PRETREATMENT OF SUGAR BEET BAGASSE FOR ENHANCE BIOGAS PRODUCTION

BİYOGAZ ÜRETİMİNİN ARTIRILMASI İÇİN ŞEKER PANCARI KÜSPESİNİN ÖN ARITIMI

LUİS ALFREDO CARMONA ARRİETA

PROF. DR. AYŞENUR UĞURLU
Supervisor

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Assoc.Prof. Dr. Ferda MUTLU

Head ....

Prof. Dr. Ayşenur UĞURLU

Supervisor ....

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Member ....

Assoc. Prof. Dr. İlknur Durukan TEMÜGE

Member ....

Assoc. Prof. Dr. Üyesi Hatice ŞENGÜL

Member ....

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ABSTRACT

PRETREATMENT OF SUGAR BEET BAGASSE FOR ENHANCE BIOGAS PRODUCTION

Luis Alfredo CARMONA ARRIETA

Master of Science, Department of Renewable Energies

Supervisor: Prof. Dr. Ayşenur UĞURLU

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World energy consumption is increasing depending on the rise in demand which threatens humanity with a soon shortage of fossil fuels. Therefore, the research is focused on alternative sources of energy such as the renewable ones. Biogas production is regarded as an alternative source of energy from various kinds’ biomass feedstock or wastes which can also have negative effects on the environment if not properly disposed. Gradually, biogas has been gaining a new pace in the market due to the efficiency and the lack of technology modification, when compared with the other renewable energy sources.

The problems encountered in anaerobic digestion degradation are caused by the complex and tough structure of lignocellulosic biomass. Its efficient disruption is required to increase the hydrolysis rate and biogas production. One way of enhancing biogas production from lignocellulosic biomass is implementing pretreatments to the substrate before the anaerobic digestion process for increasing the biodegradability. The physical, chemical and biological pretreatments are widely studied to improve the biomethane yield. The biomethane production can be enhanced by 25% and >100% with various pretreatment applications. Combinations of pretreatments are also applied.

The selection of pretreatment method is always related to application procedure, time, energy and chemical costs. Besides, the effectiveness of chemical reagents, possible corrosion or degradation is also considered due to the concentrations used.

In this study, the effects of physical and chemical pretreatments, and their combination were investigated on the degradation of lignocellulosic structure of sugar beet pulp, and (ii) the effect of pretreatment methods on the methane production by performing batch Biochemical Methane Production tests (BMP).

By considering the previous facts in the present investigation, raw sugar beet (RSB) and exhausted sugar beet (ESB) were studied. Sugar beet pulp has 22-30% cellulose, 22-30 hemicellulose, 24-32% pectin and 1-3% lignin content. Acid pretreatment with dilute H_2SO_4 and alkaline pretreatment with dilute NaOH were used as the chemical pretreatment methods. The physical pretreatments were done under microwave and autoclave.

Under the scope of the physical pretreatment the specific methane production potential (SMP) resulted in 328.90 mL CH_4/gVS_{feed}, which increased by 40% with microwave radiation (300 Watts) for 2 minutes. The alkaline pretreatment with 2% NaOH (at 25°C, 48 hours) achieved 286.96 mL CH_4/gVS_{feed}, which represents an increase by 45%. Furthermore, physicochemical pretreatment elevated the biomethane production potential by 65% when the sugar beet was exposed to 2% acid at 120°C during 30 minutes in autoclave as 387.82 mL CH_4/gVS_{feed}. Besides, a mixed substrate (70 pretreated and 30 un-pretreated) could enhance SMP by 60% as 376.11 mL CH_4/gVS_{feed} (RSB + Acid 2% + MW 300Watts for 2 min) or 280 mL CH_4/gVS_{feed} (ESB + Alkaline 2% + Autoclave 120°C, 30 min).

The applied pretreatment methods increased the biogas production from raw sugar beet (RSB) and exhausted sugar beet (ESB) by 40 – 65%. Also, it opened a new approach to investigate with the mixed substrate combination, which can reduce the cost implementation of the pretreatments in the industrial scale.

Keywords: Lignocellulosic residues, Raw Sugar Beet, Exhausted Sugar Beet, Pretreatments, Anaerobic Digestion, Biogas Production.

ÖZET

BİYOGAZ ÜRETİMİNİN ARTIRILMASI İÇİN ŞEKER PANCARI KÜSPESİNİN ÖN ARITIMI

Luis Alfredo CARMONA ARRIETA

Yüksek Lisans, Temiz Tükenmez Enerjiler Bölüme

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Dünya enerji tüketimi sön dönemlerde fosil yakıt kıtlığı ile insanlığı tehdit eden talebin artmasına bağlı olarak artmaktadır. Bu nedenle araştırma, yenilenebilir enerji kaynakları gibi alternatif enerji kaynaklarına odaklanmıştır. Biyogaz üretimi, çeşitli türlerdeki biyokütle besleme stoğundan veya uygun şekilde atılmadığı takdirde çevre üzerinde olumsuz etkileri olabilecek atıklardan üretilen alternatif bir enerji kaynağı olarak kabul edilmektedir. Kademeli olarak diğer yenilenebilir enerji kaynaklarına kıyasla biyogaz, pazardaki verimlilik ve teknoloji değişikliği eksikliğinden dolayı yeni hız kazanmaktadır.

Anaerobik bozulmada karşılaşılan problemler, lignoselülozik biyokütlenin karmaşık ve sert yapısından kaynaklanmaktadır. Hidroliz oranını ve biyogaz üretimini arttırmak için etkin bir bozulma gerekmektedir. Lignoselülozik biyokütleden biyogaz üretimini arttırmanın bir yolu olarak biyolojik olarak parçalanmayı arttırmak için anaerobik sindirim işleminden önce alt tabakaya ön arıtmalar uygulanmaktadır. Biyometan verimini arttırmak için fiziksel, kimyasal ve biyolojik ön arıtımılar yaygın olarak incelenmektedir. Biyometan üretimi, çeşitli ön arıtmalar uygulamalarıyla %25 ve

>%100 oranında arttırılabilmektedir. Çesitli ön arıtım kombinasyonları da uygulanmaktadır.

Ön arıtma yönteminin seçimi uygulama prosedürü, zaman, enerji ve kimyasal maliyetlerle ilgilidir. Ayrıca, kullanılan reaktifler nedeniyle kimyasal reaktiflerin etkinliği, olası korozyon veya bozulma da göz önünde bulundurulmaktadır.

Bu araştırmada, şeker pancarı küspesinin lignoselülozik yapısının bozulması üzerine fiziksel ve kimyasal ön arıtımıların etkileri ve bunların kombinasyonları, ve (ii) Biyokimyasal Metan Üretimi (BMP) testlerinin uygulanmasıyla ön arıtma yöntemlerinin metan üretimine etkisi incelenmiştir.

Mevcut araştırmadaki önceki veriler göz önüne alınarak, ham şeker pancarı (RSB) ve bitkin şeker pancarı (ESB) incelenmiştir. Şeker pancarı küspesi %22-30 selüloz, %22-30 hemiselüloz, %24-32 pektin ve %1-3 lignin içeriğine sahip olmaktadır. Kimyasal ön arıtma yöntemleri olarak, seyreltik H2SO4 ile asit ön arıtma ve seyreltik NaOH ile alkalik ön muamele kullanılmışdır. Fiziksel ön arımalar, mikrodalga ve otoklav altında yapılmaktadır.

Fiziksel ön arıtma kapsamında spesifik metan üretim potansiyeli (SMP) 328.90 mL CH4 / gVSfeed ile sonuçlanmaktadır, bu da 2 dakika boyunca mikrodalga radyasyonuyla (300 Watt) %40 artmaktadır. %2 NaOH (25 ° C'de, 48 saatte) ile alkalin ön arıtma, %45'lik bir artışı temsil eden 286.96 mL CH4 / gVSfeed elde edildi. Ayrıca, fizikokimyasal ön arıtma sırasında şeker pancarı 30 dakika boyunca 120 ° C'de %2 asite maruz bırakıldığında 387.82 mL CH4 / gVSfeed olarak otoklavda biyometan üretim potansiyelini %65 artmaktadır. Üstelik, karışık bir substrat (önceden arıtma edilmiş 70 ve önceden arıtma edilmemiş 30), SMP'yi %60 oranında 376.11 mL CH4 / gVSfeed (2 dakika için RSB + Asit %2 + MW 300Watts) veya 280 mL CH4 / gVSfeed (ESB + Alkalin 2% + Otoklav 120 ° C, 30 dak) arttırabilmektedir.

Uygulanan ön arıtma yöntemleri, ham şeker pancarı (RSB) ve bitkin şeker pancarı (ESB) kaynaklı biyogaz üretimini %40 - 65 oranında artırmıştadır. Aynı zamanda, karışık substrat kombinasyonuyla araştırma yapmak için endüstriyel bir ölçekte ön arıtmaların maliyetini düşüren yeni bir yaklaşım açmaktadır.

Anahtar Kelımeler: Lignoselülozik artıklar, Ham Şeker Pancarı, Bitkin Şeker Pancarı, Ön Arıtmalar, Anaerobik sindirim, Biyogaz Üretimi.

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AD	Anaerobic digestion
BMP	Biochemical methane potential
CHP	Combined heat and power
ESB	Exhausted sugar beet
FLW	Food loss and waste
FSW	Food supply chain
FTIR	Fourier transform infrared
HHV	High heating value
HRT	Hydraulic retention time
LCFA	Long chain fatty acids
LHV	Lower heating value
LHW	Liquid hot water
M	Molar
RDS	Rate determining step
RSB	Raw sugar beet
SCFA	Short chain fatty acids
SMP	Specific methane potential
P	Pressure
T	Temperature
t	Time
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids

1. INTRODUCTION

Residues of different agricultural activities are an issue that must be considered for the sustainability of the renewable energies sources in a country. Therefore, it can mitigate, somehow, the importation of energy. For instance, Turkey is a country which around 50% of its land is used for agricultural activities; its economy is 6.7% based on agriculture. A similar panorama is observed in Colombia, South American country, where the land used for agricultural purposes is around 40%, representing 7.4% of the national economy [1].

In the same order of ideas, thinking in the best suitable alternative for producing energies from the agricultural residues, Biogas appears as a good choice. Biogas production, as a field in the renewable energy sources, is one of the most interesting ones, that due to its easy set up with the current technology that uses natural gas, it requires no change in the technology whether the gas to use comes from natural or from a bio source one.

Previous studies were developed using different lignocellulosic sources to produce biogas. Sugar cane has been the most studied one. Through the scientific magazines, it can be easily confirmed that not only sugar cane as a residue has been studied, but also as a fresh raw material. Therefore, bibliography about the effect of pretreatments aimed to biogas production is mainly focused on that product, which gives a starting point to set different paths for future investigations.

In the following study, it will be explained step by step the whole process for producing biogas from sugar beet bagasse. It starts analyzing the residue from the source of its main productive process: sugar production. Subsequently, the characterization of the bagasse, the pretreatments for enhancing the biogas production and also the considerations in the anaerobic digestion process will be shown. With all these information summarized, it would be easier to explain the experimental part in the laboratory and come up with the conclusions and recommendations of the study made, which will be the final part of this investigation.

1.1 Renewable Energies’ Role in the Current World

After several years using fuels derived from fossil sources, humanity has realized that they are not unlimited and unexhausted. Around the world, experts have talked and studied the implications of consuming the conventional carburant in the way it is currently done. That is how many initiatives have come up to move slowly from the conventional fuels to the new generation ones.

Therefore, international agreements have been released to generate a conscious consumption of the resources and the emissions that they cause. The first of them was the Kyoto Protocol which had its first part in 1997, it was agreed the legally binding emission reduction targets that developed countries should work with towards the climate change. A second part of this agreement came up in 2013 with a deadline in 2020, but some developed or in developing process countries decided not to be part of this second period. Despite the dissertation, some other countries kept their word and are still working towards reaching the goals established [2].

The Paris Agreement (2016) is the latest one, at least fifty-five countries agreed on controlling and limiting their emissions of greenhouse gases to conserve the temperature under conditions that can be control in a near future, holding the increase in the global average temperature to well below 2°C above pre-industrial levels and pursuing efforts to limit the temperature increase to 1.5°C above pre-industrial levels, recognizing that this would significantly reduce the risks and impacts of climate change. It is still under construction, so there are several modifications after its first version, the idea is that some other countries join the commitment and make it more serious and feasible around the world [3].

Not only governmental initiatives are contemplated under the climate change mitigation, but also private ones as the one called “The Solution Project”, where a group of researchers did roadmaps to predict the energy consumption in 2050 under the current conditions of our daily life for 139 countries, Figure 1.1. As a complementing part, they calculated also the amount of energy required to all energy sector: transportation, heating/cooling, industry, agriculture/forestry/ fishing. However, the second calculation was done only using 100% renewable resources (Wind, Water and Solar). Therefore, the highlight of the study is a decrement of 42,5% on the total amount of energy that is used nowadays. That is sustained with the idea of changing our life habits and starting a changing from home which is moved to the industry, but requires a general awareness of the society as well [4].

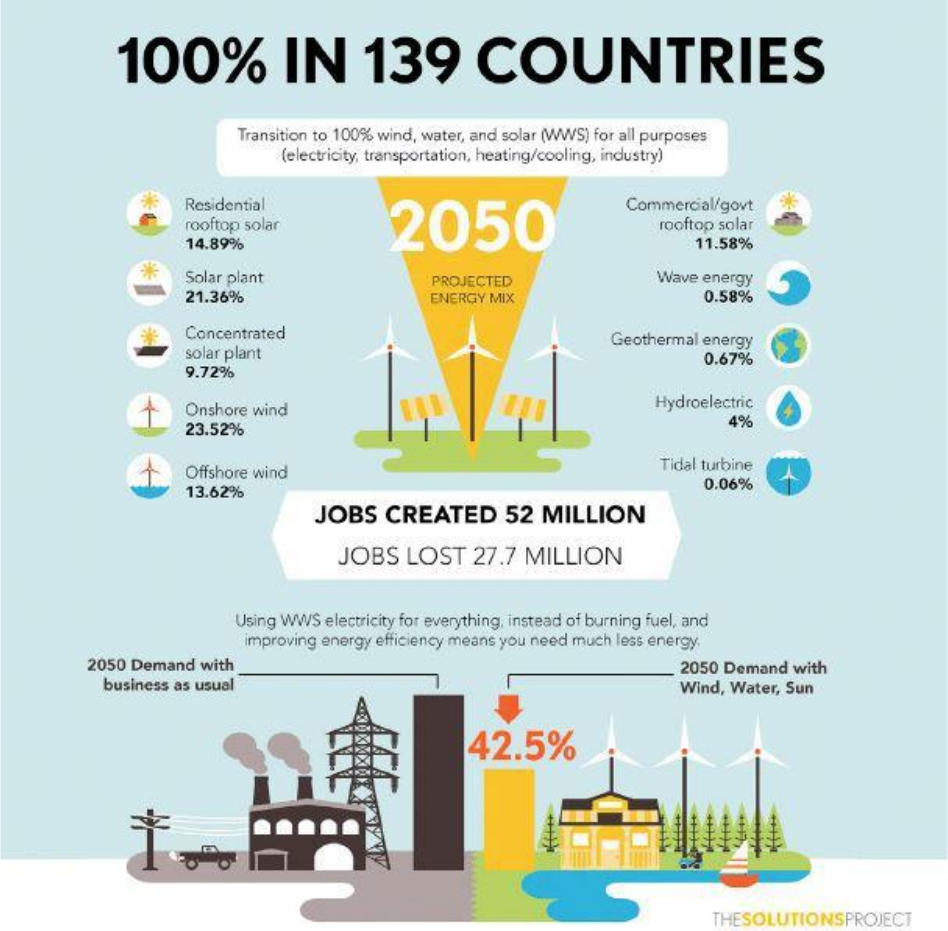


Figure 1. 1 100% Renewable energy consumption in 139 countries.
Source: https://www.thesolutionsproject.org

Despite of the no inclusion of the biogas and biomass energy sources, projects like this one give some kind of hope to think about a society that can work/live with 100% renewable energies. That would create conscious in the humanity and moreover in the way we are living our lives. It is necessary a change in the way we aim the different investigations planed, we do need to switch our mentality from the first line.

1.2 General Information about Colombia and Turkey: Energy Production

Colombia is a country located in the northern part of South America, where the country has the privilege of having one sea and an ocean, Caribbean and Pacific respectively, and being part at the same time of the big Amazon forest (8% of participation). In the other hand, we find Turkey which its position between Europe (3%) and Asia (97%) gives it strategically participation in the Mediterranean and Black sea as well [1].

Colombia has around 40% of its land dedicated to agricultural activities for producing coffee, cut flowers, bananas, rice, tobacco, corn, sugarcane, cocoa beans, oilseeds, etc. These represent 449,871.6 km2 and with 23% of the population dedicated to these labors, 11’660,770. In the side of Turkey its area dedicated to agricultural activities is around 50% for producing tobacco, tea, cotton, grain, olives, sugar beets, hazelnuts, etc. These represent 385,610 km2 and 25% of the population working for it, 19’556,750 [1].

Table 1. 1 Comparison of Land Distribution: Turkey and Colombia [5].

Country	**Country** [km^2]	**Land** [km^2]	**Agricultural** [km^2]	**Forest** [km^2]	**Population**
Turkey	785,350	769,630	385,610	116,126	78’227,000, with 25% Rural.
Colombia	1’141,749	1’109,500	449,871.6	585,284.6	50’699,000, with 23% Rural.

Figure 1. 2 Map of Colombia (Right side) and Turkey (Left side).
Source: https://www.worldatlas.com

Moreover, in Turkey the emissions of CO2eq are up to 2,592.255 gigagrams (2’592,255 Ton) while in Colombia are around 289.8951 gigagrams (289,895.1 Ton), for the year of 2016. The largest distributions are given by:

-Manure left on pasture:	30.3% – Turkey	22.2% – Colombia
-Enteric fermentation:	35.8% – Turkey	60.3% – Colombia
-Synthetic fertilizers:	22.2% – Turkey	5.10% – Colombia

In both countries, the field that provides the highest emission of carbon dioxide (seen as an equivalent value of) comes from the enteric fermentation which includes different type of fermentative processes that are developed to produce dairy consumption products as are cheese and yogurt.

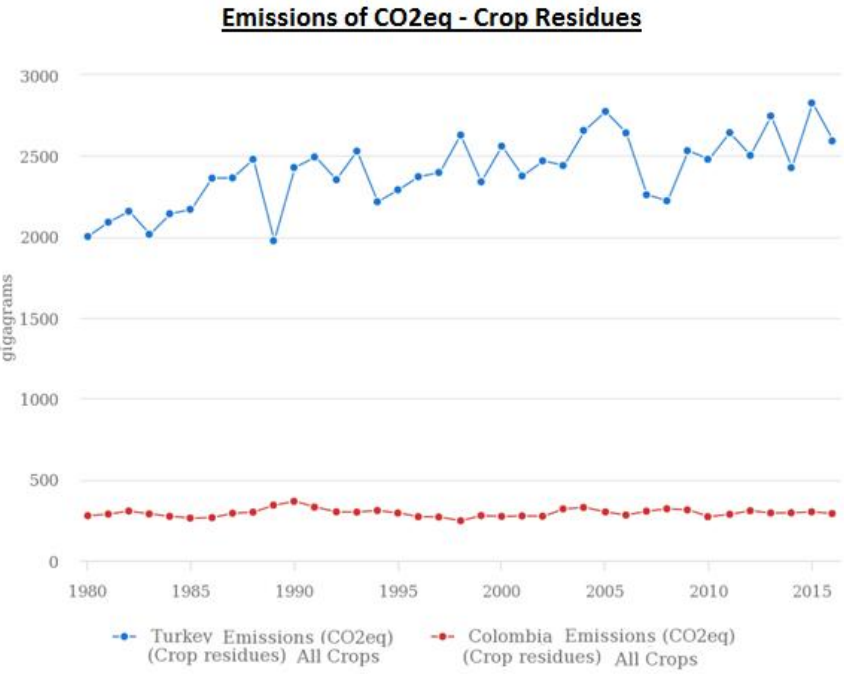


Figure 1. 3 CO2eq Emissions of Crops Residues [5].

1.3 Residues and Energy Potential

1.3.1 Turkey

Food waste is defined as “the food appropriate for human consumption, being discarded or left to spoil at the consumer level, regardless of the cause”. Although the term “food loss” encompasses “food waste” it mostly refers to losses that occur at the production, postharvest and processing stages in the FSC (Food Supply Chain). Therefore the generic term ‘‘food loss and waste (FLW)” is generally used to embrace all the steps in the FSC and refers to the edible fraction [6,7].

The total FLW from the agricultural production step in the FSC in Turkey was found to be approximately 13.75 million tons, which accounts for 11.9% of the food produced, Figure 1.4. The world FLW from agricultural production amounts to 412.9 million tons.

Fragmented farms and lack of cooperation are generally stated as the main reasons for agricultural food losses. The insistency of farmers using traditional methods, unwillingness to use new agricultural knowledge and new technologies, the old age of Turkish farmers, since young people have a tendency to migrate from rural areas, are also among the causes of poor farming practices [8].

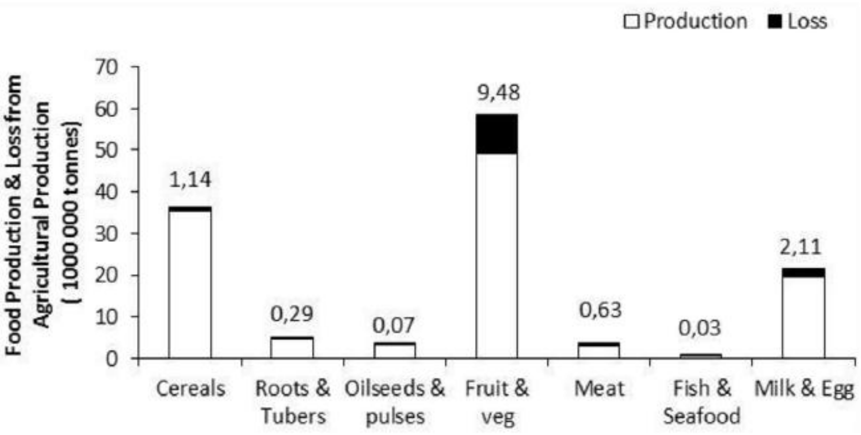


Figure 1. 4 Agricultural food production and losses in Turkey [8].

The biogas potential of Turkey from the FLW residues has been studies for several different researches in order to establish an accurate measurement of the real situation, thus a resume is shown in Table 1.2 where can be seen the theoretical and technical potential in the biogas field. There, Agro-industrial sector marks an important issue because has the lowest potential, 16.8 PJ/year, but has the highest percentage of viability shown with 88.10%.

The previous statement makes sense by basing the analogy on the idea that in the industries where this different agro-industrial products are used, also residues are gathered at the same time, thus it makes it easier to dispose as raw material in the biogas process. There is not necessity to transport the residues to be treated. For instance, one of those agro-industrial residues is the sugar beet bagasse, which has significant value in the national market for being the one that provides the sugar to the products that require it in their formulation [9].

Table 1. 2 Calculated biogas potentials based on sectors in Turkey [8].

Sector	**Substrate**	**Theoretical Biogas Potential [PJ/year]**	**Technical Biogas Potential [PJ/year]**	**Percentage of viability [%] ***
Agricultural – Livestock	Cattle manure	107.8	42.1	54.22%
	Poultry Manure	36.6	36.2	
Agricultural residues	Straw of cereals	276.7	27.7	11.86%
	Sugar beet leaf	17.5	4.4	
	Tomato waste	11.1	4.1	
Energy crops	Energy crops on fallow land	325.1	81.3	25%
Agro-industrial residues	Meat production residues	0.5	0.2	88.10%
	Cheese-waste water	2.7	2.4	
	Sugar beet press cake	5.0	4.5	
	Molasses (sugar production)	3.3	2.9	
	Olive press cake	1.3	1.2	
	Olive mill waste water	1.3	1.2	
	Juice residues (Pomace)	1.8	1.6	
	Draff (Bio-ethanol production)	0.9	0.8	
Municipal waste	Municipal waste	22.0	11.0	50%

**This concept does not appear in the original reference, made for the purposes of this study.*

Due to its importance and availability as a residue, sugar beet bagasse is suitable to work with and direct the study to enhance the production of biogas. Thus, this study aims to start with the pretreatments that can help to reach a better performance during the production of gas from this natural source.

1.3.2 Colombia [10]

Nowadays, Colombia is the country in Latin America with the third largest population (after Brazil and Mexico), the fourth largest gross domestic product –GDP– (609 billion US$ 2011, after Brazil, Mexico and Argentina) and the fifth largest primary energy demand (37 Mtoe). Unfortunately, a combination of widespread corruption, ineffective policies, weak institutions and armed conflict has hindered better wealth distribution. Unlike neighboring countries, Colombia has experienced a 50-year armed conflict, the longest-running armed conflict in the Western hemisphere, characterized by widespread violence, political instability, disregard for the rules of law and aggression against the civilian population.

In the year of 2017 Colombia signed its peace agreement with the oldest guerilla around the world, which had been fighting in internal conflicts against the government. After this iconic situation, the national government must fulfill the lack in many aspects of the society due to the stagnancy that left this internal war. One of those points that must be checked carefully is the use of renewable sources for the mitigation of environmental contaminants.

Colombia is today's 10th largest global producer of ethanol, 5th largest global producer of palm oil and first in Latin America. Colombia is also characterized by a vast biomass energy potential that remains untapped. Studies have recently estimated a theoretical potential ranging between 5 and 18 Mtoe, where a fraction ranging between 1 and 10 Mtoe might be technically available at current conditions for energy exploitation.

The current use of biomass for energy purposes in Colombia can be divided into three main categories:

1. It is used in the form of wood and charcoal as a traditional fuel for cooking and water heating (most predominant use).

2. It is used in the form of cane bagasse and palm oil residues as a fuel in boilers and cogeneration power plants to provide heat and power.

3. It is used after conversion in the form of bioethanol and biodiesel as road transport biofuels.

Other forms of using biomass for energy purposes have been explored less, sometimes even showed as pilot projects with varying degrees of success: use of landfill gas and biogas for in situ heat or power production, biomass gasification and combustion in reciprocating engines and methane collection from wastewater treatment plants for heating.

The energy potential from the agricultural residues in Colombia is shown in Table 1.3, where can be seen that 190,317 TJ/year are offered by the wastes coming from cane (both sugar and panela), from this value the 69% proceeds from bagasse.

In spite of the production of sugar beet in Colombia is low compared with the sugar cane one, 34,350 Ton (for 2012) which represents 0.9% of the total cane production, the idea of generating biogas from its waste could be interesting for the management of this type of residues, which based on the efficiency showed for the energy potential in cane production, it would be around 1,202 TJ/year for the sugar beet bagasse.

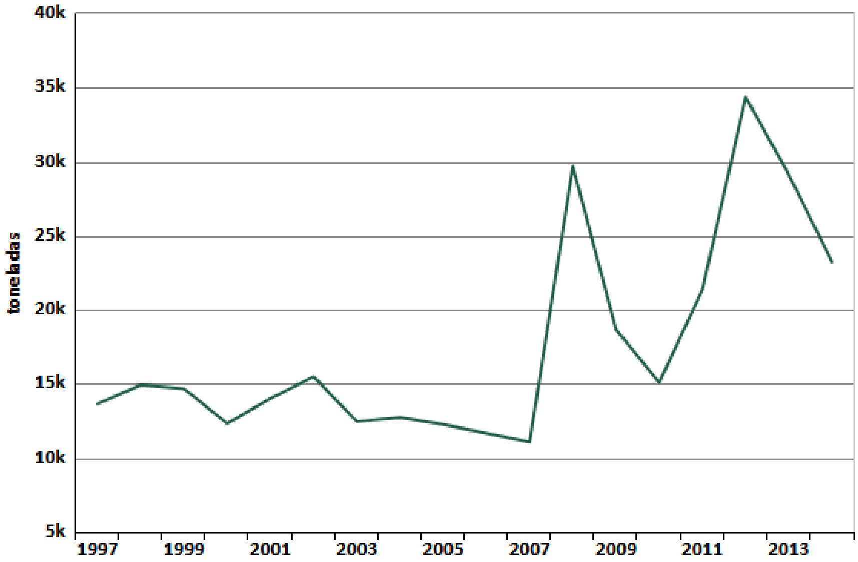


Figure 1. 5 Sugar beet production in Colombia. *Source: https://www.knoema.es*.

Table 1. 3 Energy potential of agricultural wastes in Colombia [11].

Crop	Product, 2012 [Ton]	Agricultural Waste	Waste, 2012 [Ton]	Energy potential [TJ/year]
Palm	1’137,984	Stone	246,714	3,428
		Fiber	712,946	8,845
		Rachis	1’206,490	8,622
Sugar cane	2’681,348	Leaves and Top	8’741,194	42,761
		Bagasse	7’186,013	78,814
Panela Cane	1’284,771	Bagasse	4’817,888	52,841
		Leaves and Top	3’250,469	15,901
Coffee	1’092,361	Pulp	2’327,929	8,354
		Husk	224,262	3,870
		Stem	3’303,299	44,701
Corn	1’206,467	Stubble	1’126,840	11,080
		Cob	325,746	3,389
		Skin	254,564	3,863
Rice	2’318,025	Fuzz	5’447,359	19,476
		Husk	463,605	6,715
Banana	1’834,822	Rachis	1’834,822	788
		Stems	9’174,108	5,172
		Non-Acceptance	275,223	484
Plantain	3’201,476	Rachis	3’201,476	1,374
		Stems	16’007,378	9,024
		Non-Acceptance	480,221	844

1.4 Biogas production from Sugar Beet

Biogas can be produced from many natural sources, but in the scope of this study it will be based on the production from sugar beet bagasse, which at the same time it will be gotten from the sugar production process. In the following steps, it will be shown how sugar beet bagasse in each sub-process works for having a better understanding of the production of biogas from this agricultural residue, which eventually will become the raw material for the upcoming processes.

1.4.1 Sugar Production: Sugar Beet as a Residue

There is not exact point when the production of sugar was set; some they say that this technique was taken from the Polynesia, but some others say that it was taken from Indians over 500 B.C. At the begging the production was made from sugar cane gotten from India and expanded with the Persian Empire, thus was introduced to Europe and got its market. "The white gold" was a luxury that few could enjoy [12].

It was in the XVII century when sugar beet started to be used into the sugar industry as a natural source and replace the sugar cane as raw material in the productive process. Due to the weather conditions, it is more rentable to produce sugar from sugar beet in Europe and Middle East, and in the other side, cheaper in America and Asia the production from Sugar cane [13].

The general sugar production process is shown in Figure 1.6, which can be analyzed in an overview as: Reception of raw material, productive process, dispositions of residues and storage and sell of the main product. In the scope of this research the important part is *the disposition of residues*, focused more exactly in the sugar beet exhausted pulp, but it is accurate to know about the general productive process as [14,15] :

1.4.1.1 Sugar Beet Sowing and Harvest

Normally, sugar beet is planted in spring and is harvested from September. During this period, it has nearly 200 days to pick up nutrients from the sun and the soil and grow as an optimal tubercle. Then, it is collected from the fields and transported to the factory. In this point, the sugar content is between 16 and 20%.

1.4.1.2 Beet Receiving and Extraction

After the arrival of the beet to the factory, it is washed to separate the contaminants that can bring from the fields and slice it into pieces to make easier the following processes. The sliced sugar beet is now mixed with hot water in diffusion towers to start the sugar extractive process, carried at 70°C. Thus, there will be a raw juice with 98% of so-called non-sugars. Normally, the spent beet pulp (*Bagasse*) is utilized to produce animal feed.

1.4.1.3 Juice Purification and Evaporation

The purification of the raw juice is done separating the non-sugar substances present in this liquid, and thanks to calcium carbonate and carbon dioxide, a clarified juice is obtained which has a 16% in sugar content. For that reason, it is necessary to remove the water that this juice contains. After evaporation process, the new thick juice has a 70% in sugar content and it is ready to continue its purification.

1.4.1.4 Crystallization

This thick juice is sent to a boiling station. At a certain point during this process, sugar crystals start to form and a new mixture of crystals and syrup is generated. Therefore, this mixture is filtered and is cooled before reaching forward steps.

1.4.1.5 Centrifugation

In the centrifuges, crystals are separated from the syrup by centrifugal forces. At this point, sugar gets a white color due to the simultaneous washing process that occurs inside the centrifuge. After this process, there are two products available: sugar and molasses. The main product, sugar (mostly sucrose $C_{12}H_{22}O_{11}$), can be re-process to be purified and getting high-quality sugar or simply been sold as its initial presentation, it all depends on the necessity of the market. On the other hand, molasses are used to produce animal feed and also are utilized as a raw material in the yeast and alcohol industry.

1.4.1.6 Storing and preparing varieties of sugar

Before storing, sugar need to be dried and cooled. This is the time where it will wait until it meets the customer requirements, which could be in individual packages or sold loose.

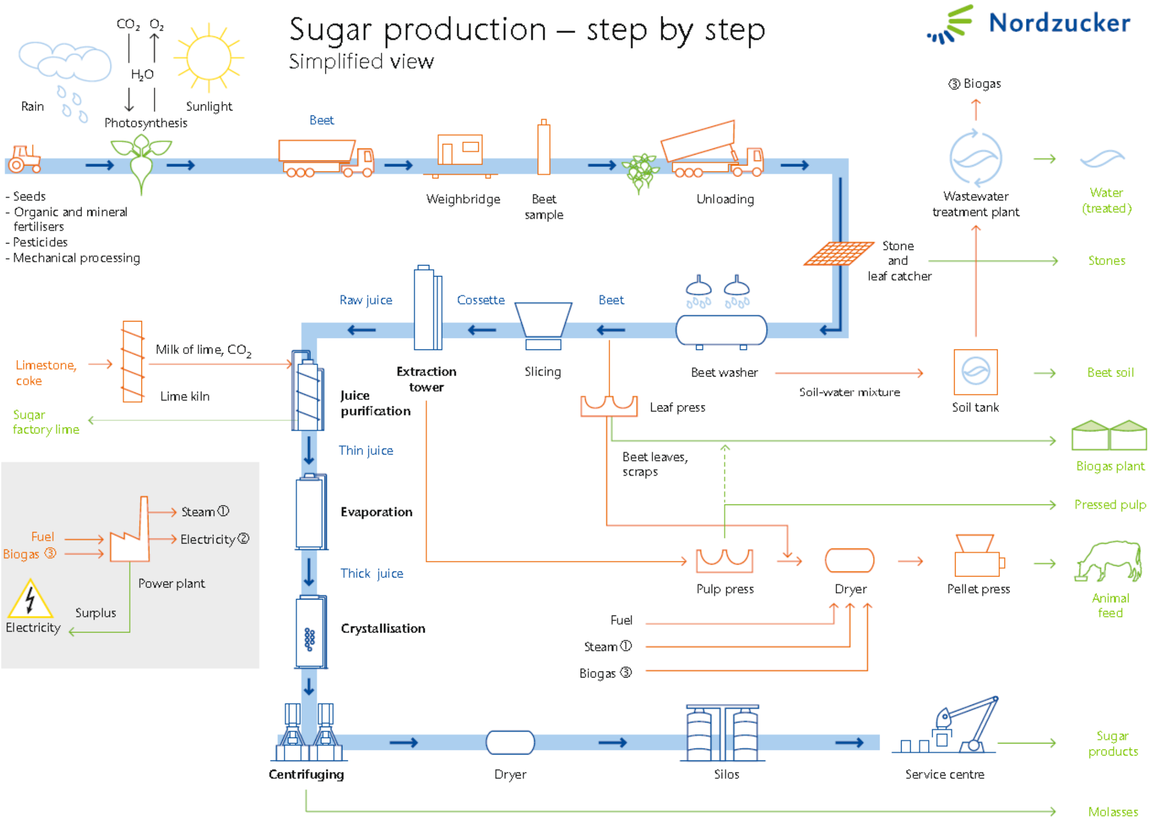


Figure 1. 6 Sugar production: Step by Step [14]. *Source: Nordzucker AG.*

1.5 Sugar Beet Bagasse Characterization: Composition

Sugar beet is sown and harvested, and then transported to the factories to start the sugar productive process. Some of the important components of this plant are:

1.5.1 Leaves

With the presence of sunshine and the chlorophyll in its leaves, the sugar beet plant converts carbon dioxide (CO_2), water (H_2O) and minerals (from the soil: Nitrogen (N), Potassium (K), Calcium (Ca), Magnesium (Mg), amount others.) into sugar components. This process is most known as photosynthesis [15].

1.5.2 The head

The head of the sugar beet plant is where the leaves start from. It does not contain any sugar material useful for the sugar productive process, that is why is removed and disposed as fertilizer or animal feed [15].

1.5.3 Body and roots

The heavier part of the plant is where sugar is stored. The body (or root) of the suagr beet contains a high concentration of sugar. The light areas are those in which the concentration of sugar is partially high [15].

Figure 1. 7 Sugar beet. *Source:* http://www.geofertilizer.com

The sugar beet plant (*Beta vulgaris saccharifera*) consists of 75% water with a sugar concentration of 16 to 20%. Compared with the sugar produced from sugar cane, this beet gives the highest yield among sugar producing plants. A standard sugar beet is 20 – 30 cm long and has an average weight of 800 gr [15].

Some researchers are characterized the sugar beet pulp as part of their studies and some results are shown in Table 1.4 and Table 1.5, both focused on different type of characteristics, which can give an idea about the techniques and initial parameters that must be measured for starting labors. Products as Cellulose, Lignin and Hemicellulose interfere in the process with its crystallinity, polymerization and the degree of acetylation, making the degradation process slow or impossible to carry on. Therefore, the idea is to alter these components to enhance the biogas production from this Lignocellulosic waste.

Table 1. 4 Main Characteristics of sugar beet pulp [16].

Parameter	Value
Total Solids, TS [g/kg]	234.82 +/- 2.02
Volatile Solids, VS [g/kg]	223.39 +/- 1.99
Ash [g/kg]	10.87 +/- 1.0
Chemical Oxygen Demand, COD [gO2/kg TS]	988.04 +/- 18.24
pH	5.25 +/- 0.01
Volatile Fatty Acid, VFA [gCH3.COOH/kg TS]	5.28 +/- 0.43
Total Kjeldahl Nitrogen, TKN [g/kg TS]	98.00 +/- 1.20
Phosphorus [g/kg TS]	2.56 +/- 0.04
Cellulose [%TS]	30.0 +/- 2.4
Hemicellulose [%TS]	26.8 +/- 1.82
Pectin [%TS]	24.2 +/- 2.1
Lignin [%TS]	4.1 +/- 1.6

Cellulose corresponds to about 40 – 60% of the total biomass weight, hemicellulose 15 – 25%, and lignin (15 – 25 %), see Figure 1.8. Cellulose is a linear, mostly crystalline polymer whose structural unit is glucose. Hemicellulose is an amorphous polymeric structure shorter than cellulose, mainly sugars as xylose and glucose. Lignin is a complex non-crystalline macromolecule, composed of a variety of aromatic constituents as sinapyl, coniferyl, and coumaryl alcohols. Lignin and hemicellulose are responsible for keeping cellulose fibers attached and give the biomass its appearance [17].

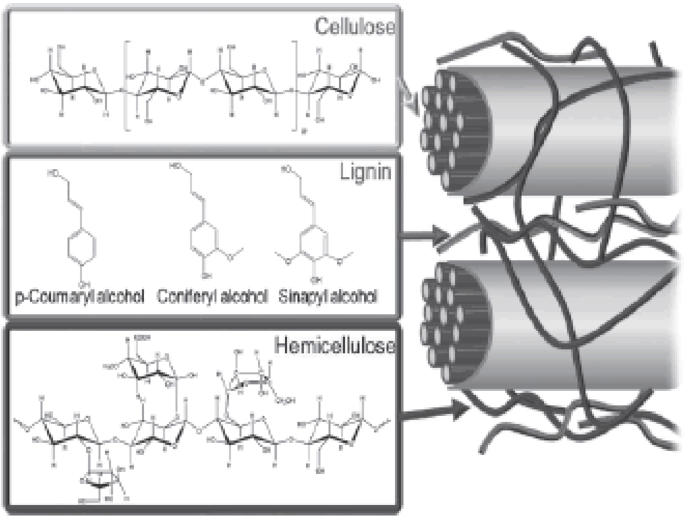


Figure 1. 8 General structure of lignocellulosic materials [17].

The modification of these chemical component structures result in improving the biogas production. Thus, a pretreatment that help to work with them is necessary into the process.

Table 1. 5 Hydrolysate Composition from sugar beet pulp [18].

Saccharide	**Concentration [g/dm^3]**
Sucrose	1.88 +/- 0.81
Xylose	4.21 +/- 0.19
Galactose	8.36 +/- 0.23
Arabinose	4.28 +/- 0.16
Maltose	8.63 +/- 0.29
Rhamnose	7.24 +/- 0.26
Glucose	19.33 +/- 0.86
α-D-Glucuronic Acid	4.21 +/- 0.22
Fructose	7.63 +/- 0.18

1.6 Pretreatments for Biogas Production

The definition of pretreatment refers to all the previous processes that are done before starting the main productive process. In this case, pretreatments will be all the processes previous the anaerobic digestion which is the main process for this investigation. In that order of ideas, the pretreatments for lignocellulosic biomass are defined in three groups: Physical, Chemical and Biological pretreatments [19].

1.6.1 Physical Pretreatments

Most of the time, these pretreatments consider modification of the physical biomass product with methods that change the physical size of the sample. In this group can be considered: Comminution (e.g. milling and grinding) and extrusion. But some other times, techniques as steam-explosion (auto-hydrolysis), liquid hot water (hydro-thermolysis) and irradiation (ultrasound and microwave), are also included in this group for not interacting directly with chemical compounds [19].

1.6.2 Chemical Pretreatments

In these types of pretreatments are considered chemicals that modify physically or chemically the components of the lignocellulosic biomass. Is the most study type of pretreatments, but it has been referred few times to biogas production most of the studies have been focused on the bio-ethanol production. There is an elevate risk to have inhibitors in the medium, owing to the concentration of the reagents used during the pretreatment step. For that reason, the use of chemical in pretreatment stages is delimited by the counter effect that can cause over the whole AD process, unless no traces of them are ensured in the digester [19].

1.6.3 Biological Pretreatments

In general this field is guided to work with fungal, microbial consumption and enzymatic pretreatments, where the process aims to produce higher amounts of biogas by increasing the accessibility to cellulose, hemicellulose and lignin. Therefore, the high lignin removal rate offered an increase of the process yield and makes this type of pretreatments the most used in industry, but also a bit limiting due to the long time of interaction needed to modify the lignocellulosic components [19].

Table 1. 6 Pretreatments lignocellulosic wastes for biogas production [19].

Pretreatment	Conditions	Results
Physical: **Liquid hot water, LHW**	T: 100 – 230 °C P: 0.1 – 2.8 MPa t: Few minutes to hours	7 – 220% increase of methane yield.
Chemical: **Acid**	Chemicals: H_2SO_4, HCl, HNO_3, H_3PO_4, Acetic Acid and Maleic Acid. Chemical loading: 1 – 4% (g/g dry matter), organic acid can be higher, AA: 35 – 80 %. T: T_{Amb} – 170 °C t: Few minutes to hour or even 30 days	Positive effect in most cases with 20 – 200% increase of methane yield. Negative effects also occurred in very few cases (e.g. 2% H_2SO_4 on rapeseed).
Biological: **Microbial consortium**	T: 20 – 55 °C t: 12 h – 20 days Autoclaving or no autoclaving of feedstock before inoculation; Aerobic condition	Methane yield improvement by 25 – 96.63 %.

In Table 1.6 three different types of pretreatments are shown: Physical, Chemical and Biological. In each of them, composition and conditions of the process and their results referred to the biogas yield are summarized. For instance, the use of LHW can increase up to 220% the methane yield from a lignocellulosic biomass, this is the highest increase reported in literature for physical pretreatments. Apart from the methane yield, also it is necessary to talk about effectiveness; how worthy it is.

1.7 Biogas Production: Anaerobic Digestion (AD) Process

As a general definition we have that the anaerobic digestion (AD) is the process in which the different organic materials are decomposed to generate or produce gas, mainly methane (CH_4), with the absent or no need of oxygen. Or in a more technical definition:

"*Biogas is produced by anaerobic bacteria that degrade organic material to biogas in four steps: hydrolysis, acidification, production of acetic acid and production of methane. The product of the digestive process, raw biogas, consists of 50–75% methane, 25–50% carbon dioxide and 2–8% other gases such as nitrogen, oxygen and trace gases (e.g. hydrogen sulfide (H_2S), ammonia (NH_3) and hydrogen).*" [20]

The general AD scheme is shown in Figure 1.9 where the different steps for transforming the organic components into gases are defined.

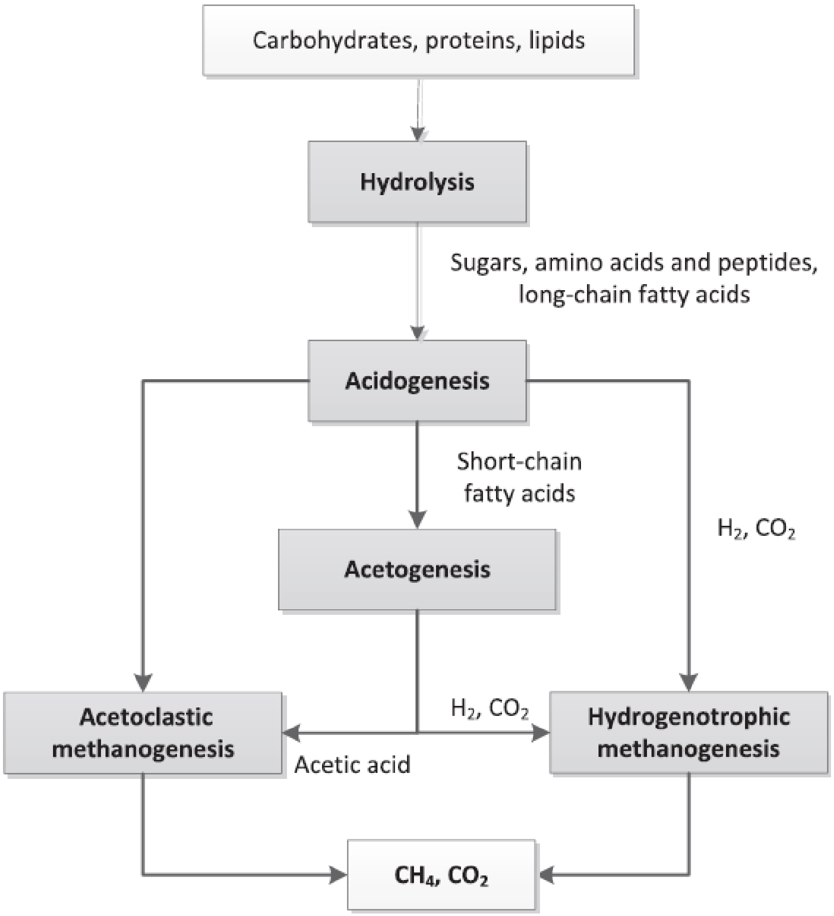


Figure 1. 9 Process flow during anaerobic digestion [19].

Complex components that are fed into the system start bit by bit a process of degradation into more simple components until becoming the desired gases that are expected from this digestion. At the end of the AD, two main products are gotten: Biogas and Digestate. The first one is a mixed of several gases, mainly methane, as it was explained before. While digestate is the decomposed substrate which is mainly used as fertilizer due to the high content of nutrients, but before that, it needs to pass a quality control to avoid pathogens and ensure safe incorporation to the fields [21].

Whole along the process and sub-processes exist different considerations for enhance the methane production and in the same time the efficiency of the AD and its generation of biogas. However, what is true is that there is no common point to establish the rate-determining step (RDS) of this digestive process. Some authors agreed that for lignocellulosic substrates the RDS is hydrolysis. That statement is supported with the necessity to decompose the cellulose, hemicellulose and lignin, long chain components, into more easily degradable components as sugars and fats [19,20,21].

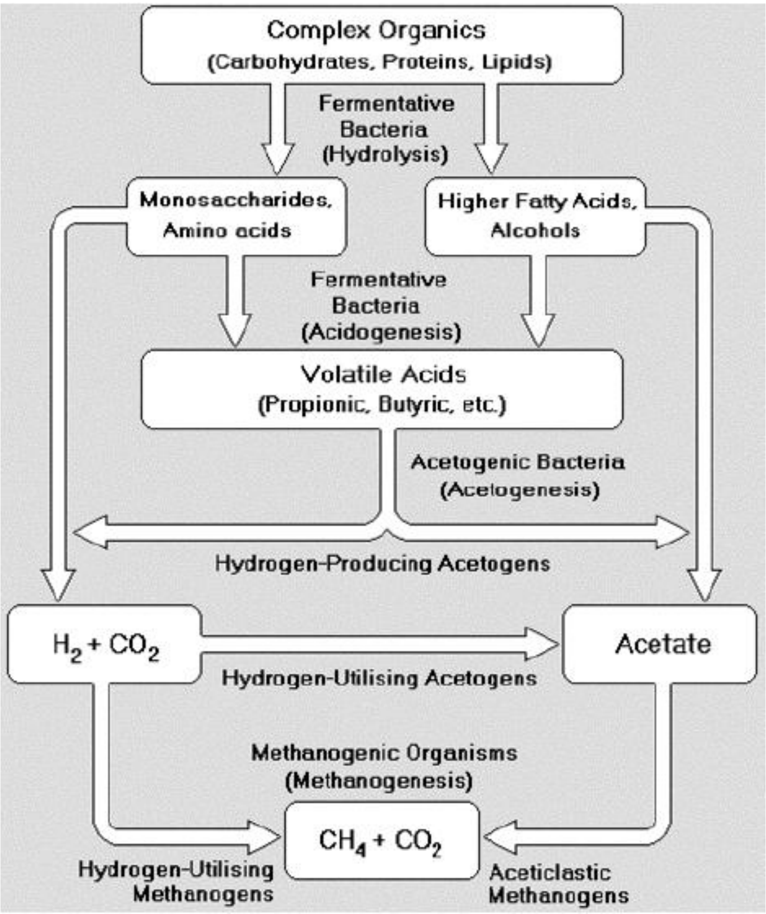


Figure 1. 10 Metabolic pathway and microbial groups involved in AD [22].

1.7.1 Anaerobic Digestion Process: Hydrolysis

In this step long chain compound are decomposed to short chain monomers or oligomers by fermentative bacteria. For instance, proteins, lipids and carbohydrates are hydrolyzed to amino acids, long chain fatty acids and sugars, respectively. A variety of enzymes excreted by fermentative bacteria make possible this degradation, these exoenzymes decompose non-dissolved materials. Some of the exoenzymes are: lipase, cellulase, cellobiase, xylanase, amylase and protease. The hydrolysis stage is very sensible to the temperature and its variations, that is why fluctuation during this process might be avoided [21,23].

As hydrolysis is the first step in the AD process, for some type of substrates, hydrolysis becomes the RDS, but it is not due to the poor presence of enzymes or their activity, it is more because the difficulty of them to find free accessible area and start its degradation process to short chain monomers. For that reason, there is a necessity to implement, as a previous step, pretreatments that can include physical, chemical or a mix of them (physico-chemical) interactions to make easier the access of these enzymes into the solid substrate structure and achieve higher decompositions [23].

1.7.2 Anaerobic Digestion Process: Acidogenesis

The products from the hydrolysis are then converted to some other products under the presence of the fermentative Acidogens bacteria. Here, simple sugars, amino acids and fatty acids are mainly reduced to acetate, carbon dioxide and hydrogen (70%), also volatile fatty acids (VFA's) and some alcohols (30%). Besides, there are some traces of lactic acid, ammonium, hydrogen sulphide, amount others. Acidogenesis is the fastest stage in the AD process, the anaerobic conversions are reached more rapidly due to the high bacteria growth rates, between ten and twenty times compared with the other sub – process. For that reason, if the AD process is not correctly equilibrated with the necessary alkaline supplements, a drop in the pH is observed after the produced acids consume the alkalinity supplied [21,23].

Nevertheless, the main products of this sub-process depend mainly on the hydrogen concentration inside the digester. If hydrogen is used efficiently by the Methanogenic bacteria, the main product of the Acidogenesis will be acetate and the AD will continue under normal – standard parameters. In contrast, if there is a high concentration of hydrogen in the medium, owing to a methanogens retarded effect, there will be affinity for the production of propionate and butyrate, or even some others products as lactate and alcohols. Those intermediate products are undesirable and should be avoided due to their inhibitory or retardant effect over the whole AD system [23].

1.7.3 Anaerobic Digestion Process: Acetogenesis

VFA and the alcohols produced in the Acidogenesis step are oxidized into acetate, carbon dioxide and hydrogen. These new components will be the substrates fro forward steps. For instance, hydrogen is an important substrate for Methanogenesis stage. However, the concentration of hydrogen apart from increasing the pressure of the system also inhibits the Acetogenic bacteria activity. Acetogenesis and Methanogenesis occur simultaneously where hydrogen becomes a critical component both as product and as substrate [21].

Acetogenesis is highly dependent sub-process on Acidogenesis. If the hydrogen concentration on the Acidogenesis stage is kept under partial pressure of $10^{-4} – 10^{-6}$ atm, the production and consumption of hydrogen is controlled and does not interfere at all with the Acetogenesis chemical reactions. Therefore, the Acetogens will have the required energy to convert the short chain fatty acids (SCFA) to acetate, carbon dioxide and hydrogen gas. However, long chain fatty acids (LCFA) are retardant of Acetogenesis, which will limit the conversion to gases in Methanogenesis by consuming the energy for the degradation of the SCFA [22,23].

1.7.4 Anaerobic Digestion Process: Methanogenesis

In this last AD step, methane is produced in conjunction with carbon dioxide, nitrogen, oxygen, hydrogen sulfide and some ammonia (called biogas as a total), all this thanks to the Methanogenic bacteria. In this sub-process, 70% of the methane produced comes from the conversion of acetates, and the remaining 30% is supplied by the hydrogen and carbon dioxide concentration in the medium. As it was mentioned before, there is not clarity about the RDS of this process, thus Methanogenesis is also considered a critical step in AD due to its slow chemical reaction [21].

Methanogens are classified in two groups: aceticlastic methanogens and hydrogenotrophic methanogens. The first group of bacteria convert acetate to methane, amount other gases, and the second group does the same from hydrogen using carbon dioxide as its main reagent into the chemical reaction. Aceticlastic methanogens produce 70% of the methane of the AD, but its bacteria growth rate is slow (days) compared with the hydrogenotrophic, which produce 30% of the methane of the AD with a bacteria growth rate of 4 – 12 hours. That is why sludge with high aceticlastic methanogens is preferred to start-up reactors in industry [22,23].

1.8 Biogas Production: The Parameters Affecting Anaerobic Digestion (AD)

The production of biogas is sensible to the different parameters that are present in the medium. Some of the most important parameters for anaerobic bacteria are: Concentration of oxygen, temperature, pH, nutrients, agitation and the concentration of inhibitors. Some of the previous parameters have more effect over the efficiency than the others. For instance, the presence of oxygen can deviate the digestion process and produce some other non-desirable gases as carbon dioxide [21].

1.8.1 The Parameters Affecting AD: Temperature

Temperature is one of the main parameter in AD, its selection can have great effect in the amount of biogas produced, as well as in the hydraulic retention time (HRT) into the process. Thus, there are three bands defined where microorganisms can work properly, Table 1.7. Therefore, the right selection of the temperature of AD would have not only effect over the HRT, but also in the sensibility of the system for getting perturbed under any small variation of this parameter. For instance, a +/-1°C fluctuation in the AD temperature for the thermophilic stage, will destabilize the bacteria performance and will take some other time to reach its high production of biogas again. Meanwhile, for the mesophilic band this fluctuation can be up to +/-3°C [21].

On the other hand, biogas production gets affect by the temperature chosen. For that reason, the temperature needs to be chosen depending on the substrate used for the process. As the HRT depends on temperature, the production of biogas, and therefore of methane, increases with temperature and a higher amount is produced in less time, making the AD process faster, Figure 1.11. That high production of biogas does not depend on the short retention time, it does on the growth of the Methanogenic bacteria in the medium, which vary their proliferation depending on the temperature set, Figure 1.12 [21,22].

Table 1. 7 Thermal stage and hydraulic retention time (HRT) [21].

Thermal stage	Temperature	HRT
Psychrophilic	< 25 °C	70 – 80 days
Mesophilic	25 – 45 °C	30 – 40 days
Thermophilic	45 – 70 °C	15 – 20 days

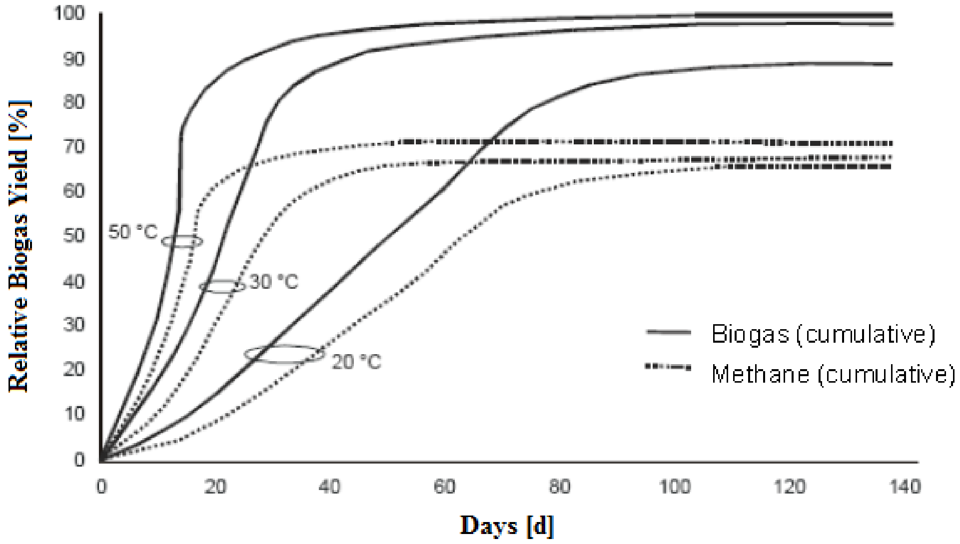


Figure 1. 11 Relative biogas yield depending on temperature and retention time [21].

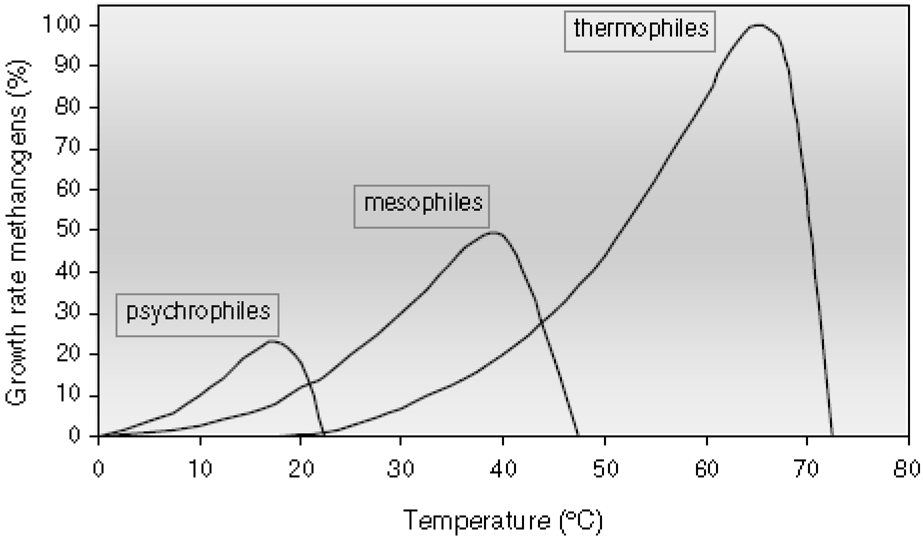


Figure 1. 12 Influence of temperature on methanogens growth rate [22].

1.8.2 The Parameters Affecting AD: pH – Value

The pH – value is maybe the most critical parameter in AD, a small fluctuation in it can inhibit the growth of the bacteria that are present in the medium. This value measures the acidity or alkalinity of the solution, thus is how it gets affect by the solubility of the different gases that are produced constantly. Therefore, the partial pressure of carbon dioxide must be avoided, due to the variation that causes into the AD. That is why a buffer agent is added to the system. For example, sodium bicarbonate is added to counteract the concentration of acid or alkaline components present in solution. Unfortunately, sodium bicarbonate has a limit buffer capacity and when it is surpassed, the pH fluctuations in the system occur drastically. The AD process will never reach a stable state and will be inhibited [21].

For the mesophilic stage the pH value is between 6.5 and 8.0 units, but it has a critical effect under the value of 6 and over 8.3. In the thermophilic digestion, the pH value is higher than in the mesophilic case. That is supported by the fact that the solubility of carbon dioxide in water decreases with the temperature increase. Thus, thermophilic AD keeps a more stable pH under the scope of CO_2 inhibition [21].

1.8.3 The Parameters Affecting AD: Presence of Inhibitors

There are many different components that have inhibitor behavior on AD. In some cases, the inhibitory effect depends on the substrate, it means that a specific component can inhibit the biogas production for some biomass, but it can behave as a neutral agent for some other ones. For instance, the presence of intermediate components as volatile fatty acids (VFA: Acetate propionate, butyrate) can decrease the pH – value and inhibit the biogas production in the AD. However, the VFA inhibitor effect is not always easily detect, because of the buffer capacity of the sodium bicarbonate. Sometimes, the excess of VFA's in the system is lately determined and the AD inhibited [21].

Ammonia (NH_3) is an important nutrient for the AD process, but its excess in the digester can cause an inhibitory effect for the methane production. Proteins are an important source of ammonia for digestion. Methanogenic bacteria are highly sensitive to the high concentration of free ammonia in solution. The ammonia concentration in solution must be under 80 mg/L to be beneficial for the AD and not affect the process.

Free ammonia is an important parameter for the thermophilic stage, because its concentration is proportional to high temperatures [21].

1.9 Biogas as an Energy Source

Biogas is a mixed of several gases produced in a digestive process. For the anaerobic digestion process the composition of biogas is mainly: methane (CH_4: 55 – 75 vol%), carbon dioxide (CO_2: 25 – 45 vol%), hydrogen sulfide (H_2S: 0 – 1.5 vol%) and ammonia (NH_3: 0 – 0.05 vol%). That gas is also saturated with some other components as water vapor, dust particles and some traces of hydrogen (H_2), nitrogen (N_2), carbon monoxide (CO) and some other halogens which depend on the feedstock used. A standard biogas fuel value, based on the previous composition, is 22 – 30 MJ/Nm3 (Higher Heating Value; HHV) and 19 – 26 MJ/Nm3 (Lower Heating Value, LHV) [24].

There are several uses for biogas. Depending on its final use, there are some quality standards that must achieve. For instance, heat production in gas heater/boiler systems requires a low quality of biogas to work. In here, it is suitable a reduction of hydrogen sulfide concentration (H_2S < 1000 ppm) to avoid corrosion in the equipments utilized. In contrast, combined heat and power (CHP) systems and for upgrading to natural gas or fuel gas require higher quality of the biogas to utilize. For that reason, the removal of the trace components mentioned before, play an important role to the final dispose of the gas obtained. That removal process might include different unitary processes where absorption and membrane separation are the most implemented ones [24,25].

Nowadays, the implementation of biogas as a renewable energy source has gained its own market within the European countries, where in the 90's several biogas production plants were constructed to take advantage of the solid or liquid wastes and convert them value-added product. Therefore, in the 21st century the growth of new biogas plants is still a latent topic in Europe which keeps adding adepts to the existed ones, Figure 1.13. Germany, Italy and France contribute to these numbers with 10 846, 1 555 and 717 working plants in each country, respectively. Despite the reduction of new plants in Europe, where its glorious year was 2010 with +69%, the capacity of the new constructions has increased substantially [24,25].

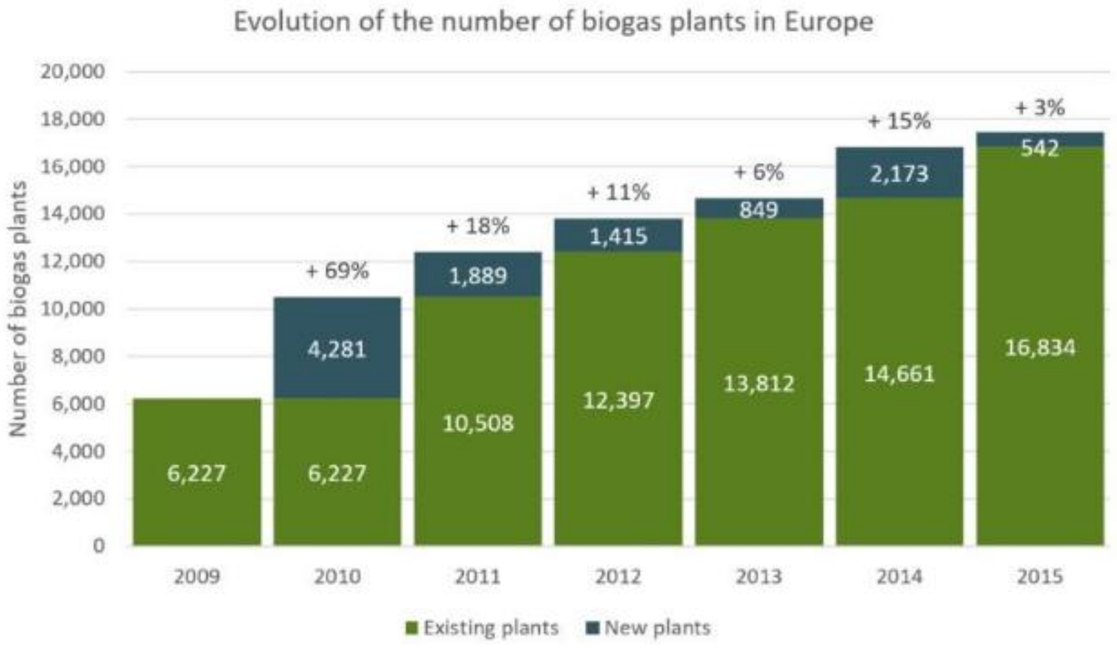


Figure 1. 13 Evolution of the number of biogas plants in Europe [25].

In the world market, the panorama is also encouraging. Year by year, the construction of biogas plants are implemented whole around the world. The production of biogas has passed from 13.2 Billion m^3 (2000) to 60.8 Billion m^3 (2016), it means an increase of more than 300% in less than twenty years, Table 1.8. Europe is the main producer of that amount with 54%, followed by Asia and America with 30% and 14%, respectively Figure 1.14. Not only the biogas production increases year by year, but also the technologies to reach higher efficiencies and the jobs in this type of industry are augmenting through the time [26].

Table 1. 8 World biogas production in 21st century [26].

Year	Biogas [Billion m^3]	Biogas [EJ]
2000	13.2	0.28
2005	23.1	0.50
2010	38.7	0.84
2015	60	1.30
2016	60.8	1.31

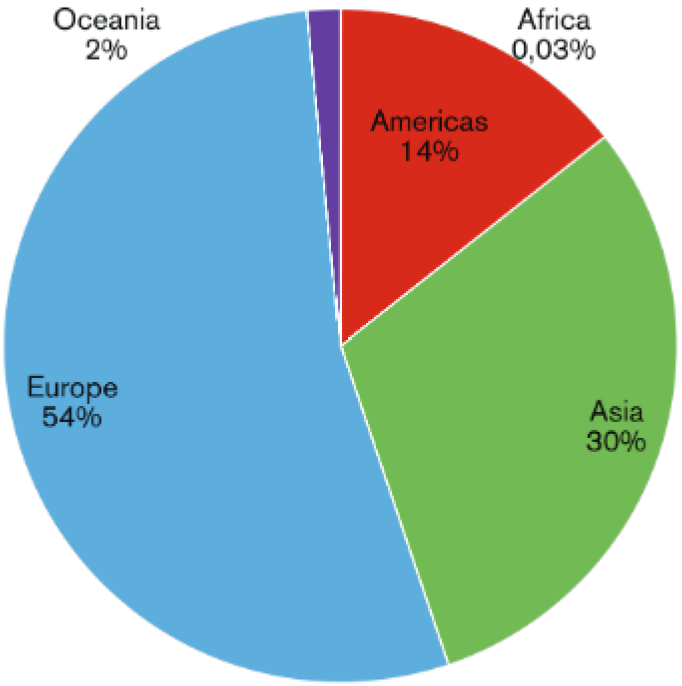


Figure 1. 14 World biogas production by continent in 20016 [26].

2. METHODS AND MATERIALS

Through this section, all the methods and the methodology used during the investigation process are shown. Therefore, steps such as the reception and the pretreatments of the raw material are specified, that with the intention of giving a general idea about the different considerations that were taken into account to set the experiments in this project. Besides, the biochemical methane potential tests are considered to have a better understanding of the real effect that the pretreatments cause over the biogas production.

2.1 Sugar Beet Reception and Preparation

2.1.1 Reception

The sugar beet for the investigation was provided from a local farm where this plant is sown near Ankara, Turkey. After the reception, it was cleaned and the remaining soil, leaves, roots and damaged parts were discarded.

2.1.2 Preparation

By using a commercial house blender, the substrate was cut until reach small pieces of approximately 4 mm^2. Moreover, after that gridding process, the raw material was divided into two sub-groups: One will be the raw sugar beet (**RSB**) that contents high amount of sugars, also known as sugar beet pulp.

In the other group, raw sugar beet was heated during 60 minutes at 70 °C to extract all the sugar that it contents, and simulate the industrial process of sugar extraction. Thus, we can use an exhausted/boiled sugar beet (**ESB**), representing the waste that is normally gotten from the sugar industry, also known as sugar beet bagasse.

2.2 Pretreatments

Under the scope of this project, the pretreatments applied to the lignocellulosic materials are chemical and physical methods or a combination of them. The chemical pretreatments were conducted under acidic and alkaline conditions, meanwhile the physical ones were performed with the reduction of size of the raw root (grinding:

explained in the sugar beet preparation section) and with the supply of heat from external sources.

2.2.1 Chemical Pretreatments

The chemical reagent used for the acid pretreatments was the sulfuric acid, H_2SO_4 (95 – 97 % purity), which was diluted to have the desired concentrations. While, in the alkaline side, sodium hydroxide, NaOH (98 – 100.5 % purity), was used to perform counterpart of the experiments. The resume is shown in Table 2.1. In here, low concentrations (M: Molar) where used with high ratio between solid and liquid (L/S: 20) and short time of interaction (10-15 minutes), and high concentrations (% percentage) were used with the same ratio, but longer time of interaction: 48 hours.

Table 2. 1 Variables for the chemical pretreatment processes.

Substrate (S)	Liquid (L)	Concentration	Ratio L/S	Time
1. Raw Sugar Beet – RSB **2.** Exhausted Sugar Beet – ESB	-Water -Acid: H_2SO_4 -Alkaline: NaOH	0.2 M 0.3 M 0.4 M	20 S: 2.5 g L: 50 mL	Short time: 10 – 15 minutes
		2 % 4 %	30 S: 1.67 g L: 50 mL	Long time: 48 hours

The acid or alkaline solution, depending on the case, was mixed with the lignocellulosic material to perform the pretreatment. After the pretreatment studies, the samples were neutralized (pH: +/-7) before BMP test, filtered by using a vacuum pump and the liquid phase was separated from the solid phase. Therefore, there was a treated lignocellulosic material (solid treated substrate, insoluble residues) and a liquid phase (liquid substrate, filtrates) which is used to determine the presence of soluble sugars after the pretreatment. The procedure is followed as it is shown in the Figure 2.1. Both, liquid and solid substrates were used to perform the biochemical methane potential process.

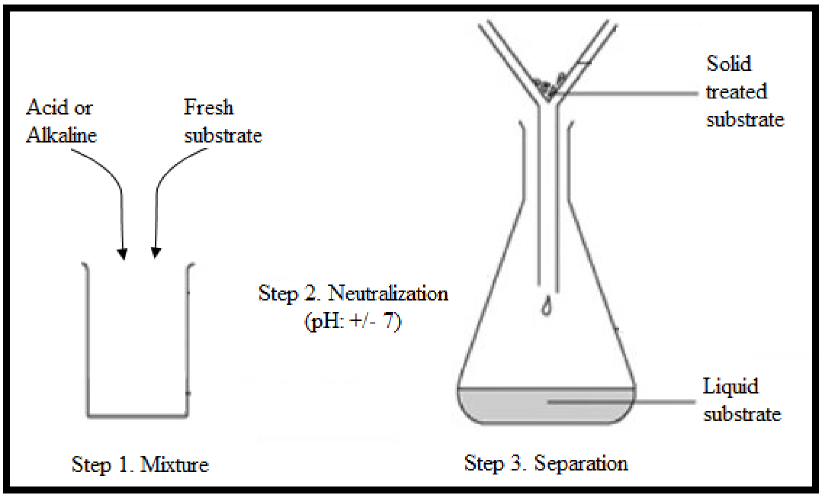


Figure 2. 1 General procedure for the chemical pretreatments.

2.2.2 Physical Pretreatments

The size reduction of the raw material is a necessary activity to have a better performance of the pretreatments over the lignocellulosic biomass [27]. Besides, there are two systems for heat supply: microwave radiation and autoclave. The general procedure of this type of pretreatments is shown in Figure 2.2, where the first step is the mixture of the fresh substrate (solid) with the liquid phase. Thus, after the pretreatment there is a solid treated substrate and the liquid substrate phase.

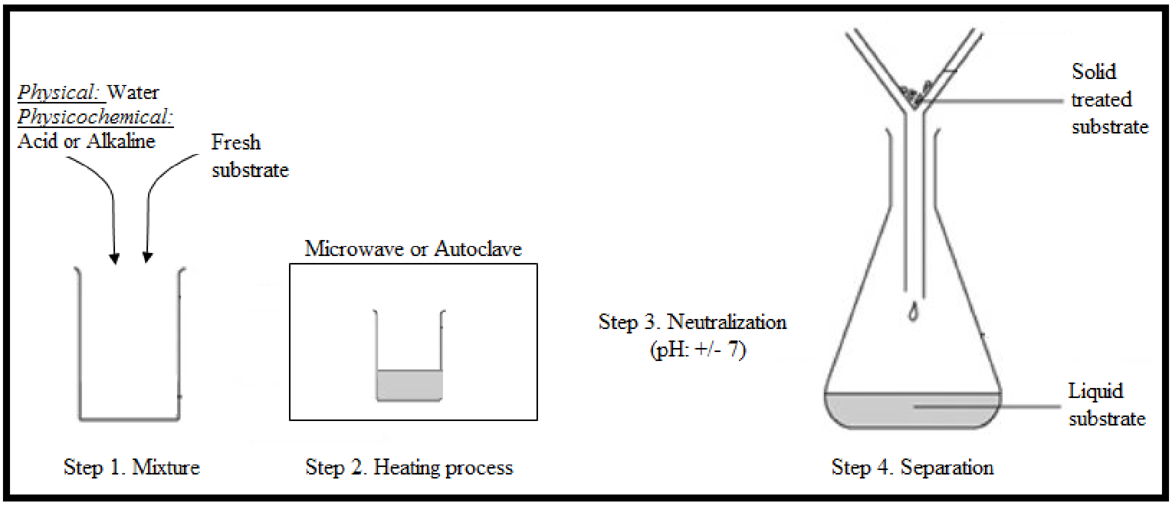


Figure 2. 2 General procedure for the physical/physicochemical pretreatments.

2.2.2.1 Microwave Pretreatment

For the microwave pretreatment, a commercial microwave was used with a pre-set power of 300W and the variable was the exposure time: 1 and 2 minutes (see Table 2.2). Microwave radiation is widely used to pretreat lignocellulosic biomass due to the effectiveness that offers for disrupting the cell wall. Also, it can enhance brakeage of the lignocellulosic structure of components like hemicellulose which accelerate the hydrolysis stage [28].

Table 2. 2 Variables for the microwave assisted pretreatments.

Substrate (S)	Liquid (L)	Concentration	Ratio L/S	Power	Time
1. Raw Sugar Beet – RSB **2.** Exhausted Sugar Beet - ESB	-Water	0.2 M	20 S: 2.5 g L: 50 mL	300 Watts	0 min
	-Acid: H_2SO_4	0.3 M			1 min
	-Alkaline: NaOH	0.4 M			2 min

2.2.2.2 Autoclave Pretreatment

For the autoclave thermal pretreatment, the supplied autoclave in the laboratory was used to perform the experiments where time was also the variable of the process: 30, 60 minutes. The set temperature was 120 °C with a solid – liquid ratio of 20 (see Table 2.3). Thermal supply is one of the most use physical pretreatment, so it makes it easy to compare with other type of feedstock studied under the biochemical methane potential. Studies show different conditions to work under this type of pretreatment. Thus, based on literature 120°C is the set temperature due to the results achieved [29,19].

Table 2. 3 Variables for the pretreatments with autoclave pretreatment.

Substrate (S)	Liquid (L)	Concentration	Ratio L/S	Temperature	Time
1. Raw Sugar Beet – RSB **2.** Exhausted Sugar Beet – ESB	-Water -Acid: H_2SO_4 -Alkaline: NaOH	2 % 3 % 4 %	30 S: 1.67 g L: 50 mL	120 °C	0 min 30 min 60 min

2.3 Biochemical Methane Potential Process

2.3.1 Sludge Reception and Preparation

The seed sludge used for the different experiments was collected from a local plant which produces biogas from the animal manure located in the rural area of the city. In there, the company collects all the manure from cows and chickens, to benefit from that waste and produce biogas. After the reception, it was kept in the fridge until the use and five days before the set up of the tests, it was pre-heated at 37°C with some buffer agent (Sodium bicarbonate, $NaHCO_3$) and activated.

2.3.2 Biochemical Methane Potential Tests

The batch biochemical methane potential (BMP) tests were carried under mesophilic conditions: 37°C, neutral pH 7 +/- 0.5 and 30 – 40 days of time process (HRT) or until the biogas production ceased. All the experiments were done by duplicate to have more accurate approximations of the values. The incubator used kept the samples under the temperature specified and also could supply the necessary required agitation to have the liquid mixture under suspension, 60 rpm.

The experiments were set in 100 mL glass bottles, with a total volume of substrate – sludge (1:1) of 60 mL, which also gave the necessary free space to store the gas produced day by day. Besides, to each of the bottles was added some amount of sodium bicarbonate ($NaHCO_3$, 99% purity) as a buffer agent. The substrate is composed of 5%

solid substrate and 95% of the liquid substrate (***Test 1.***) obtained from the pretreatment processes, as it is shown in Table 2.4.

On the other hand, some other experiments were carried under the similar conditions mentioned before, but in this set (***Test 2.***) the liquid substrate (filtrates) gotten from the pretreatments performed, were discarded and water was used instead. Those experiments were done with the intention of discard any possible inhibitor effect that can occur from the reagents used in the pretreatment phases. Besides, the pretreatments conditions (Temperature, time and concentration of reagents) performed under this test, were based on the best results gotten from the previous test developed [29].

After each bottle was filled, it was closed with a rubber cap and sealed with rubber cork. Therefore, the process is considered under batch conditions, because there was not any addition or removal of substrates until the end of the tests where there is no significant gas production.

Table 2. 4 Composition of each of the BMP's experiments.

Concept	**Quantity**
Seed Anaerobic Sludge	30 mL
Substrate:	30 mL
Solid substrate (insoluble residues)	*5 % w/v*
Test 1.: Liquid substrate (filtrates) ***Test 2.: Distilled Water***	*95 % w/v* *95 % w/v*
Buffer: Sodium bicarbonate, $NaHCO_3$	20 mg
Free space: Biogas storage	40 mL
Total volume	100 mL

2.3.3 Monitoring of the BMP Tests

The BMP process was followed by an indirect method; the gas production was monitored using a manometer which helped to measure the total pressure of each bottle (Figure 2.3). During the first week, the data was collected daily, after the second week and until the end, it was done every other two or three days. After each pressure measurement, the biogas produced in each bottle was analyzed for CO_2 content. After the analysis, the biogas was released to have again the empty space for future gas production, avoiding any possible inhibition from the previous biogas already produced.

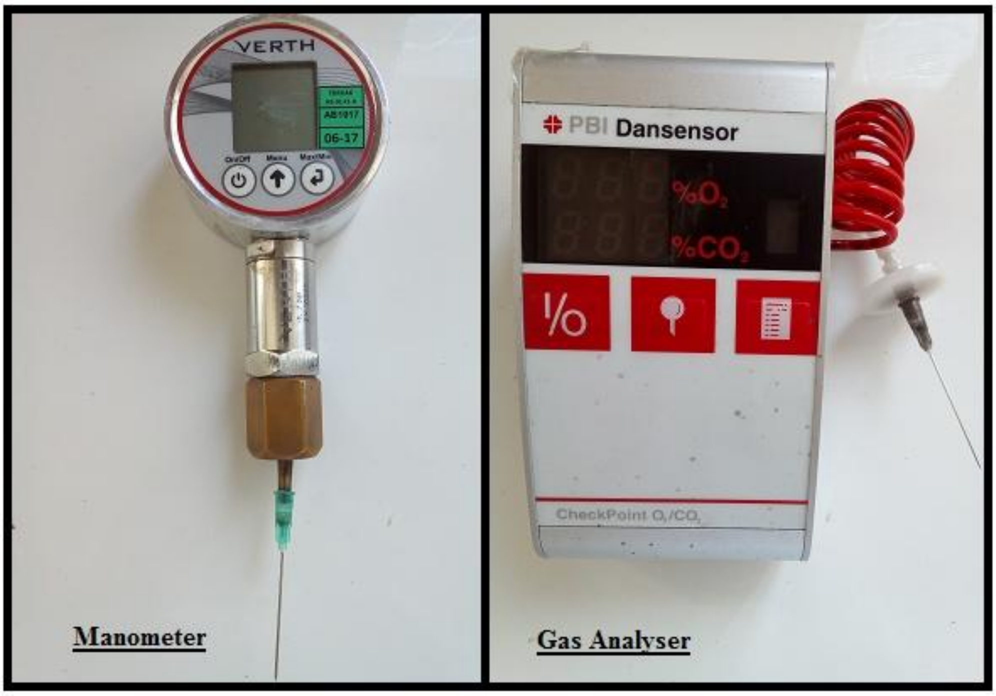


Figure 2. 3 Instruments used for the following of the samples in the BMP tests.

2.4 Analytical Methods

The measurement of Total Solids (TS) and Volatile Solids (VS) were performed under the conditions specified by the relevant standard methods of the APHA [30], using the necessary equipments of the laboratory. Not only pH and conductivity of the samples were measured by the relevant APHA [30] considerations, but also with the specifications given by the supplier of the laboratory pH-meter were taken into account.

The soluble sugar determination was done by the specifications given by Miller [31], which includes steps such as the preparation of the reagents with dinitrosalicylic acid, followed by the thermal process to develop the color of each of the samples, and finishing with the use of a spectrophotometer to determine the amount of sugar present in the aliquots taken at 575 nm.

2.4.1 Fourier Transform Infrared Spectroscopy Analysis

The Fourier Transform Infrared (FTIR) analysis was made under the standard procedure, which includes mixing 1 mg of the ground lignocellulosic sample with 99 mg of KBr (spectroscopic grade) which is an inert component. After that, the mixture is compressed at 1.5 ton pressure in a disk form and placed in the spectrophotometer to get the spectra from this equipment. This analysis was made by the personnel from the Chemical Engineering department of the Hacettepe University [32].

3. RESULTS & DISCUSSION

The analysis of the results is divided into two main parts which are the availability of sugars in solutions and the BMP process results. In either case the results depend on the type of pretreatment applied: Chemical, physical and physicochemical (combination of chemical and physical). In the pretreatments, optimum contact time is important in this study. Acid and alkaline pretreatments as well as microwave and autoclave assisted heating were investigated for the solubility of lignocellulosic contents.

3.1 General characterization of the substrates

In the scope of this investigation, two main substrates were used for biogas production. The sugar beet was used in two different forms: The first one is the raw sugar beet (RSB) without any chemical or physical modification (until the pretreatment can take place), and the other one is the exhausted sugar beet (ESB). This second substrate suffered a previous extractive process, where the natural sugars that it contains were removed by heating the sugar beet for certain time in order to resemble the industrial process that it suffers in the sugar factories.

Therefore, the substrates mentioned before will have different characteristics, but no far from each other. The total solids and volatile solids contents are similar to each other. Also, the seed sludge characterization is given in Table 3.1.

Table 3. 1 Analysis of the substrates and the sludge used.

Concept	TS [%]	VS [%]
Raw sugar beet – RSB	22.15	99.39
Exhausted sugar beet - ESB	15.42	99.61
Seed Anaerobic Sludge	9.30	96.66

On the other hand, the FTIR analysis is used to understand the chemical variation of the functional groups. In this case, the substrates were studied under the wavenumbers between 4000 and 600 cm^{-1}. It showed that there is a slightly difference between the RSB and ESB. It means that the sugar extraction (ESB), not only exhausted the biomass from its monosaccharide, but also it modified its functional groups, Figure 3.1.

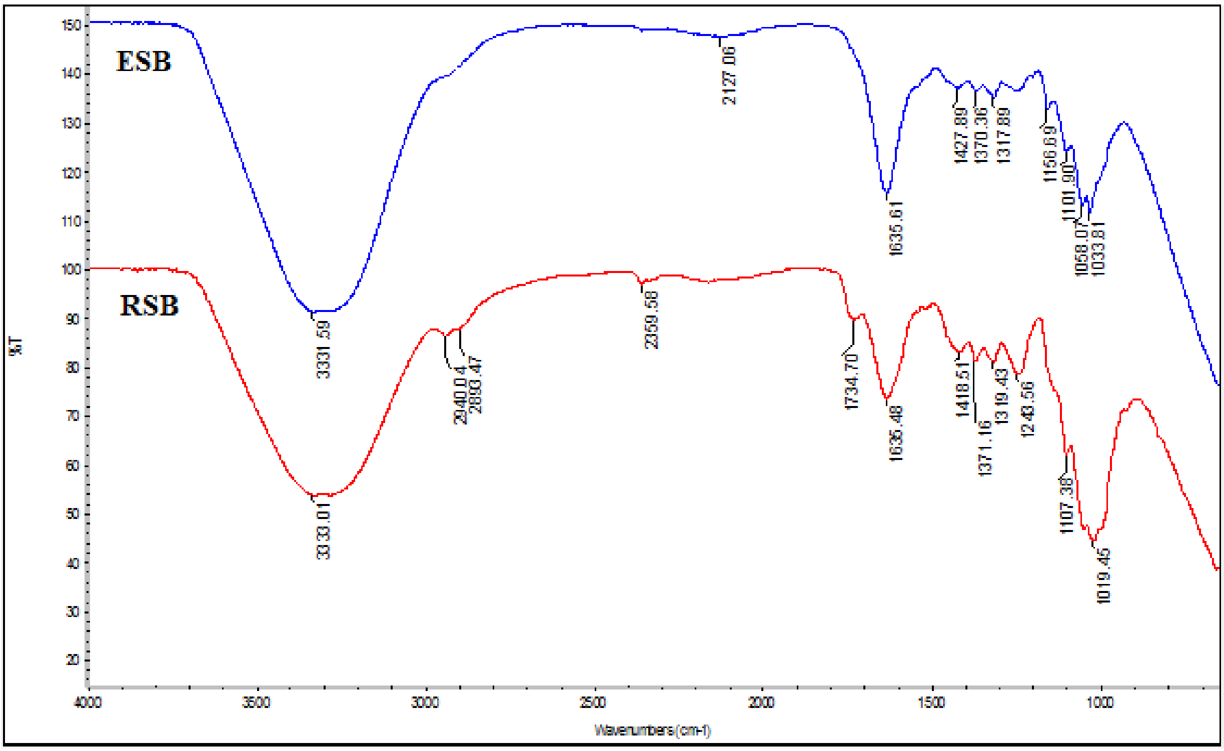


Figure 3. 1 FTIR spectrum for RSB (lower line) and ESB (upper line).

From the FTIR spectra there are some surface modifications that should be considered for the analysis. In the RSB infrared spectra, the OH bond at 3333 cm^{-1} represents the OH groups in cellulose. While the CH aliphatic stretching peak at 2940 and 2893 cm^{-1} represent the CH_2 and CH_3 groups present in cellulose and hemicellulose, respectively. On the other hand, ESB has a higher content of cellulose at 3331 cm^{-1} than RSB. However, at the second wavenumber coordinate, there is not presence of radicals that suggest the presence of cellulose and hemicellulose [32,33].

Furthermore, the RSB spectrum shows the ester bond groups at 1734 and 1243 cm^{-1} which are a signal of the ester connection that exists between carbohydrates and lignin. However, both of those two bonds disappear for the ESB analysis, suggesting the breakage of those linkages. It shows that the pretreatment could disrupt ester linkages between carbohydrates and lignin. Moreover, the weak phenolic hydroxyl bond signal at

1370 cm^{-1} in the ESB spectrum, also suggests that the lignin structure was disrupted during the sugar extraction process [34,35].

In general, RSB as a lignocellulosic biomass is represented by twelve groups (or bonds) in its structural surface, as it is observed in the FTIR spectra. All of them contribute to generate the lignocellulosic characteristics that this biomass has. RSB, as a fresh raw substrate, has more components that are linked between them and give it its stiff aspect. In contrast, the thermal sugar extraction over ESB disrupts the bonds between the lignocellulosic components and is represented by ten groups (or bonds) in the FTIR spectra. Even, the ESB groups are weaker than in the fresh substrate. For that reason, the thermal extraction could be considered as a pretreatment to collapse the tough links before anaerobic treatments [32].

From literature is suggested that the structural modification showed by the FTIR analysis also reflects a modification over the crystallinity of lignocellulosic biomass. The cellulose crystallinity is reduced by the pretreatments, which is likely to affect positively the AD. On the other hand, an increase of crystallinity is expected from the pretreated substrate due to the degradation and disruption of the amorphous components from the lignocellulosic structure. The crystallinity index (CI) which measures the ratio between amorphous components of cellulose, is likely reduce by the pretreatments that are applied over the lignocellulosic substrates [32].

In addition to the FTIR, analyses show that the lignocellulosic structure is modified by the effect of the pretreatments over the biomass. The effect of acidic and alkaline pretreatments can dislocate and fragment the cell wall. Thus, hemicellulose and lignin content in the sugar beet can be disrupted to monomers. However, from literature it is suggested that there is also a risk of relocation of these components, which is not beneficial for the AD [32].

3.1 Sugar Solubility after Chemical Pretreatments

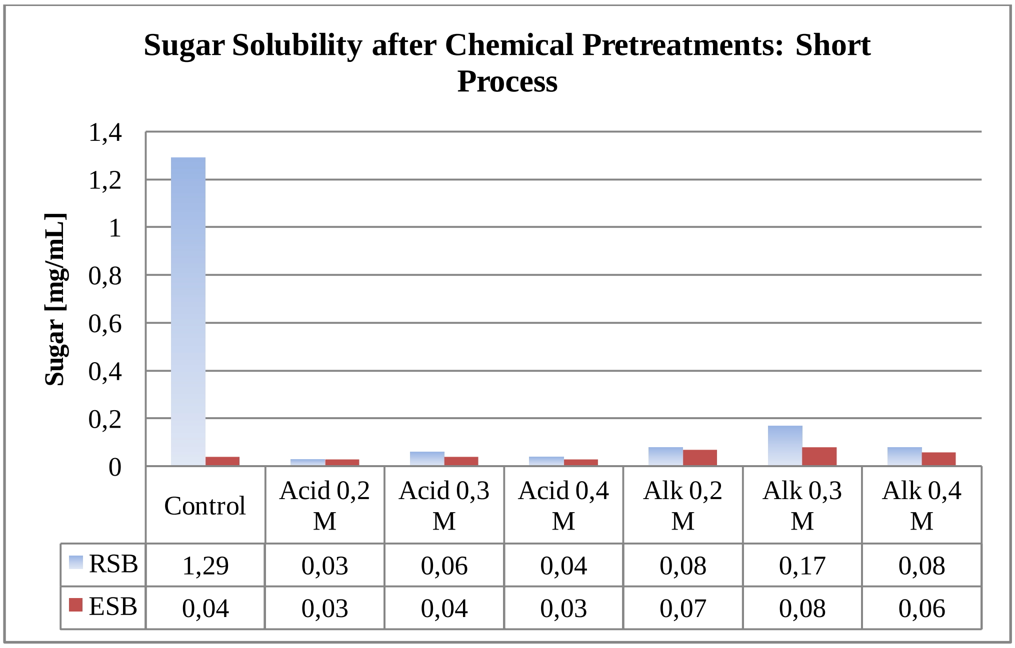
The solubility was measured as reducing sugar (measured as glucose) present in solution after each pretreatment. By considering the characteristic of each of them, the values depend on the type of pretreatment developed.

For the chemical pretreatments experiments, the reagents used were sulfuric acid and sodium hydroxide. Here, the variables were the contact time of the pretreatment and the acid/alkaline amount, S/L ratio in the process. Where the results are as follow:

3.1.1 Sugar Solubility after Chemical Pretreatments: Short Time Duration Process

As it was mentioned before, the duration of the pretreatment with short contact time was selected as between 10 – 15 minutes. The fresh substrate was mixed with the chemical agent to interact: Water, Acid or Alkaline. The reducing sugar results are shown in Figure 3.2. For this case, the control is mixed with water.

Despite the high concentration of sugar released in solution with the sample of RSB (Raw Sugar Beet) in water, 1.29 mg/mL, samples show that the concentration of sugar in solution have a tendency to be more available within the alkaline solutions. The soluble sugar concentrations for the ESB (Exhausted Sugar Beet) samples are lower than that for the RSB, due to the previous extractive process applied. The higher concentrations in solution were gotten with the acid or alkaline concentration of 0.3M, which coincide for both of the samples: RSB and ESB.



	Control	Acid 0,2 M	Acid 0,3 M	Acid 0,4 M	Alk 0,2 M	Alk 0,3 M	Alk 0,4 M
RSB	1,29	0,03	0,06	0,04	0,08	0,17	0,08
ESB	0,04	0,03	0,04	0,03	0,07	0,08	0,06

Figure 3. 2 Sugar solubility after chemical pretreatments, short time contact.

The short time contact process with chemical reagents seems to work better for ESB than for RSB. In the case of RSB, the alkaline medium does not favorable to release more sugars; in contrast it reduces its initial concentration. The initial sugar that RSB possesses could be transformed into new components enhanced by the alkalinity in the medium like the lignin disruption. The concentration of sugar released from ESB with the alkaline pretreatments, is higher than the blank (water). Thus, the alkaline medium enhances the disruption of some lignocellulosic component and increase the soluble sugar concentration. This exhausted substrate does not possess initial soluble sugars, thus it does not lose its initial concentration.

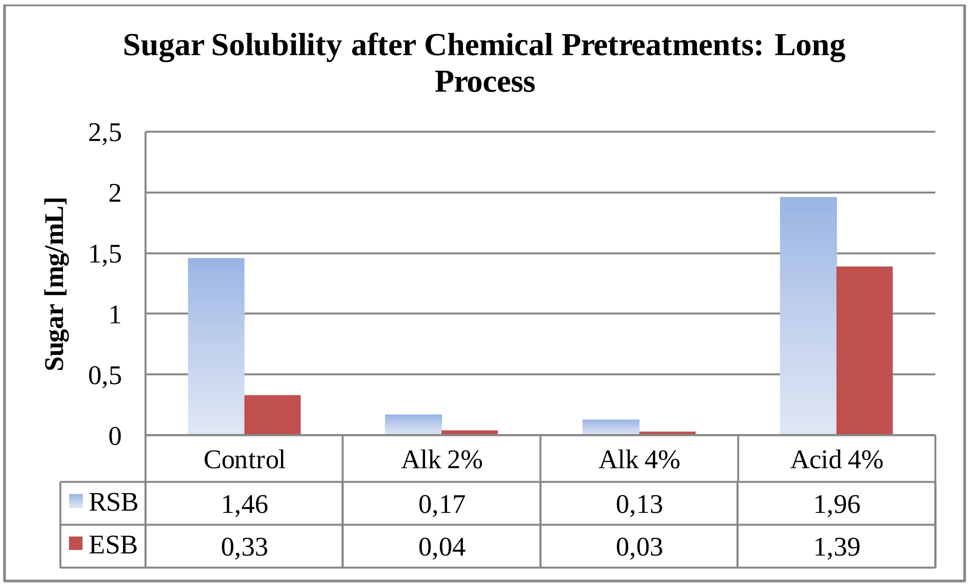
On the other side, acid pretreatments were not effective in releasing sugar for RSB neither ESB. In the case of RSB, the initial soluble sugar concentration could become new components under the acidity conditions. But, even after the acid pretreatment, the reagent did not disrupt the lignocellulosic structure to release sugars. A similar behavior was observed with the ESB substrate, where no change from the initial sugar concentration was gotten.

3.1.2 Sugar Solubility after Chemical Pretreatments: Long Time Duration Process

The duration of the long time pretreatment process was around 48 hours, time where the fresh substrate was mixed to interact with the chemical agent (acid or alkaline) or with only water (control), depending on the each case. The determination of the soluble sugars is shown in Figure 3.3.

Based on literature, the alkaline pretreatment, at low temperatures, has significant effect over the substrate solubilization than the acid reagent itself. For that reason, the acid 2% was not performed, but pretreatment with 4% H_2SO_4 resulted in a high amount of soluble sugars in the solution. The soluble sugar amounts were 1.96 mg/mL and 1.39 mg/mL, for RSB and ESB respectively. For the alkaline pretreatment, low concentration of the reagent was effective on releasing lower amount of soluble sugars were released compared to acid pretreatments: RSB with 0.17 mg/mL and ESB with 0.04 mg/mL. In addition, the substrates mixed with sole water resulted in 1.46 mg/mL sugar released from RSB and 0.33 mg/mL from ESB.

Therefore, for the long contact time is found as more convenient to have higher concentration of soluble sugar, thus the results of reducing sugars in solution were approximately 35% higher for RSB and 320% higher for ESB, compared with the sample treated with only water. When the short contact experiments are performed, the interactions time is not enough to disrupt the hemicellulose and the lignin in the lignocellulosic structure, while the long contact time shows that acid can break down the component from the structural matrix, but the alkaline generates other intermediate components as salts that are not measure for this study.



	Control	Alk 2%	Alk 4%	Acid 4%
RSB	1,46	0,17	0,13	1,96
ESB	0,33	0,04	0,03	1,39

Figure 3. 3 Sugar solubility after chemical pretreatments, long time process.

3.2 Sugar Solubility after Physical Pretreatments

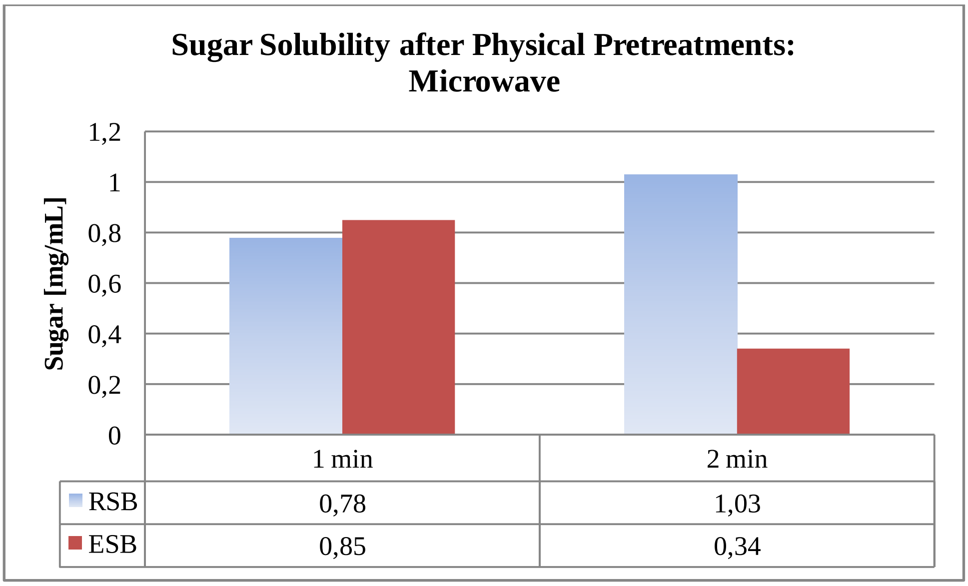
The variable in the physical pretreatments is the way of how the heat is transmitted to the samples. Two options were studied: microwave pretreatment and the autoclave process.

3.2.1 Sugar Solubility after Physical Pretreatments: Microwave Assisted Process

Under these experiments, some samples were exposed to microwave radiation (300 W) during one or two minutes, depending on the case, to supply the necessary energy and help to release the sugars from the substrate (Figure 3.4). In this section, samples were mixed only with water to see its effect under microwave radiation.

Both raw and exhausted SB were mixed with water (S/L: 20) and kept in microwave for 1 and 2 minutes periods and the soluble sugar amounts were measured. It was seen that higher amount of sugar was released from RSB samples as 1.03 mg/mL. While in the case of the ESB samples, the highest solubility was achieved in one minute: 0.85 mg/mL. These results compared with the sample without microwave radiation gotten in the chemical pretreatments for the control in short time process, are 20% lower and 2000% higher for the samples of RSB and ESB mixed with water, respectively.

It is likely because microwave radiation can debilitate the cell wall and increase the concentration of the soluble sugar with the release reached disrupting the lignocellulosic structure. Besides, it has a significant effect over the hemicellulose brakeage into other monosaccharide like xylose and without affecting substantially the cellulose structure. Lignin can be also affected and hydrolyzed into other components by the radiation [28,36,37].



	1 min	2 min
RSB	0,78	1,03
ESB	0,85	0,34

Figure 3. 4 Sugar solubility after physical pretreatments, microwave process.

3.2.2 Sugar Solubility after Physical Pretreatments: Autoclave Process

During the autoclave process, the samples were exposed to a temperature of 120°C, where the time for the pretreatment process was thirty minutes or an hour. The soluble sugar in solution is shown in Figure 3.5.

In this case, the soluble sugars values for RSB samples were 0.64 mg/mL and 0.2 mg/mL for thirty and sixty minutes contact times, respectively. While the ESB samples resulted in 0.06 mg/mL (for thirty minutes exposure) and 0.12 mg/mL (for sixty minutes exposure) soluble sugars values. The release of the sugars from RSB samples is more effective at shorter time, while in for the ESB longer contact times required to extract soluble sugars. However, since ESB was already exposed to a sugar extractive process, the solubility of sugars was low. These results compared with the results without microwave in the chemical pretreatments for the control in short time process are 50% lower and 200% higher for the samples of RSB (at 30 min in autoclave) and ESB (at 60 min in autoclave), respectively.

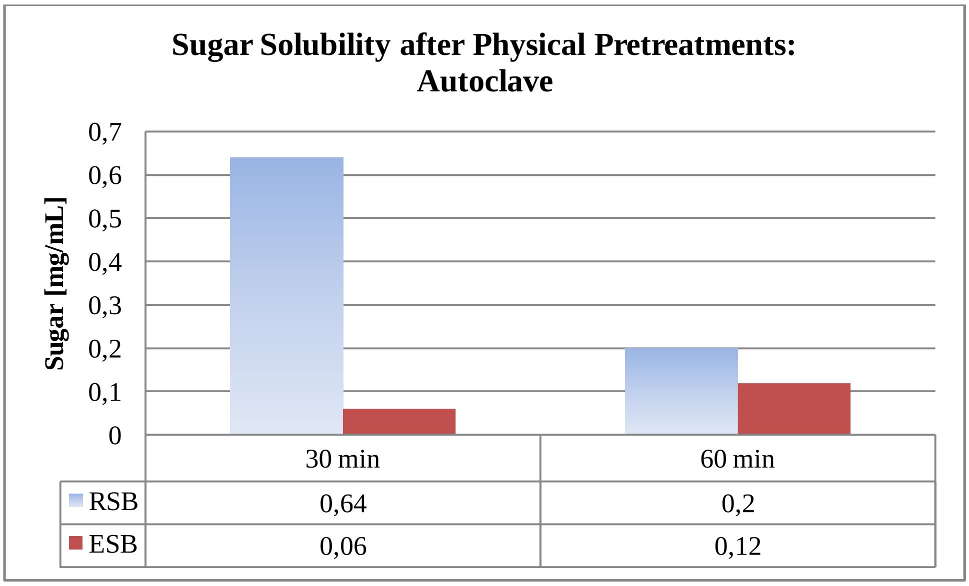


Figure 3. 5 Sugar solubility after physical pretreatments, autoclave process.

However, the results of the pretreatments studied with autoclave are lower compared with the values obtained in the microwave pretreatment. The interaction of the microwave radiation is more effective for extracting the sugar contained in the solid substrates. Microwave increased the sugar solubility up to 38 – 74% for RSB samples and 82 – 85% for ESB higher than the autoclave results. The thermal radiation of the autoclave equipment could be stronger than the microwave radiation. For that reason, the interaction with the lignocellulosic component might modify structurally the substrate, but at the same time the thermal pretreatment can degrade the monosaccharide released. In consequence, the soluble sugar is lower at higher temperatures. From literature it is suggested that there must be a reduction in pretreatment time at higher temperatures to avoid the sugar degradation [16,38].

3.3 Sugar Solubility after Physicochemical Pretreatments

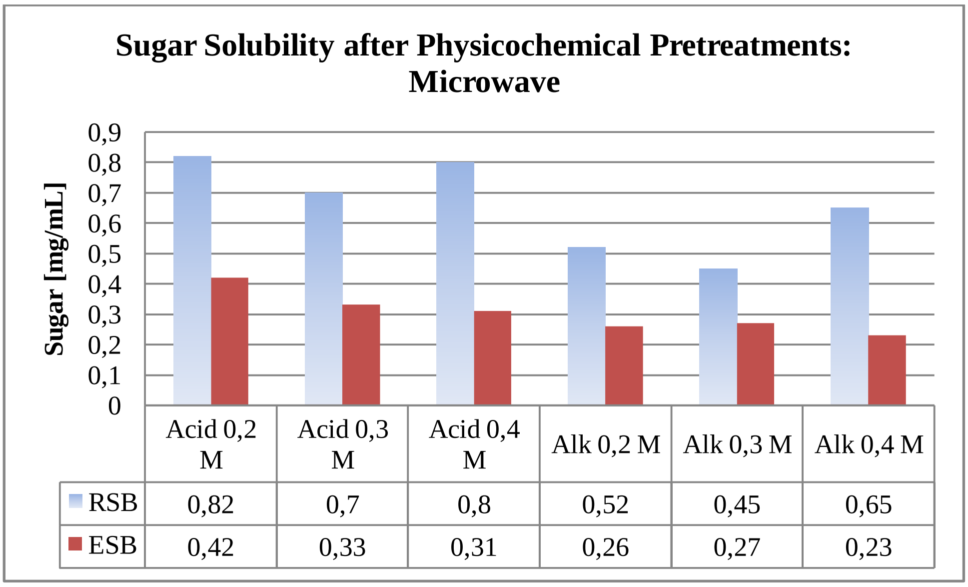
The physicochemical pretreatments are the combination of the chemical reagents with the heat assisted processes. Thus, acid or alkaline was mixed with the substrate before being exposed to the microwave or thermal radiation.

3.3.1 Sugar Solubility after Physicochemical Pretreatments: Microwave

In this part of experiments, samples of fresh substrate were mixed with acid or alkaline. After that, they were exposed to the microwave radiation (300 Watts) for two minutes; the time in this case, was not a variable. The amounts of sugars available in solution were determined and are shown in the Figure 3.6.

The higher results are gotten with the RSB samples, within acid solutions. By rounding numbers, it seems that high concentrations (0.4M) of acid or alkaline solutions offered higher amounts of sugars in solution. Contrary, the ESB samples showed that low concentrations (0.2M) of acid or alkaline can offer the best values of availability for this type of substrate.

In RSB samples the higher soluble sugar concentration was observed with the acid pretreatment, where 0.2M H_2SO_4 gave the higher soluble sugar: 0.82 mg/mL. In the ESB substrate acid pretreatment also released higher sugar at the low concentration of the acid (0.2M) resulted in higher amount of sugar in solution as 0.42 mg/mL. However, the soluble sugar released from RSB under the acid pretreatment + MW was 20% lower than the control of this type of pretreatment (MW + water, 2 min). Furthermore, the soluble sugar released from ESB under acid pretreatment plus microwave radiation increased 24%. In this case, the acidity conditions disrupted the ESB substrate structure to solubilize sugars under the microwave radiation.



	Acid 0,2 M	Acid 0,3 M	Acid 0,4 M	Alk 0,2 M	Alk 0,3 M	Alk 0,4 M
RSB	0,82	0,7	0,8	0,52	0,45	0,65
ESB	0,42	0,33	0,31	0,26	0,27	0,23

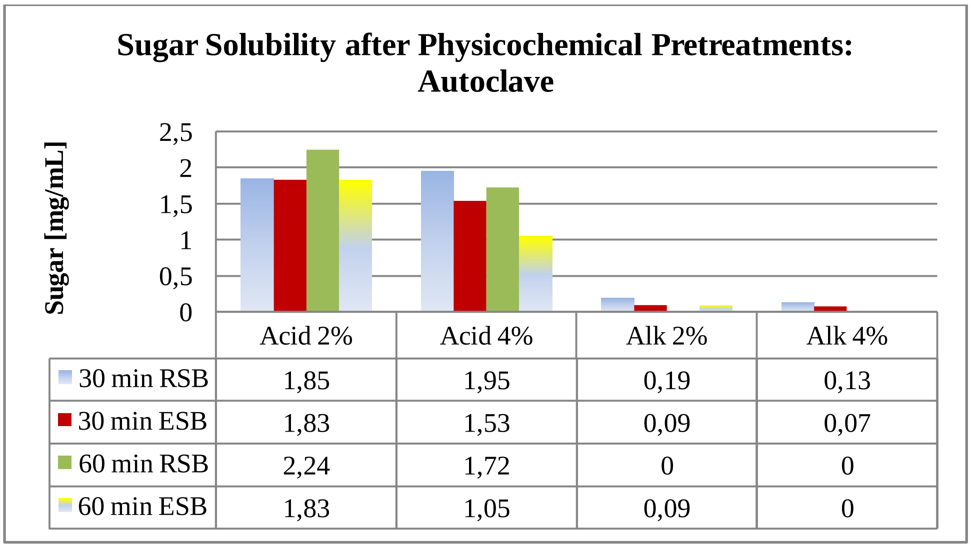
Figure 3. 6 Sugar solubility after physicochemical pretreatments: microwave.

The combination of alkaline pretreatment with microwave radiation is not effective to release sugars. Both RSB and ESB obtained lower soluble sugars than the blank. The acid pretreatment can disrupt certain amount of sugars from the lignocellulosic components which is observed in the results. However, it is likely that the alkaline pretreatment has a higher effect on the lignin disruption under microwave radiation, but it is aimed to the production of intermediate components and the relocation of them over the same lignocellulosic structure. For that reason, the soluble sugar concentrations are low for the physicochemical pretreatments under microwave radiation [28].

3.3.2 Sugar Solubility after Physicochemical Pretreatments: Autoclave

In the physicochemical pretreatment by the autoclave equipment the contact time was thirty and sixty minutes of thermal exposition, at 120°C. Based on literature, the acid pretreatments tend to work better and solubilize sugars in high temperature presence and exposition time. That was why, there was no reason to perform the alkaline pretreatments at sixty minutes. In short exposition time for autoclave process (thirty minutes), both, acid and alkaline were set to have a comparative point, see Figure 3.7.

In general, based on the results of Figure 3.7, the autoclave process benefits the acid reagent to release more sugars into solution. Both for RSB and ESB, low concentrations of the acid reagent (2%) helped to have higher values of sugar. In contrast, for the alkaline component it seems that the high temperature is not beneficial at all; even the results are lower when it comes to compare with the microwave assisted process.



	Acid 2%	Acid 4%	Alk 2%	Alk 4%
🟦 30 min RSB	1,85	1,95	0,19	0,13
🟥 30 min ESB	1,83	1,53	0,09	0,07
🟩 60 min RSB	2,24	1,72	0	0
🟨 60 min ESB	1,83	1,05	0,09	0

Figure 3. 7 Sugar solubility after physicochemical pretreatments: autoclave.

Furthermore, comparing some of the values of concentrations gotten from the physical and physicochemical pretreatments in the autoclave process, the second ones seem to be better. At thirty minutes with RSB, the increment is around 190 and 205% for acid solution, while at sixty minutes it is between 760 and 1020%. For the ESB case, also in acid solution, is 2450 – 2950 % for thirty minutes and 775 – 1425 % for sixty minutes. It is clear that working with the thermal radiation offered by the autoclave equipment, acid is a good reagent to work with.

3.4 Effect of Chemical Pretreatments on Biogas Production – Test 1

Chemical pretreatments were studied to measure the BMP (Biochemical Methane Production), during more than a month or until biogas production ceased. The biomethane production under the mesophilic conditions of the batch anaerobic digestion process was considered to generate the following analysis.

3.4.1 Effect of Chemical Pretreatments: Short Time Duration Process

The average daily values of the samples are shown in the following figures as a cumulative amount of biomethane produced. Results for the samples without any pretreatment are shown in Figure 3.8 to define the control of the system. It shows that the biomethane production from the RSB is higher than ESB, the difference is around 15%. RSB resulted in 236.66 SMP mL CH_4/gVS_{feed} and ESB up to 200.05 SMP mL CH_4/gVS_{feed}, where 70% of the biogas was produced within the first 15 days of AD.

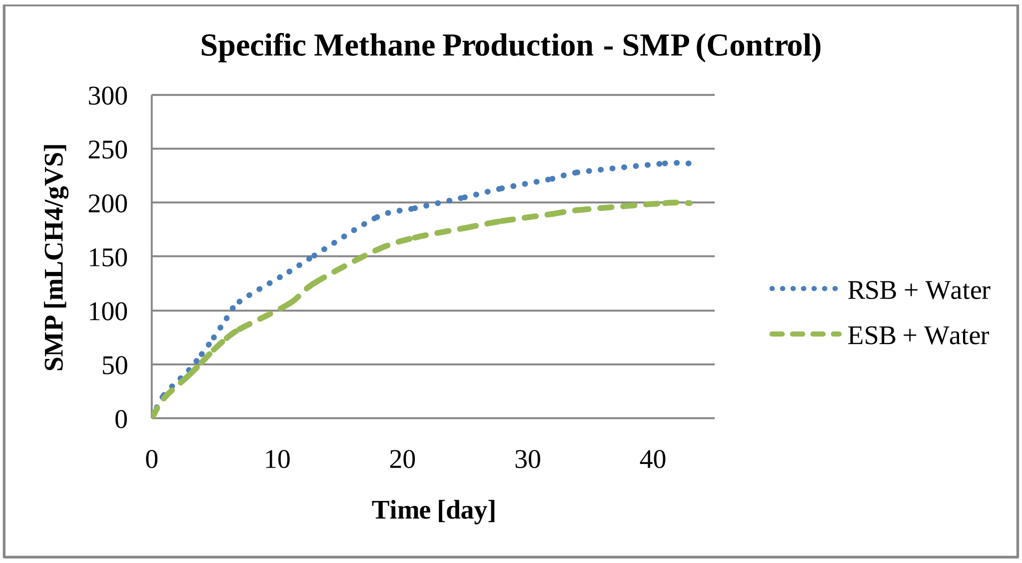


Figure 3. 8 Cumulative specific methane production, Control.

Figure 3.9 shows the SMP potential from RSB and ESB after acid pretreatment, with short time contact (10 – 15 minutes). Higher methane production was observed for ESB as 112.65 mL CH_4/gVS_{feed} for acid 0.4 M. Meanwhile ESB under acid pretreatment (0.2 M) could achieve SMP 100.88 mL CH_4/gVS_{feed}. Acid pretreatment affects negatively the biomethane production. The inhibitors in solution affect microorganisms present in the medium and inactivate the inoculum. The inoculum could survive the first 7 days of the anaerobic process, where 70% of the produced biogas was achieved, but after that period of time, the production decreased.

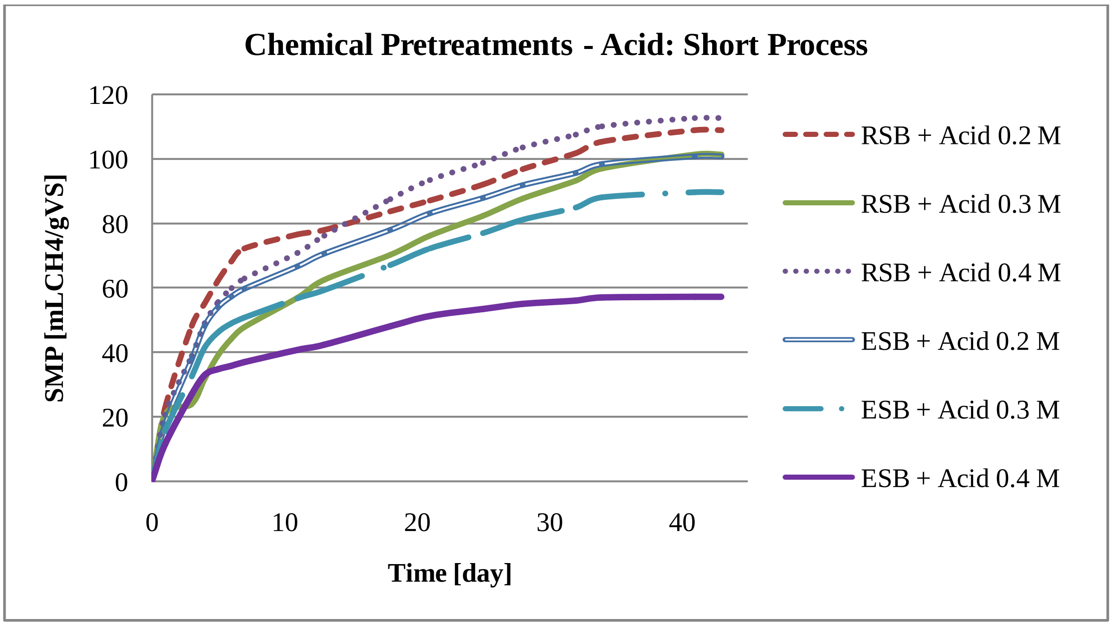


Figure 3. 9 Effect of Chemical pretreatments on SMP – Acid: Short.

The alkaline pretreatment over ESB at short contact time is shown in Figure 3.10. Thus, it is beneficial to have lower concentrations (0.2 M), instead of high (0.4 M). The cumulative SMP achieved is 136 mL CH_4/gVS_{feed}, 122.82 mL CH_4/gVS_{feed} and 113.47 mL CH_4/gVS_{feed} for the concentrations of 0.2M, 0.3M and 0.4M respectively. This amount of SMP is higher than the acid pretreatment. Alkaline pretreatment has a better effect at lower temperatures. Alkaline pretreatment disrupted the lignocellulosic structure in all the concentrations applied. At high concentrations, the lignin decomposition creates new components which could interfere with the bacteria due to the relocation or poison effect over the medium. Thus, the low alkaline concentration could achieve higher amount of biogas produced due to the balance between lignin disruption and contaminants in solution.

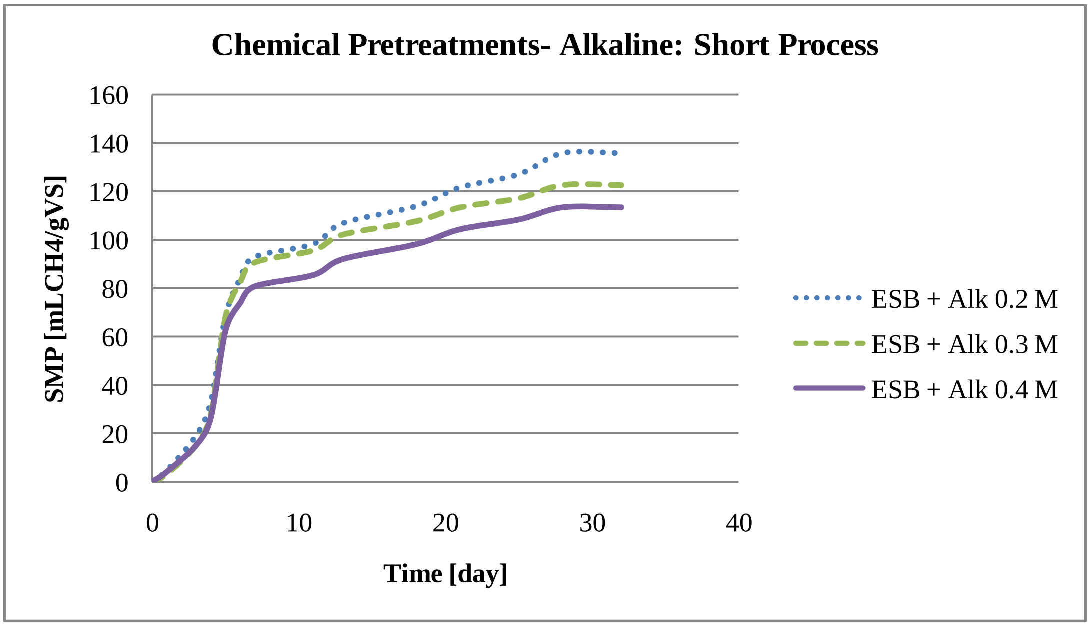


Figure 3. 10 Effect of chemical pretreatments on SMP – Alkaline: Short.

In Table 3.2 are shown the SMP resulted from chemical pretreatments in short contact process. There, the differences between acid and alkaline pretreatments can be easily perceived. RSB had a reduction in the biomethane produced up to 52 – 57%, while acid pretreatment over ESB also reduce the production by 50 – 71% and up to 32 – 43% for alkaline pretreatment.

The short contact process is not accurate to work with filtrates. The presence of inhibitors in the medium does not allow microorganism proliferation. Besides, the acid or alkaline residues from the pretreatment could perturb the bacteria and inhibit the medium after the first week of the anaerobic process. Therefore, the production of other gases is stimulated and the ratio between CH_4 and CO_2 drops drastically in comparison with the process control. Thus, the biomethane production was reduced by 30 – 50% in this type of pretreatment.

The biogas produced from carbohydrates usually contains biogas with 50% CH_4 and 50% CO_2. Lower CH_4 percentage shows that the system is inhibited. Also, lower CH_4 content will make the process unfeasible with lower calorific value. For that reason, the CH4/CO2 ratio is an important parameter to consider when pretreatments are applied over lignocellulosic substrates.

Table 3. 2 Effect of chemical pretreatments on SMP, short time process.

Sample	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**	**Sample**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Acid: 0,2M	108.90	0.586	ESB + Acid: 0,2M	100.88	0.755
RSB + Acid: 0,3M	101.51	0.521	ESB + Acid: 0,3M	89.81	0.457
RSB + Acid: 0,4M	112.65	0.464	ESB + Acid: 0,4M	57.31	0.574
-	-	-	ESB + Alk: 0,2M	136	0.864
-	-	-	ESB + Alk: 0,3M	122.82	0.786
-	-	-	ESB + Alk: 0,4M	113.47	0.903

3.4.2 Effect of Chemical Pretreatments: Long Time Duration Process

For long contact tests, the substrate was mixed with the respective chemical component to perform the chemical pretreatment. And then, the mixed solution was left under environment conditions with no modification of temperature neither pressure. Thus, two days after (48 hours), the normal procedure of neutralization, vacuum filtration and storage was accomplished.

Figure 3.11 shows the cumulative SMP for chemical pretreatment under long contact. The maximum production for the RSB substrate resulted under the alkaline 2% pretreatment, which cumulated 99.94 mL CH_4/gVS_{feed}. That was followed by the contribution made by the sample pretreated with water which after 48 hours resulted in SMP 69.56 mL CH_4/gVS_{feed}. The production time was reduced by 7 days, where 80% of the produced biogas was achieved.

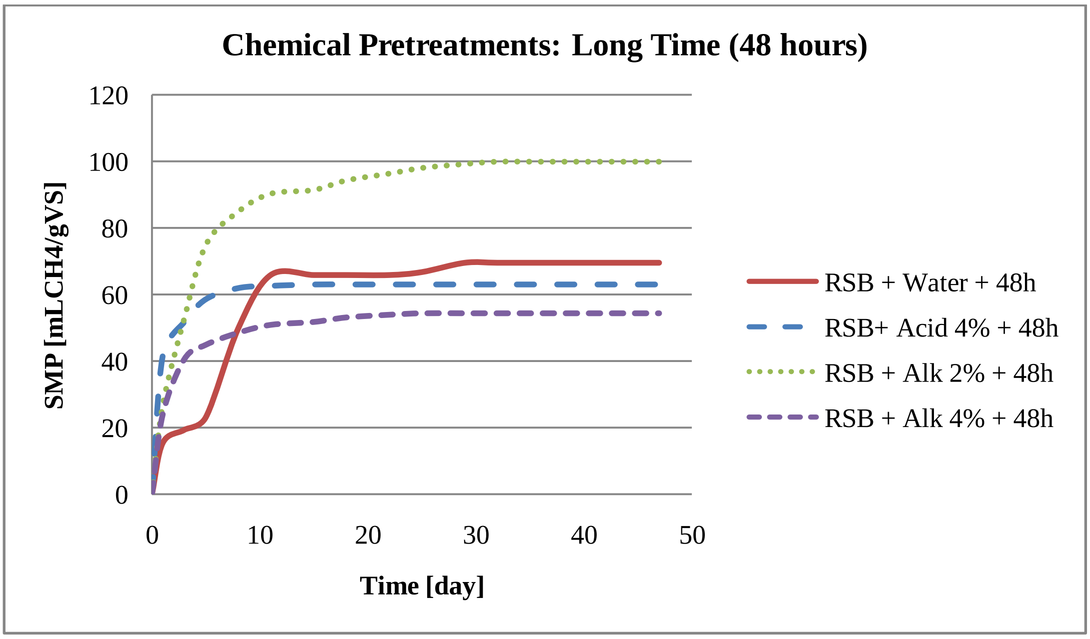


Figure 3. 11 Effect of Chemical pretreatments on SPM – RSB: Long Duration.

On the other hand, ESB substrate pretreated with water during 48 hours resulted in cumulative SMP as 144.69 mL CH_4/gVS_{feed}. In this group of experiments, alkaline 2% pretreatment resulted in cumulative biomethane potential as 46.67 mL CH_4/gVS_{feed}. Figure 3.12 shows the negative effect that chemical pretreatment has over the ESB substrate when 48 hours contact the degradation of hemicellulose and lignin can contaminate the medium due to the excess of intermediate components produced.

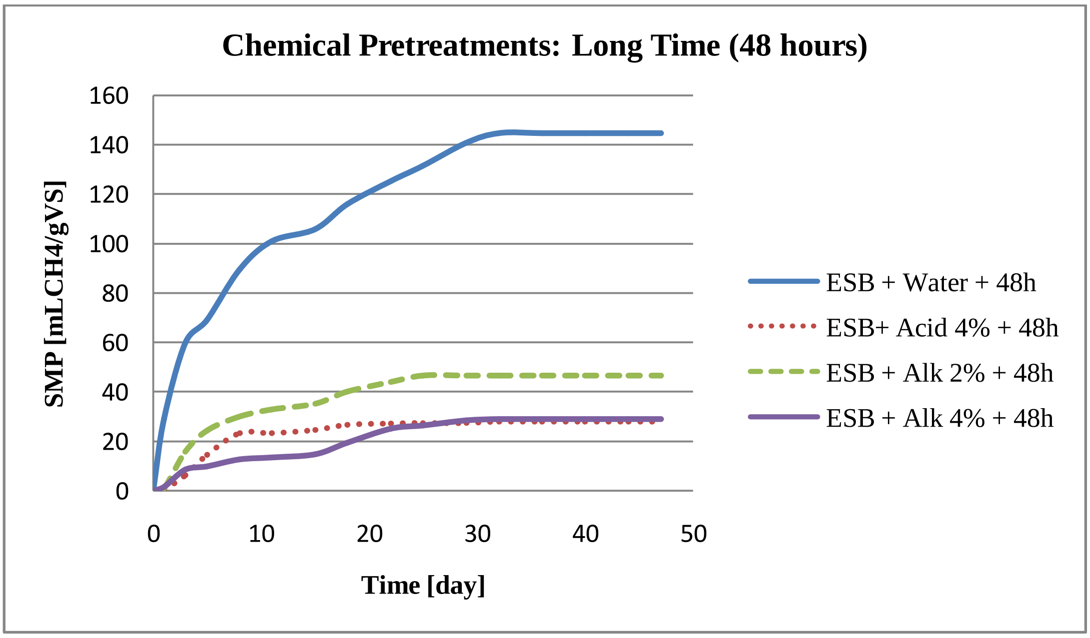


Figure 3. 12 Effect of Chemical Pretreatments on SMP – ESB: Long Duration.

Table 3. 3 Effect of chemical pretreatments on SMP, long time process.

Sample	SMP $mLCH_4/gVS_{feed}$	CH_4/CO_2	Sample	SMP $mLCH_4/gVS_{feed}$	CH_4/CO_2
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Water	69.56	1.345	ESB + Water	144.69	0.919
RSB + Acid: 4%	63.12	0.269	ESB + Acid: 4%	27.89	0.825
RSB + Alk: 2%	99.94	0.357	ESB + Alk: 2%	46.67	0.739
RSB + Alk: 4%	54.37	0.538	ESB + Alk: 4%	29.01	0.601

For both cases, RSB and ESB, the production of biomethane was lower than the process control. The inhibitor compounds played a drastic role in the performance of this pretreatment. For that reason, not only the activity of the microorganisms was disturbed by the remaining excess of acid or alkaline in solution, but also by the presence of some components that were released in the presence of water after the duration of the 48 hours (low ratio between CH_4 and CO_2), Table 3.3.

These results are in concordance with the reported in literature, where components as xylose can degrade easily in the presence of acid conditions when there is long contact. Therefore, in the AD process xylose starts to degrade into some other components as furfural, formaldehyde, formic acid, crotonaldehyde, acetaldehyde among others. All of them are likely to create an inhibitor effect over the whole system modifying the pH-value which inactivates the anaerobic bacteria [29,36].

3.5 Effect of Physical Pretreatments on Biogas Production – Test 1

3.5.1 Effect of Physical Pretreatments: Microwave Assisted Process

In this type of tests the substrates were mixed with water and then exposed to microwave radiation. The effect of the pretreatment is observed in Figure 3.13, where the higher results of cumulative biomethane in the batch process resulted with the longer contact time. Therefore, RSB and ESB samples with two minutes of radiation reached SMP as 328.9 mL CH_4/gVS_{feed} and 282.76 mL CH_4/gVS_{feed}, respectively. While for the one minute process RSB resulted in 246.85 mL CH_4/gVS_{feed} and ESB in 193.89 mL CH_4/gVS_{feed}, as it is shown in Table 3.4. 70% of the biogas obtained was produced within 10 days. Thus, bacteria have longer time to proliferate and consume the nutrients in the medium for longer time which resulted in higher biogas production.

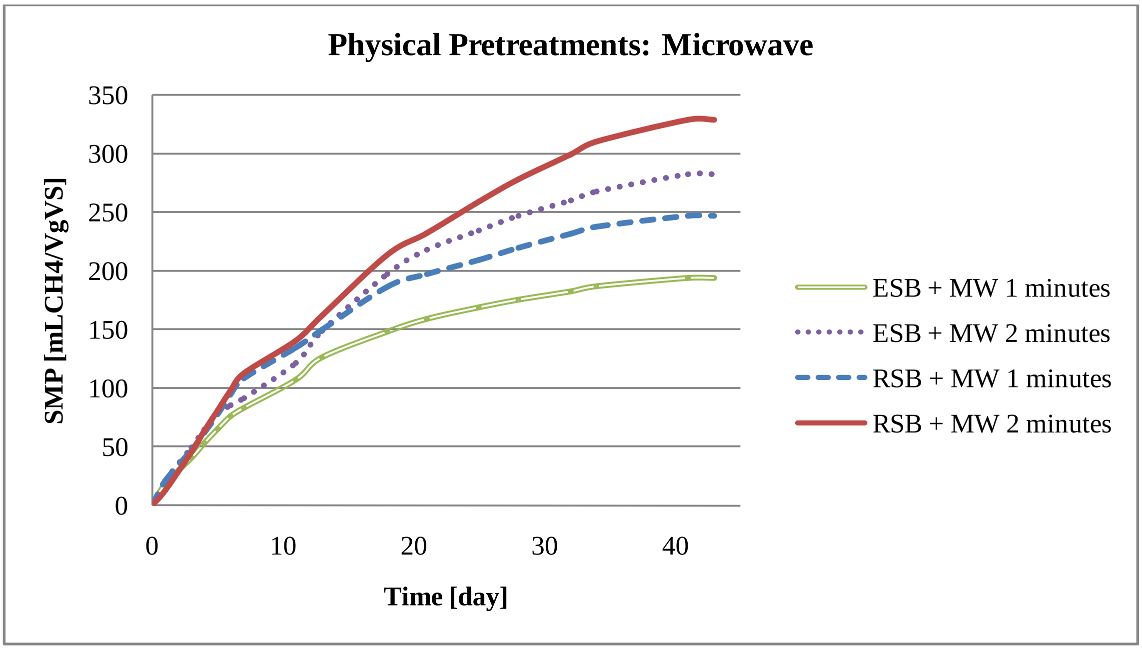


Figure 3. 13 Effect of physical pretreatments on SMP: microwave.

Table 3. 4 Effect of physical pretreatments on SMP, microwave.

Sample	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**	**Sample**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Water + MW 1 min	246.85	1.413	ESB + Water + MW 1 min	193.89	1.252
RSB + Water + MW 2 min	328.90	1.286	ESB + Water + MW 2 min	282.76	1.010

By comparing the results showed in Table 3.4, it is observed that the biomethane produced from the RSB samples with one and two minutes of microwave radiation was increased by 5 – 39 %. On the other hand, for ESB there was a decrease by 3% with one minute exposition, but an increase up to 40% for the two minutes radiation. Therefore, the quality ratio (CH4/CO2) is upper than one, which means that the biomethane production potential is favored rather than the carbon dioxide during the AD.

Biodegradability depends on various parameters like the substrate type, pretreatment applied and digestion time as well as hydrolytic. Pretreatment affects solubility. On the other hand, hydrolysis is no longer a rate limiting stage after the pretreatment of lignocellulosic substrate. However, it is important to avoid inhibition due to accumulation of VFA's and intermediate compounds after pretreatment.

3.5.2 Effect of Physical Pretreatments: Autoclave

The thermal supply for this type of pretreatments was achieved with the presence of water. Moreover, the variation in this process was the time, under which the samples were exposed thirty (30) or sixty (60) minutes at 120°C. Those conditions make this process a bit harsh for the lignocellulosic components (hemicellulose and lignin), but also make them decompose to cellulose and have an easier biodegradation under the anaerobic conditions of the process, as it can be observed (Figure 3.14).

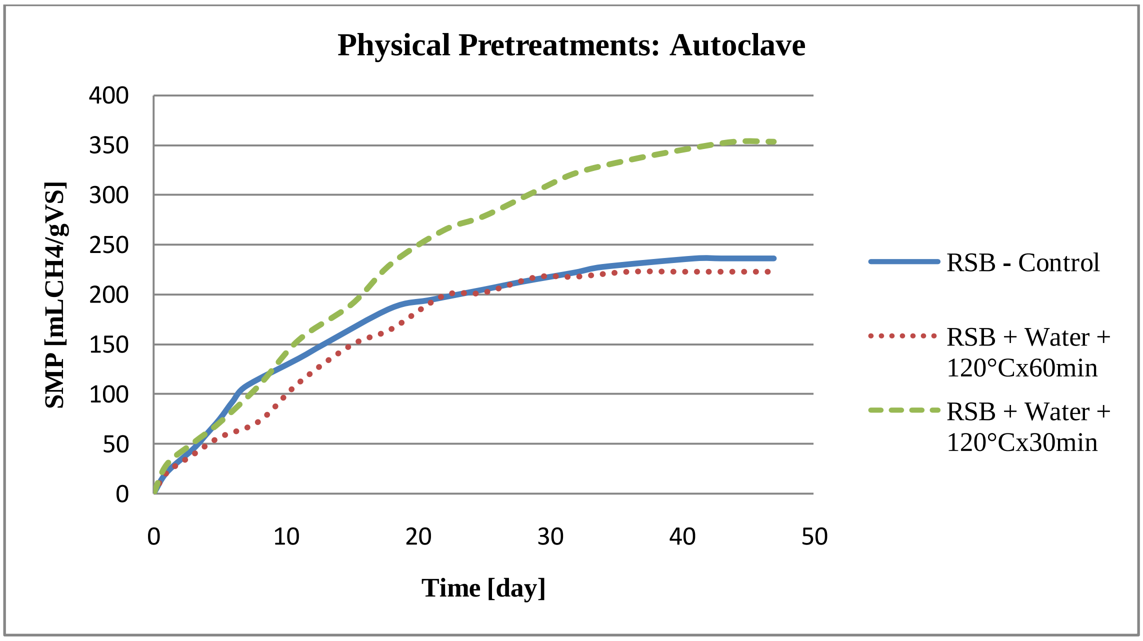


Figure 3. 14 Effect of physical Pretreatments on SMP – RSB: Autoclave.

In the case of RSB, the sample mixed with water and exposed during thirty minutes at 120°C resulted in the higher amount of SMP potential for this substrate as 353.39 mL CH_4/gVS_{feed}. For the sixty minutes exposition sample, the biomethane produced was similar to the amount resulted in the process control of this AD: 223.24 mL CH_4/gVS_{feed}. The amount of biomethane resulted from the sample exposed at 120°C during 30 minutes was increased by 50%. It means that the physical pretreatment affects positively the SMP potential for this type of substrate (Figure 3.14).

For ESB substrate, the higher amount of biomethane production potential under physical pretreatment resulted from the sample exposed at 120°C during sixty minutes as 208.61 SMP mL CH_4/gVS_{feed}. It means that it increased by 5% the SMP potential compared with the process control. On the other hand, the sample exposed at 120°C during thirty minutes to the thermal pretreatment resulted in 200.68 SMP mL CH_4/gVS_{feed}, which is almost the same amount that resulted from the process control (Figure 3.15).

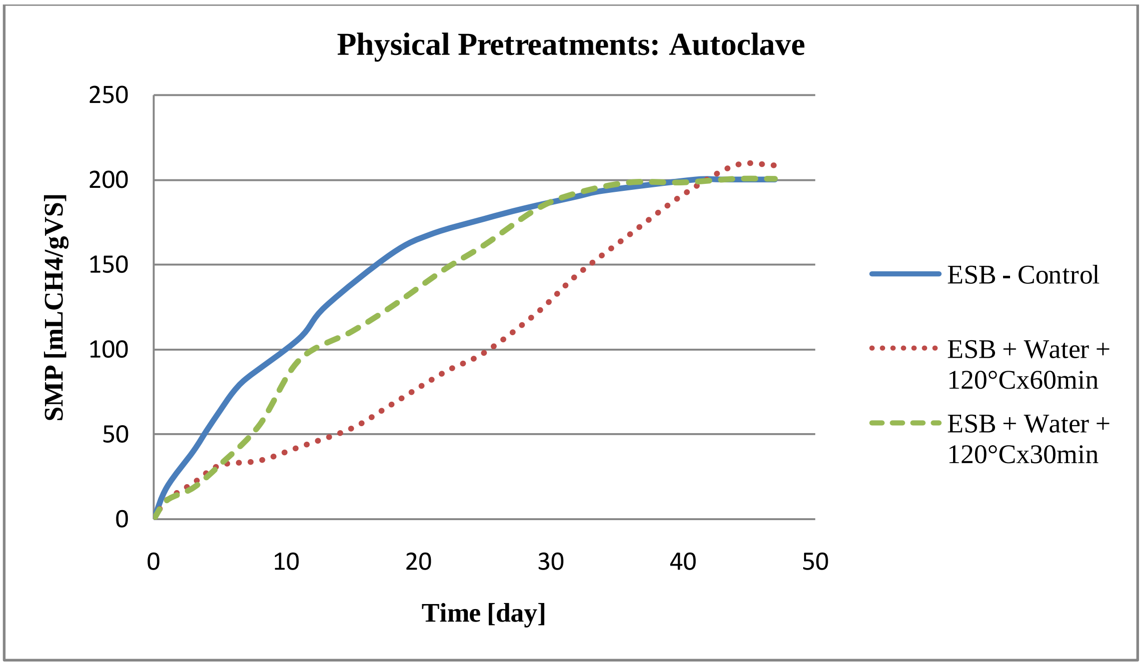


Figure 3. 15 Effect of physical Pretreatments on SMP – ESB: Autoclave.

In general, the thermal exposure for the substrates analyzed improves the production of biomethane at 120°C. For the case of RSB, it can increase by 50% due to the release of some monosaccharide that are inside of the lignocellulosic structure. Meanwhile, for ESB the increase only reaches 5% due to the lack of monosaccharide inside its lignocellulosic matrix. ESB substrate is already exhausted from the sugars that are converted to biogas. Thus, the SMP potential is lower than the achieved by RSB (see Table 3.5). Also, the 70% of biogas was produced within 18 days which suggests longer proliferation of bacteria in the medium. Despite the high production of biomethane under these thermal conditions, the ratio between CH4 and CO2 shows that at this temperature there are inhibitors that alter the quality of the biogas produced, which is also a critical parameter for the AD.

Table 3. 5 Effect of physical pretreatments on SMP, autoclave.

Sample	**SMP** mLCH_4/gVS$_{feed}$	**CH_4/CO_2**	**Sample**	**SMP** mLCH_4/gVS$_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Water + Autoclave 30 min	353.39	1.188	ESB + Water + Autoclave 30 min	200.68	1.306
RSB + Water + Autoclave 60 min	223.24	1.429	ESB + Water + Autoclave 60 min	208.61	1.054

3.6 Effect of Physicochemical Pretreatments on Biogas Production – Test 1

3.6.1 Effect of Physicochemical Pretreatments: Microwave

The acid pretreatments under the microwave radiation were only performed with the ESB substrate (see Figure 3.16). Within the acid pretreatment the higher SMP potential resulted from acid 0.2 M as 88.52 mL CH_4/gVS$_{feed}$. From literature it is suggested that the inactivity of the anaerobic bacteria can result from the presence of inhibitors as hydroxymethylfurfural (HMF) and furfural which affect negatively the inoculum activity and the death of the microorganism in the medium [29].

The alkaline pretreatment resulted in a higher amount of SMP potential than the acid pretreatment (Figure 3.17). The highest biomethane production in this pretreatment was achieved by alkaline 0.2M as 164.43 mL CH_4/gVS$_{feed}$. For the alkaline 0.3M and 0.4M resulted in 120.15 mL CH_4/gVS$_{feed}$ and 105.72 mL CH_4/gVS$_{feed}$, respectively (Table

3.6). It is likely that the alkaline pretreatment also was inhibited by some components which were released from the decomposition of hemicellulose and lignin under alkali conditions. However, the lower alkaline concentration could have lower inhibition. The time to produce 70% of the cumulative SMP was reduced to 7 days [29].

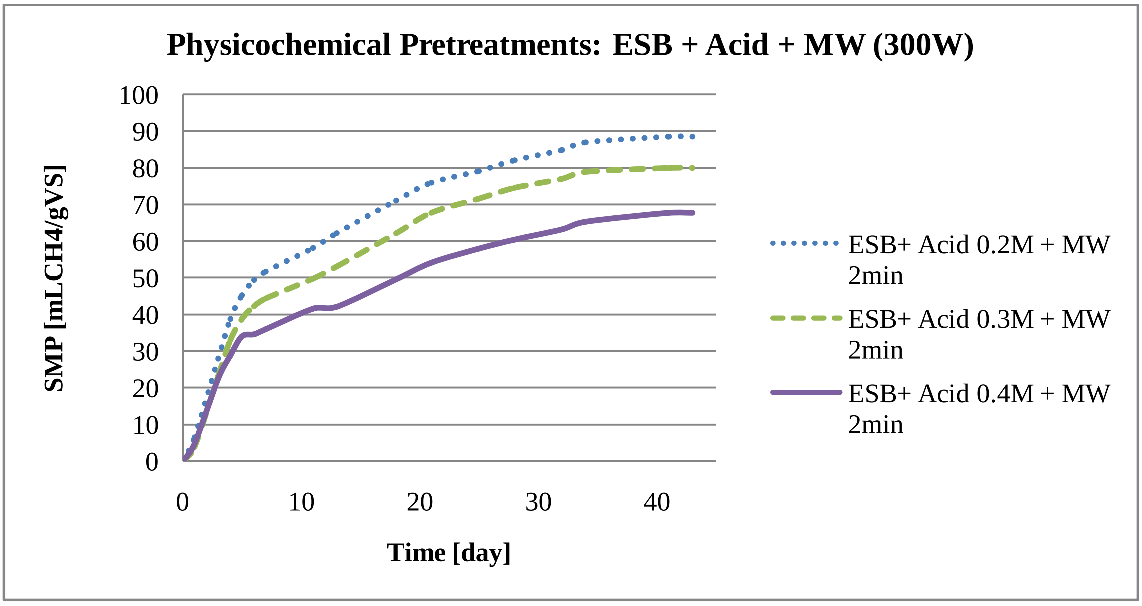


Figure 3. 16 Effect of physicochemical pretreatments on SMP, microwave.

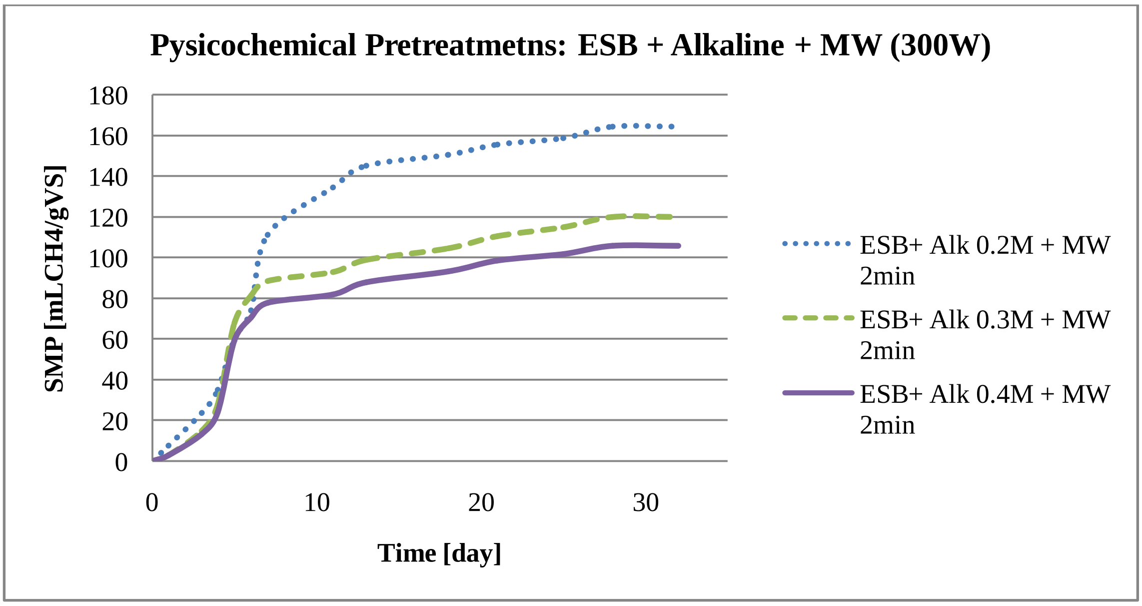


Figure 3. 17 Effect of physicochemical pretreatments on SMP, microwave.

As it is observed in Table 3.6, the use of microwave radiation for the samples treated under acid or alkaline pretreatment does not avoid the production of the inhibitors that alter negatively the biomethane production potential. Microwave pretreatment over the lignocellulosic samples resulted in high SMP potential. However, microwave pretreatment in addition with chemical reagents it is not a viable alternative for the enhancement of biomethane.

Table 3. 6 Effect of physicochemical pretreatments on SMP, microwave.

Sample + MW 2min	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**	**Sample + MW 2min**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**
ESB - Control	200.05	1.386	ESB - Control	200.05	1.386
ESB + Acid: 0,2M	88.50	0.639	ESB + Alk: 0,2M	164.43	0.880
ESB + Acid: 0,3M	80.03	0.745	ESB + Alk: 0,3M	120.15	0.766
ESB + Acid: 0,4M	67.67	0.783	ESB + Alk: 0,4M	105.72	1.020

3.6.2 Effect of Physicochemical Pretreatments: Autoclave

The experiments for the physicochemical pretreatments with thermal supply were performed after mixing the substrates with the chemical reagents. Then, this mixture was exposed at 120°C in autoclave. Time was variable of the experiments, where thirty and sixty were the conditions studied.

Figure 3.18 shows the cumulative biomethane potential for RSB samples during the AD process. The higher amount of biomethane produced resulted from the alkaline 2% pretreatment which was exposed at 120°C during 30 minutes as 170.17 SMP mL CH_4/gVS_{feed}. Meanwhile, ESB resulted in 60.66 mL CH_4/gVS_{feed} after the alkaline 2% pretreatment under similar conditions. On the other hand, autoclave pretreatment at 120°C during 60 minutes resulted in a similar cumulative SMP 68.40 mL CH_4/gVS_{feed} for ESB. The physicochemical pretreatment affects negatively the enhancement of biomethane for both substrates at 120°C during 30 or 60 minutes.

For the acid pretreatment the SMP potential resulted was similar to alkaline pretreatment. The thermal supply inactivates the microorganism proliferation and the biomethane produced is low. The higher SMP potential was obtained from acid 2% pretreatment as 120.80 mL CH_4/gVS_{feed}.

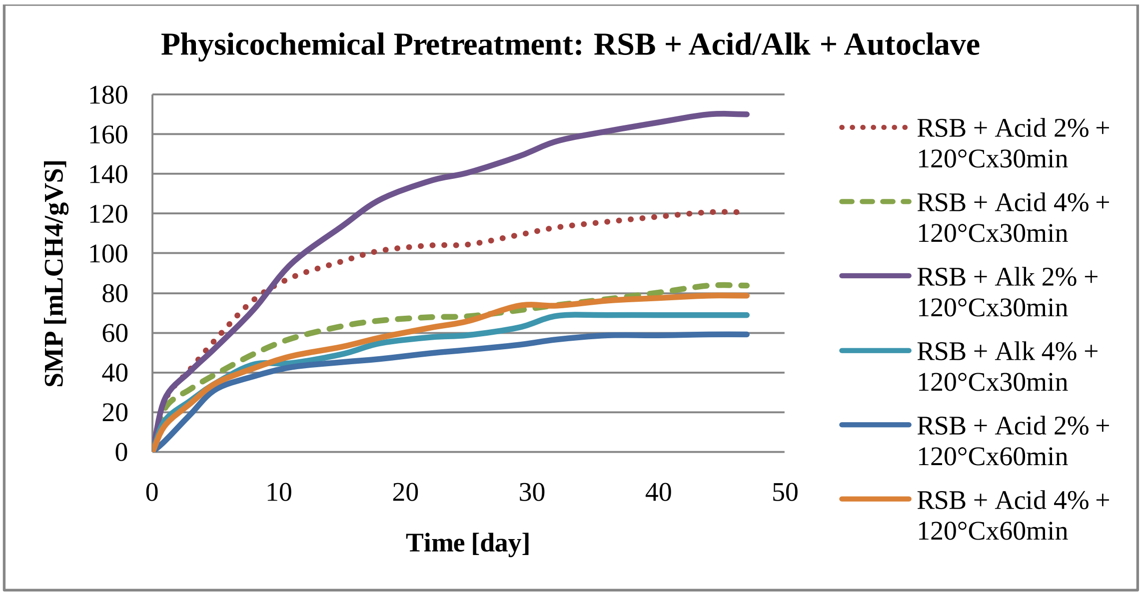


Figure 3. 18 Effect of physicochemical pretreatments on SMP, autoclave.

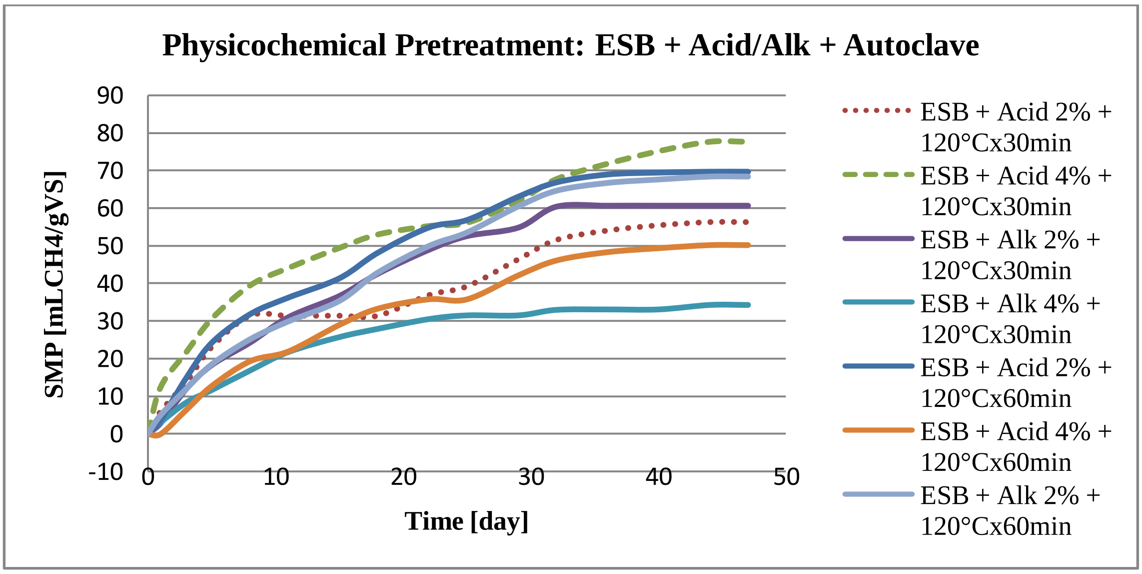


Figure 3. 19 Effect of physicochemical pretreatments on SMP, autoclave.

For both cases, RSB and ESB, the physicochemical pretreatments under thermal radiation made by autoclave did not enhance the biomethane production during anaerobic process. It is likely that the concentration of inhibitors in solution made this process impossible to stabilize the alkaline or acid conditions for the microorganism's proliferation. For that reason, all the amounts produced are lower than the produced by process control (Table 3.7). However, the time to produce 60% of the cumulative SMP was reduced by 10 days [29].

Table 3. 7 Effect of physicochemical pretreatments on SMP, autoclave.

Sample **+ Autoclave 30min**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4 /CO_2**	**Sample** **+ Autoclave 60min**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4 /CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Water	353.39	1.188	RSB + Water	223.24	1.429
ESB + Water	200.68	1.306	ESB + Water	208.61	1.054
RSB + Acid: 2%	120.80	0.667	RSB + Acid: 2%	59.10	0.670
RSB + Acid: 4%	83.70	0.584	RSB + Acid: 4%	78.63	0.643
RSB + Alk: 2%	170.17	0.701	-	-	-
RSB + Alk: 4%	68.79	0.589	-	-	-
ESB + Acid: 2%	56.33	0.627	ESB + Acid: 2%	69.59	0.466
ESB + Acid: 4%	77.58	0.634	ESB + Acid: 4%	50.18	0.784
ESB + Alk: 2%	60.66	0.688	ESB + Alk: 2%	68.40	0.524
ESB + Alk: 4%	34.25	0.879	-	-	-

It is suggested from literature that pretreatments under tough conditions normally degrade the components derived from hemicellulose and lignin. Some of the main products are phenolic and heterocyclic compounds, which due to the high temperatures and long reaction time, can degrade to vanillin, vanilic alcohol, furfural and hydroxymethylfurfural (HMF). Many of them are toxic to the medium and affect the microorganism proliferation. For instance, the phenolic acid concentration is normally balanced by the AD agents itself. However, in alkali or acidic mediums, the phenolic acid released at high temperature altogether with p-hydroxy-benzoic acid unbalances the pH of the reactor affecting the biomethane production [16,39,40].

3.7 Effect of Chemical Pretreatments on Biogas Production – Test 2

In this part of the experiments the studies were conducted with acid 2% and alkaline 2% pretreated samples. The pretreated samples were neutralized before BMP tests.

3.7.1 Effect of Chemical Pretreatments: Short Time Duration Process

Figure 3.20 shows the tendency that biomethane production has during the AD process of RSB and ESB exposed to acid and alkaline pretreatments. The short chemical pretreatment is found beneficial especially for the alkaline samples. Higher methane productions were observed for RSB and ESB as: 214.10 and 286.96 mL CH_4/gVS_{feed}, respectively. The acid pretreatment seems to affect negatively the system and the biomethane productions were lower, 169.08 mL CH_4/gVS_{feed} for RSB and 183.68 mL CH_4/gVS_{feed} ESB. Also, the time for reaching 60% of biogas is different, alkaline pretreatment took 10 days, while acid pretreatment 7 days.

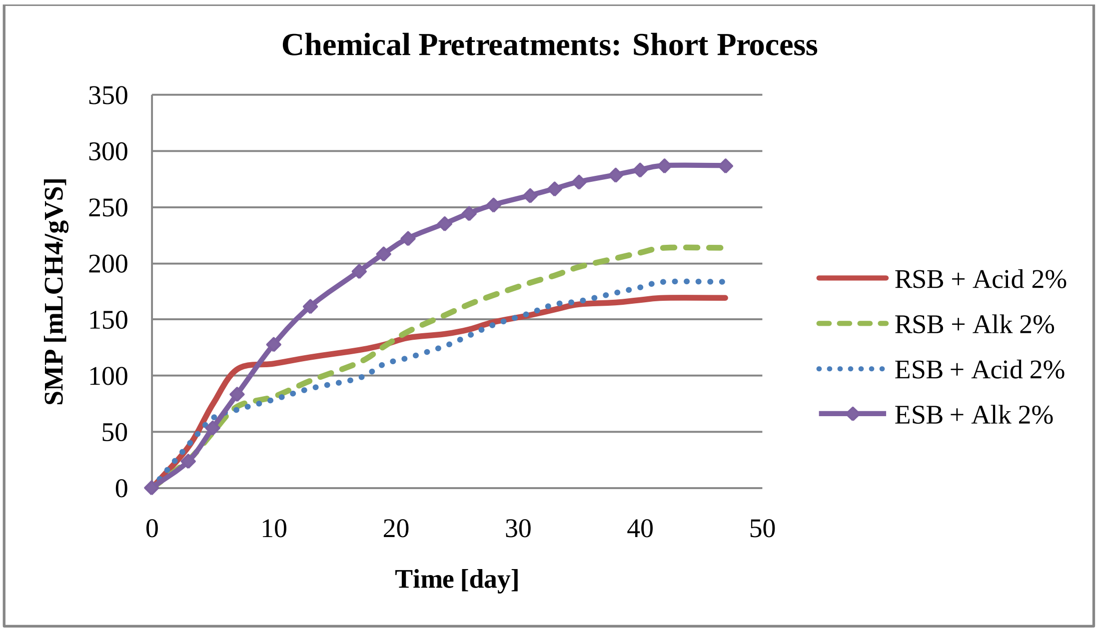


Figure 3. 20 Effect of chemical pretreatment on SMP: Short process – Test 2.

Table 3. 8 Effect of chemical pretreatments on SMP, short process – Test 2.

Sample	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**	**Sample**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Acid: 2%	169.08	0.856	ESB + Acid: 2%	183.68	0.929
RSB + Alk: 2%	214.10	0.833	ESB + Alk: 2%	**286.96**	0.809

When the general performance of this pretreatment compared with the control of the process, it is unfavorable for RSB where a decrease of 10 – 28% is observed. The acid application over ESB decreases the biomethane production in 8%, but the alkaline reagent increases the gas production up to 40%, Table 3.8. The alkaline reagent had a better interaction with the lignocellulosic substrates and without energy supply can decompose, under certain limit, the structural components that enhance the biomethane production. However, the quality of the gas produced is not high (CH4/CO2), there is a presence of some other gases that are not the desirable and can affect the biomethane increase.

3.7.2 Effect of Chemical Pretreatments: Long Time Duration Process

Biogas production from raw sugar beet (RSB) has an interesting behavior through the AD process. Acid, alkaline pretreatments had a similar effect on solubility and similar biomethane productions: 212.38, 212.55 CH_4/gVS_{feed}, respectively, Figure 3.21. On the other hand, pretreatment had a different effect on ESB. Methane productions were 241.53 mL CH_4/gVS_{feed} for acid, 225.70 mL CH_4/gVS_{feed} for alkaline and 203.23 mL CH_4/gVS_{feed} for the control samples. Acid pretreatments showed higher BMP potentials.

The effect of pretreatment on RSB for longer contact time has no significant effect on biogas production. It has an enhancement on biogas production only by 10%. For the ESB substrate the improvement was between 2 – 20%, depending on the case.

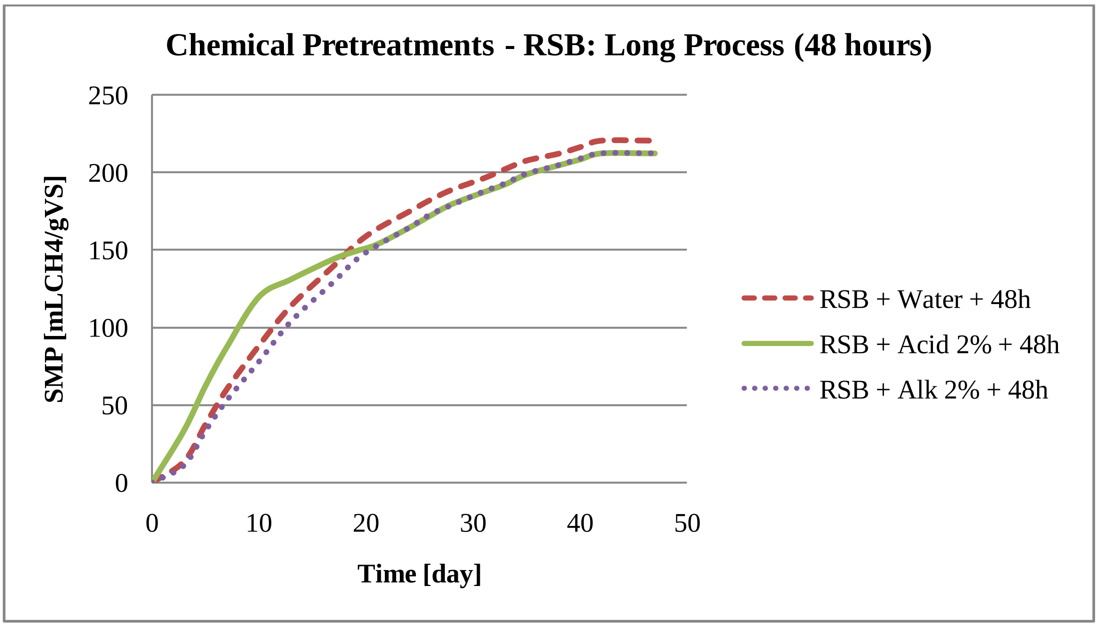


Figure 3. 21 Effect of chemical pretreatments on SMP, RSB – Test 2.

The quality within the long time chemical process was found better compared with the short contact periods. The samples pretreated with water and alkaline got values over the unity which can show a good ratio between the biomethane produced and the other gases in the medium. But, still the acid reagent samples show a lower biogas quality which alerts about the presence of inhibitors that deviate the digestion to undesirable gases and affect the total performance of the BMP, Table 3.9.

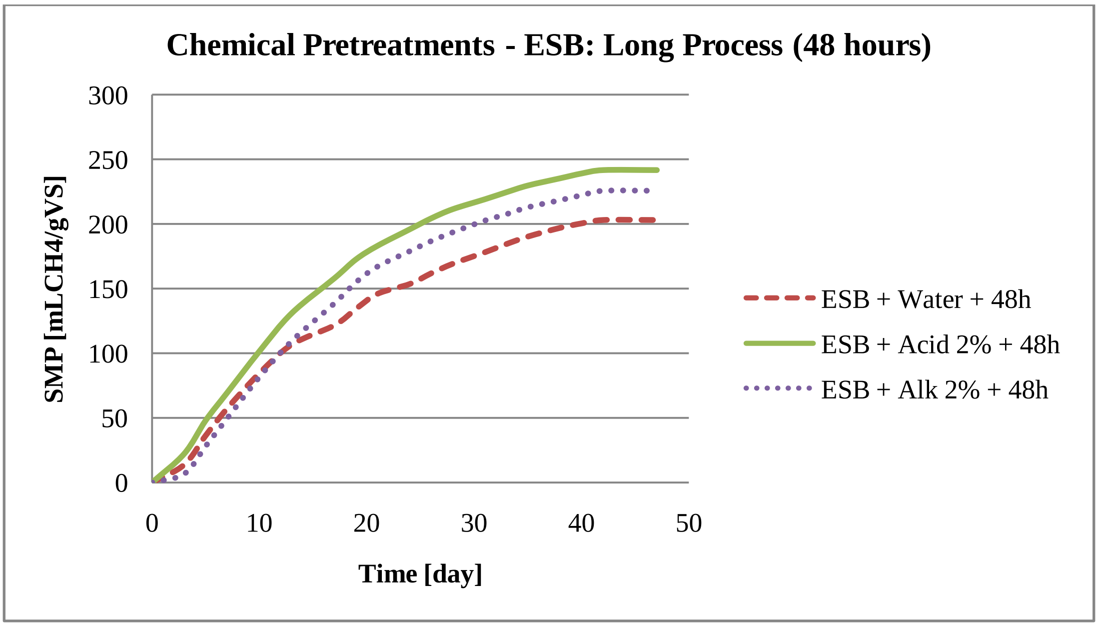


Figure 3. 22 Effect of chemical pretreatments on SMP, ESB: Long process – Test 2.

Table 3. 9 Effect of chemical pretreatments on SMP, long process – Test 2.

Sample	**SMP** mLCH_4/gVS$_{feed}$	**CH_4/CO_2**	**Sample**	**SMP** mLCH_4/gVS$_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Water	220.60	1.035	ESB + Water	203.23	1.150
RSB + Acid: 2%	212.38	0.795	ESB + Acid: 2%	**241.53**	0.847
RSB + Alk: 2%	212.55	1.036	ESB + Alk: 2%	225.70	1.102

3.8 Effect of Physical Pretreatments on Biogas Production – Test 2

The physical pretreatments are performed under the microwave heat assisted process and the thermal autoclave supply with a distilled water medium. In the microwave tests, 2 minutes and 300 Watts were the constant conditions, while for the autoclave equipment were 120 °C and 30 (thirty) minutes. For both cases, the tendency and final results of the BMP under these pretreatments are shown in Figure 3.23 and Table 3.10, respectively.

3.8.1 Effect of Physical Pretreatments: Microwave Assisted Process

Under the microwave radiation RSB and ESB biomethane accumulated production was 285.52 and 240.47 mL CH_4/gVS$_{feed}$, respectively. Both cases produced more than the control of each, it means that the microwave assisted process with 2 minutes of radiation enhance positively the biomethane production of these samples. This pretreatment increased the RSB biomethane production in 20%, surprisingly the ESB enhance was increased in the same rate. The microwave radiation helps the structural composition of the lignocellulosic components to be more expose for the microorganism that can attack it and decompose into the intermediate necessary compounds in the process. Despite the biomethane favorable production, the gas quality of this pretreatment does not surpass the unity and shows the presence of some other gases that can have a contrary effect over the AD, Table 3.10.

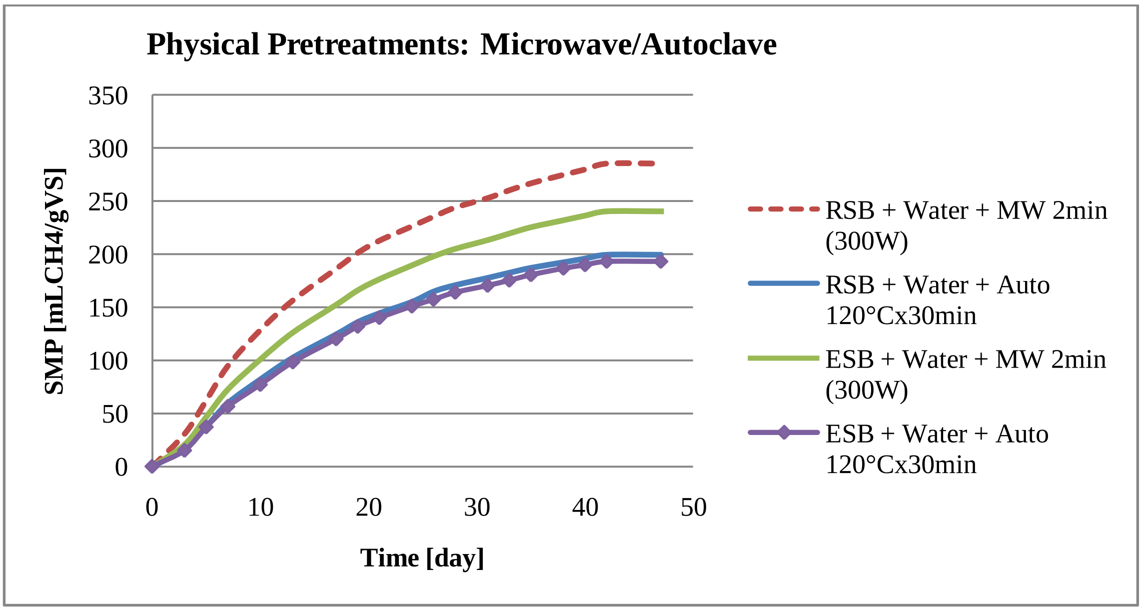


Figure 3. 23 Effect of physical pretreatments on SMP: Microwave/Autoclave -Test 2.

Table 3. 10 Effect of physical pretreatments on SMP: MW / Auto – Test 2.

Sample	**SMP** mLCH$_4$/gVS$_{feed}$	**CH$_4$/CO$_2$**	**Sample**	**SMP** mLCH$_4$/gVS$_{feed}$	**CH$_4$/CO$_2$**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + MW 2 min, 300W	**285.52**	0.813	ESB + MW 2 min, 300W	240.47	0.987
RSB + Autoclave 120°Cx30min	199.57	1.010	ESB + Autoclave 120°Cx30min	193.35	1.041

3.8.2 Effect of Physical Pretreatments: Autoclave

The thermal supply in the autoclave pretreatments for RSB and ESB resulted in 199.57 and 193.35 mL CH$_4$/gVS$_{feed}$, respectively. This pretreatment affected negatively the biomethane production, or in other words it did not interact strongly enough with the lignocellulosic substrates to modify their structure and aid to an easier degradation under this conditions. The reduction of biomethane produced with RSB was 15%, while for the ESB sample it was 5% compared to control. In contrast, the gas quality was good for a biomethane and carbon dioxide ratio, Table 3.10.

3.9 Effect of Physicochemical Pretreatments on Biogas Production – Test 2

The physicochemical pretreatments were performed with chemical reagents with a stable concentration of 2% and not only under microwave radiation, but also under thermal supply in the autoclave equipment. For the microwave the radiation was 300 Watts for 2 minutes, while in the autoclave it was 120 °C for 30 (thirty) minutes. Furthermore, in these physicochemical pretreatments some pretreated substrates were mixed with fresh un-pretreated substrates. Therefore, the samples marked with 70/30 represent 70% of pretreated substrate and 30% un-treated.

3.9.1 Effect of Physicochemical Pretreatments: Microwave

The physicochemical interaction with microwave radiation over the RSB pretreated samples 211.32 mL CH_4/gVS_{feed} for acid and 254.57 mL CH_4/gVS_{feed} for alkaline pretreatments samples. The acid pretreatment did not enhance the biomethane production as expected, but the alkaline pretreatment enhanced methane potential by 8%. On the other hand, the studied biomethane production potential with ESB was 217.23 and 170.07 mL CH_4/gVS_{feed}, for acid and alkaline pretreated samples respectively. For this case, the acid pretreatment had positive effect on biogas production and it increased by 10% while the alkaline pretreatment resulted in 15% decrease in specific methane potential, Figure 3.24. Both cases, the time for achieving 60% of the produced biogas was 13 days, bacteria proliferates for longer time.

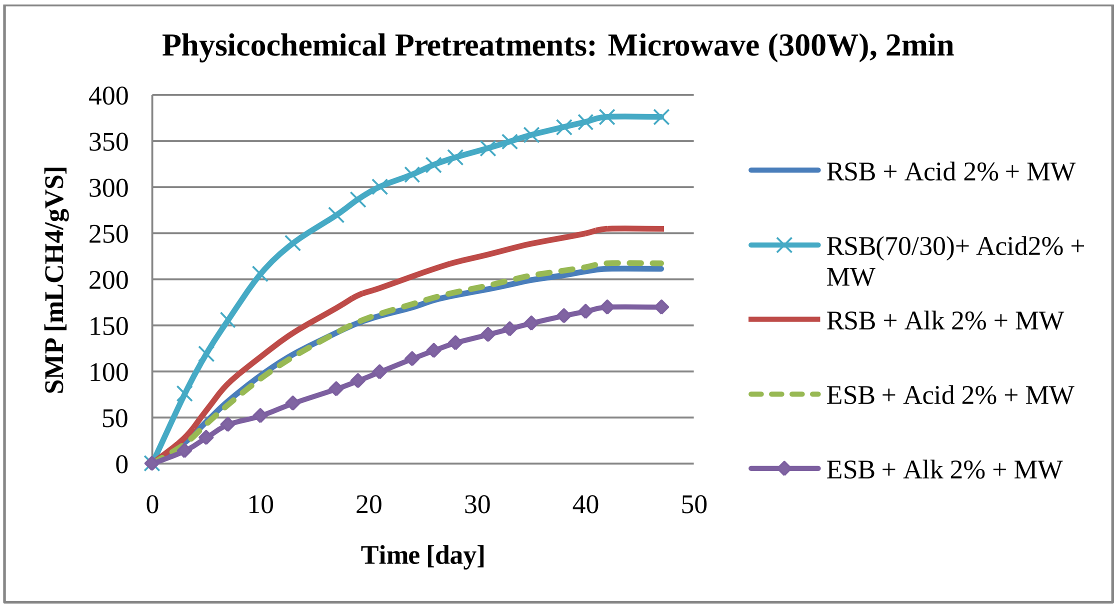


Figure 3. 24 Effect of physicochemical pretreatments on SMP: Microwave – Test 2.

Table 3. 11 Effect of physicochemical pretreatments on SMP, Microwave Test 2.

Sample + MW 2 min	**SMP** mLCH_4/gVS$_{feed}$	**CH_4/CO_2**	**Sample + MW 2 min**	**SMP** mLCH_4/gVS$_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Acid: 2%	211.32	0.654	ESB + Acid: 2%	217.23	0.759
RSB (70 pretreated / 30 un-pretreated) + Acid: 2%	**376.11**	0.627	-	-	-
RSB + Alk: 2%	254.57	0.856	ESB + Alk: 2%	170.07	1.047

The BMP test with the mixed substrate (70 pretreated / 30 un-pretreated), which was pretreated with 2% acid, had a specific biomethane production potential of 376.11 mL CH_4/gVS$_{feed}$. This increase represents 80% compared with the production achieved by the sample which was set with 100% of pretreated substrate. If the SMP is compared with the control of the system, it has an increase of 60%. The behavior of this mixed substrate is supported by the idea of having some available sugars in solution at the beginning of the AD, which helps the microorganism to start their decomposition process faster. Thus, 60% of SMP was achieved in 10 days of AD. Soluble sugar is not present in the 100% – pretreated sample because was removed with the filtrates after the pretreatment process, Table 3.11.

3.9.2 Effect of Physicochemical Pretreatments: Autoclave

The pretreatment of RSB substrate by physicochemical – autoclave had a harsh interaction with the samples. The effect of thermal autoclave pretreatment was clearly observed over the lignocellulosic material, the autoclave assisted acidic pretreatment resulted in higher SMP. Thus, acid and alkaline pretreated samples achieved specific biomethane potentials up to 387.82 and 222.32 mL CH_4/gVS$_{feed}$, respectively. The acid pretreatment increased the biomethane production by 65%. However, for the alkaline pretreatment sample SMP was reduced by 5%, the effect was not significant Figure 3.25. The 60% biogas production time was also 10 days.

For the ESB substrate in the physicochemical pretreatments that were assisted by autoclave also resulted in a higher amount of SMP with the samples treated under acidic conditions. Acid pretreatment resulted in 276.20 mL CH_4/gVS_{feed} and alkaline pretreatment 212.83 mL CH_4/gVS_{feed}. The acid interaction increase 38% the biomethane production for the ESB samples, while under the alkaline presence it was 6%. So the effect of acid pretreatment was more pronounced when assisted by autoclave Figure 3.25.

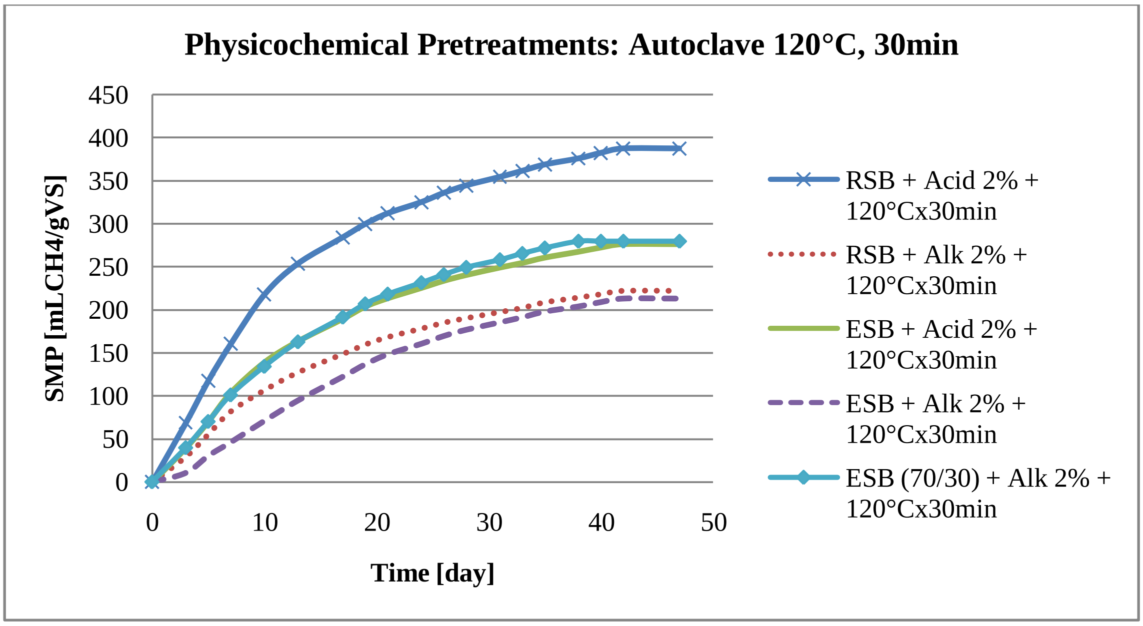


Figure 3. 25 Effect of physicochemical pretreatments on SMP: Autoclave – Test 2.

The mixed substrate (70/30) sample was treated with alkaline 2% and its biomethane accumulated production was 280 mL CH_4/gVS_{feed} (with 13 days to produce 60%), which means an increase 30% of compared with alkaline pretreated sample, increased by 40% compared the control. It shows that the sugar availability in solution at the beginning of the AD process helps the microorganism to enhance the biogas production in the system. The destruction of lignocellulosic structure is essential for biogas production from sugar beet (Table 3.12).

The physicochemical pretreatments enhance biogas production by 40 – 60 % when the lignocellulosic structure is degraded into its components as cellulose, hemicellulose and lignin. The interaction of a chemical reagent and the thermal radiation resulted in higher concentrations of biomethane during the AD. This general enhancement is mostly achieved within acidic pretreatment; the alkaline pretreatment is found not very effective in breakdown of the complex structures of sugar beet as the acidic medium does [29].

Table 3. 12 Effect of physicochemical pretreatments on SMP, Autoclave Test 2.

Sample + 120°C,30min	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**	**Sample + 120°C,30min**	**SMP** $mLCH_4/gVS_{feed}$	**CH_4/CO_2**
RSB - Control	236.66	1.824	ESB - Control	200.05	1.386
RSB + Acid: 2%	**387.82**	0.667	ESB + Acid: 2%	276.20	0.781
RSB + Alk: 2%	222.32	0.961	ESB + Alk: 2%	212.83	1.188
-	-	-	ESB (70 pretreated / 30 un-pretreated) + Alk: 2%	280	0.824

3.10 Discussion of the Results

Due to its morphology, sugar beet is a lignocellulosic substrate which needs the decomposition of the cellulose, hemicellulose and lignin that make part of its structure. All these previous components can be degraded into monosaccharide to produce fermentable sugars trough anaerobic digestion process. That is how, pretreatments play an important role in the release of soluble sugars from the main lignocellulosic structure and helps anaerobic digestion (AD) process, increasing the amount of biomethane gas due to higher available soluble substrate [41].

From literature it is suggested that acid pretreatments can disrupt the lignocellulosic structure and breakdown the hemicellulose rigid composition to release more degradable component. On the other hand, lignin can weaken under alkaline conditions, which make its structure more fragile and easier to hydrolyze at the beginning of the anaerobic process. However, in both cases the intermediate components can poison the medium and avoid the bacteria growth [16,28].

It is likely that the most important components of hemicellulose are glucuronoarabinoxylans and the xylose, arabinose, and glucose. Unfortunately, chemical components as xylose can be easily degraded to components like furfural, formaldehyde, formic acid, crotonaldehyde, lactic acid, acetaldehyde, dihydroxyacetone, which can inhibit the proliferation of the microorganism in the digestion process and affect the final performance of the biochemical methane production (BMP) [36,37].

Due to the rigid structure that posse the lignocellulosic materials, grinding is an important must-do physical pretreatment that improves biodegradability of that type of biomass increasing the contact with bacteria. Studies show that smaller particle size can enhance the biogas production up to 20% and even the biomethane production until 25%. Those improvements can be achieved by physical pretreatments. Therefore, in combined pretreatments (Physical, chemical and biological), higher than 100% increase in biomethane production can be possible [27].

From literature it is suggested that while decomposing the lignocellulosic materials, some other inhibitors can be produced which can disrupt the anaerobic digestion process. These intermediate products can affect the microbial activity of the BMP process. Moreover, the use of chemical pretreatment resulted in lower specific methane potential (SMP) production, compared with the experiments that only included water as liquid phase. Even, by using chemical pretreatment assisted with microwave or thermal radiation (physicochemical pretreatments), the biogas production was inhibited by the remaining excessive presence of alkaline or acid components substrate which could not be removed after the rinsing. However, it is important for the anaerobic digestion (AD) process that at Acidogenesis stage VFA acids are produced. For that reason, some reports show that in the presence of mannitol and in the absence of n-butyric acid at this stage of the process, the intermediate products of the reactions can lead to inactivate the acidogenic bacteria and disrupt AD. Therefore, some researchers considered that the pH-value of the process is more important than the temperature and should be carefully monitored during the whole AD process [42].

The agitation of the reactors also plays an important role to consider. It is likely that the hydrolysis of the components can be promoted and contact between bacteria and substrates increase with a homogenized content and temperature in the reactor. For instance, Methanogenesis can be disturbed and the methanogens do not have time to attack the acetate, hydrogen and carbon dioxide already produced to be converted into biogas. Thus, the required intermediate components cannot consume as they are produced and the AD cannot proceed and biogas cannot be produced [43].

The enhancement achieved with the autoclave pretreatment is supported by researchers who showed that thermal supply over sugar beet can increase the specific methane production by 50% at 120°C for 20 minutes (SMP from 285.42 to 432.84 mL CH_4/gVS_{feed}). Those results are similar to the present investigation where under the same conditions the improvement resulted from 236.66 to 353.39 SMP mL CH_4/gVS_{feed}. There are also some investigations which incorporate mechanical (grinding) and enzymatic (at 50°C for 4 days) pretreatment over sugar beet. For that case, the biomethane production was enhanced by 60%: from 277.3 to 452.1 SMP mL CH_4/gVS_{feed}. A similar increase was achieved under physicochemical conditions with 2% acid pretreatment expose to autoclave 120°C for 30 minutes: from 236.66 to 387.82 SMP mL CH_4/gVS_{feed} [16,27].

It is important to note that the chemical pretreatment has different performance in the anaerobic digestion to biogas depending on their type. For instance, samples pretreated with acid at high temperatures (120°C, 30 min), had a higher production of biomethane. On the other hand, the alkaline samples had a better performance at lower temperature (25°C, 48 hours), but with a longer contact time to react with the lignocellulosic components. Previous investigations over wheat straw showed that alkaline pretreatment at low temperature (20°C) and long contact time (48 hours) can enhance by 20% the biomethane production: from 260 to 313 mL CH_4/gVS_{feed}. Under similar conditions (2% alkaline, 25°C during 48 hours), SMP from ESB was enhanced by 40%: from 200.05 to 286.96 mL CH_4/gVS_{feed} [29,44].

In the AD process, the incorporation of the filtrates from the pretreatment plays an important role for the biogas production yield. Some studies with enzymes show that there is a high tolerance to mitigate the negative effect that can have the chemical reagents used in the pretreatments over the whole AD. But also, in the cases where enzymes are not included in the pretreatments, it is more recommended not to add the filtrates in the reactor, which will have an unfavorable effect over the biomethane production due to the inhibitor components that can result from the degradability of the hemicellulose and the lignin. Previous studies over wheat straw and sugarcane bagasse showed that only thermal pretreatment benefit the biomethane production when there are filtrates. On the other hand, when the pretreated substrates are incorporated to the BMP test (without filtrates), the biomethane production is enhanced by 30% under alkaline pretreatments. In the present study, the alkaline pretreatment could increase the SMP by 5%, but the acid pretreatment up to 35%: from 200.05 to 276.20 mL CH_4/gVS_{feed} [29,45].

4. CONCLUSIONS

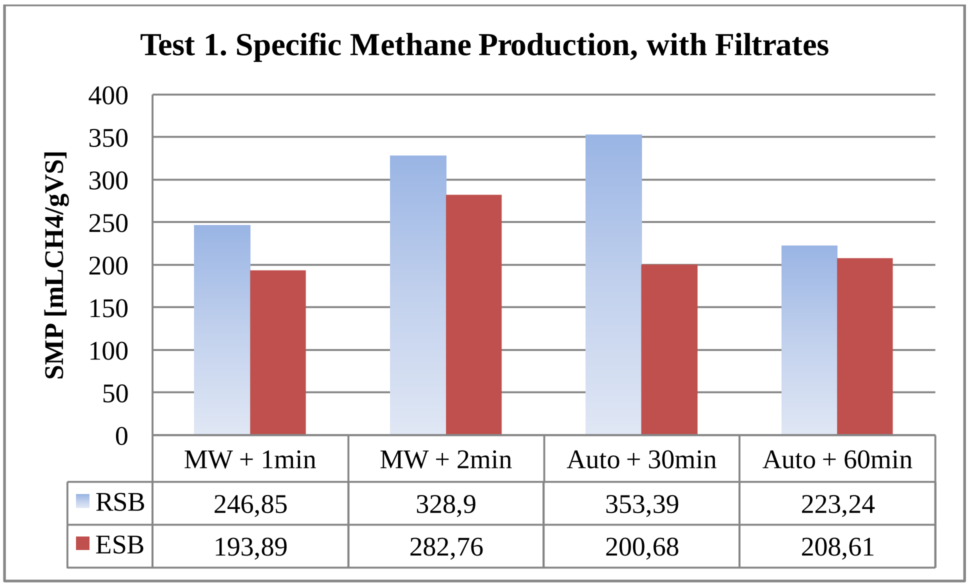
In the scope of this investigation, biogas production from sugar beet was investigated. In order to enhance the biogas production chemical, physical and physicochemical pretreatments were applied to decompose its lignocellulosic structure which can improve its AD. Therefore, raw sugar beet (RSB) and exhausted sugar beet (ESB, exhausted from sugars in an extractive process) were exposed to several pretreatments; chemical (Acid: H_2SO_4 and Alkaline NaOH) and physical (Microwave and Autoclave) type, and also a combination of these pretreatments: Physicochemical.

At first, the analysis of the effect that each one of the pretreatments had over the substrates was measured by the availability of sugars in solution that each of them could generate. Thus, chemical and physical pretreatments did not have much effect over the increase of the sugars in solution. However, the physicochemical pretreatments – Autoclave could increase the soluble sugars concentration at 120°C for 30 min. From the RSB substrate it could increase the soluble of sugar by 30 – 70% and for ESB was 2500 – 4500 %. In both substrates it was observed that under acidic pretreatment (H_2SO_4), there was a stronger attack to decompose the lignocellulosic structures.

In the second part of the analysis, the biochemical methane potential (BMP) was measured within an anaerobic digestion (AD) process. In order to have a better comprehension of the system, the investigation was carried with and without using the ***filtrates*** which is the liquid phase obtained after the pretreatments, it is reached in soluble sugars. For that reason, there are two main parts of this set of experiments:

Test 1. With filtrates: Filtrates possess the sugars in solution that were extracted from the solid substrates. In theory, it is a better medium for the proliferation of microorganisms due to the high concentration of saccharide that they can digest and start the biogas production faster. The soluble part is easy to convert to methane with no hydrolysis stage. Eventually, these microorganisms present in the reactor can also start the degradation of complex structure that the lignocellulosic substrates have and aim the anaerobic process to the production of biomethane.

The filtrate also contains a high concentration of residual chemical reagents used in the pretreatments and it can disrupt the digestion and inhibit Methanogenesis in the AD. Therefore, only the experiments where no chemical agents were used in the pretreatment, could achieve higher concentrations of biomethane during the process.



	MW + 1min	MW + 2min	Auto + 30min	Auto + 60min
🟦 RSB	246,85	328,9	353,39	223,24
🟥 ESB	193,89	282,76	200,68	208,61

Figure 4. 1 Specific Methane Production with Filtrates – Test 1.

RSB exposed to 1 and 2 minutes of microwave radiation (300 W) can enhance the biomethane production between 5 – 40%, or in the case of autoclave pretreatment (120°C, 30 min) can increase up to 50% the SMP. On the other hand, the biomethane produced by ESB substrate can enhance SMP by 40% within 2 minutes microwave radiation (300 W), while with the autoclave pretreatment (120°C, 60 min) the improvement would be 5%. Thus, autoclave pretreatment at 120°C for 30 minutes resulted in achieving a higher SMP Figure 4.1.

Test 2. Without filtrates: The liquid phase is fresh distilled water. The main consideration that these experiments have is that the substrates will perform depending in the structural modification that the pretreatments could achieve over the lignocellulosic structure. There are no soluble sugars at the beginning of the digestion process as the filtrate was discarded and replaced by distilled water.

It was observed that acid and alkaline pretreatments could enhance the biogas production during the anaerobic digestion. The acid treated substrate had higher concentration of biomethane production when the pretreatment was carried under high temperatures (120°C), in combination with physicochemical pretreatments: Microwave and Autoclave. While the alkaline treated substrate had a better performance to achieve higher concentrations in low temperatures (25°C), chemical pretreatments: Short (10 – 15 minutes) and long (48 hours) time contact process.

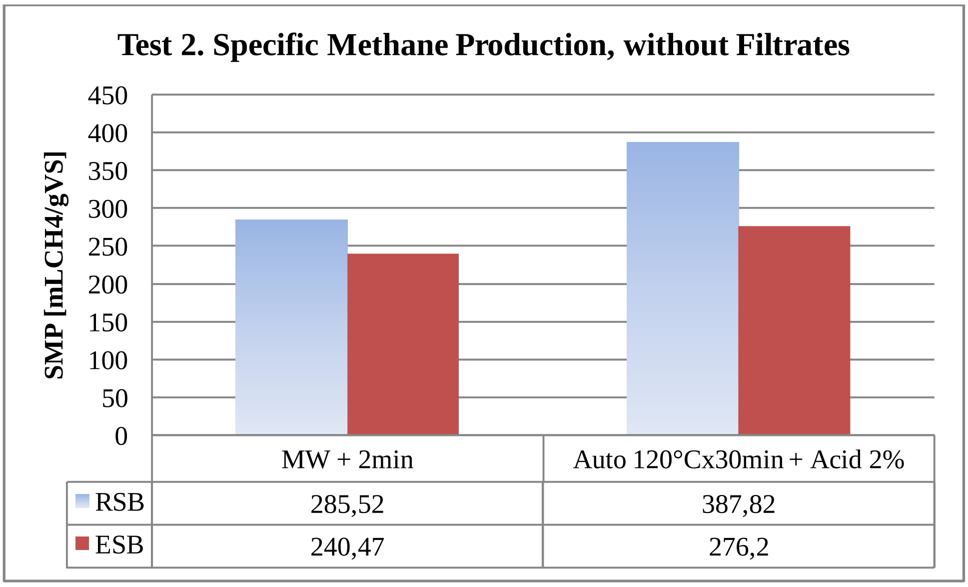


Figure 4. 2 Specific Methane Production without Filtrates – Test 2.

RSB substrate exposed to microwave radiation for 2 minutes could enhance SMP by 20%, and by 60% when the samples were exposed to autoclave pretreatment (120°C, 30 min). ESB samples exposed to microwave radiation during 2 minutes could enhance SMP by 20% and by 40% when it was autoclave pretreatment (120°C, 30 min). Thus, autoclave pretreatment at 120°C for 30 minutes resulted in achieving a higher SMP Figure 4.2.

Within the Test 2 procedure, some samples were considered as mixed substrate. These samples are a mix between treated and untreated substrates. Thus, 70/30 refers to 70% of pretreated substrate and 30% to un-pretreated substrate. The experiments under this condition were chosen randomly: one for RSB and one for ESB substrate. For both cases, the mixed substrate could increase the biomethane production when it is compared to its non-mixed substrate peer: for RSB it represents almost 80% of enhancement and for the ESB sample 30%, which in the overall process shows an improvement of 60% and 40%, respectively, Figure 4.3.

The advantage of working with the mixed substrate system is not only about the positive effect that it causes to enhance the biomethane production, but also it refers to a reduction in the cost that can involve the implementation of pretreatments in to the process. It would not be necessary to treat a hundred percent of the initial substrate, it would be enough to pretreat with a certain percentage of the biomass. On the other hand, the untreated substrate could provide the initial available sugars that microorganisms require to start their AD. Thus, higher amounts of biogas are achieved. In this way hydrolysis would not be a limiting stage for biogas production.

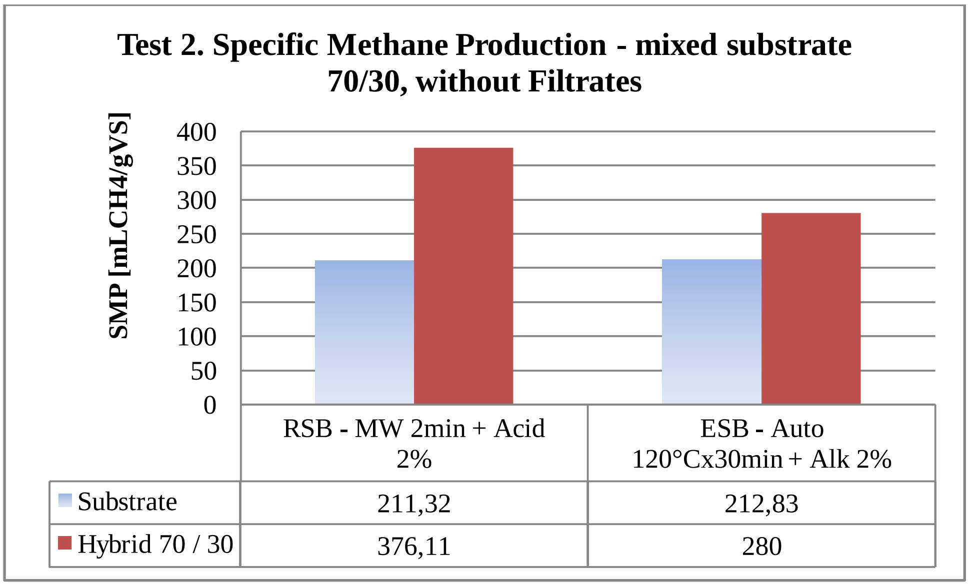


Figure 4. 3 SMP without Filtrates, mixed substrate 70/30 samples.

Sugar beet was studied under the scope of physical, chemical and physicochemical pretreatments to enhance the biomethane production. Not only were the different effects that those pretreatments could cause over the lignocellulosic substrate analyzed, but also a new mixed substrate formulation was proposed to avoid the slow adequacy of the microorganisms in the medium and enhance the yield of the system by having a faster proliferation since the beginning of the digestion process. Of course, this idea needs to have deeper experiments to guarantee the future good results over the lignocellulosic substrate studied.

In conclusion:

1. Sugar beet is a good biomass for biogas production, not only as a raw substrate but also as an exhausted lignocellulosic substrate residue from the sugar industry. The

specific methane production achieved was enhanced by the different pretreatments applied over sugar beet. For that reason, the present investigation showed an alternative when there is an excess of exhausted sugar beet in sugar factories and the enhancement of biogas production is a suitable option to designate that extra biomass.

2. The soluble sugars in solution, due to the pretreatments applied over sugar beet, were increased by 1.96 mg/mL and 1.39 mg/mL for RSB and ESB, respectively, when acid pretreatment took place: H_2SO_4 4%, T: 25°C, t: 48h. Also, physicochemical pretreatments (H_2SO_4 2% + Autoclave 120°C for 30min) resulted in 1.85 mg/mL (RSB) and 1.83 mg/L. Alkaline pretreatments generated other intermediate non-saccharide components.

3. Pretreatments had an effect over the time of the anaerobic digestion to achieve 60 – 70% of SMP produced. The higher biogas concentrations resulted from the experiments which took 10 – 15 days. Therefore, within that time bacteria could proliferate and degrade the lignocellulosic components during that time. Experiments with shorter time did not achieve high amount of biogas. Microorganisms were deactivated after 7 days of digestion and the inhibitors poisoned the system.

4. The biogas production is not proportional to the soluble sugar in solution at the beginning of the anaerobic digestion. When filtrates were included into the BMP tests, the higher cumulative biogas production was achieved by the physical pretreatments. For that reason autoclave pretreatment at 120°C for 30 minutes increased the SMP up to 353.39 mLCH_4/gVS, meanwhile microwave pretreatment up to 328.9 mLCH_4/gVS, at 300Watts for 2 minutes. When acid or alkaline pretreatments were used, the biogas production resulted in similar or lower amounts than the process control.

5. When filtrates were not used in the BMP tests, chemical and physicochemical pretreatments produced higher amounts of biogas. The alkaline pretreatment enhanced the specific methane production by 286.96 mLCH_4/gVS, when NaOH 2% for 10 minutes was used. While for the physicochemical pretreatments with H_2SO_4 2% and autoclave 120°C for 30 minutes resulted in 387.82 and 276.20 mLCH_4/gVS for RSB and ESB, respectively. The alkaline pretreatment worked better at low temperatures and short contact time, meanwhile the acid pretreatment increased the results at high temperatures.

6. When the mixed substrate (70 pretreated – 30 un-pretreated) was used under the physicochemical pretreatments conditions, it resulted in higher biogas production. RSB (70/30) exposed to H_2SO_4 2% at 120°C for 30 minutes in autoclave enhance the specific methane production up to 376.11 $mLCH_4/gVS$. On the other hand, under alkaline conditions ESB (70/30) exposed to NaOH 2% at 120°C for 30 minutes in autoclave resulted in 280 $mLCH_4/gVS$. Mixed substrate not only enhanced the biogas production when compared with the process control, but also it achieved higher productions when compared to the 100% pretreated substrate which used the same conditions. Furthermore, mixed substrates could reduce the budget expenses on pretreatments when these types of configuration face industrial applications.

7. In both cases, with and without filtrates, the chemical and physical pretreatments could modify the lignocellulosic structure of the sugar beet. However, it is not only about the cell wall disruption with the pretreatments developed, it is also important to consider the intermediate components that resulted from the hemicellulose and lignin brakeage. The chemistry of those intermediate components would help positively or negatively the anaerobic process, and plus the relocation that could happen over the pretreated substrate.

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Name Surname: LUİS ALFREDO CARMONA ARRİETA
Student No: N16123712
Department: RENEWABLE ENERGIES
Program: RENEWABLE ENERGIES – MASTER DEGREE
Status: ☑ Masters ☐ Ph.D. ☐ Integrated Ph.D.

ADVISOR APPROVAL

APPROVED.

Prof.Dr. Aysenur Ugurlu
(Title, Name Surname, Signature)

CURRICULUM

Name and Surname : Luis Alfredo Carmona Arrieta

Place of Birth : Cartagena, Colombia

Date of Birth : 28/06/1988

Marital Status : Single

Correspondence Address : Neyzen Tevfik So. 15, Maltepe/Ankara.

Telephone : +90 537 944 96 20

Email Address : luiscarmonaiq@gmail.com

Foreign Language : Spanish, English and Turkish

EDUCATION

Undergraduate : Chemical Engineering
Universidad Nacional de Colombia
2008 – 2013

Master’s Degree : Renewable Energies
Hacettepe University
2016 – 2019

JOB EXPERIENCE

2013 – 2014	Chemical Engineer – Industrias Protón Ltda.
2014 – 2016	Chemical Technician – Telas Lafayette SAS.