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Evaluation of Biogas Production through Anaerobic Digestion of Aquatic Macrophytes in a Brazilian Reservoir

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Authors' contributions:

This work was carried out in collaboration among all authors. Author RNDSP designed the study, wrote the protocol, performed the experiments, managed the analyses of the study, wrote the first draft of the manuscript and effect all necessary corrections. Authors AVGV and PR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aquatic macrophytes are important components of aquatic habitats. However, the overgrowth of aquatic plants can cause severe problems for the management of bodies of water. As a result, these plants must be removed and disposed of as waste. However, the usage of this biomass as a substrate in biogas plants would appear to be more beneficial. The present work deals with the anaerobic digestion (AD) of macrophytes species that cause inconvenience to power generation at hydroelectric plant in Minas Gerais - Brazil. The study examines the following macrophytes species; *Salvinia molest Oxycarium cubense*, *Eichhornia crassipes*, *Pistia stratiotes* and *Brachiaria*. The experiments were carried out as stainless steel reactor with temperature, agitation and pressure control. As pre-treatment of macrophytes was used heat treatment at 120°C and pressure of 1.6 atm. The maximum methane content was 60% during 40 days digestion time, for Brachiaria of higher lignin content. The result obtained, mainly with Brachiaria demonstrates the efficiency of pre-treatment for the lignocellulosic samples.

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1. INTRODUCTION

Bioenergy represents energy from biomass and plays an important role in promoting renewable alternatives. This way, Brazil is a world reference for the use of this type of energy. This is because 43.5% of its energy matrix comes from renewable resources, and the world average is only 14.2%. If we consider only what is used for electricity production, the percentage rises to 81.7%, since the main source of supply of the electricity sector comes from hydroelectric plants throughout the country. Historically, Brazil invests a lot in this system due to the abundance of available water resources. The burning of non-fossil organic materials, such as sugarcane bagasse, alcohol, wood, etc., causes the generation of energy through biomass. In this case, we are particularly interested in macrophytes as raw material. One of the main points of the use of this energy generation system is in the use of agricultural waste that, theoretically, would be discarded and in the possibility of cultivation. Biofuels (biogas, bio alcohol, biodiesel, among others) are obtained in the transformation of these organic wastes. Although the burning of these materials release-polluting gases, it is still considered a source of clean energy, because all CO_2 generated is sequestered in the cultivation of organic materials [1,2].

Aquatic macrophytes are, for the most part, terrestrial vegetables that, throughout their evolutionary process, have adapted to the aquatic environment, so they have some characteristics of terrestrial vegetables and a great capacity to adapt to different types of environments. In many bodies of water, undesirable macrophytes exhibit excessive growth, generating adverse effects on the aquatic environment, which accelerates the eutrophication process. Eutrophication is a natural process resulting from the accumulation of organic matter resulting from the excessive accumulation of organic matter from sewage and the development of algae. In hydroelectric reservoirs, the uncontrolled growth of macrophytes causes problems in the turbines, forcing frequent discharges by the spillway to the exit of the plants, generating water waste and decreased electrical production. These plants need to be controlled or removed from many aquatic environments.

To control aquatic macrophytes that are unbalanced in the environment, manual or mechanized withdrawal is preferably applied [3]. In order to take advantage of this biomass, it can be used biogas production from anaerobic mono or co-digestion [4,5].

It is also possible to carry out a pre-treatment (chemical, physical or biological) of the substrates, in order to promote the disruption of the structure of lignocellulose and increase the surface, improving biodegradability through cell wall rupture [6]. In this way, access to microorganisms to the substrate, increasing the potential for biogas production [7,8].

In view of this, in order to obtain a more sustainable development and also to provide a destination for the biomass macrophytes, the anaerobic biodigestion of these residues is shown to be a viable alternative by promote nutrient recycling, produce bio fertilizer (or digestate, a by-product of the process) and also to generate sustainable energy through biogas [9-11].

Compared to the mono digestion, anaerobic co-digestion has great potential to maximize biogas production and therefore has grown interest in the search for new waste to act as co substrates [12-14].

Lignocellulosic materials are the best sources used for biofuel production, such as biogas, and include residues from agriculture and forests, energy crops, and municipal and food waste. In Europe, almost 72% of the feedstock used in the anaerobic digestion (AD) process for biogas production come from the agricultural sector, such as energy crops, manure, and other agricultural residues. The main issue of using Lignocellulosic (LC) biomass for the biogas production is biomass recalcitrance, which represents biomass resistance to chemical and biological breakdown.

The external and internal structures of the plant, its lignin content and many other factors determine how well the anaerobic bacteria in the biogas process can digest the plant. Lignin content and the available surface area are considered the two most important factors [15, 16]. A pretreatment used to break the structures and increase the surface area. Many pretreatments have previously been evaluated; mechanical, thermal, acid, alkaline, biological and others [17,18]. Acidic and alkali pretreatments have shown of great promise in biomass solubilization because of their multiple advantages, for example, a simple device, ease of operation, high methane conversion efficiency and low cost. Acidic hydrolysis is performed using acids such as HCl, H_2SO_4, H_3PO_4, and HNO_3 while alkali pretreatment usually employs several alkaline solutions, inducing NaOH, KOH, $Ca(OH)_2$, $Mg(OH)_2$, CaO, and ammonia. [19].

In the biogas process, bacteria digest organic matter under anaerobic conditions and produce methane and carbon dioxide as final products. It is a multi-step reaction where at least three groups of different bacteria are involved. The process is often divided into four main steps (Fig. 1). Hydrolysis. The first step is called the hydrolysis, where hydrolytic bacteria excrete enzymes that break insoluble polymers to soluble oligomers and monomers. In this step carbohydrates are converted into sugars, lipids are broken down to fatty acids and proteins are split into amino acids. The hydrolysis is often known to be the rate-limiting step in the anaerobic digestion (AD) process [20]. In the second step, Acidogenesis, the smaller units from the hydrolysis can be taken up through the bacterial cell wall and are digested in the second step by acidogenic fermentative bacteria. Some of the products from this fermentation (mainly acetate, CO_2 and hydrogen) can be utilized directly in the fourth step; methanogenesis. Products like alcohols and volatile fatty acids on the other hand must be oxidized in the third step by acetogenic bacteria [20,21]. Acetogenesis. The acetogenic step is where alcohols and volatile fatty acids produced during the fermentation are converted to acetic acid, carbon dioxide and hydrogen, which is used in the last step (methanogenesis). These acetogenic bacteria live in symbiosis with the methanogenic organisms [22]. Methanogenesis. This is the final step where methanogens convert acetic acid, hydrogen and CO_2 to methane, CO_2 and some trace gases [22].

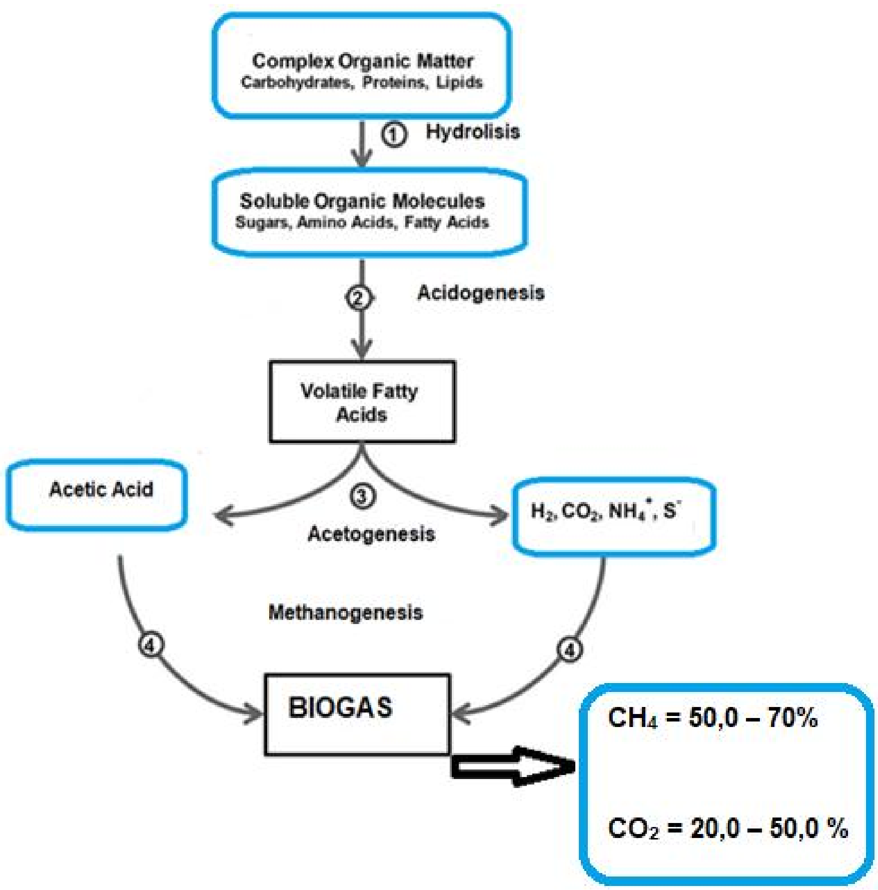

Fig. 1. Stages of anaerobic digestion

Biogas obtained in this way is, therefore, a renewable form of energy that may contribute to mitigate environmental pollution [22], and simultaneously can be utilized to produce electricity, heat, or fuel for vehicles. However, the obtaining biogas from lignocellulosic biomass is difficult, because lignocellulose is recalcitrant to microbial or enzymatic biodegradation, due to its structure and composition [23].

Lignocellulosic biomass is composed mainly by cellulose, hemicellulose, and lignin. Cellulose is a biopolymer formed of crystalline and amorphous parts, while hemicelluloses are amorphous and have heterogeneous complex structures formed by different polymers such as pentoses, hexoses, among others. Hemicellulose serves as a connection between cellulose and lignin; and, therefore, it provides more rigidity [24]. Lignin is an amorphous aromatic hetero polymer whose function is to provide support and impermeability to the plant as well as resistance to microbial attack and oxidative stress [23] Due to this structural complexity, lignin is difficult to break down.

A number of different pretreatment techniques involving physical, chemical, and biological approaches have been investigated over the past few decades, but there is no report that systematically compares the performance of these pretreatment methods for application on Lignocellulosic biomass for biogas production.

2. MATERIALS AND METHODS

2.1 Study Area

The Barra do Brauna Hydroelectric Power Plant (UHE), is located on the Pomba River, and is geographically located in the municipality of Recreio, State of Minas Gerais. The Pomba River is born in the Serra Conceição, belonging to the Mantiqueira chain, its extension is 265 km, and flows into the Paraiba do Sul River, between the municipalities of Cambuci and Itaocara, in the state of Rio de Janeiro. The Basin has a drainage area of 8,616 km^2, with relatively uniform land use and occupation, covering about 35 municipalities in Minas Gerais and 3 municipalities in Rio de Janeiro, where a population of approximately 618,000 inhabitants lives. To further aggravate the situation of the water quality of the Pomba River basin, the absence of domestic sewage treatment, released "in nature" directly into the watercourses, promoted the deterioration of the quality of the water in the basin. In addition, the river is constantly degraded by the release of industrial effluents. Its use for dilution of waste from the industries has caused strong contamination of the aquatic biota of the basin. The variations in water quality of the medium Pomba River it happened by the presence of a group of nutrients associated with domestic sewage and diffuse pollution, caused by the release of domestic sewage into the watercourse.

The loading of part of the fertilizers used in agricultural crops and the large load of residential and industrial sewage have led the watercourse of the Pomba River and its reservoirs to a condition of imbalance, characterized by the great availability of nutrients, leading to exaggerated and undesirable growth of a varied aquatic vegetation. Mainly *Oxycarium cubense*, *Salvinia molesta*, *Pistia stratiotes* and *Eichhornia crassipes*, which are considered the most worrisome, due to their fluctuating way of life and their high reproductive index, compose the species of macrophytes dominant and shapers of floating communities in the reservoir. It is worth mentioning the abundance of *Brachiaria*.

2.2 Aspects of Macrophytes Proliferation

The uncontrolled growth of macrophytes in hydroelectric reservoirs are causes for problems in power generation equipment, forcing frequent discharges by spillway to the exit of plants, generating water waste and decreased energy production. The accumulation of plants in the turbine grids causes from clogging to deformation or rupture of these grids, which makes it inevitable to interrupt the production of electricity for the replacement of the damaged grid. For the replacement of a single grid, each generating unit with about 110 MW is unavailable for about 40 hours. Moreover, the rot of submerged vegetation in the reservoir area produces hydrogen sulfide that corrodes the turbines, and its replacement is necessary.

The control of the proliferation of these plants is complex and makes mitigating actions necessary in order to avoid major problems with the case of reservoir silting, as already observed in many cases. In Brazil, lakes and rivers are important because they provide water and support agricultural and industrial production systems, human consumption, recreation, navigation, irrigation, fishing, in addition to the generation of electricity. The loading of part of the fertilizers used in agricultural crops and the large load of residential and industrial sewage have led courses and reservoirs to a condition of imbalance, characterized by the great availability of nutrients, accelerating the growth of undesirable aquatic vegetation, including algae and higher plants.

The occurrence of aquatic plants in hydroelectric reservoirs is a problem of increasing importance. Some hydroelectric plants already have their efficiency compromised by the high infestation of aquatic plants, which in some cases presents approximately 20% of its surface covered by these plants, causing major problems to their use by the company and the local community. There are few options for controlling aquatic plants in Brazil. The only alternative used is mechanical control. Like any other method of control of aquatic plants, this has advantages and limitations.

The operations related to mechanical control can be occurs into four stages: removal of plants from rivers, canals or lakes; transport of plants still in the water body; transfer of this material to the terrestrial environment; transport and disposal of the collected material. Alternative technologies for the use of this biomass present in order to avoid the simple disposal of the material in landfills, or even, because it is burning in the open. Its use for biogas production can be control proliferation in hydroelectric reservoirs; there are no records in the literature.

The article presents the development of studies on biogas production from the bio digestion of aquatic plants of the macrophytes type, aiming in general to the generation of electricity and the development of hydrogen synthesis technologies from purified Biogas extracted from the processes of anaerobic bio digestion of the vegetation cover of hydroelectric generation reservoirs.

The macrophytes collected at various points of the dam and under various coordinates as shown below:

"*Brachiaria*" located on the banks of the reservoir at the point on the coordinates:

S21°23' 47.4" / W042°30' 41.9".

"*Salvinia molesta*" located on the banks of the reservoir at the point on the coordinates:

S21°23' 03.3" / W042°29' 07.2".

"*Oxycarium Cubense*" located on the banks of the reservoir at the point on the coordinates:

S21°23' 50.3" / W042°30' 42.5".

"*Pistia stratiotes*" S21°26' 36.4" / W042°25' 14.5".

"*Eichhornia crassipes*" S21°26' 51.4" / W042°24' 27.2".

Fig. 2 shows the various types of macrophytes used in this work.

2.3 Aspects of Macrophytes Retreat

In order to reduce their abundance, the large biomasses of aquatic macrophytes have been combated using mechanical, chemical and biological control, because there is still no specific legislation for their management in an open system. In mechanical control, plants need to be collected, transported and deposited in an appropriate location, which makes the process costly and with short efficacy, because in a short time the reservoirs are recolonized. Manual withdrawal is efficient only in smaller, shallower environments.

The chemical control of aquatic macrophytes occurs with the use of herbicides. It is a widely used method worldwide, but in Brazil, the only herbicide registered in the Ministry of Agriculture, Livestock and Supply (MAPA) and the National Health Surveillance Agency (ANVISA) for the control of aquatic macrophytes of the species in hydroelectric reservoirs, is the Fluridone. Other active ingredients was been tested in closed systems, but their use is not allowed in the country.

The efficiency of chemical control will depend on water quality factor such as turbidity, pH, electrical conductivity and temperature.

The alternative that used most in Brazil is mechanical control with the removal of plants from the water body and used, mainly due to environmental restrictions to other methods and the immediacy of results [25]. Operations related to mechanical control occurs into four phases: Removal of plants from rivers, canals or lakes; Transport of plants still in the water body; Transfer of this material to the terrestrial environment, Transport, and disposal of the collected material [26]. Although the mechanical control presents some advantages, such as punctual action in the infested areas and non-contamination of the environment with chemical and toxic compounds, there is concern regarding the collected material to be discarded, given the large amount of biomass involved in this process. Fig. 3 illustrates the mechanical withdrawal of macrophytes.

Salvinia

Oxycarium cubense

Pistia stratiotes

Brachiaria

Eichhornia crassipes

Fig. 2. Aquatics macrophytes related to the study in Barra do Brauna reservoir

Fig. 3. Mechanical removal of macrophytes

2.4 Analytical Methods

2.4.1 Chemical oxygen demand (COD)

COD is the amount of oxygen necessary to oxidize the organic compound (OC) completely to CO_2, H_2O and NH_3. On can to measure, COD via oxidation with potassium dichromate ($K_2Cr_2O_7$) in the presence of sulfuric acid and silver, and is expressed in mg/ℓ. Thus, COD is a measure of the (O_2 equivalent of the organic matter, as well as microorganisms in the wastewater. If the COD value is much higher than the BOD5 value, the sample contains large.

2.4.2 BOD- Biochemical oxygen demand (BOD)

(BOD) is the amount of oxygen, expressed in mg/l or ppm that bacteria can take from water when they oxidize organic matter. The carbohydrates, proteins, petroleum hydrocarbons and other materials that comprise organic matter get into the water from natural sources and atom pollution. The BOD value is commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20°C and is often used as a surrogate of the degree of organic pollution of water.

2.4.3 Total nitrogen

Organic and inorganic nitrogen compounds are transformed to nitrate according to the Koroleff method by treatment with an oxidizing agent in a thermo reactor. In a solution it was acidified with sulfuric acid and phosphoric acid, it reacts with 2, 6-dimethylphenol nitrate (DMP) to form 4-nitro-2, 6-dimethylphenol, which is determined photo metrically.

2.4.4 Calorific power

For a rational and appropriate use of the material is necessary to know its energy properties. An excellent parameter for assessing fuel energy power is calorific power, defined as the amount of heat energy released during the complete combustion of a mass or volume unit depending on of the combustible material [27]. The higher the calorific value, the greater the energy contained. Every fuel has two types of calorific power, one called higher calorific power and the other lower calorific power. In this work, we are interested in determining the lower calorific value through IKA 2000 equipment.

2.5 Anaerobic Digestion

To perform the anaerobic digestion process, employed a 20-liter steel reactor with stirring, pH indicator, pressure gauge. The temperature remained constant by a thermostatic bath and equal to 35°C. This reactor appears in Fig. 4.

Fig. 4. Digestion reactor

Initially put 3 kg of crushed macrophytes in a diluted industrial blender with a small amount of water, the latter just to facilitate the formation of a paste with the macrophytes. This macrophytes paste was pre-treated in an autoclave at 120^0C and 1.6 atm for one hour.

To this paste, Fig. 5, were added 6 liters of water and 600 ml of inoculum from a reactor where the pig slurry was digested for several months.

Fig. 5. Macrophytes paste

After the reactor supply, the pH and the measurements of COD, BOD and nitrogen in various forms were measured.

The reaction mixture was kept at constant temperature for up to 40 days. Eventually the system was shaken to facilitate homogenization of the reaction mixture.

The composition of biogas CH_4, CO_2, CO measured using a biogas analyzer.

2.6 Preparation of Macrophytes Paste

The experiments were performed using a 2.0-liter stainless steel reactor. The reactor included a reactor body, heater, attemperator, and stirrer. For all experiments, a 1200 ml feedstock (sludge and water mixture) was loaded into the reactor. The experiment operating temperature was 120°C, the pressure of HTP was 1.6 atm., and the reaction time was 60 min. The samples in the reactor were mixed using an agitator stirring at 220 rpm. The steam was discharged from the pressure-reducing valve and the sludge was removed after the completion of pre-treatment reaction.

The Fig. 6 shows the various segments related to the use of macrophytes until their use in the production of biogas.

The pre-treatment of recalcitrant lignocellulosic biomass is an alternative to improve the biogas yield in the BA process.

There are different pre-treatment techniques that can be applied, such as physical treatments, chemical and biological or a combination thereof [28,29].

Among the objectives of pre-treatment are: The disruption of layer of lignin that protects cellulose and hemicellulose (in order to make fermentable sugars more accessible to bio digestion, as illustrated in Fig. 6) and the decrease in cellulose crystallinity, increasing its porosity [28].

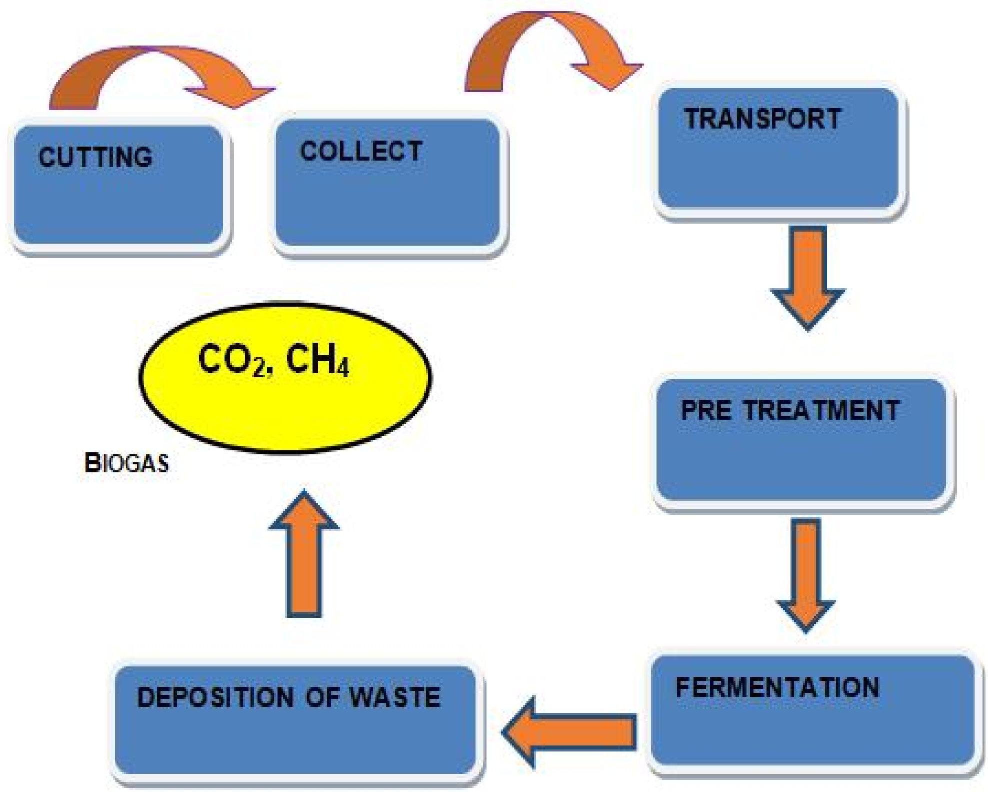

Fig. 6. Steps involving the collection of macrophytes and biogas production

3. RESULTS AND DISCUSSION

The efficiency of digestion anaerobic is influenced by some critical parameters, thus it is crucial that appropriate conditions for anaerobic microorganisms are provided. The growth and activity of anaerobic microorganisms is significantly influenced by conditions such as exclusion of oxygen, constant temperature, pH value, nutrient supply, stirring intensity as well as presence and amount of inhibitors. The methane bacteria are fastidious anaerobes, so that the presence of oxygen into digestion process must be strictly avoided.

Factors, which affect the production of biogas, are: Temperature b) Organic Loading Rate c) pH Value or Hydrogen Ion Concentration d) Hydraulic Retention Time e) Stirring or Agitation of the Content of Digester f) C/N ratio

The main factors in the production of biogas:

1. Temperature: Temperature inside the bio digester requires control in order to achieve the desired production. The bacteria responsible for bio digestion are very sensitive to sudden variations in temperature; small temperature variation already sufficient to cause the death of most of the digesting bacteria. In this work on the aquatic macrophytes, the temperature was the 35°C.
2. Type of waste: The type of organic matter used is also essential since it is necessary to maintain a favorable carbon / nitrogen ratio and the amount of volatile solids must be large enough to guarantee good productivity (case of macrophytes).
3. Hydraulic Retention time (TRH): In this project, we work the maximum time 40 days for the reaction development.
4. pH: The pH must also be controlled, because Methanogenic bacteria are inactive at acidic ph. The pH varies of 6.8–7.3).
5. Carbon / nitrogen ratio: The relationship between the amount of carbon and nitrogen in the substrate is essential for the formation of organic acids transformed by Methanogenic bacteria into biogas. The ideal ratio is around 1:30 or 1:20.
6. Water quantity: The bio digester works by hydraulic load and, therefore, it needs a certain amount of water to function. The dilution ratio was (macrophytes)/ (H_2O) = 1:3.
7. The Lignocellulosic material the process of bio digestion of macrophytes will depend on much of the type of pretreatment. Thus, the heating 120°C use for one hour, the pressure was 1.6 atm.

Table 1 presents the results obtained with the various types of macrophytes in relation to oxygen chemical demand, biochemical oxygen demand, and nitrogen amounts in the forms of total nitrogen (N), nitrate (NO^{3-}) and nitrite (NO^{2-}).

Significant amounts of nitrogen can be with the macrophytes *Oxycarium cubense.*

It should be noted that the COD and BOD values measured only at the reactor inlet. The values when withdrawn after 40 days have not been determined. The lowest COD / BOD ratio occurs with the macrophytes *Oxycarium cubense*, 10.26, while the highest ratio occurs with *Pistia stratiotes*, 50.0. Lignocellulosic components including hemicellulose, cellulose and lignin are the three major components of plant cell walls, and their proportions in biomass crops.

The Table 2 presents the results of the quantitative analysis for the different types of macrophytes used in this work. The evolution of Biogas production as function for the various types of macrophytes can be seen in Fig. 7 to 11. Wille in Fig. 12 this evolution was only about methane production.

Table 1. Chemical analysis of macrophytes

Biomass	COD m g/l	BOD m g/l	N m g/l	NH_3 m g/l	NO_3^- m g/l
Eichhornia crassipes (EC)	579	42	11.6	14.1	51.6
Salvinia (SA)	407	30	10.5	12.7	46.5
Pistia Stratiotes (PS)	2152	43	14.8	18.1	65.8
Brachiaria (BC)	238	09	80.0	85.3	100.3
Oxycarium cubense (OC)	2258	220	85.6	96.2	113.5

According to the results, it can be verified that the macrophytes present in their constitution different compositions of cellulose, Hemicellulose and lignin. The macrophytes with higher lignin content being the *Brachiaria* and *Oxycarium Cubense*. As shown above the lignin content is directly matched with anaerobic digestion. Thus, lignin impairs the digestion process, as it is resistant to attack by microorganisms. The macrophytes *Brachiaria* and *Oxycarium Cubense* also have the highest values of calorific value.
By checking the biogas yield, we can see that the methane yield after about 40 days is the Brachiaria with the highest lignin content and 60% in methane. The macrophytes *Oxycarium Cubense* present 46% in methane and has the second highest lignin content. The efficiency of the process is related to the heat treatment of the macrophytes. The macrophytes *Pistia Stratiotes* has 56% de methane, the *Salvinia* 30%, and *Eichhornia Crassipes* 43%.

During a heat pretreatment process, the residual biomass of lignocellulosic origin is heated, and a part of the hemicellulose is hydrolyzed forming acids. These acids in turn catalyze additional hemicellulose hydrolysis [30] Thus, hydrolyzed material can be converted more easily, through anaerobic digestion, to other by-products such as methane gas [31].

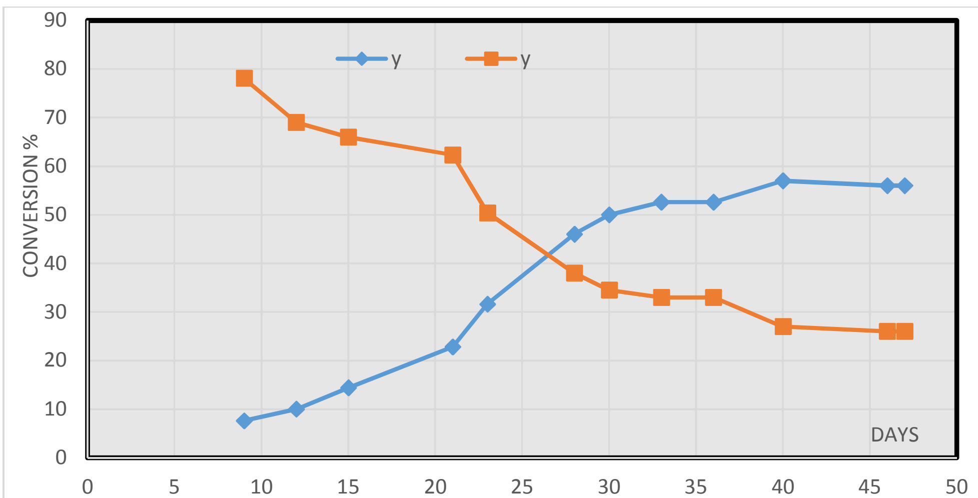

Fig. 7. Methane and CO_2 yields of macrophytes *Pistia stratiotes* (*PS*) as a function of time

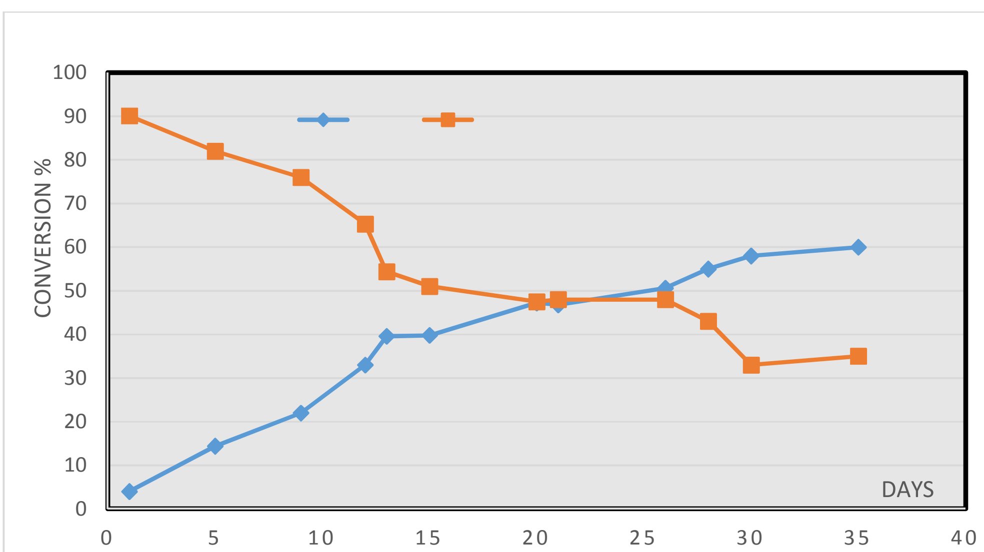

Fig. 8. Methane and CO_2 yields of macrophytes *Brachiaria* (*BA*) as a function of time

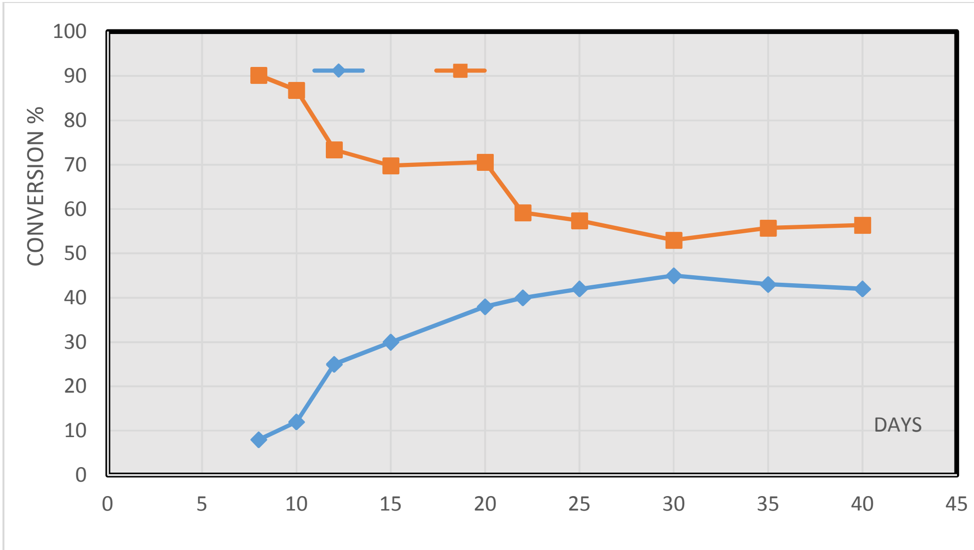

Fig. 9. Methane and CO_2 yields of *macrophytes Eichhornia crassipes* (*ES*) as a function of time

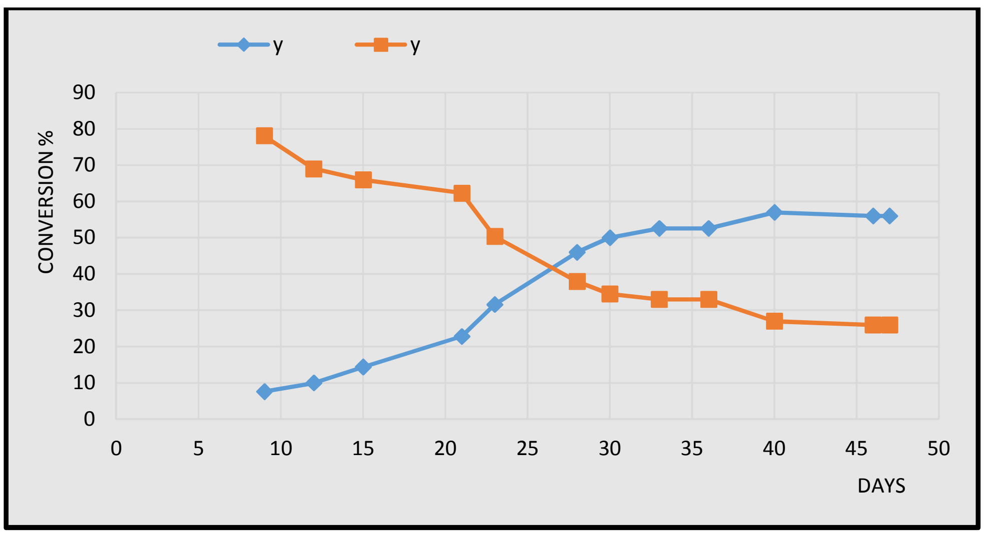

Fig. 10. Methane and CO_2 yields of macrophytes *Pistia stratiotes* (*PS*) as a function of time

Table 2. Chemical composition of macrophytes

Macrophytes	Cellulose %	Hemicellulose %	Lignin %	Calorific Power (kJ kg^{-1})
Pistia stratiotes	16.47	16.91	15.70	12.182
Salvinia	29.16	10.16	13.70	10519
Brachiaria	39.41	27.45	27.39	17.473
Eichhornia crassipes	21.63	25.94	12.12	14.630
Oxycarium cubense	35. 6	14.8	25.20	17.914

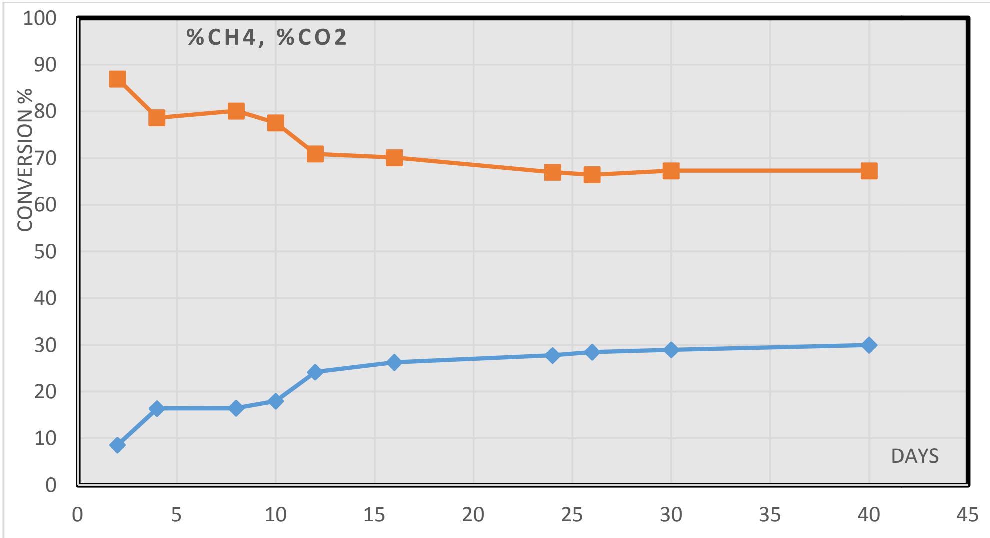

Fig. 11. Methane and CO_2 yields of macrophytes *Salvinia* (*SA*) as a function of time.

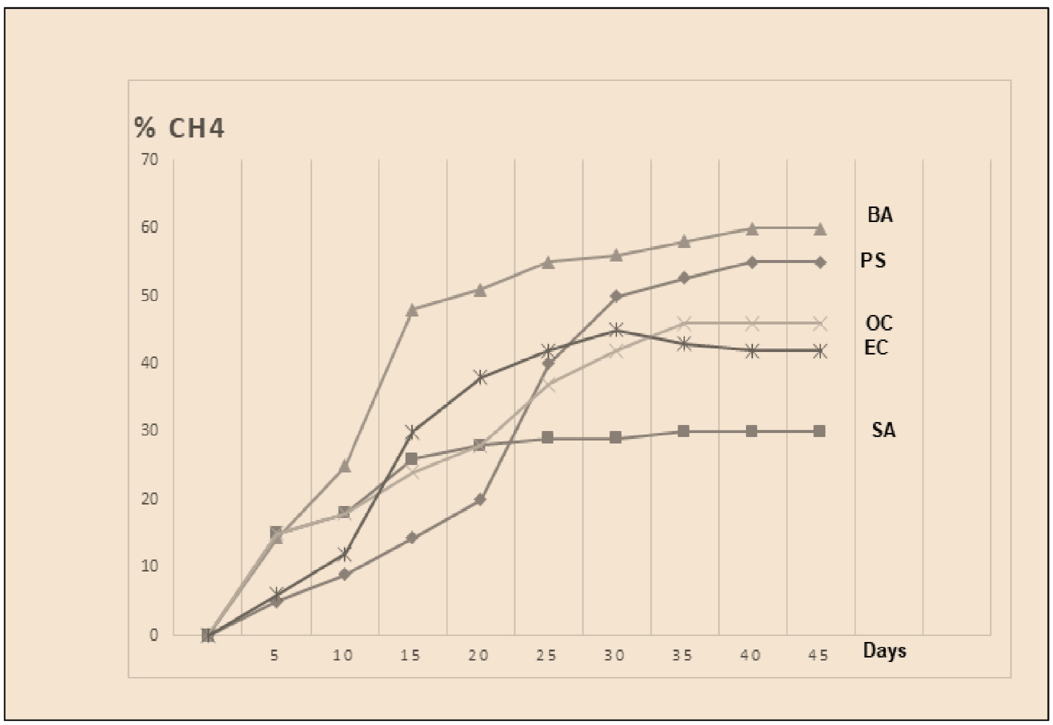

Fig. 12. Methane yields of macrophytes as a function of time

4. CONCLUSION

The pre-treatment of recalcitrant lignocellulosic biomass is an alternative to improve the biogas yield in the anaerobic digestion process. There are different pre-treatment techniques that can be applied, such as physical treatments, chemical and biological or a combination thereof. Among the objectives of pre-treatment are the disruption of layer of lignin that protects cellulose and hemicellulose (in order to make fermentable sugars more accessible to bio digestion, and the decrease in cellulose crystallinity, increasing its porosity. In this work, physical treatment with heating at 120°C and 1.6 atm. was used.

Anaerobic digestion (AD) is a biochemical process that converts the organic matter present in various types of wastes in:

(1) Biogas (rich in methane, used for heat and/or electricity generation); (2) bio solids (microorganisms grown on the organic matter and fibers, used as soil conditioner); and (3) liquor (dissolved organic matter, recalcitrant to AD, used as liquid fertilizer). AD has been described as one of the most energy-efficient and environmentally friendly technologies for energy production.

The macrophytes *Brachiaria, Oxycarium cubense, Eichhornia crassipes, Pistia stratiotes*, and *Salvinia* were efficient in biogas production after pretreatment at 120°C and 1.6 atm. Although the Brachiaria had the highest content of lignin, the one showed the highest content in methane, 60%, after 40 days. This fact proves the efficiency of the pre-treatment employed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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