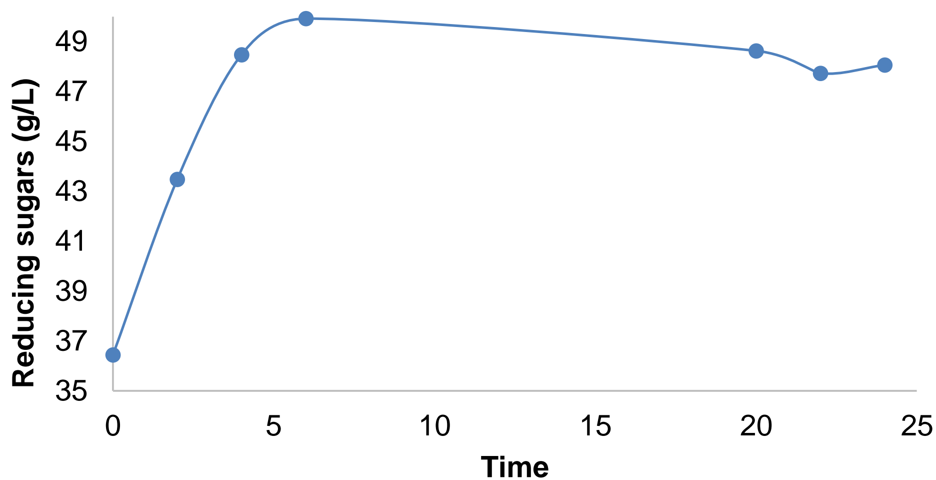

Figure 4-1. Sugars production during enzymatic hydrolysis of the plantain peel

As can be seen in the figure during the first 10 hours, the main production of reducing sugars occurs, after this time the concentration is almost constant, for this reason the hydrolysis is not maintained for more than 24 hours. These results are affected by the concentration of cellulose in the plantain peel which is not very high in comparison with other lignocellulosic raw materials, so the enzyme does not require so much time to completely hydrolyze the cellulose in sugars. The total yield of sugars for the peel after applying all the pretreatments is 2.4 g reducing sugar/g plantain peel.

For the enzymatic hydrolysis carried out to whey, results are presented in the **Figure 4-2**. Although an increase in the production of reducing sugars can be observed, the measures are not entirely correct, since the method for the determination of these sugars as already described above was the DNS, which determines the presence of all the reducing sugars

present in a solution. For this case, lactose, as well as the glucose and galactose that comprise it, are reducing sugars, so that all can be determined by this method. Therefore, it is not possible to know the exact amount of lactose that was hydrolyzed and the glucose that was produced. However, for fermentations this method of sugar determination continues to be used, since it allows to determine the consumption of sugars.

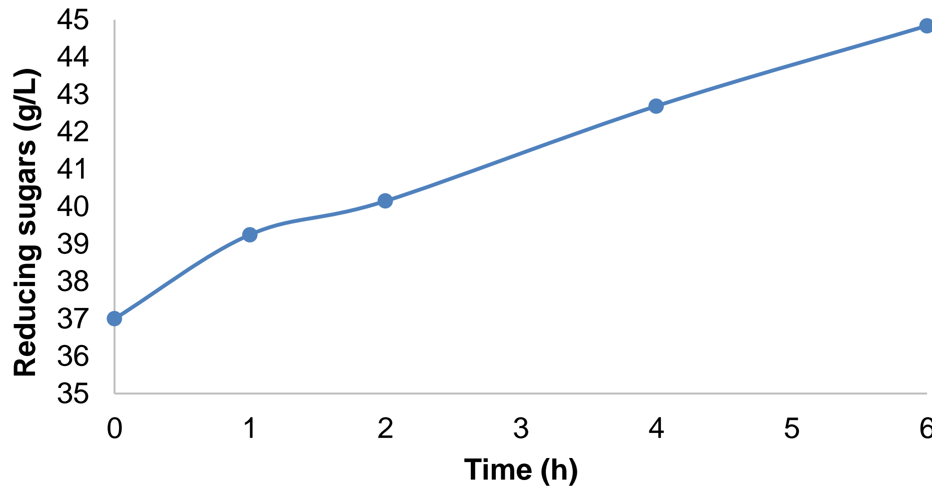

Figure 4-2. Sugars production during enzymatic hydrolysis of the milk whey.

4.2.4 Deproteinization with heat pretreatment

Upon receiving the whey from the local company Normandy, its pH was measured and the suspended solids were determined to see if it was necessary to carry out an initial filtering process. The pH was registered at a value of 4.02, which is why it is considered an acid whey and due no suspended solids were observed, it was not necessary to carry out any filtration processes. **Table 4-5** shows the results obtained using conventional heat pretreatment.

Table 4-5. Protein concentration before and after heat pretreatment

	Initial	Final	Percentage of removal
Conc. (g/L)	13.75	12.02	12.55%

This process allows a removal of 12.55% of the protein. This value is low and the amount of protein that still remains in the whey can affect the growth of the microorganism, also

when sterilization is done there is precipitation of more protein, which can generate floccules that trap the microorganism and prevent its growth and development during fermentation.

4.2.5 Deproteinization with ultrafiltration

For this pre-treatment, as in the previous one, the pH of the whey was taken and the procedure was carried out. **Table 4-6** shows the results obtained.

Table 4-6. Protein concentration before and after ultrafiltration pretreatment

	Initial	Final	Percentage of removal
Conc. (g/L)	13.75	6.44	53.19%

This pretreatment allows the removal of more than 50% of the protein present in the whey. Therefore, this pretreatment is considered the best to carry out the fermentation with the *Clostridium acetobutylicum*. Additionally, this pretreatment requires a smaller number of operation units than the previous one, which could mean a lower energy requirement..

4.3 ABE fermentation

This section presents the results obtained during the fermentations carried out with each of the raw materials. Initially the results obtained in a preliminary work carried out in the Biotechnology Institute of the Universidad Nacional Autonoma de Mexico are presented, afterwards the results are presented for the fermentations carried out in vials and in bioreactor. Additionally, the results for fermentations with glucose as a substrate are presented.

4.3.1 Preliminary experiments

As preliminary experiments, a fermentation was carried out from plantain peel with autohydrolysis as the only pretreatment. **Figure 4-3** shows the development of the fermentation.

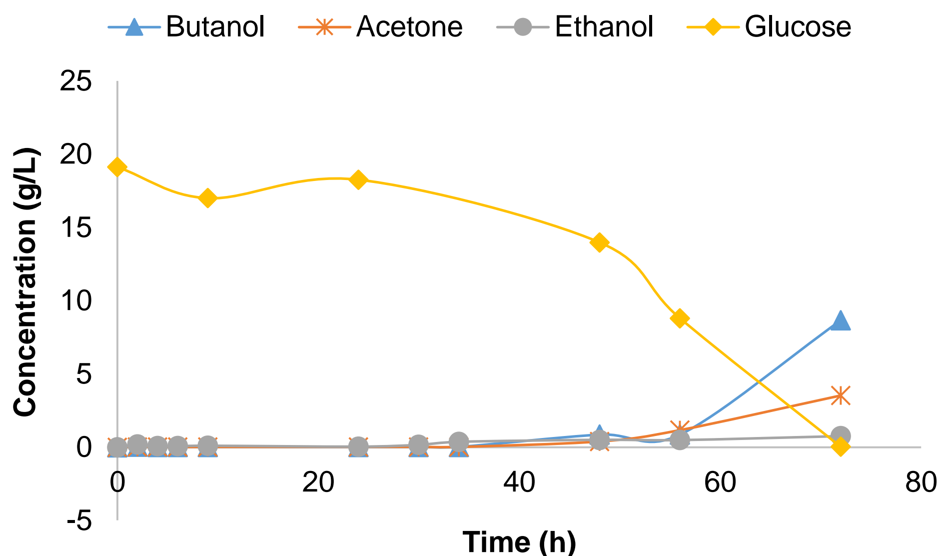

Figure 4-3. Solvents production and glucose consumption in the plantain peel hydrolyzate fermentation

At the beginning of the fermentation, the glucose consumption is low. This is because it is an adaptation phase of the microorganism. At the same time, it can be seen that there is no production of any of the solvents. After approximately 30 hours, there is a significant glucose consumption and an increase in the production of solvents until reaching a final production of 8.66 g/L of butanol, 3.53 g/L of acetone and 0.75 g/L of ethanol (12.94 g/L of ABE). With this experiment, it was found that the strain has the capacity to produce solvents from the glucose obtained from the plantain peel. The overall yield of solvents in this fermentation was 0.68 g ABE/g glucose, while the yield for butanol was 0.45 g butanol/g glucose. The literature reports a large number of studies with the strain of *Clostridium acetobutylicum* ATCC 824, Ennis [126] reports a production of 3.69 g/L of butanol from glucose in synthetic medium. Lin and Blaschek [127] report a production of 12.6 g/L of butanol from 16% extruded corn broth. Jang et al. [43] report a concentration of 6.1 g/L ABE from 10% hydrolyzed lignocellulosic organic waste.

4.3.2 Glucose fermentation

Initially, different fermentations were carried out with glucose as a substrate to know the behavior of the microorganism and the factors that could affect the fermentation. The first factor that was evaluated in the preparation of the P2 medium is the lack of oxygen. For

this purpose, resazurin is very important because it allows to know when the medium was prepared correctly and thus ensure the complete anaerobiosis required. **Figure 4-4** shows how is the medium with presence and absence of oxygen after being prepared. To achieve the desired conditions, it is necessary to take into account that the medium should be removed immediately after boiling and cooled as quickly as possible, so it is very important to put it in the bath with ice while nitrogen is bubbling. The medium must be heated to reduce the solubility of oxygen in the medium and thus be able to move it more easily with the nitrogen bubbling.

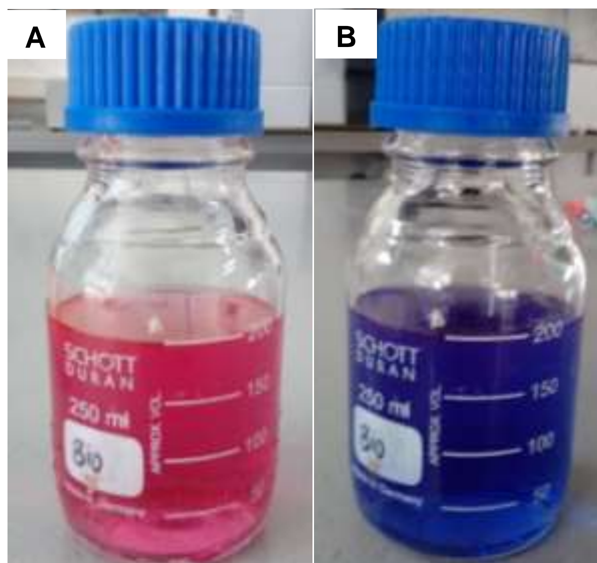

Figure 4-4. Color indication of resazurin for a medium with (A) and without oxygen (B)

After achieving the correct preparation of the medium, it was decided to evaluate the rubber plugs available in the laboratory to ensure that they were impermeable to oxygen and nitrogen. However, they were not completely impermeable and the medium remains with a slight pink tone after being autoclaved. After this, blue butyl rubber plugs were tested and it was possible to show that there was no transfer of oxygen or nitrogen through them. With the medium ready to be inoculated, the first experiments were made. These were carried out without the addition of p-aminobenzoic acid to the medium due to lack of availability in the laboratory. In these experiments the growth of biomass was very low and the consumption of glucose was limited to less than one gram per liter. For this reason, after reviewed the literature it was observed that the majority of media formulated for this type of microorganisms includes this compound in its preparation [128]–[130]. The fermentations were carried out again with the addition of this acid and the growth of the biomass in the

vials was evident. Therefore, it was concluded that, despite their low concentration in the culture medium, the vitamins are of great importance for the correct growth of this microorganism (*C. acetobutylicum* ATCC 824).

The step to follow was the evaluation of the appropriate inoculum age for fermentation. For this, three bottles were inoculated and left at 37 °C and 90 rpm for 12, 24 and 48 hours, then fermentation vials were inoculated and left for 72 hours at the same conditions. At the end of this time, the glucose consumption was determined, which was 3.8, 7.1 and 9 g / L, respectively. Thus, it was decided to use an incubation time for the inoculum of 48 hours, since it has a higher biomass concentration and a very long adaptation stage in the fermentation is not required. With all this information, fermentation with glucose as a substrate in vials was carried out. **Figure 4-5** and **Figure 4-6** show the consumption of glucose, the growth of biomass and the production of solvents during fermentation.

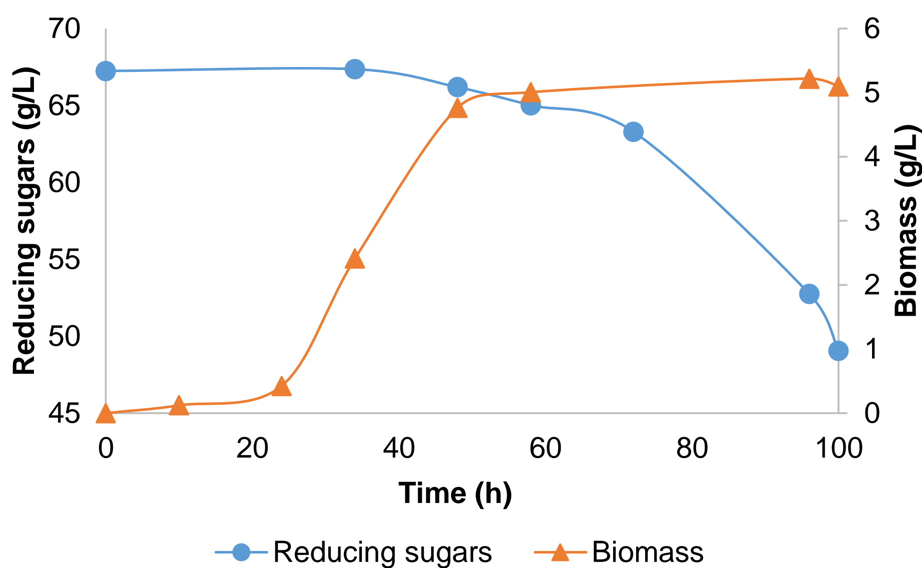

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Figure 4-5. Biomass growth and sugars consumption in the fermentation with glucose in vials.

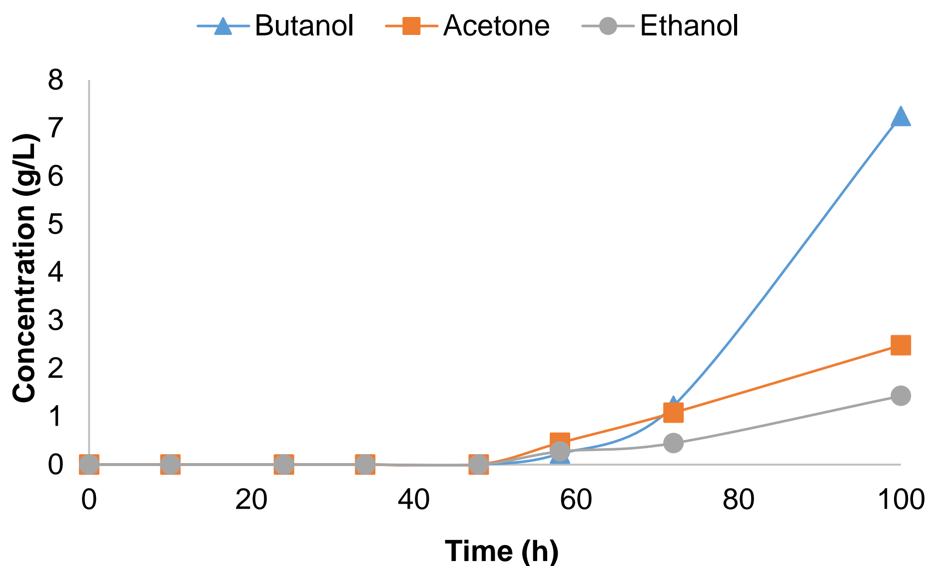

Figure 4-6. Solvents production in the fermentation with glucose in vials.

The microorganism did not consume all the glucose present in the culture medium, this can occur mainly because the microorganism reaches the stationary phase where it initiates the highest production of solvents, which increase its concentration to such an extent that the microorganism feels inhibited and it does not present substrate consumption. For this experiment, the adaptation phase lasts around 24 hours in which glucose consumption is minimal. The stationary phase starts at 50 hours where there is evidence of increased glucose consumption. Finally, a biomass concentration of 5 g/L and a final concentration of reducing sugars of 49 g/L are reached. In the graph of the solvents, it can be observed that the production of these begins to be considerable after 40 hours, just when the stationary phase is reached. In this experiment, a greater amount of butanol is obtained, followed by acetone and finally ethanol. The final concentration of these was 7.24, 2.48 and 1.43 g/L, respectively. These values are close to those obtained in the initial experiment with plantain peel. The total yield of solvents was 0.61 g ABE / g of reducing sugars.

After having finished the experiment in vials, they were carried out in a 2 L bioreactor. For this, it was necessary to make some small changes in the protocol used for the vials. First, the medium was prepared without using resazurin as an anaerobic indicator, since the bioreactor cannot be hermetically sealed, so there would be oxygen and nitrogen exchange with the medium. To ensure an anaerobic medium, nitrogen was bubbled in for one hour before inoculation and then for 24 hours. Also, the dissolved oxygen was measured to

observe its absence in the medium. **Figure 4-7** and **Figure 4-8** show the results obtained for this experiment.

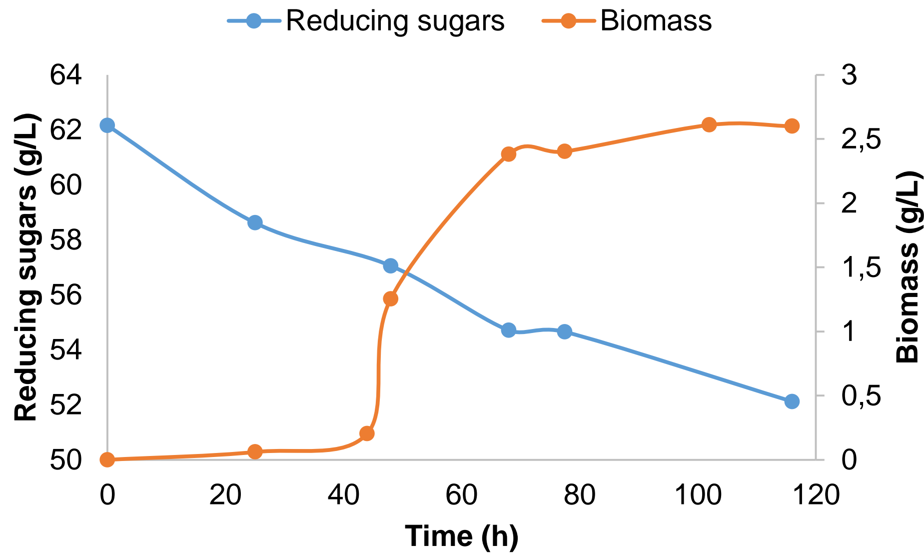

Figure 4-7. Biomass growth and sugars consumption in the fermentation with glucose in the bioreactor.

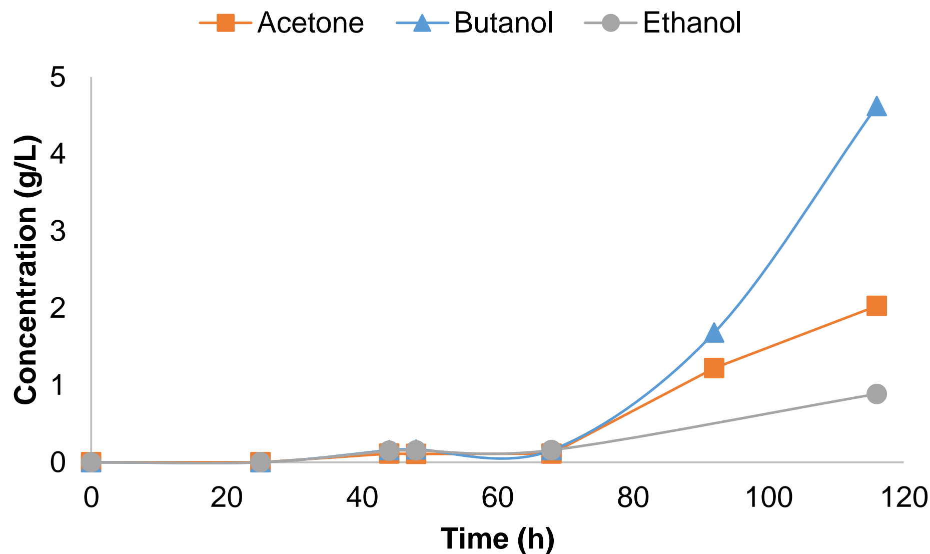

Figure 4-8. Solvents production in the fermentation with glucose in the bioreactor.

For this case it can be observed that there was a lower glucose consumption and therefore a lower growth of biomass. The factors that may have affected the microorganism were the

initial 24 hours of fermentation during which there was constant bubbling of nitrogen, in addition to the type of agitation since for the vials it was an orbital agitation while in the bioreactor it is by means of an agitator with blades. The change of these parameters for fermentation influences the adaptation and growth of the microorganism, affecting the final results obtained. In this case, the adaptation phase ends a little after 40 hours while the solvent production starts after 60 hours. As in biomass, the production of solvents is lower, being 4.62 g/L of butanol, 2.03 g/L of acetone and 0.89 g/L of ethanol. The yield of solvents is 0.75 g ABE/g reducing sugars. Despite having obtained lower final amounts, the yield is a little higher since more solvent is obtained per gram of reducing sugars consumed.

4.3.3 Plantain peel hydrolyzate fermentation

In the case of plantain peel hydrolysates as well as glucose, the experiment was carried out first in vials and then in the bioreactor. The hydrolysates were treated with much asepsis, since to avoid losses of sugars they were not autoclaved. Before being used for the fermentation, they were heated to boiling and then they were bubbled for half an hour with nitrogen to ensure the anaerobic medium and thus be able to perform the inoculum. After carrying out the enzymatic hydrolysis a hydrolyzate is obtained with a very small particle size of solid that covered the pores of the filter paper, which could not be filtered under vacuum to leave the liquid fraction free of solids. Instead, a conventional strainer was used to separate the solids from larger size and the liquid fraction obtained in this step was used. These solids were a great inconvenience for the determination of the biomass in these experiments, because before starting a sample was taken to be the blank and thus subtract it from the following samples to know the real value of the biomass. However, it was not possible to ensure that in all the samples the same amount of plantain peel solids will be removed, so when performing the biomass calculations negative values were obtained and the trend could not be determined. It is for this reason that the growth of the biomass is not shown for the experiments carried out with plantain peel. **Figure 4-9** shows the results of sugar consumption and solvent production for the fermentation of plantain peel in vials.

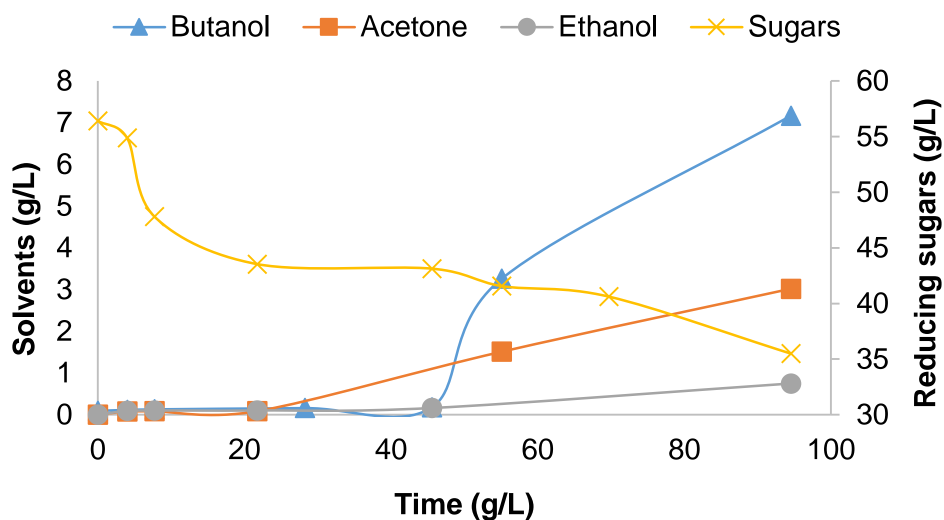

Figure 4-9. Sugars consumption and solvents production in the fermentation with plantain peel hydrolyzate in vials.

As with glucose, the consumption of sugars was not total, however, it was greater (20.9 g/L). Unlike in the previous case in which the three solvents have a higher production at almost the same time. The final production of acetone, butanol and ethanol was 3.02, 7.17 and 0.75 g/L, with a solvent yield of 0.52 g ABE/g reducing sugars. The obtained values are similar to those obtained for the reference case with glucose in vials. For the experiment performed in the bioreactor, the results are presented in **Figure 4-10**.

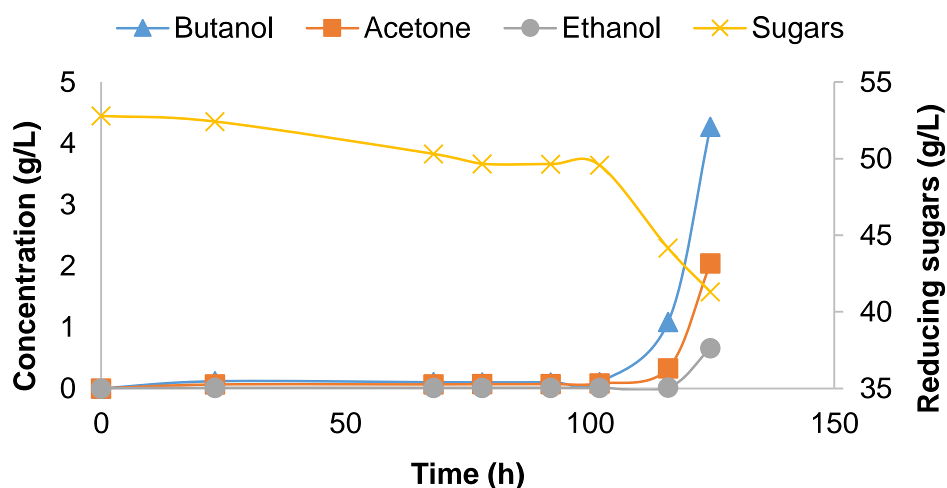

Figure 4-10. Sugars consumption and solvents production in the fermentation with plantain peel hydrolyzate in the bioreactor.

In the experiments carried out in the bioreactor it can be said that the microorganism had a longer adaptation phase, since the consumption of sugars is not considerable until 100 hours as well as the production of solvents. The same phenomenon was obtained for glucose with a lower concentration of solvents, being 2.04 g / L of acetone, 0.66 g / L of ethanol and 4.27 g / L of butanol. The total production of solvents was 0.58 g ABE / g reducing sugars.

4.3.4 Milk whey hydrolyzate fermentation

For the experiments carried out with the whey hydrolyzate, the same trends are obtained in the results. **Figure 4-11** and **Figure 4-12** show the growth of biomass with the consumption of glucose and the production of solvents, respectively. Referring to the biomass, it can be found that the stationary phase was not reached, this means that the fermentation must have been longer. This can be due to the fact that part of the sugars present in the medium are lactose, a sugar that the microorganism has the capacity to consume for the production of solvents. However, since it was not grown in a medium with the presence of this sugar, it requires more time to adapt to it and consume it.

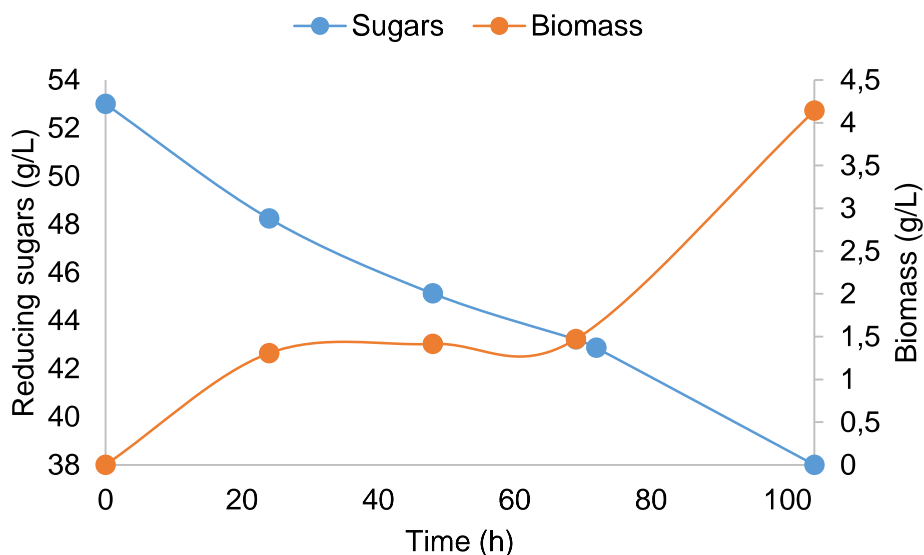

Figure 4-11. Biomass growth and sugars consumption in the fermentation with milk whey hydrolyzate in vials.

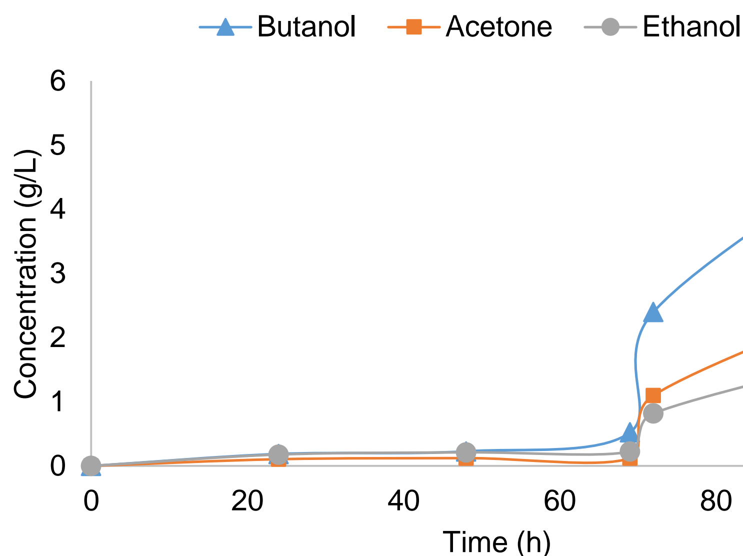

Figure 4-12. Solvents production in the fermentation with milk whey hydrolyzate in vials.

The production of solvents is evidenced from the 70 hours. This time is longer than the time required in the experiments in vials with glucose and plantain peel. The final concentration of acetone, ethanol and butanol was 2.82, 1.88 and 5.31 g/L, respectively, for a yield of 0.66 g ABE/g of reducing sugars.

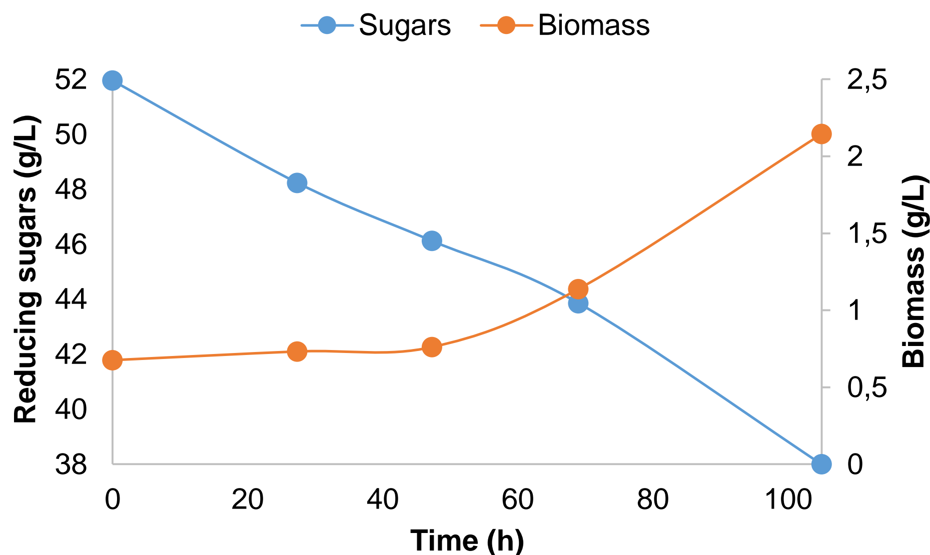

Figure 4-13. Biomass growth and sugars consumption in the fermentation with milk whey hydrolyzate in the bioreactor.

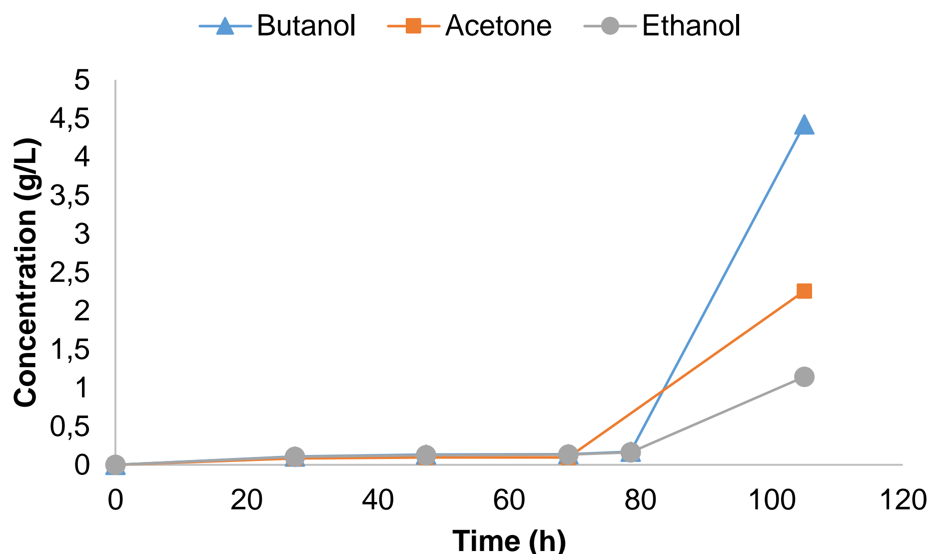

Figure 4-14. Solvents production in the fermentation with milk whey hydrolyzate in the bioreactor.

Figure 4-13 and **Figure 4-14** show the development of fermentation in the bioreactor with whey hydrolyzate. They have a similar behavior to the previous ones, since the solvents take a few more hours to start producing than in the vials. As in the experiment in the vial, the biomass does not reach a stationary phase. As in all cases sugars are not consumed completely. For this experiment a final concentration of acetone of 2.26 g/L, ethanol of 1.14 g/L and butanol 4.42 g/L was obtained, clearly the production of acetone is very similar to that obtained in the vials. The yield was 0.56 g ABE/g of reducing sugars. It was the only case in which a lower yield was obtained in the bioreactor than in the vials. This may be linked to the presence of lactose in the medium that makes the adaptation of the microorganism difficult.

As results, of the experiments carried out, the final concentration of each of the acids that can be produced during the development of the ABE fermentation was measured. **Table 4-7** presents the results.

Table 4-7. Final acids concentrations

Fermentation	Acetic acid	Butyric acid	Lactic acid
Glucose - Vial	0	0.28	0

Glucose - Bioreactor	0	0.81	0
Plantain Peel - Vial	2.46	0.39	0
Plantain Peel - Bioreactor	2.38	0.79	0
Milk Whey – Vial	1.12	0.65	0.48
Milk Whey - Bioreactor	1.20	1.31	0.59

For the fermentations with glucose, only butyric acid was detected. In the vials a lower concentration was obtained than in the reactor. The same for the other two substrates. In the plantain peel, a very high concentration of acetic acid was measured in both experiments. While lactic acid was only detected in the whey, but this does not mean that it has been produced necessarily by the microorganism, because whey has this acid in its composition. Those acids that are in higher concentrations as is the case of the experiment with whey in bioreactor means that there is the possibility of obtaining higher concentrations of the solvent corresponding to these secondary metabolites.

4.3.5 Experimental separation

In this section, the experimental separation of the solvents from the culture medium was evaluated with the scheme presented in **Figure 4-15**.

Figure 4-15. Experimental separation assembly

This assembly did not allow the separation of any of the components present in the mixture. The main inconvenient was the large amount of water and the low concentration of solvents. Additionally, the boiling temperatures of the components and the azeotropes present in the mixture were very close, so it was not possible to maintain the temperature of the mixture controlled. For this reason, to have an idea of the separation routes of this complex mixture, the use of topological thermodynamics was proposed as shown below.

4.4 Comparisons with other cases from literature

To know the differences between the results obtained in this work with others, it is necessary to make a comparison with the data reported in the literature. In this case, **Table 4-8** different concentrations obtained from solvents are reported during the ABE fermentation with different strains of *Clostridium acetobutylicum*, for lignocellulosic residues, glucose and whey.

Table 4-8. Yields reported in the literature for different *Clostridium acetobutylicum* strains.

Strain	Substrate	ABE Yield	Reference
ATCC 824	Glucose	B: 4.62 g/L A: 2.03 g/L E: 0.89 g/L	This work
ATCC 824	Plantain peel hydrolyzate	B: 4.27 g/L A: 2.04 g/L E: 0.66 g/L	This work
ATCC 824	Milk Whey hydrolyzate	B: 4.42 g/L A: 2.26 g/L E: 1.14 g/L	This work
ATCC YM1	Glucose 40 g/L	B:8.09 g/L A:2.7 g/L E:0.14 g/L	[55]
ATCC 824	Glucose 45.5 g/L	ABE: 8 g/L	[131]
P262	Sago starch	B: 16 g/L ABE: 18 g/L	[56]

ATCC 824	10% domestic organic waste (4.6 g/L of glucose)	B: 3 g/L ABE: 3.3 g/L	[57]
ATCC 824	Deproteinized milk whey	B: 7.13 g/L A: 0 g/L E:5.11 g/L	[132]
ATCC 824	Milk Whey	ABE: 9.2 g/L	[131]

B: Butanol, A: Acetone, E: Ethanol, ABE: total solvents, RS: reducing sugars

With the data reported in **Table 4-8**, it can be concluded that the results obtained in the development of this work were satisfactory since for each of the raw materials used very similar results were obtained to those reported in the literature. It is also very important to take into account that the strains of each microorganism can present different yields of the raw material used. An example is the strain P262 of the *Clostridium acetobutylicum* presented in the table that has the capacity to produce 16 g/L of butanol from sago starch, almost double the concentration obtained in the fermentations of this work. On the other hand, for lignocellulosic materials Amiri et. al [47] reported a yield of 104.5 g ABE/kg pine and in this work for the lignocellulosic material used, a yield of 192.36 g ABE/kg of plantain peel was obtained. Equally for the whey, values slightly lower than those reported in the literature were obtained, even though the results were good. With this it can be concluded that this strain presents good yields for the production of ABE from industrial waste such as plantain peel and milk whey.

4.5 Bioreactor design

This section presents a brief design of a bioreactor with the calculation of the most relevant parameters of the fermentation, and a sizing of a bioreactor of 1 m^3 with a fermentation capacity of 0.8 m^3. The energy required for stirring it at 90 rpm is also calculated. **Table 4-9** presents the most relevant parameters obtained using the data from the fermentations carried out in the bioreactor.

Table 4-9. Fermentations parameters

Parameter	Symbol	Units	Glucose	Plantain Peel	Milk Whey
Specific growth rate	μ	h^{-1}	0.032	---	0.006

Products yield	$Y_{p/s}$	g ABE/g reducing sugars	0.760	0.580	0.561
Productivity	P	$g\ l^{-1}\ h^{-1}$	0.065	0.056	0.074

As previously mentioned it was not possible to determine the biomass for the experiments carried out with plantain peel that is why the specific growth rate for this case is not presented. This speed relates the growth of the cells with the nutrients present in the medium, this shows that the composition of the medium changes as the substrate is being consumed and the products generated. The experiment with glucose has a higher rate of growth than with the whey, this means that there is more biomass production per unit of time, this may be due to the different compounds present in the milk whey that prevent the microorganism from adapting more easily to the medium and consume it to generate biomass. On the other hand, as expected the experiment with glucose is the one that presents a higher yield of products, followed by the plantain peel and the milk whey. Finally, the productivity in each test was determined. This parameter indicates the quantity of solvents produced per unit of volume per unit of time. In this case, the whey has a higher productivity than the plantain peel. This is because more solvents are obtained in a shorter time, without forgetting that the whey did not reach the stationary phase. It is demonstrated that despite the microorganism has difficulties in adapting to the environment, it is capable of producing similar amounts of solvents as in the other cases.

After this, a brief dimensioning was carried out using the correlations presented in **Table 3-4**, for the parameters represented in **Figure 3-1**. The **Table 4-10** presents the results obtained for a reaction volume of 0.4 m^3. Taking into account that is necessary 40 kg of plantain peel for each run, and 400 L of milk whey for each run

Table 4-10. Bioreactor sizing

Parameter	Description	Value (m)
H	Liquid height	0.798
D_t	Total diameter	0.798
J	Baffle width	0.066
D_a	Agitator diameter	0.266
E	Agitator distance from the bottom	0.266
L	Palette width	0.066
W	Palette height	0.159

Finally, the power required by the agitator for each of the three fluids studied was calculated. The results are presented in **Table 4-11**.

Table 4-11. Density and power required for each fluid.

	Glucose	**Plantain peel**	**Milk Whey**
Density (g/mL)	1.001	1.043	1.030
Power (HP)	0.790	0.820	0.810

As the densities of the three fluids is very close, the energy required for each case is also very similar. This preliminary dimensioning of a bioreactor allows to know the geometry and power needs to continue to a second stage where a detailed design of each parameter required for this type of fermentation could already be carried out.

4.6 Platform simulation

This section presents the results obtained from the simulations of each of the proposed platforms with each raw material evaluated in the experimental section. Initially, the results of the topological analyzes carried out for the separation stages of the platform are presented. Then the flows and purities obtained from each of the added value compounds are presented in the technical analysis. Subsequently, the results of the economic analysis are shown, showing the distribution of plant costs as well as the influence of the scale and the value of the products on the net present value.

4.6.1 Thermodynamic-topological analysis

The results obtained following the propose procedure are in this section.

Mixture Acetone-Butanol-Ethanol-Water

1. Azeotropes Identification

Approach of phase equilibria

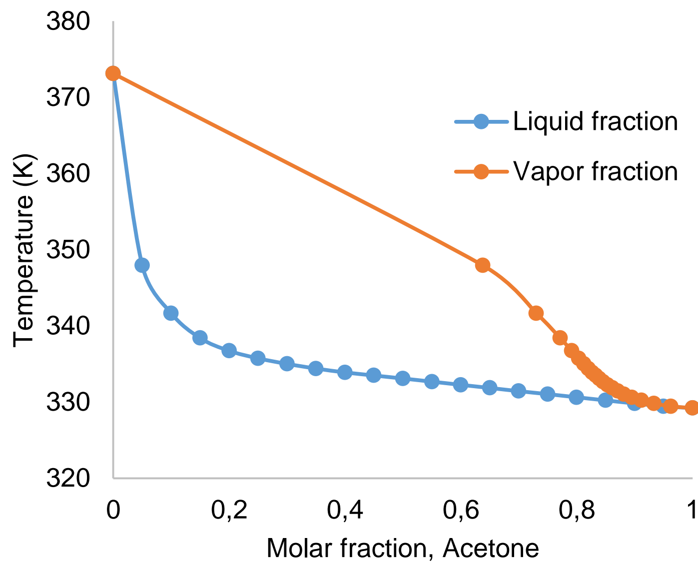

Figure 4-16. LVE Acetone – Water [133]

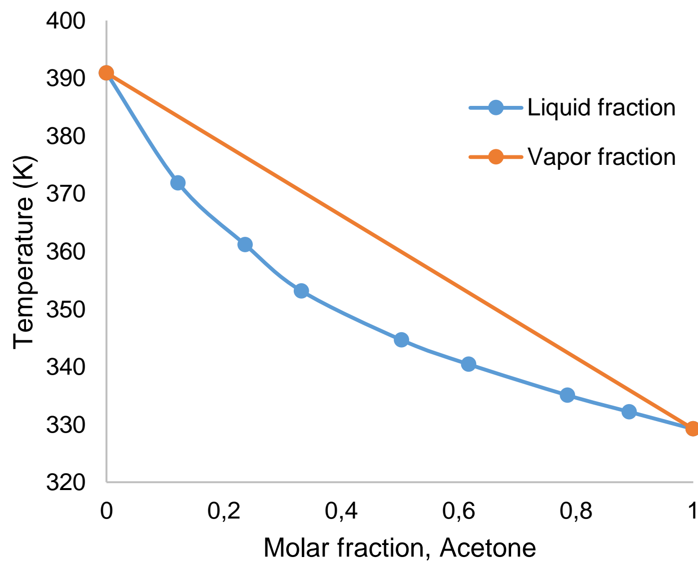

Figure 4-17. LVE Acetone – Butanol [134]

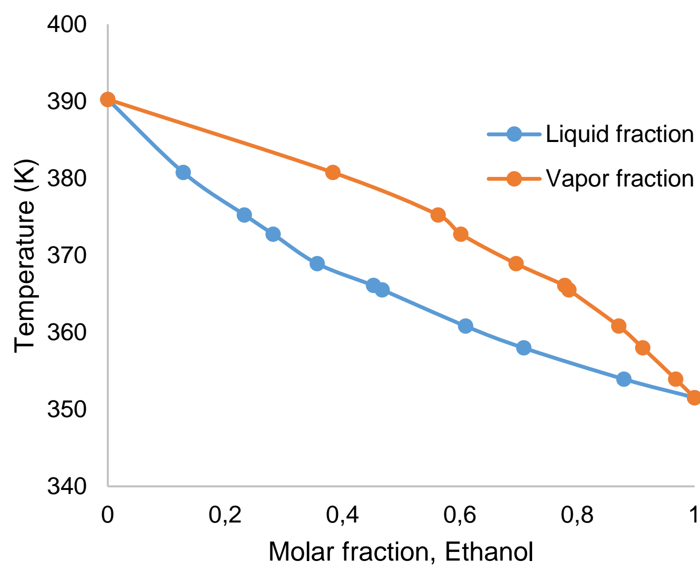

Figure 4-18. LVE Butanol – Ethanol [135]

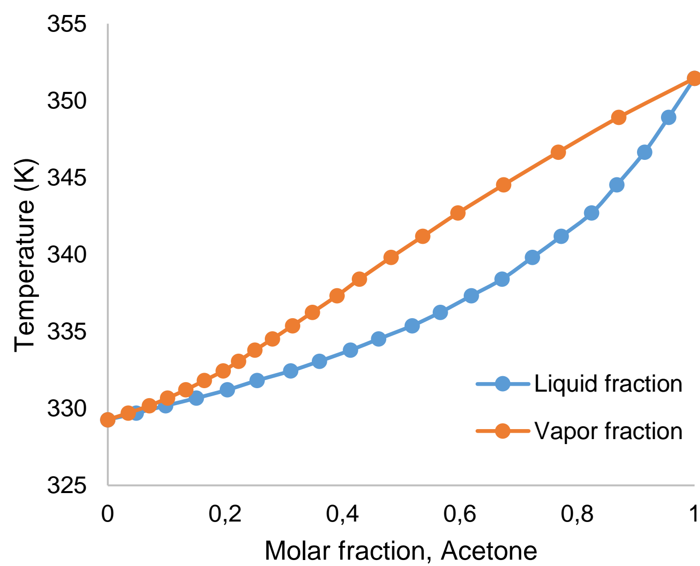

Figure 4-19. LVE Acetone – Ethanol [136]

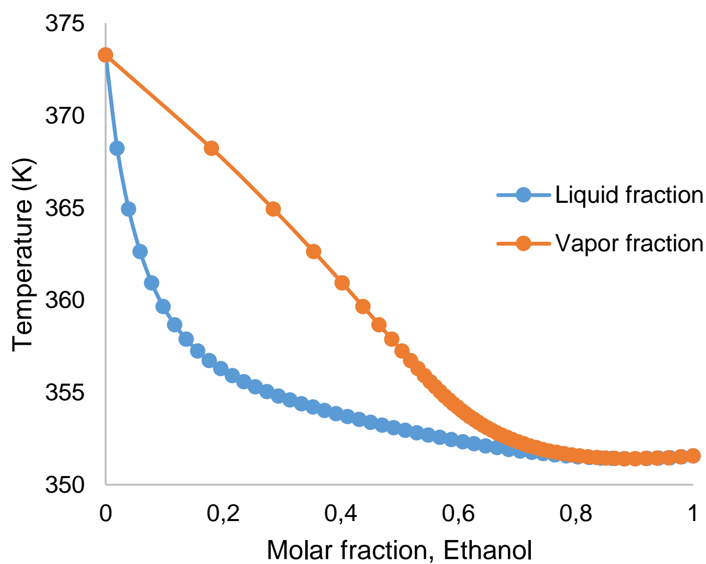

Figure 4-20. VLE Ethanol – Water [137]

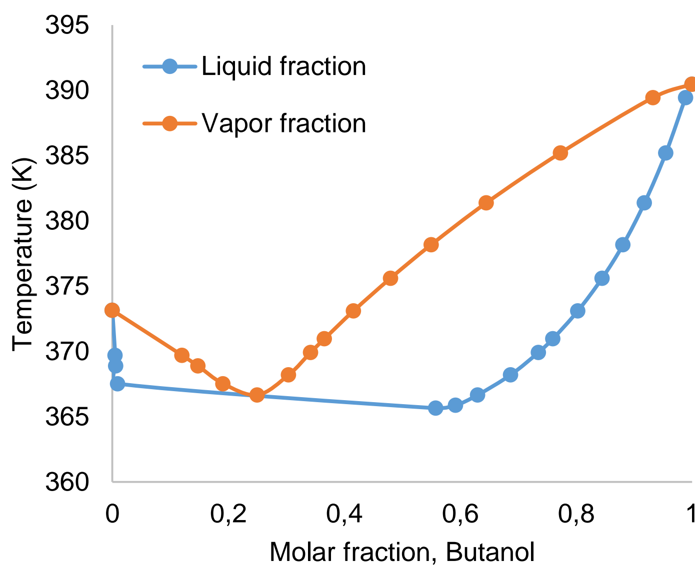

Figure 4-21. VLE Butanol – Water

These diagrams allow us to know that the mixture presents a liquid-liquid equilibrium between butanol and water, in addition two azeotropes between butanol-water and ethanol-water are formed. The azeotropes characteristics are presented in **Table 4-12.**

Table 4-12. Azeotropes characteristics

	Species	Molar compositions	Temperature	Characterization
Azeotrope 1	Water Ethanol	0.105 0.895	78.15 °C	Saddle
Azeotrope 2	Water Butanol	0.759 0.241	91.72	Saddle

2. Topological characterization of the sample

To have an initial overview of the behavior of this mixture, the extended diagram was made and it is presented in **Figure 4-22**.

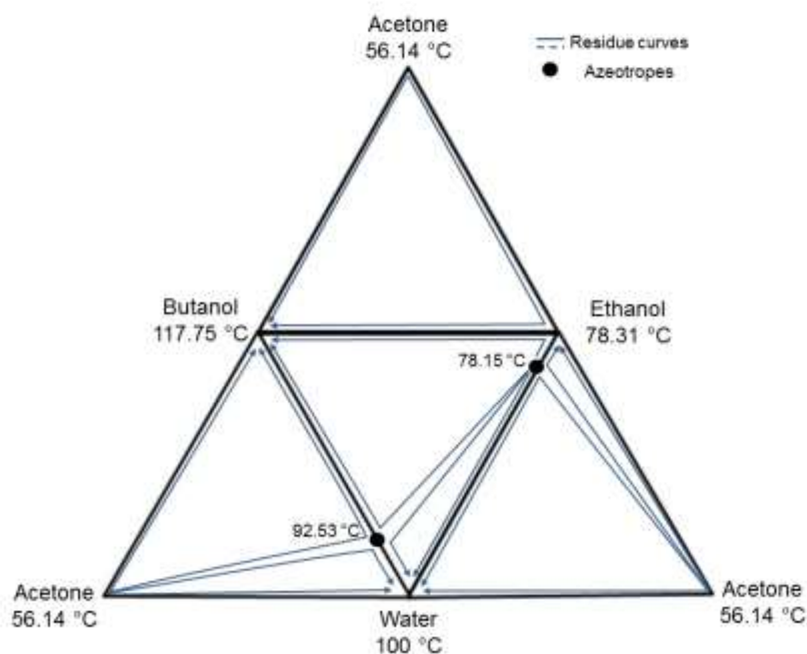

Figure 4-22. Extended residue curves map for the mixture Acetone – Butanol – Ethanol – Water. P=1 atm.

This diagram shows the interaction between components in the mixture and as in each of the ternary diagrams that an azeotrope is present, a separatrix is formed. Thus, it is possible to point out that this system has a line which delimits the area of the mixture and divides it

into two, depending on the feed composition, the best separation route should be evaluated. **Figure 4-23** shows the quaternary mixture diagram with its respective residue curves.

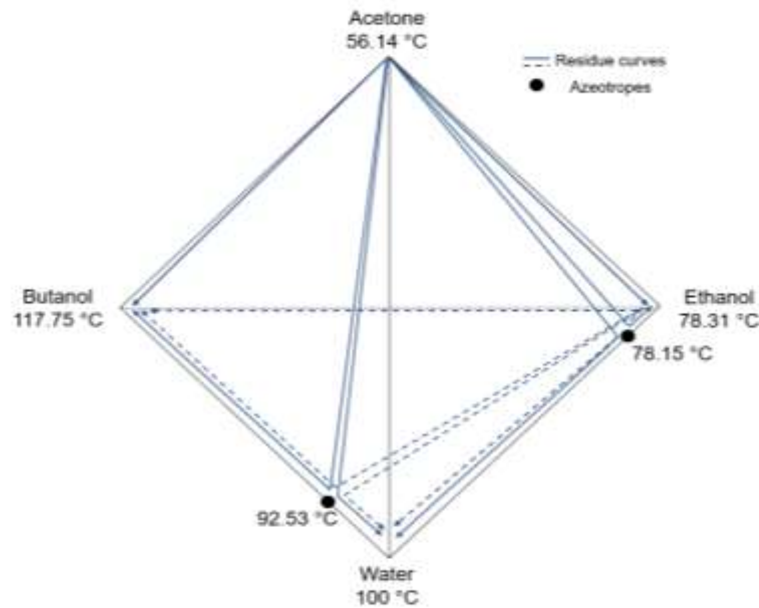

Figure 4-23. Residue curves map for the mixture Acetone – Butanol – Ethanol – Water. P=1 atm.

The region formed at the front of the diagram is delimited by the azeotropes between butanol and ethanol with water and for acetone vertex and the water vertex generate a smaller region. The second distillation region formed at the back of the figure is the most important region, since it is delimited by the ethanol, butanol and acetone vertexes, in addition the azeotropes present in the mixture are show in **Figure 4-24**.

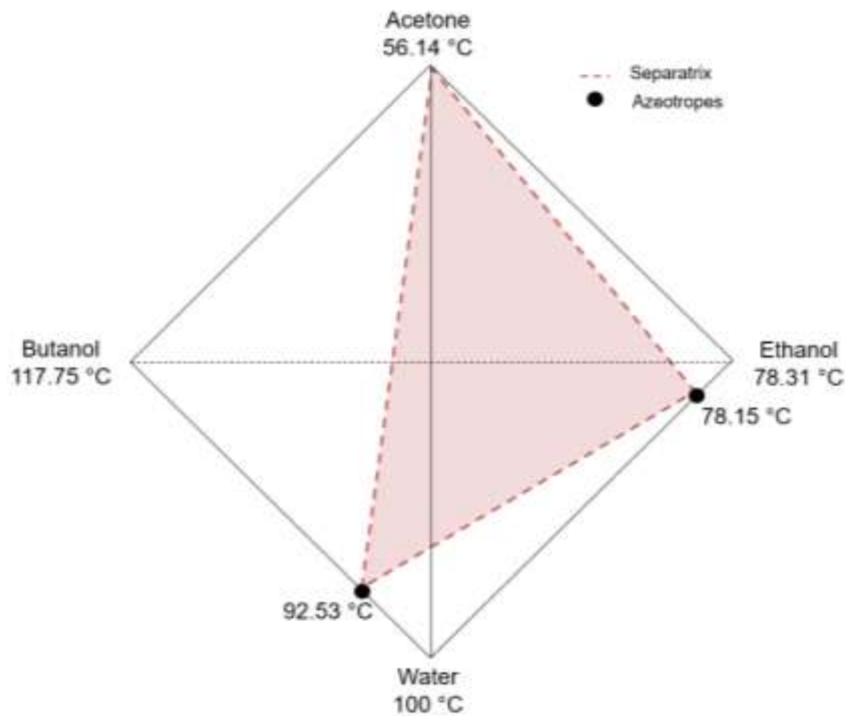

Figure 4-24. Separatrix for the mixture Acetone – Butanol – Ethanol – Water. P=1 atm.

3. Separation in regime ∞/∞: The initial mixture obtained during fermentation has 0.97 of final molar fraction of water, which makes difficult to analyze the separation system. For this reason, based on the information reported by Sánchez et. al [138], the stream is send into a distillation column to separate as much water as possible and, from the ABE and water streams obtained, the thermodynamic analysis is started. To obtain butanol from this mixture, different separation stages must be proposed. Initially, the feed point is located at composition of 0.64 water, 0.22 butanol, 0.12 acetone and 0.02 ethanol. This point is located in the second distillation zone, since a direct balance line is drawn to separate the acetone. With the feed flow it is possible to find distilled and bottoms flows using the lever rule. Likewise, to know the initial estimates that will be used in the simulations, the residue curve that unites the distillate with bottoms is drawn, segmenting it in a number of stages where compositions are obtained. The initial estimates in relation to the number of stages used in the simulated distillations are taken as reported by Sánchez et. al [138]. **Figure 4-25** shows the separation path in the quaternary diagram of the mixture.

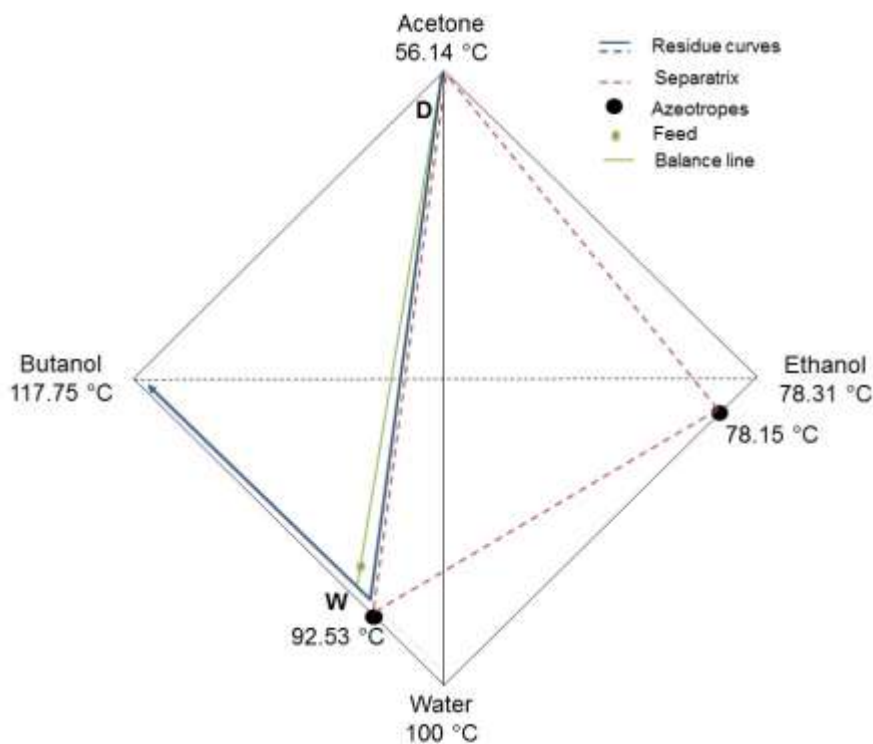

Figure 4-25. Acetone separation path. P=1 atm

In this first distillation column, pure acetone is obtained as distilled component while a stream bottoms with a composition of 0.73 water, 0.25 butanol and 0.02 ethanol is collected. The same procedure is carried out, but with a ternary diagram, where the feed composition is the bottoms from column mentioned above.

Table 4-13. Distillation tower 1 liquid fraction estimates for the simulation

Distillation tower 1				
Stage	Acetone	Butanol	Ethanol	Water
1	0,000	0,250	0,020	0,730
2	0,130	0,210	0,020	0,640
3	0,280	0,170	0,010	0,540
4	0,400	0,150	0,010	0,440
5	0,580	0,090	0,010	0,320
6	0,720	0,070	0,010	0,200
7	0,850	0,040	0,010	0,100
8	1,000	0,000	0,000	0,000

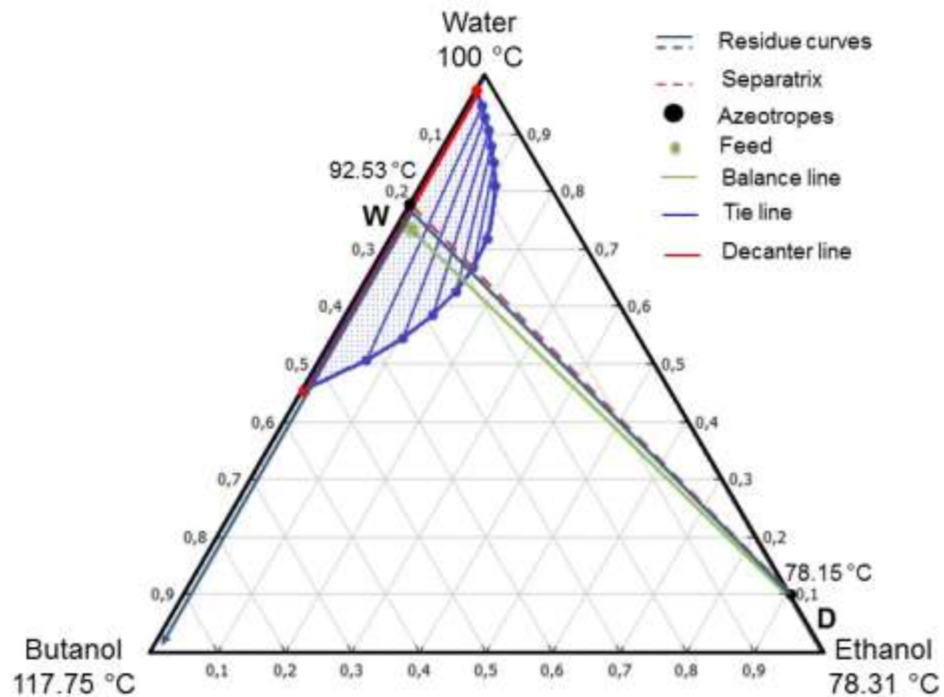

Figure 4-26. Ethanol and butanol separation path.

Figure 4-26 shows how the feed composition is very near to the butanol-water line. However, it is possible to draw a balance line for obtaining the azeotrope of ethanol with water. To obtain the initial estimates, the same procedure is performed with the residue curve. In this case, the residue curve is so near to the separatrix, thus, the concentrations of it can be assumed as the initial estimates. Likewise, the diagram of the liquid-liquid equilibrium that is formed between water and butanol is presented. The bottom composition is inside the dome, so it is necessary to enter this mixture into a decanter to form the two phases, obtaining the marked compositions by the extremes of the red line represented in the triangle. The phase with the lowest water content, it will be fed to the third and last column to obtain pure butanol. For this last column, it is fed with the stream from the decanter and a line is drawn that joins the azeotrope of butanol-water and the vertex of butanol, to obtain this component through bottoms. In the following tables is presented the initial estimates for each of the distillation columns.

Table 4-14. Distillation tower 2 liquid fraction estimates for the simulation

Distillation tower 2							
Stage	Butanol	Ethanol	Water	Stage	Butanol	Ethanol	Water
1	0,000	0,900	0,100	13	0,130	0,400	0,470
2	0,005	0,870	0,125	14	0,130	0,370	0,500
3	0,010	0,840	0,150	15	0,140	0,330	0,530
4	0,015	0,780	0,205	16	0,160	0,280	0,560
5	0,020	0,750	0,230	17	0,162	0,240	0,598
6	0,060	0,700	0,240	18	0,170	0,200	0,630
7	0,062	0,660	0,278	19	0,180	0,160	0,660
8	0,070	0,620	0,310	20	0,181	0,120	0,699
9	0,090	0,570	0,340	21	0,190	0,080	0,730
10	0,091	0,540	0,369	22	0,210	0,030	0,760
11	0,100	0,490	0,410	23	0,260	0,000	0,740
12	0,120	0,460	0,420				

Table 4-15. Distillation tower 3 liquid fraction estimates for the simulation

Distillation tower 3					
Stage	Butanol	Water	Stage	Butanol	Water
1	0,250	0,750	21	0,634	0,366
2	0,269	0,731	22	0,653	0,347
3	0,288	0,712	23	0,672	0,328
4	0,308	0,692	24	0,692	0,308
5	0,327	0,673	25	0,711	0,289
6	0,346	0,654	26	0,730	0,270
7	0,365	0,635	27	0,749	0,251
8	0,384	0,616	28	0,768	0,232
9	0,404	0,596	29	0,788	0,212
10	0,423	0,577	30	0,807	0,193
11	0,442	0,558	31	0,826	0,174
12	0,461	0,539	32	0,845	0,155
13	0,480	0,520	33	0,864	0,136
14	0,500	0,500	34	0,884	0,116
15	0,519	0,481	35	0,903	0,097
16	0,538	0,462	36	0,922	0,078
17	0,557	0,443	37	0,941	0,059
18	0,576	0,424	38	0,960	0,040
19	0,596	0,404	39	0,980	0,020

20	0,615	0,385	40	1,000	0,000

After finishing the main mixture, the same procedure is carried out for the other three mixtures that are in the process.

Mixture Butyl Acetate – Acetic acid - Water

1. Azeotropes Identification

Approach of phase equilibria

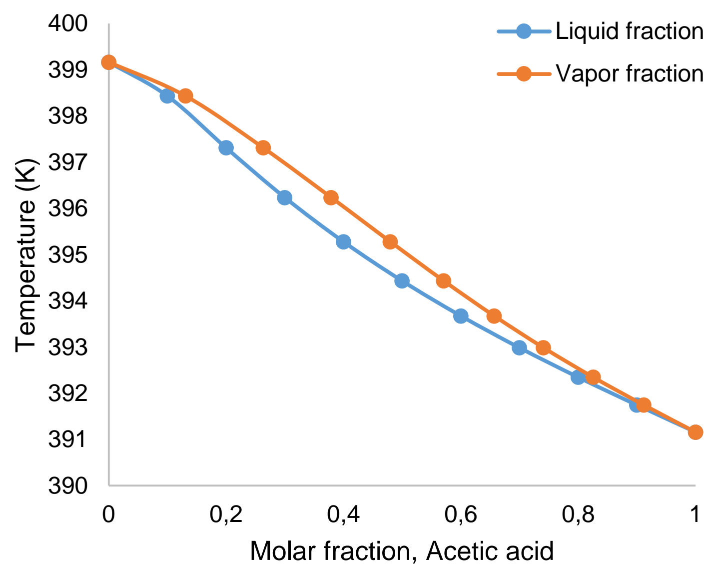

Figure 4-27. VLE Butyl Acetate – Acetic acid [139]

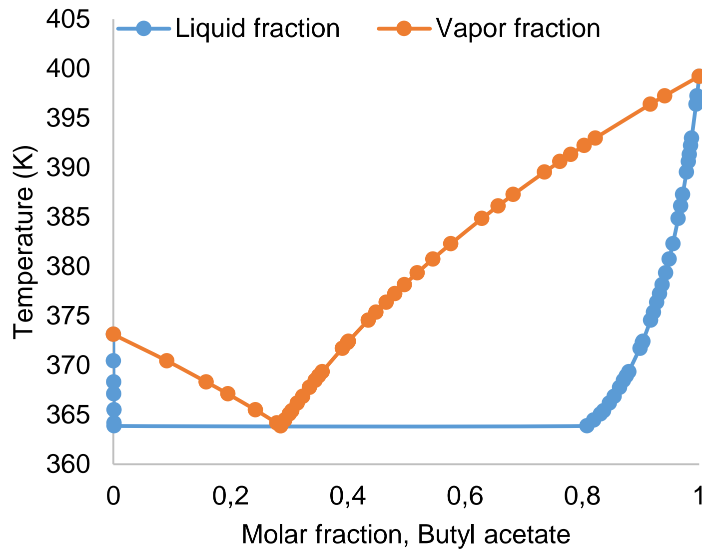

Figure 4-28. VLE Butyl Acetate – Water [140]

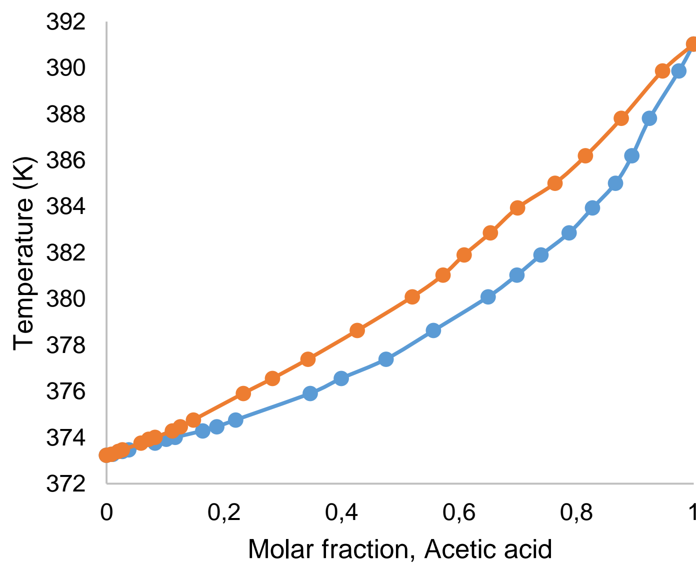

Figure 4-29. VLE Acetic acid – Water [141]

The mixture has an azeotrope between the water and the butyl acetate. In addition, these components have a liquid – liquid equilibrium.

2. Topological characterization of the sample and 3. Separation in regime ∞/∞.

Figure 4-30 shows the ternary diagram for this mixture, in which the liquid-liquid equilibrium and the only azeotrope can be appreciated. Likewise, this mixture does not have any separatrix so it only has a separation zone.

The feeding point is inside the dome of the liquid - liquid equilibrium so initially, it is necessary to enter the mixture into a decanter, after this, the organic phase is fed to a distillation tower to obtain the butyl acetate by bottoms.

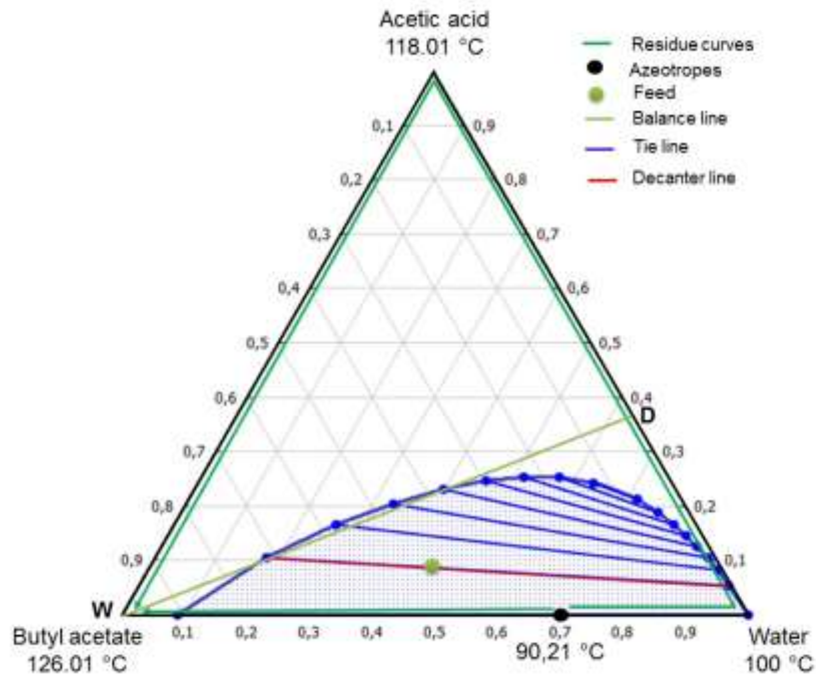

Figure 4-30. Ternary diagram and separation path for the mixture butyl acetate – acetic acid – water

Mixture Butyl Acrylate – Acrylic acid - Water

1. Azeotropes Identification

For this mixture, not all the binary diagrams were found reported in the literature, so only those reported are analyzed.

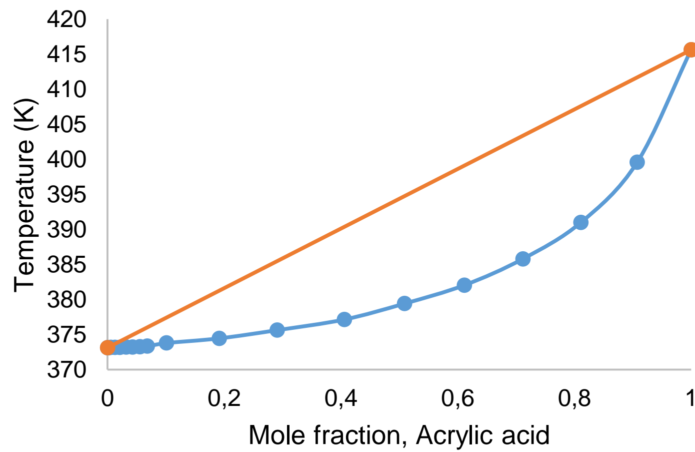

Figure 4-31. VLE Water – Acrylic acid [142]

2. Topological characterization of the sample and 3. Separation in regime ∞/∞.

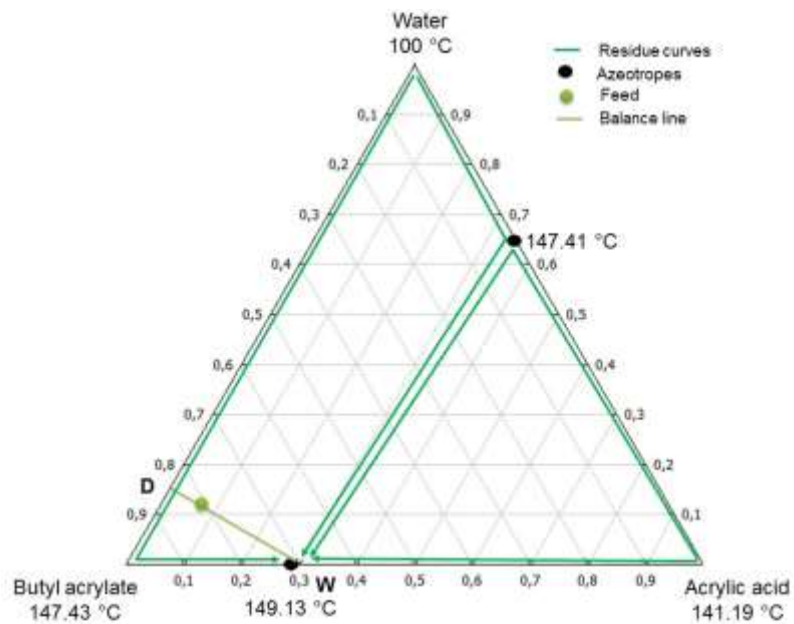

Figure 4-32. Ternary diagram and separation path for the mixture butyl acrylate – acrylic acid – water

In the production of butyl acrylate, a mixture of this component with water and acrylic acid is obtained. As can be seen in **Figure 4-32**, this mixture has two azeotropes. The first azeotrope is a mass composition of water of 0.65 and acrylic acid of 0.35 to 147.41 ° C, this point is characteristic saddle type. The second azeotrope occurs at a mass fraction of

butyl acrylate of 0.7 and acrylic acid of 0.3 to 149.13 ° C and is of the stable node type. When carrying out the residual curves corresponding to the mixture in the ternary diagram, two distillation regions are observed. The feeding point is located within the distillation region delimited by both azeotropes the vertex corresponding to water and the apex of butyl acetate. After locating this point the balance line is drawn, and it is found that by bottoms the azeotrope will be obtained in water and acrylic acid and by distillate a butyl acrylate at a concentration of around 0.85.

Mixture Butyl Propionate – Propionic acid - Water

1. Azeotropes Identification

For this mixture, not all the binary diagrams were found reported in the literature, so only those reported are analyzed.

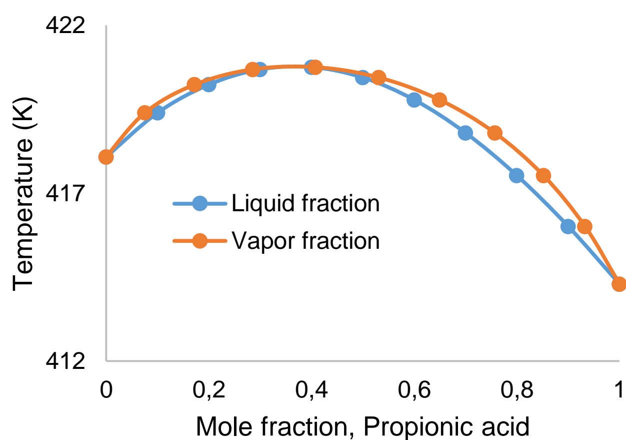

Figure 4-33. LVE Propionic acid – Butyl propionate [143]

2. Topological characterization of the sample and 3. Separation in regime ∞/∞.

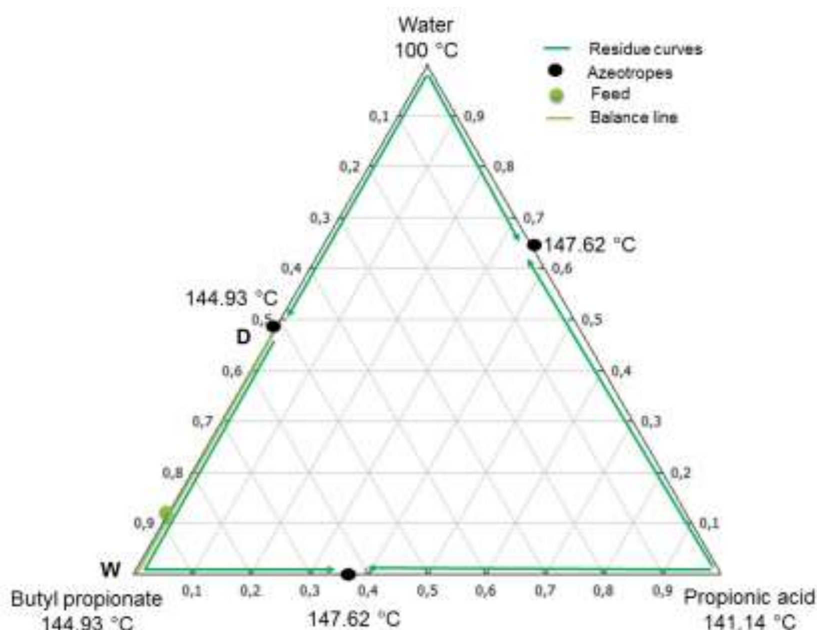

Figure 4-34. Ternary diagram and separation path for the mixture butyl propionate – propionic acid – water

In the process of obtaining butyl propionate there was a total conversion of the propionic acid so that a stream with a mass composition of 0.9 of butyl propionate and 0.1 of water is obtained. As seen in **Figure 4-34** this mixture has three binary azeotropes. The azeotrope formed between water and propionic acid is at 147.62 ° C at a composition of 0.35 and 0.65 respectively at 147.62 ° C. The azeotrope between propionic acid and butyl propionate is at a composition of 0.65 and 0.45 respectively, at a temperature of 147.62 ° C. The third azeotrope is formed between water and butyl propionate at a mass composition of 0.5 of each component at 144.93 ° C.

4.6.2 Technical analysis

For the technical analysis, simulations of plantain peel and milk whey were performed for a feed flow of 4 ton/h. This flow was chosen taking into account an increase in milk whey production for 2017 based on the data previously reported for the last years. The same value was chosen for the plantain peel so that they were comparable In the pretreatment and fermentation stages, the yields obtained in the experimental section were used. After this, taking into account the yields, the amount of glucose obtained per gram of plantain peel was calculated and the relation was made to know the feeding flow and its

concentration for the glucose simulation. This in order not to feed the same 4 ton/h of glucose alone since the compound flows would be too large and not very comparable. For this, a feed of 100 ton/h of a solution of glucose and water was made, with a glucose fraction of 0.019 (the one obtained for the plantain peel), that is, a flow of 1.9 ton/h. After this, the results presented in **Table 4-16** were obtained.

Table 4-16. Flows and mass fraction for the added value compounds for each raw material.

	Glucose		**Plantain Peel**		**Milk Whey**	
	Flow(kg/h)	Mass Frac	Flow(kg/h)	Mass Frac	Flow(kg/h)	Mass Frac
Acetone	500.033	0.982	516.856	0.977	54.454	0.979
Ethanol	87.059	0.626	5.038	0.704	1.465	0.733
Butyl acetate	369.646	0.954	112.521	0.972	10.362	0.969
Butyl acrylate	404.093	0.999	139.817	0.997	12.083	0.999
Butyl propionate	395.819	0.999	116.213	0.999	10.019	0.999
Electricity	NA		533.4 kW		NA	

NA: Not Applicable

The processes with glucose and plantain peel as raw materials present similar values for each one of the obtained compounds. However, for the milk whey, the flows are very low. This is because when 4 ton/h of milk whey is fed to the process, no other component is added in large quantities and a certain concentration of sugars is obtained. While in the case of the plantain peel for the same flow of 4 ton/h, a 10 times greater flow of water must be added to carry out the pretreatments. Where the same concentration of sugars will be obtained as in the milk whey but in one volume 10 times higher. During the pretreatment, it is necessary to add water increasing the total flow. The amount of cellulose and hemicellulose that can be converted into glucose are almost one ton. While in the milk whey it is only the lactose, therefore in the case of the plantain peel there is greater availability of sugars, therefore, a greater amount of butanol can be obtained. Based on this, a flow ten times higher of milk whey should be used to obtain flows similar to those of the plantain peel and glucose.

In all three cases, the only compound that cannot be obtained with a mass fraction greater than 0.9 is ethanol. As can be seen in the flows the amount of ethanol generated during the fermentation is very low in relation to the other components, in addition to having a concentration lower than that used in the industry, for this reason half of the value reported in the literature is used. In addition, due to the large amounts of water, very large refluxes are required for the purification of this compound, which is why it is not possible to obtain higher purities, since the increase of reflux in a tower means greater energy consumption for the process. For the other components, considerable flows are obtained with respect to the amount of raw material fed. In addition, it is important to take into account that the process with plantain peel allows to obtain electricity from the solids residues.

4.6.3 Economic assessment

4.6.3.1 Butanol production

Initially the economic evolution for the production of butanol was made from each of the raw materials and thus to know the production value of this. The economic distribution for each case is presented in **Figure 4-35**.

Figure 4-35. Distribution of costs in the butanol production process from each raw material at a scale of 4 ton/h

As can be seen in the figure for the three cases, the utilities are those that have the highest cost in all three cases. Followed by the depreciation of capital and raw materials. Likewise, the process with glucose has much lower values since it does not require pre-treatment stages. After this, the butanol production value was calculated from each raw material, the results are presented in **Table 4-17**.

Table 4-17. Butanol production costs for each raw material

Glucose	Plantain peel	Milk Whey
1.82 USD/kg	1.41 USD/kg	19.52 USD/kg

As can be seen in the table, the plantain peel is the raw material that allows butanol to be obtained at a low cost, a value very close to that of glucose. However, the value for the whey is too high, this is due to the yields presented with this raw material that allow obtaining less quantity of product. Glucose despite having more economical values is a very expensive raw material so looking for alternatives to obtain it as waste and in this case we can say that the plantain peel is an excellent alternative. In addition, it should be taken into account that the yields of this work were used here, but in the literature there are higher reported yields that would allow obtaining better results in production costs.

4.6.3.2 Platforms

In this analysis, the distribution of costs of the processes according to the scale was initially evaluated, in order to know the influence of the capacity of the plant in each of them. The parameters evaluated in the distribution of costs include the value of all the raw materials required during the process, the cost of the utilities that each equipment requires, the maintenance required in the plant, the labor costs, as well as the general costs, administrative costs and capital depreciation. For the calculation of this analysis, the initial flow proposed for each raw material is taken as a base and some minor scales are evaluated to know the behavior of these parameters.

The **Figure 4-36**, **Figure 4-37** and **Figure 4-38** represent the percentage of influence of each of the parameters evaluated in the total production costs for glucose, plantain peel and milk whey respectively.

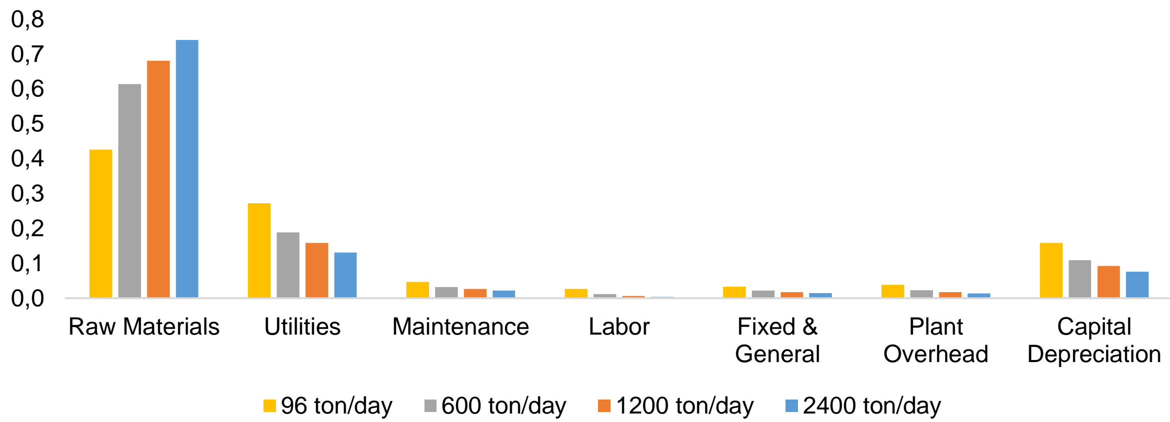

Figure 4-36. Cost distribution for different plant capacities for the process with glucose

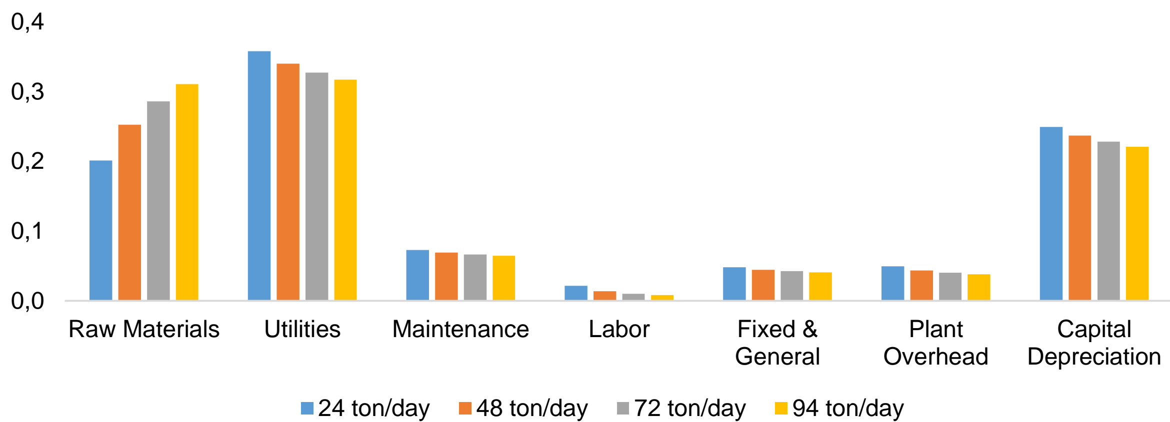

Figure 4-37. Cost distribution for different plant capacities for the process with plantain peel

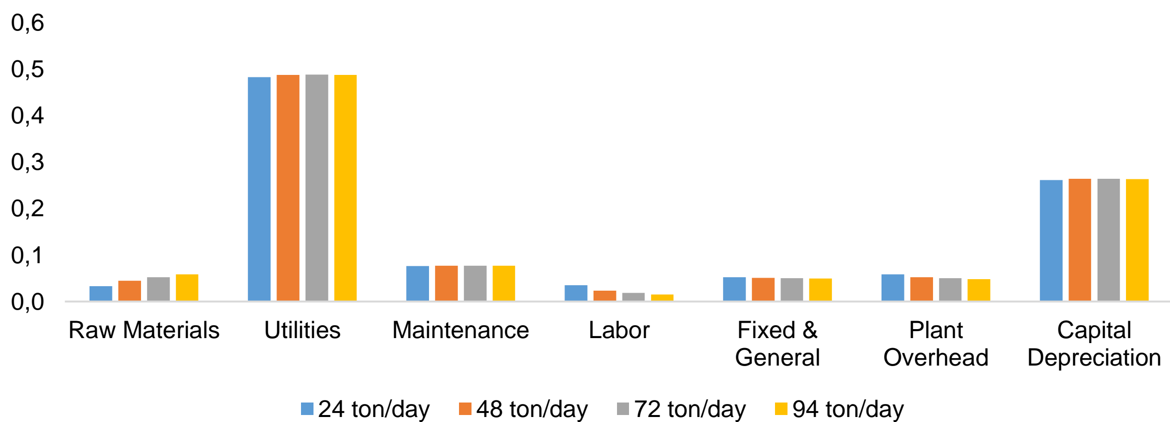

Figure 4-38. Cost distribution for different plant capacities for the process with milk whey

The three cases evaluated present a very similar cost distribution and the same trend as the scale increases. As can be seen on a larger scale, the influence of the cost of raw materials is greater, since as the capacity of the plant increases, more of these inputs are required. In the same way the percentage of influence of the utilities decreases, but not necessarily because less quantity is required, but because when increasing the percentage of costs of the raw material the other costs are redistributed, generating that the costs of the utilities have less representation in production costs. The milk whey has a higher cost (3 USD/ton) than that of the plantain peel (1 USD/ton), because specialized transportation is required because it is a liquid waste. In spite of this, the process with milk whey is the one that has less influence in general of the raw material cost, due it is the only one that must be purchased. In the process with the plantain peel the water is required too, which increases the costs. On the other hand, the process carried out with glucose has the least influence on utilities. This is due to the fact that the other two raw materials involve pre-treatment stages with high temperatures, which make it necessary to use a greater flow of these. In general, the other parameter with the greatest influence on total costs is depreciation, which also decreases with the increase in plant capacity. The other parameters have a similar behavior and are not as representative, as the scale increases they decrease so much that in some cases they are very close to zero.

After the evaluation of the distribution of costs, an analysis of the net present value at different scales is made, to know the time in which the investment made is recovered. This time is when the net present value is equal to zero, that is, the curve crosses the x-axis. From this point on, the process is viable. It also allows to know the time it will take the process to reach this point, and to know if it is less than the projected time for the process than. **Figure 4-39**, **Figure 4-40** and **Figure 4-41** present the results obtained for glucose, plantain peel and milk whey respectively. Initially an analysis of each process was done increasing the magnitude orders of the scale, fixing arbitrary values. However, when evaluating the milk whey process, it was found that for the same values used with the other two raw materials, this process still did not have a NPV of zero, so it was necessary to establish higher scale values for this process .

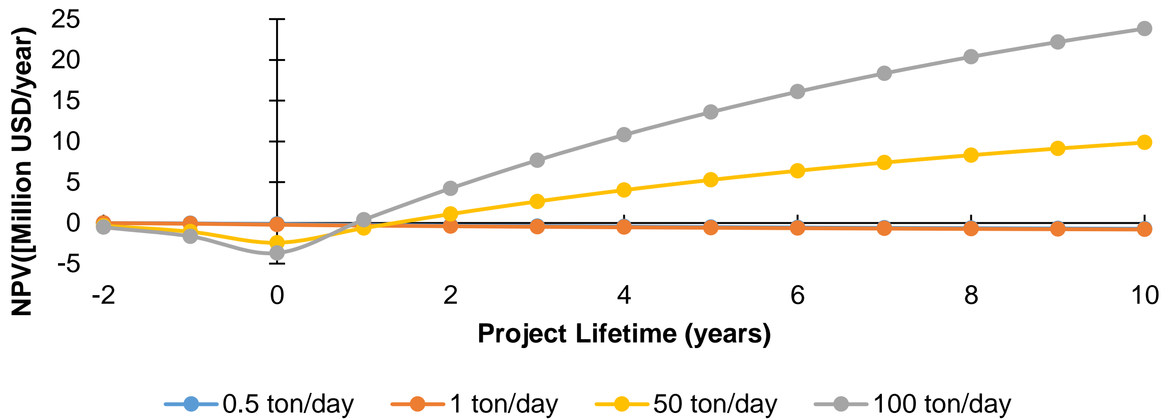

Figure 4-39. VPN over project lifetime for the process with glucose

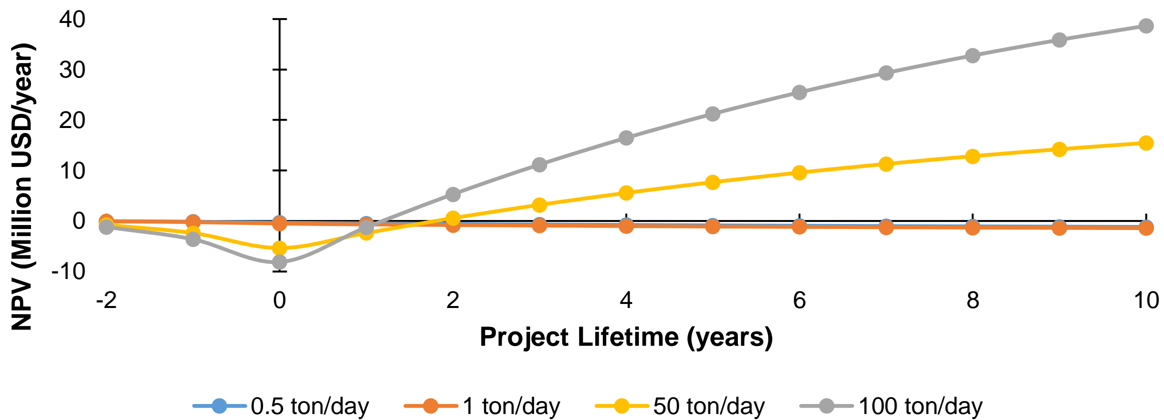

Figure 4-40. VPN over project lifetime for the process with plantain peel

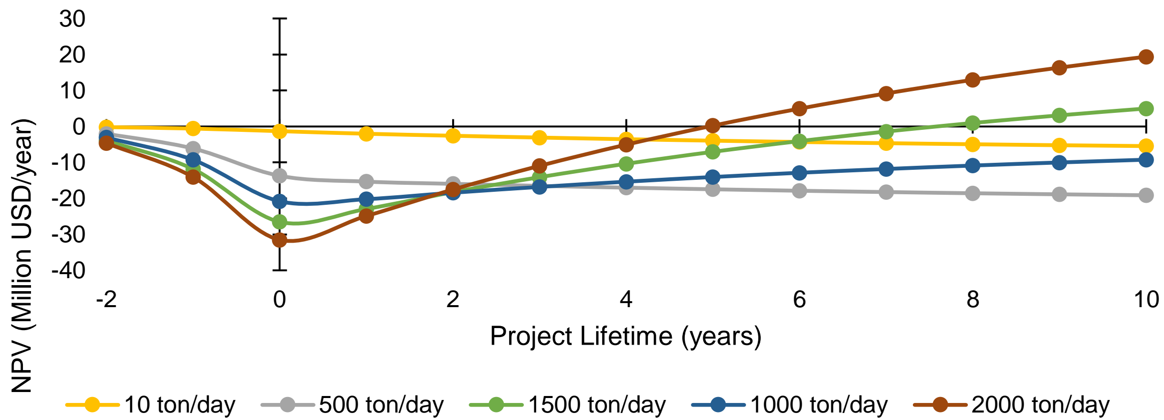

Figure 4-41. VPN over project lifetime for the process with milk whey

The graphs show that when the raw material of the process is glucose or plantain peel, around 50 tons per day are required for the process to reach the NPV equal to zero. for this scale two years of operation are required to generate profits. However, for the milk whey it is required around 1500 tons per day for the process to be viable in 8 years. This is due to what was analyzed previously because for the same flow the same quantities of products are not reached, so the profits generated by the sale of these are very low and do not cover production costs. In this way, the plantain peel as a raw material is a waste with greater economic viability for the production of value-added products such as butyl acetate, butyl acrylate and butyl propionate.

Below are the results of the analyzes carried out to know the influence of the cost of selling the products. This analysis is already done for some products, as is the case of ethanol, the cost of sale is affected by market volatility. Taking into account the previous results for glucose and plantain peel was done for a capacity of 50 tons per day and for the milk whey for one of 1500 tons per day.

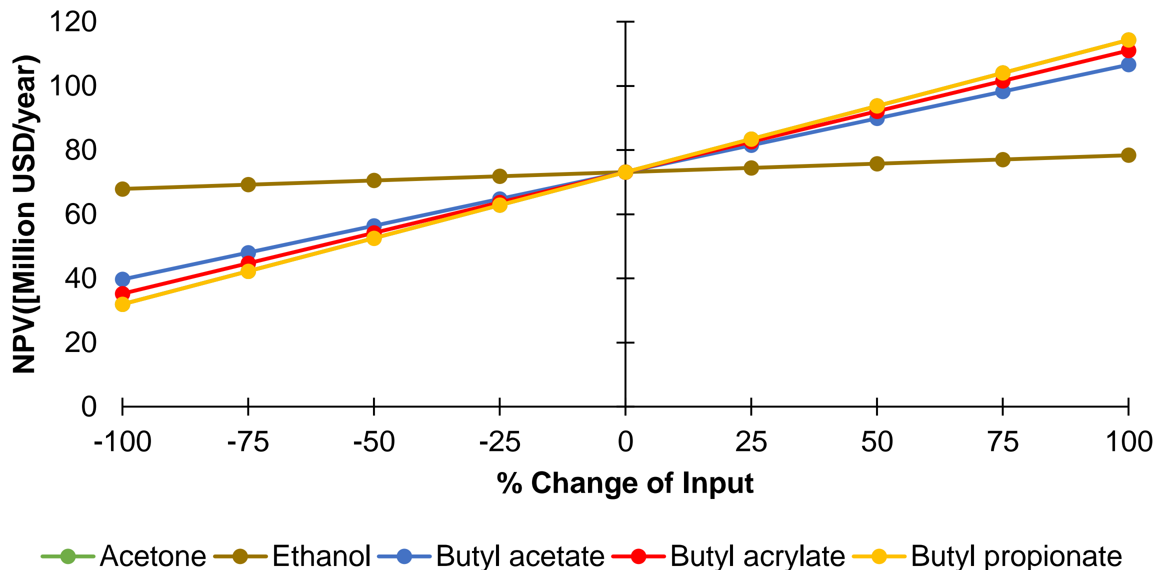

Figure 4-42. Influence of products cost in the process with glucose

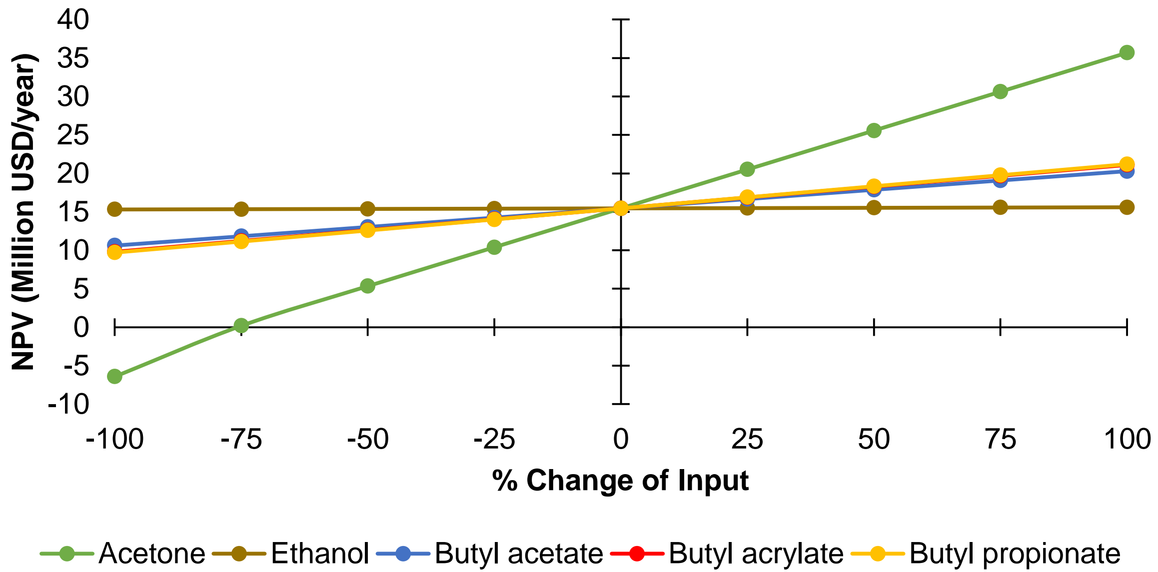

Figure 4-43. Influence of products cost in the process with plantain peel

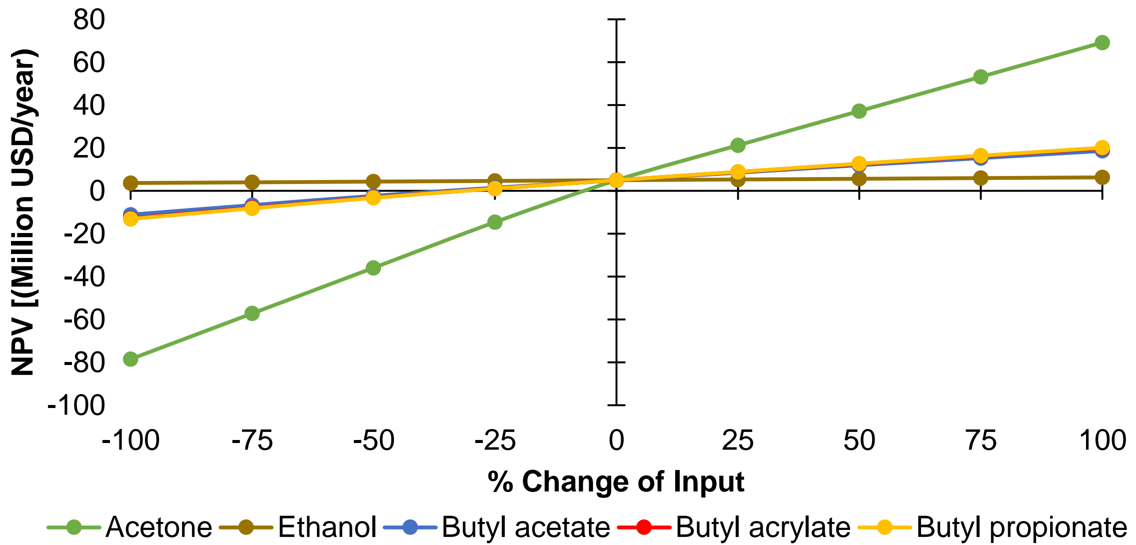

Figure 4-44. Influence of products cost in the process with milk whey

This analysis shows how a change in the sale price of each product can make the process viable or not. In the case of glucose, the process at this scale would not be affected by a change in these, since the NPV is very high. In the case of plantain peel, a reduction in the sale price of acetone of 75% would cause the process to no longer be viable, however it is very difficult to have such high reductions in the market of this solvent. Finally, for the milk

whey, the only product that would not generate a negative impact due to the change in its sale value is ethanol. On the other hand, a change of less than 10% in the price of acetone would cause the net present value to be negative, this is very likely to occur and therefore a larger scale would have to be evaluated in order not to present this problem. In the same way a reduction in the price of acetate, acrylate and propionate that is very similar to 25% would cause the same effect.

In conclusion, the process with plantain peel allows obtaining higher flows of value-added products than the whey, so it is considered the raw material with greater economic viability.

4.6.4 Environmental assessment

Below are the results obtained from the environmental analysis carried out with the software WAR GUI. **Figure 4-45** shows the graphic. As a result, it was found that only five of the eight categories evaluated generate environmental impact. These are HTPI, HTPE, TTP, ATP and PCOP, related to skin exposure and inhalation generating toxicity, photochemical oxidation and acidification. The first categories evaluate the impact generated on humans, this means that if the outlet streams are discharged into water, the consumption or contact with these can generate toxicity to people and skin affections, this is due to the presence of compounds such as solvents and acids in waste streams. In the case of HTPI, the process with glucose has the least impact for this category, since not having pretreatment stages generates less amount of contaminants. For the HTPE the three processes have a negative impact, that is to say that the inlet streams are more dangerous when being exposed to the skin or being inhaled than those of outlet. In the case of TTP, the process with milk whey is the one that generates more pollution when it is poured on the land, due to the currents that contain proteins. For the ATP it is observed that the processes with glucose and plantain peel have no impact, while for the milk whey it has a negative impact. Finally, for the PCOP category it is observed that glucose has a negative impact, while the other two processes have a positive impact. The potential environmental impact (PEI) is not only affected by the composition of the waste streams of the process. It is also affected by the energy required for the process. The latest is the reason why the process that use milk whey is more polluting. First, it requires more energy in the pretreatment, and second the outlet streams contain proteins and fat that are very contaminant.

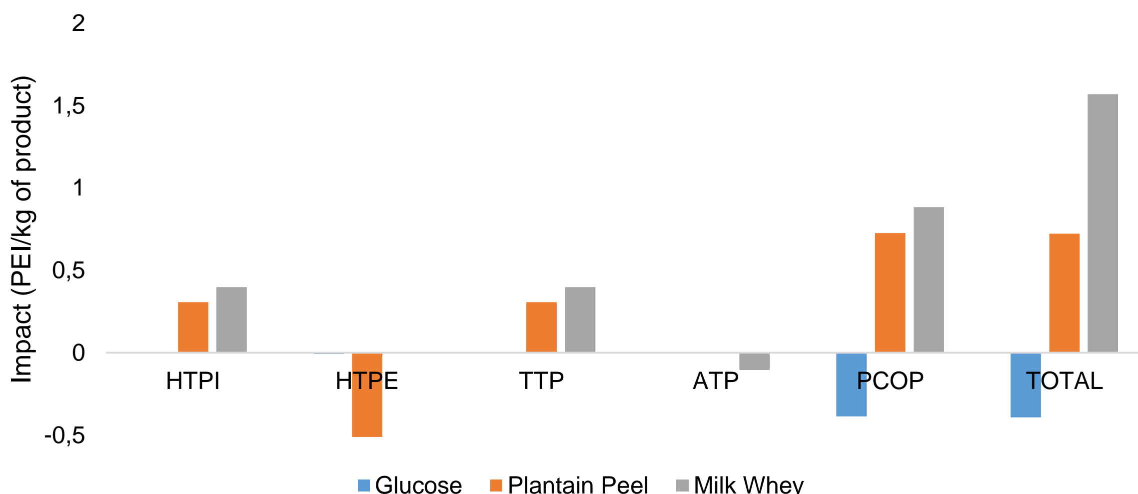

Figure 4-45. Potential environmental impact per kilogram of product

Additionally, it is necessary to take into account that the economic analysis takes into account the reuse of 90% of wastewater. This helps reduce the pollution generated in large quantities. On the other hand, the process with glucose is the least polluting because in its waste streams it does not have compounds as polluting as the protein, the fats and the solid material. In addition, since it does not require pre-treatment, it does not consume as much energy as the other two processes.

5. Other complementary work

During the development of this thesis, a research stay was made at the Univerisas Nacional Autonoma de Mexico, in the Metabolic Engineering Laboratory. In order to strengthen knowledge in fermentation, two main experiments were carried out with genetically modified strains of *Escherichia coli*, one for the production of ethanol (MS04) and the other for the production of lactic acid (JU15) both from mixtures of glucose and xylose. This chapter presents the results obtained and the approach of a biorefinery for the production of these value-added compounds.

5.1 Experimental procedure

The procedure followed for the fermentation of both microorganisms is very similar. The objective with the ethanol strain was to evaluate the influence of the concentration of biomass in the inoculum, while for the lactic acid only the experiment was carried out under certain conditions.

5.1.1 Microorganism and inoculum

The strain of correspondent *Escherichia coli* was growth in AM1 medium with 40 g/L of xylose, this medium is composed by ammonium phosphate (7.56 mmol/L), diamonic phosphate (19.92 mmol/L), potassium chloride (2 mmol/L), $FeCl_3 \cdot 6H2O$ (8.88 μmol/L), $CoCl_2 \cdot 6H_2O$ (1.26 μmmol/L), $CuCl_2 \cdot 2H_2O$ (0.88 μmol/L), $ZnCl_2$ (2.20 μmol/L), $Na_2MoO_4 \cdot 2H_2O$ (1.24 μmol/L), H_3BO_3 (1.21 μmol/L), $MnCl_2 \cdot 4H_2O$ (2.5 μmol/L), Betaine KCl (1 mmol/L) [144]. A cryovial with 1 ml of strain and 1 ml of glycerol 80% was added in 250 ml flasks with 200 ml of work volume to develop the inoculum at 37 °C and 150 rpm. After 15 hours the inoculum was centrifuged and the biomass suspended once again in new culture medium to adjust the initial biomass concentration.

5.1.2 Fermentation

The fermentation was developed in mini-fermenters with 200 ml of AM1 medium supplemented with 32 g/L of xylose, 42 g/L of glucose and 4 g/L of sodium acetate to simulate a corn stover hydrolysate. The conditions were 37 °C, 150 rpm and pH 7. The fermenters had a pH controller with KOH 2M for the ethanol experiment and KOH 6M for the lactic acid experiment. The biomass growth was measured using a spectrophotometer at 600 nm. The sugars and the lactic acid was determined by HPLC analysis (Waters U6K, Millipore Co., Milford, MA, USA). The separation system comprises an Aminex HPX-87H ion exclusion column (300x7.8 mm; Bio-Rad Laboratories, Hercules, CA), 5 mM H2SO4 solution as the mobile phase (0.5 ml/min) at 45 °C, a photodiode array detector at 210 nm (Model 996, Waters, Millipore Co) and a refractive index detector (Model 2410, Waters, Millipore Co., Milford, MA, USA). Ethanol was analyzed by gas chromatograph using n-butanol as internal standard (6850 Series GC System, Agilent, Wilmington, DE).

5.1.3 Proposed biorefinery

For the biorefinery, the scheme for obtaining ethanol presented in **Figure 5-1** is proposed. A fraction of glucose and xylose are fed to a fermenter at 30 °C to obtain ethanol. Subsequently there is a separation and dehydration stage where the ethanol is purified in a distillation tower up to 60% w/w, and then in a rectification tower the ethanol is purified to the azeotropic composition (96%). Finally, a 4 Å molecular sieve purifies the ethanol up to 99.6% w/w.

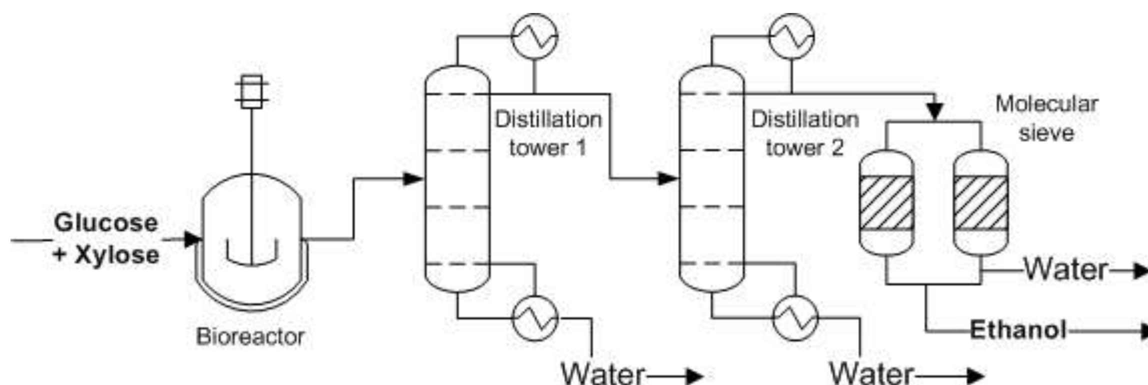

Figure 5-1. Ethanol production process

Figure 5-2 shows the proposed process for the purification of lactic acid. The separation and purification stage of lactic acid is much more complex than that of ethanol. This process requires a stage of precipitation, recovery of lactic acid, evaporation, liquid-liquid extraction and distillation.

The precipitation stage consists in adding calcium hydroxide to precipitate the lactic acid and separate it from the fermentation broth. This stage was simulated at 90 ° C according to what was reported by Da-Jeong Min et al [145]. Then the filtration is carried out and the solid fraction is mixed with a sulfuric acid solution to obtain lactic acid and calcium sulfate. After this the calcium sulphate is filtered and separated from the lactic acid stream. An organic solvent is used to remove lactic acid from water in an extraction column to remove all impurities that remain in lactic acid [146], [147]. After the extraction, the lactic acid goes through an extraction tower with water at 80 ° C as solvent. Finally, this mixture is distilled.

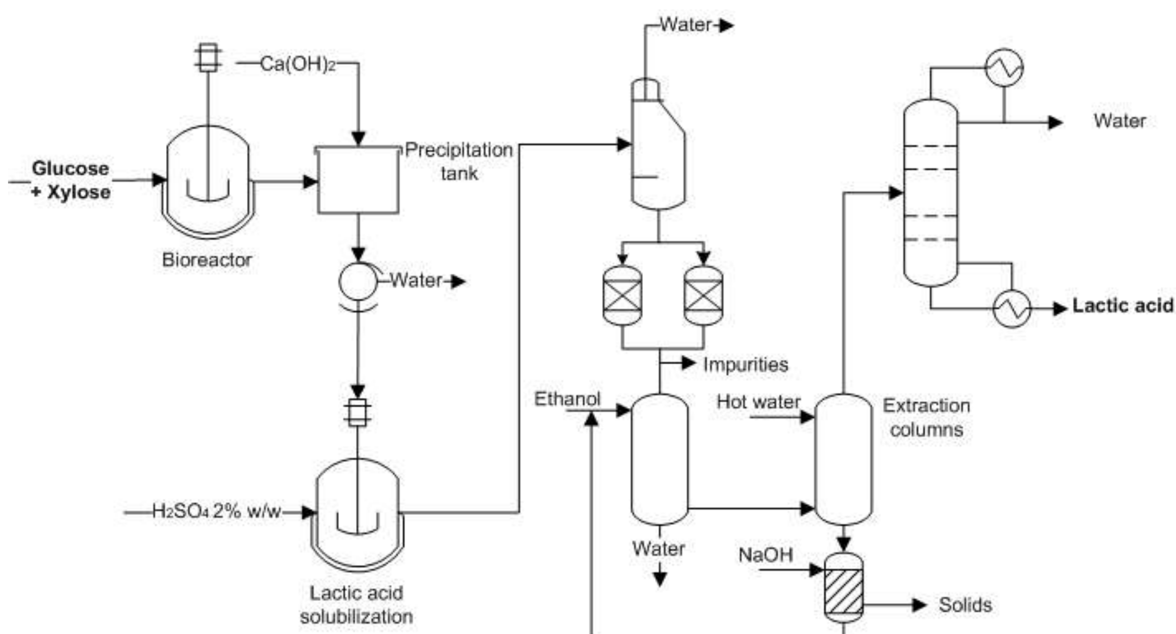

Figure 5-2. Lactic acid production process

With this proposed biorefinery, a simulation was carried out with a technical and economic analysis in the same way as described in the methodology. For this case, a feeding flow of 100 tons/day with a concentration of glucose and xylose of 40 g/L and 30 g/L respectively was used. For the economic analysis a price of 1.3 USD/kg of ethanol and 1.89 USD/kg of lactic acid was used [112].

5.2 Results

5.2.1 Ethanol fermentation

The concentrations evaluated in this work were 0.07, 0.25, 0.65, 1 and 1.68 optical densities (OD) at 600 nm (1.33 g dry cell/L at 1 $OD_{600\ nm}$).

Figure 5-3shows the growth of biomass with respect to time. This graph shows the exponential growth of the microorganism followed by the stationary phase in all cases. Depending on the initial concentration of biomass, the time to reach the stationary phase is different. When it is less, more time is required to reach this phase.

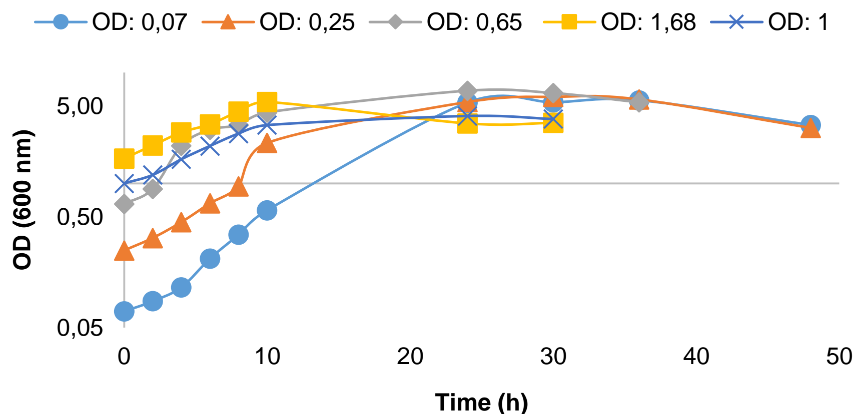

Figure 5-3. Biomass growth at different concentrations of inoculum

Equally, in this graph it can be observed that this microorganism does not have an adaptation phase, since from the beginning it has the exponential phase. This adaptation phase can also be modified by genetic engineering and allows having better production times. The exponential phase where the greatest growth of the biomass is evidenced is between 10 and 20 hours depending on the concentration of the inoculum, being the shortest time for those that have the highest concentration and the longest time for those with the lowest concentration.

Figure 5-4 and **Figure 5-5** show the consumption of sugars in each of the experiments carried out. As expected, the glucose was consumed with greater speed in the fermentation with an inoculum with OD: 1.6. However, for all the experiments at 30 hours of fermentation the glucose consumption was total.

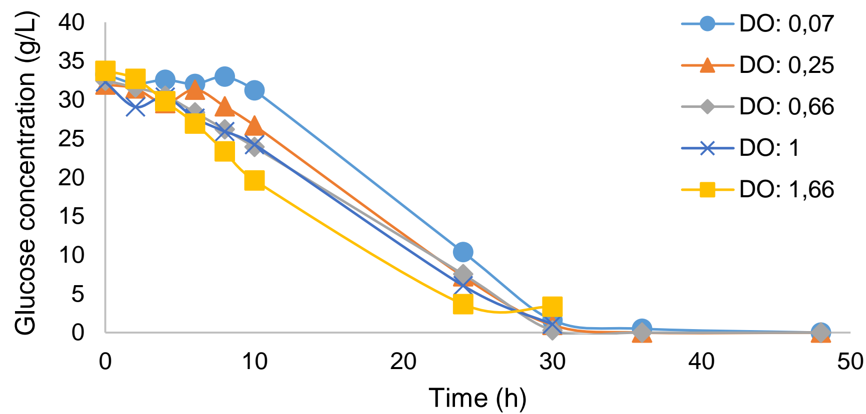

Figure 5-4. Glucose consumption at different concentrations of inoculum

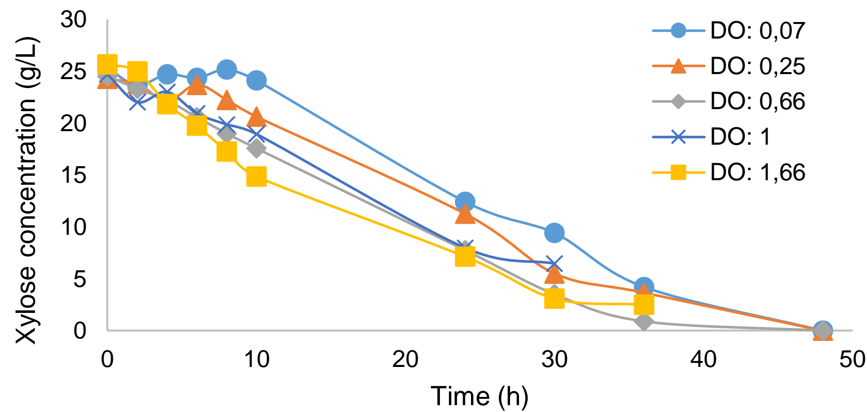

Figure 5-5. Xylose consumption at different concentrations of inoculum

Like glucose, xylose is consumed in its entirety in all fermentations after 48 hours of fermentation. Comparing both graphs, it can be observed that the microorganism is not selective to a single sugar, since from the beginning of the fermentation both are consumed. Also, that the microorganism does not depend on the presence of glucose in the medium, because it is consumed with greater speed and it is exhausted in the medium, and after that it continues without any problem the consumption of all the available xylose in the medium. In the case of sugars, inoculum concentration has a similar influence as in biomass growth, although in intermediate points for a specific time the concentration of sugars is slightly higher in the fermentation inoculated with 0.07 OD than in the inoculated with 1.66 OD, in the end the consumption of sugars is total.

In **Figure 5-6** the production of ethanol can be observed in all the fermentations carried out. Initially, those fermentations that have a higher initial concentration of cells produce a large

amount of ethanol. However, all eventually reach a concentration close to 30 g / L. The fermentation with lower initial concentration takes about one more day to reach the same concentration as those fermentations that have the highest initial concentration.

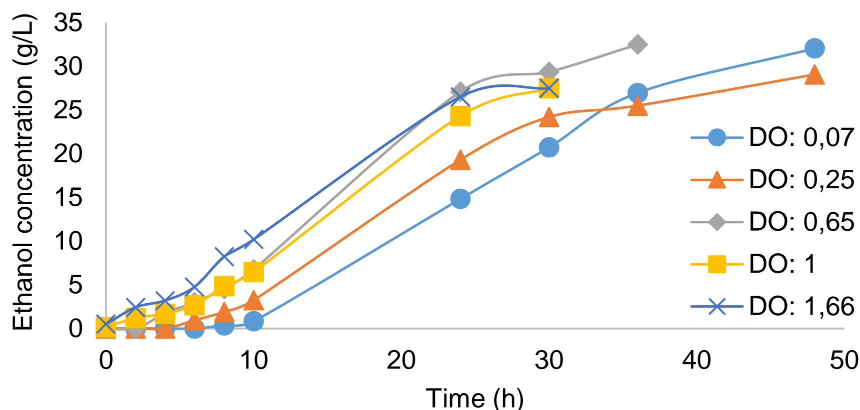

Figure 5-6. Ethanol production at different concentrations of inoculum

In order to know the development of each of the fermentations carried out, the biomass ($Y_{x/s}$) and product ($Y_{p/s}$) yields presented in **Table 5-1** were calculated.

Table 5-1. Yields of the fermentations

Inoculum OD	$Y_{x/s}$	$Y_{p/s}$
0.07	0.021	0.354
0.25	0.019	0.430
0.65	0.031	0.515
1	0.021	0.556
1.68	0.013	0.513

With regard to biomass, the fermentation inoculated with 0.65 OD was the one that obtained a higher yield and the one inoculated with 1.68 OD the one with the lowest yield. This may be due to the fact that due to the high cellular concentration with which the medium is inoculated, the production of a large number of cells is not necessary for the consumption of sugars and the production of ethanol. On the other hand, the fermentation with an inoculum of 1 OD was the one with the highest product yield, although this value is not very far from that of the inoculum of 0.65 and 1.68 OD. This is because these values can already be considered high in number of cells so there is no large production of biomass and

existing cells consume sugars for the production of ethanol. Based on this, the yields obtained for the fermentation carried out with an inoculum of 1 OD are chosen to carry out the simulation.

5.2.2 Lactic acid fermentation

In the fermentation for the production of lactic acid, the microorganism did not consume all the xylose. As can be seen in **Figure 5-7**, this strain, like MS04, consumes glucose more quickly, however, when glucose is finished, the consumption rate of xylose does not increase and becomes almost constant, leaving a remnant in the middle. It is also observed that this strain does not have an adaptation phase, on the contrary, it starts in its exponential phase and around 30 h it starts the stationary phase.

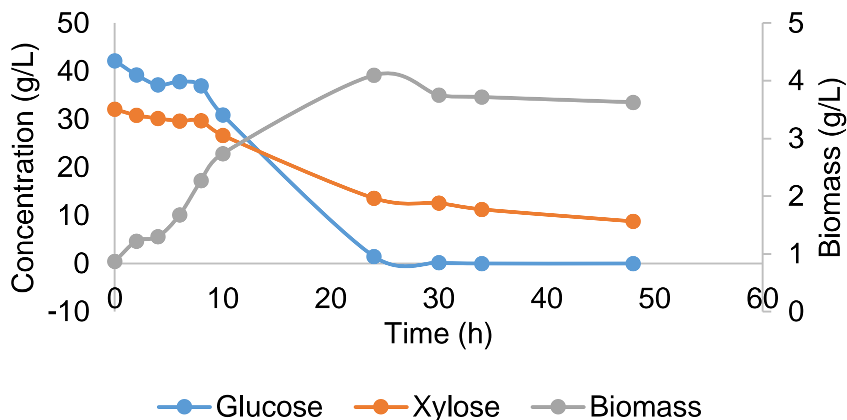

Figure 5-7. Sugars consumption and biomass growth in the lactic acid fermentation

The **Figure 5-8** shows the production of lactic acid during the fermentation. The highest production occurred during the exponential growth phase of the microorganism, after this, the production is lower. During this fermentation, pH control is very important since the product strongly acidifies the medium and inhibits the microorganism.

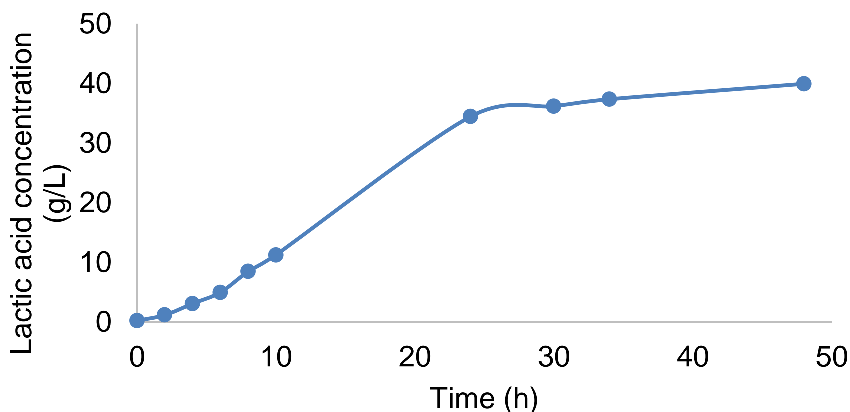

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Figure 5-8. Lactic acid production during the fermentation

Finally, the yields for this experiment were 0.6 g lactic acid/g total sugars and 0.04 g biomass/g total sugars. These results were used for the simulations

5.2.3 Techno-economic analysis of the biorefinery

In the technical analysis of this proposed biorefinery, the flows obtained from each of the value-added compounds were evaluated after the separation stages planned for a flow of 100 ton/day of glucose (30 g/L) and xylose (40 g/L). These results are presented in **Table 5-2**.

Table 5-2. Products flows

	Flow (kg/h)	Mass Frac
Ethanol	60	0.99
Lactic acid	116	0.99

As can be seen, the flow obtained from lactic acid is much greater than that obtained for ethanol. This is due to the stages of separation. In the case of ethanol, this compound passes through a first tower where as much water as possible is separated, however this water stream drains ethanol due to its solubility. After this, it enters a second tower where the azeotropic composition is obtained and where this component may be lost. On the other hand, in the case of lactic acid there are no stages where there is much loss of the component so the final flow obtained is a large percentage of that produced in the fermentation.

Figure 5-9 shows the distribution of costs obtained for the proposed platform. As it can be observed the raw materials represent more than 50% of the total costs of the plant. This is mainly due to the fact that it considers using pure glucose and xylose and additionally another series of components are required for the separation of the lactic acid. On the other hand, the amount of utilities required in the separation stages make this parameter the second most influential. The process could be economically improved by obtaining these sugars from lignocellulosic wastes present in different sectors of the country.

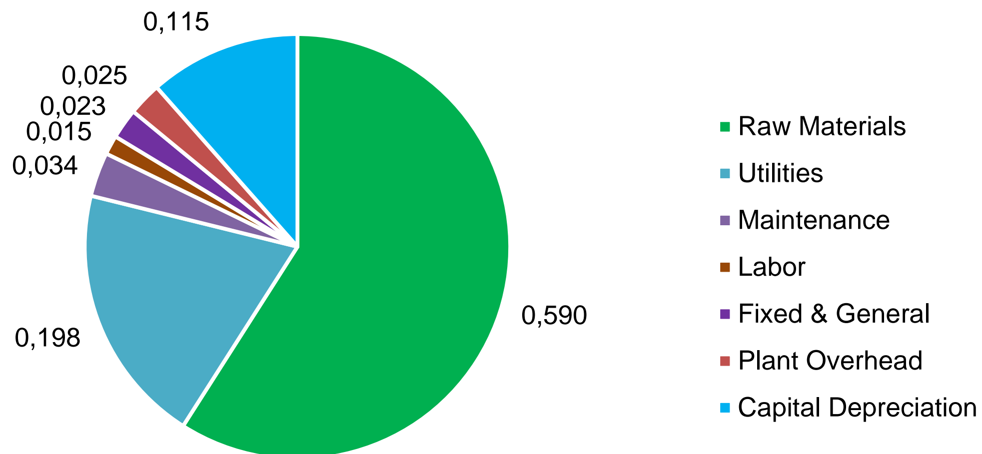

Figure 5-9. Distribution costs

5.3 Conclusion

The genetically modified microorganisms allow the utilization of all the sugars produced during a pretreatment to a lignocellulosic material. This allows to increase the economic viability of the process and the yields of the products.

6. Conclusion

- Industrial residues generated in the Colombian context such as plantain peel and milk whey are able to be used as substrates for biobutanol production through ABE fermentation. Nevertheless, the use of plantain peel as raw material for this process yields better results in terms of solvents production than milk whey due to the large amount of soluble sugars that can be obtained from it.

- The autohydrolysis treatment allows to obtain similar amount of fermentable sugars as well as, lower amount of inhibitors from the plantain peel than the diluted acid method. Therefore, this pretreatment can be postulated as the pretreatment of the biobutanol production process when different raw materials (comparable with the intrinsic characteristics of the plantain peel) are used, decreasing the capital and operational investments of the process as well as the environmental impact caused by the use of chemical components..

- The formulation of the rich vitamins medium and as well as the adaptation of the strain *Clostridium acetobutylicum* ATCC 824 to a similar conditions obtained from the hydrolysates of milk whey and plantain peel are key steps that affect greatly the course of the fermentation. Several passes are recommended before to scale-up the process aiming to increase the microorganism productivity as well as to improve the fermentation yields. Despite this, it was possible to obtain solvent concentrations very close to those reported in the literature during the realization of this work.

- The coffee growing region is one of the regions of Colombia with high potential to produce a wide variety of value-added products such as biofertilizers, biofuels, among others due to the large production of agroindustrial residues. In this way, the characterization of the plantain peel and milk whey demonstrates that both are raw materials from which sugars can be obtained for fermentation. Nevertheless, the

plantain peel has a higher percentage of material that can be transformed into these components (i.e., cellulose and hemicellulose). In this work, the plantain peel and the whey were evaluated and it was demonstrated that they are promising raw materials for obtaining these products. However, the plantain peel has advantages over the whey, since it has a higher yield of sugars per gram of raw material, allowing greater obtaining of butanol. It is transported more easily from the place where the industry is generated, while the whey being a liquid waste presents greater difficulties for its transport. And finally, it is a process with greater economic viability and less environmental pollution.

- The thermodynamic-topological analysis helps to improve and to make more accurately the design of separation schemes of a biorefinery, which allowing to know graphically the separation tendency of the mixtures at the time of purification. The above reduces the simulation errors associated to the convergence, decreasing the calculation times of the separation schemes. In the case of ABE fermentation, it was demonstrated that the main problem does not lie in the separation of butanol as the main component, but in the way of removing all the amount of water present in the culture medium. Since unlike other fermentations the concentrations reached of products are low, it requires large amounts of energy for the purification of their products.

- The basic dimensioning of a bioreactor allows to know its geometry and basic power requirements in order to obtain similar results to experimental ones at laboratory scale. However, a deeper study is necessary to achieve the complete design of a bioreactor understanding the effect of the mass and heat transfer as fundamental processes in the scale-up of the fermentation process.

- Butanol obtained from agroindustrial waste from the Colombian coffee region through ABE fermentation is a promising chemical platform since it allows obtaining a wide variety of products. The products studied in this case, such as butyl acetate, butyl acrylate and butyl propionate, can be obtained economically viable from plantain peel at scales greater than 50 tons/day, while from of the whey, scales of more than 1500 ton/day are required. All this is due to the yield of sugars of both raw materials that allows greater obtaining of butanol for the case of plantain peel.

- The biobutanol production using the sugar content derived from the plantain peel and milk whey can be compared from a techno-economic and environmental point of view when conceptual design tools are applied to evaluate it, which frames the prefeasibility of the project. Therefore, the evaluation of this process using these raw materials from a more detailed point of view is required. In this way, equipment sizing, control system evaluation, raw materials supply chain as well as the inclusion of economic indicators (i.e., IPC, wage increase per year and so forth) are necessary to give a more realistic idea about the bioethanol production in the coffee growing region.

7. Recommendations

For future work related to the production of butanol, a series of recommendations are made. Anaerobic strains, especially Clostridium strains, are difficult to manage, susceptible to minimal changes in the environment that can seriously affect their growth and even prevent it. The lack of vitamins is one of the main causes of the non-growth of this microorganism. Microorganisms should be studied thoroughly because in this thesis a low consumption of glucose was evidenced, which leads to great economic losses, since the pretreatment that must be done to the raw materials to achieve these sugars has a great cost. One of the improvements that can be made to the fermentation system is the increase of nitrogen in the medium, because the reactive limit, when this is exhausted the strain is inhibited and does not continue with its growth and solvent production.

In the case of the techno-economic analysis of the milk whey, a concentration of the sugars can be evaluated by withdrawing water from the medium in order to increase the yield. While taking into account the energy costs that this entails.

A more detailed study of the bioreactor design is recommended, which includes parameters such as the regulation of the temperature and the flow of nitrogen necessary for the growth of the microorganism.

Although the separation was evaluated, no results are presented, since the differential distillation evaluated scheme did not allow the separation of any of the solvents from the medium, due to the large amount of water. For this reason it is recommended to propose a different separation scheme that includes extraction stages that present better results in obtaining the products

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