Microbiological Characterization and Evaluation of Alternatives for the Use of Sludge From Service Stations

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The activities that involve the use of fossil fuels generally bring with them the generation of waste, mostly of a dangerous or special type, which is difficult to manage and dispose of due to its polluting capacity. In the present work, the characterization and microbiological quality of the collected waste subjected to the application of a composting process carried out in a biodigester is exposed. A sample of oily sludge contaminated with used lubricant residues was taken from the "Los Caciques" Fuel Station in the Vereda San Onofre, in the municipality of Cómbita - Boyacá; Said sample was collected in the storage chambers of the biosolids generated by the establishment during the development of activities such as vehicle washing, fuel sales and changing automobile lubricants. The sample that had a weight of 10.5 kg was taken to the biodigester, and there began a process of production of oxidizing gases of 1L/week on average, due to the presence of anaerobic microorganisms in the sample, among them some were found thermophiles, mesophiles etc. This was evidenced in the characterization carried out in 4 sessions over 1 month. This indicates that the sludge has energy generating capacity due to the characteristics of the bacteria found.

Keywords: hidrocarbons, contamination energies, alternatives, microbiology, composting, biosolids

INTRODUCTION

Poor management of contaminated waste from lubricants, hydrocarbons and chemical components such as naphthalene, fluorene, benzenes (polycyclic aromatics), among other contaminants, is an environmental and public health problem of significant importance today, due to their carcinogenic and toxic effects, in addition to the fact that "they are considered substances of difficult biodegradation and are classified as hazardous waste by the regulations established in the Basel Convention".(Vargas C & Peréz P, 2018)

"The current energy dependence on fossil fuels makes it necessary to search for solutions in favor of the reduction of atmospheric emissions generated by industrial and extractive activities, as well as the growing need to reduce the waste produced by the use of hydrocarbons. Based on this, this work focuses

on the characterization of hydrocarbon sludge, resulting from the sale of fuel at a gas station, as well as the evaluation of environmentally friendly alternatives for its utilization." .(Neisa & Silva, 2022)

This project evaluates the pollutant parameters and is developed as part of the Research Project "Obtaining Alternative Energies through Biosolids from a Gas Sales Station". Developed by two environmental engineering graduates of the Santo Tomás University Tunja Branch, together with the dean also belonging to the faculty PhD Luz Ángela Cuellar.

The purpose of this article is to identify the different microorganisms found in sludge samples from activities such as car washing, gas sales and change of automotive lubricants carried out in a gas station.

These are the subject of the study of the aforementioned project, which aims to generate alternative energies using this sludge; the first step in the project is to identify the microorganisms, which is carried out in this report.

In addition, if the project is carried out and the proposed objectives are achieved, it would provide a solution to the problem of handling waste contaminated with hydrocarbons and other harmful and/or toxic substances.

THEORETICAL FRAMEWORK

Pollution generated by waste from urban activities is one of the main problems in the world today, the management, treatment and disposal of this waste is critical to mitigate potential health risks to the environment and human health. (Banco Mundial, 2018)Likewise, "the lack of control in the discharge of polluted water into surface water bodies in Colombia is one of the problems affecting the environment".(Araque et al., 2020)

According to Decree 4299 of 2005 issued by the Ministry of Mines and Energy, automotive gas stations (EDS by its Spanish acronym) are defined as:

> An establishment that stores and distributes basic fuels used for motor vehicles, which are delivered from fixed equipment (dispensers) that directly fill the fuel tanks. Such establishments may include facilities to provide one or more of the following services: lubrication, general and/or engine wash, tire change and repair, alignment and balancing, diagnostic service, minor automotive maintenance work, sale of tires, wheels, lubricants, batteries and accessories, and other related services. (Decree 4299, 2005).

Current national legislation regarding the use of ALU and hazardous waste was taken into account. The sludge from the "Mobil - Los Caciques" EDS gas station was characterized. The methods of use were determined in accordance with the energy potential of the waste, proposing three alternatives for generating value: biogas, diesel and biofuel, making it possible to establish that the latter provided added value in its use by generating gas and liquid fuel and could also continue to feed the virtuous circle of the circular economy.

The lubrication service offered at the Gas Stations (EDS) generates a series of hazardous solid wastes in the form of sludge, sand and/or sediments polluted with different hydrocarbon fractions.

> Poor management of waste polluted with lubricants, hydrocarbons and chemical components such as naphthalene, fluorene, benzenes (polycyclic aromatics), among other pollutants, is a major environmental and public health problem today, due to their carcinogenic and toxic effects. In addition to being considered as "substances that are difficult to biodegrade and are classified as hazardous waste by the regulations established in the Basel Convention".(Vásquez et al., 2010)

The United States consumes some 7.6 million tons of lubricants per year, Japan 2.2 million, the European Union 4.7 million and Spain 40 million tons per year". Depuroil (2005) cited by (Martín-Tapia et al., 2010). "Each year, approximately 50 million gallons of lubricants are consumed in Colombia. One

part is produced by ECOPETROL and the other is imported from countries such as Venezuela and the United States". (Valencia et al., 2009)

In Colombia, the incorporation of processes under the guidelines of circular economy in the hydrocarbon sector is required because its waste constitutes a great challenge for the sustainable development of a country, due to its difficult removal and the economic, environmental and social damage it generates. In this regard, the Colombian Petroleum Association is the entity responsible for the management of used lubricating oils, and through the Used Oil Fund (FAU by its Spanish acronym) it has made use of 65% of used lubricating oils (ALU by its Spanish acronym). Data based on the estimated total ALU generation in 2011 (26,400 Gal).

Therefore, it is necessary to implement measures to mitigate the negative impacts generated by activities related to the use of hydrocarbons, which can produce hazardous waste. The circular economy is established as the strategy for the interconnection between environment, society and economy, thus promoting environmental sustainability. "Things that yesterday were considered disposable waste, today are resources for a new production process, thus closing the "virtuous circle" of the economic process." (Da Silva et al., 2019). The implementation of innovative practices for the elimination and reuse of pollutants are an important advance in recent years, techniques such as bioremediation, composting, biodigesters, among others, become alternatives to give a different use to this type of waste, in addition to the generation of products that can benefit social and economic areas of a population.

According to data from the Ministry of Mines and Energy (2019), in the Department of Boyacá there are around 206 EDS (20 of them located in the city of Tunja), certified by the Ministry of Mines and Energy, all of which have automotive lubrication services. The amount of ALU generated at those stations is unknown, although according to FAU data, 4,915 gallons were managed in 2019. acp (2019, para. 9) Based on this, the case study of the EDS Mobil - Los Caciques, located in the San Onofre de Cómbita - Boyacá, is established.

METHODOLOGY (MATERIAL AND METHOD)

Studies similar to the one we intend to carry out have been conducted at the Technological University of Pereira, where studies were carried out on bioremediation of sludge obtained from the grease traps of a gas station that operated within a transportation company.

In this study, bacterial growth and degradation capacity were monitored for 23 weeks. A bioremediation factor was used, eight mesocosms composed of high density polyethylene baskets, in which the "polluted sediments" were treated; for this purpose one (1) factor (bioremediation) and four (4) levels (100% polluted wastes; 100% polluted waste and nutrients; 60% polluted sediments + 40% soils with adapted microorganisms and 60% polluted sediments + 40% soils with adapted microorganisms and nutrients); each mesocosm was replicated for statistical purposes." (Vásquez et al., 2010)

"For the purpose of increasing the amount of hydrocarbon degrading microorganisms, a mixture of sediment from the gas station with a soil that suffered a spill of ACPM (hydrocarbons) from the Medellín-Cartago polyduct, occurred at Km 226+350, Chapinero property, entrance 14 via Cerritos-Cartago (Colombia), in an approximate area of 20 m2 (year 2009), since authors such as Vargas et al. 2016, highlight the importance of taking advantage of native microorganisms of polluted soils to carry out remediation processes." (D. Revelo et al., 2013).

At the National University of Trujillo, a study was conducted in which pyrolysis was used in a fluidized bed reactor to take advantage of the sludge from the wastewater treatment plant of Covicorti - Trujillo. The aim was to take advantage of the enormous quantities of sludge generated in the city to produce a liquid fuel for industrial applications. It is a certain point of reference for our project, since after the characterization of the sludge obtained from the grease traps of the gas station located in the city of Tunja, we intend to analyze the possibility of extracting gases from the samples, which can be used as industrial fuels.

Gasification is combustion in a low-oxygen atmosphere, which generates a gaseous stream and a solid residue with combustion properties. This reaction can be described as the achievement of several

phenomena: drying, thermal decomposition or pyrolysis, partial combustion, gas cracking, vapors and tars, and gasification of the final products. (D. M. Revelo et al., 2013)

Although the concept of generating electricity with biomass is not new, in the last decade there has been an increased interest in developing highly efficient technologies for this purpose. Biomass is particularly attractive for low capacity stations (Pannell et al., 2016)

Oily sludges are the result of solids settling (Unger, S.L; Lubowitz, H.R. 1990) in crude oil storage tanks and the separation of these solids during production. The main types of sediments found in this type of material are sands, silts and clays (Jiménez salas J. A.; Justo Alpañes J. L. 1975) and, of the above, the ones that have the greatest negative impact on production are the sands, without saying that they are the ones found in the greatest proportion.

Despite the little existing information about systems for the recovery of hydrocarbon-contaminated sludge in gas stations, Vargas (2012) carried out a project in which the thermal and catalytic cracking reaction of used lubricating oil for the production of diesel#2 was studied by using pure mesoporous aluminium-silicates doped with Zn at 1 and 2%, a study in which it was determined that the activation energy was reduced by 25% when using the synthesized aluminium-silicate catalysts. This allows the energy required to initiate the combustion process in energy systems that make use of diesel #2 to decrease, thus increasing the reaction capacity, and therefore increasing its efficiency.

A contribution by Balat (2008) sought to obtain gasoline as fuel from residual engine oil by catalytic pyrolysis. Conducting experiments with perlite and wood ash, establishing the best comparative performance of the catalysts.

> The pyrolysis liquid products from the pyrolysis of waste lubricating oil can be used as an alternative engine fuel. The yields of liquid products, referred to as "residual gasoline oil" increase the temperature and the amount of catalyst. In order to investigate the effects of catalysts on distillation yields, purified oil samples were mixed as a mass basis of 2.5%, 5.0% and 10.0 with known catalysts such as perlite and wood ash. The highest yield of the liquid product was 92.5%, which can be obtained from pyrolysis with 10% perlite. The yields of liquid products from the pyrolysis of residual lubricating oil in the presence of perlite are higher than those from wood ash catalytic runs. (Balat, 2008).

Taking into account research and compilation of information established at national and international level, it is concluded that the recovery, pretreatment and use to generate energy value, mainly in ALU, of sludge polluted with hydrocarbons is not only efficient due to its chemical value, but can also increase its fuel potential with different catalysts and even with simple treatments to eliminate solids and compounds other than hydrocarbons. Even in its use process in industrial areas, it reduces its pollutant load to the atmosphere, and does not generate increases in particulate matter.

Sample Collection

The current management of these sediments is the dehydration in drying beds and delivery to a company that immobilizes the Toxic Waste, therefore, the performance of a microbiological characterization becomes attractive. A sample was taken in sterile bottles for medical samples with a capacity of 120 mL, with prior use of gloves and/or the necessary personal protection elements. The sample was then taken to the laboratory within a maximum of 12 hours.

FIGURE 1
BIODIGESTER

Hermetic container used for the decomposition of organic matter under anaerobic conditions for the production of gas and energy.

Laboratory Handling

In order for bacteria to grow properly in an artificial culture medium, a series of conditions such as temperature, humidity and oxygen pressure, as well as a correct degree of acidity or alkalinity, were required. A culture medium must contain the necessary nutrients and growth factors and must be free of all polluting microorganisms. (Balat, 2008).

In this project, a common medium was used, which is one that "possesses the minimum components for the growth of bacteria that do not need special requirements. The best known medium of this group is nutrient agar or common agar, which results from the addition of agar to nutrient broth." (Méndez C & Echeverry S, 2015)

Agar is a solidifying element widely used for the preparation of culture media. It is completely liquefied at the temperature of boiling water and solidifies when cooled to 40 degrees. With minimal exceptions it has no effect on the growth of bacteria and is not attacked by bacteria growing on it. (Reyes A, 2016)

Nutrient Agar is a culture medium normally used as a routine for all types of bacteria. It is very useful because it remains solid even at relatively high temperatures. In addition, bacterial growth on this agar is on the surface, so that small colonies can be better distinguished. In a nutrient broth, the bacteria grow in the liquid, and appear as a thick substance, with hardly observable colonies. (Fuentes, 2004)

Procedure

When the culture was performed, it was kept in ideal conditions for bacteria. An enrichment medium is a culture medium that contains the nutrients necessary to support the growth of a wide variety of microorganisms, including some of the most demanding microorganisms. They are commonly used for harvesting different types of microbes that are present in the sample. In general, the use of enrichment media is employed to determine the absence of a given microorganism, or to detect if there is any, but in very low proportion. (González Hernández et al., 2002).

Certain organisms do not grow in ordinary culture media.

They require ingredients that promote growth, such as blood, glucose, serum, egg, among others. Enrichment media contain ingredients that enhance the stimulating qualities of the medium and promote high growth. (Sun et al., 2016)

The aim is to have an optimal development of the bacteria for their subsequent characterization by microscopic observations. And subsequent comparative literature review. Culture media have a number of components:

1. Essential: The first ones include water, organic nutrients (carbohydrates, amino acids, vitamins, etc.) and inorganic nutrients (P, Fe, N, Mg, S, etc).
2. Alternatives: (NaCl) substances, solidifying agent (agar-agar), buffers, pH indicator, etc. First the components of the medium are dissolved in distilled water. In many cases, the starting point is a commercial preparation with all the components dehydrated.

According to the teacher's instructions, the appropriate amount of water is added to achieve the desired concentration. If the medium contains a solidifying agent (agar-agar) it is necessary to heat the preparation until boiling, stirring from time to time, to assure a complete dissolution of the agar (solid and semisolid media); for liquid media it is not necessary to heat, only to stir the mixture until the complete dissolution of the same one.

The solution is then sterilized to prevent the growth of pollutants. Depending on the way the medium is to be used, the procedure will be different: a. Solid media on plate. - Close the flask with a cotton plug and cover with aluminum foil. Sterilize in an autoclave (1210C) for 15-20 minutes. Once sterile, spread in sterile Petri dishes and let stand to solidify. b. Liquid media. - Once the components have been dissolved, distribute in tubes at a rate of 2-4 ml per tube, close with an aluminum cap and sterilize in an autoclave. Procedure taken from: Microbiology Practicals Universitas Miguel Hernández

FIGURE 2
SEEDING PROCESS OF THE SAMPLE IN THE PETRI DISH WITH NUTRIENT AGAR

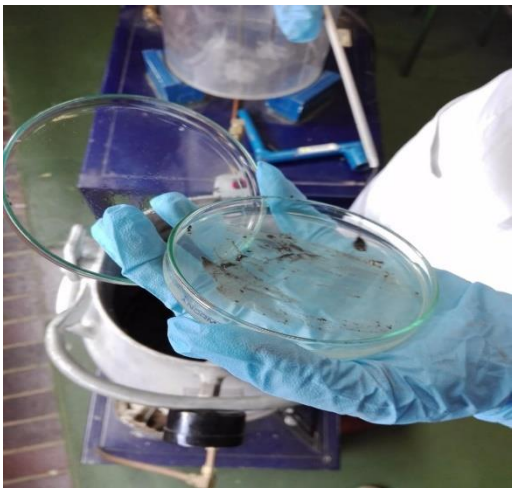
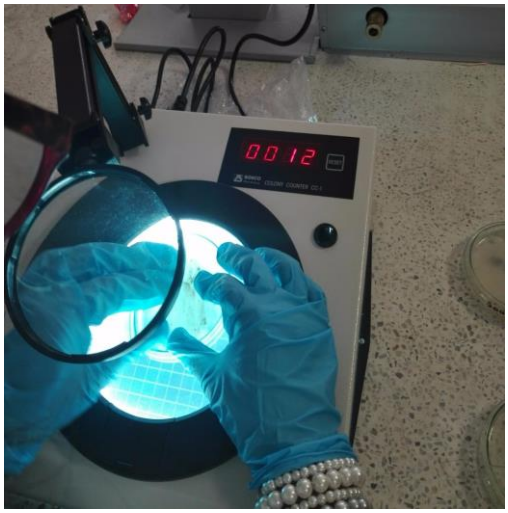
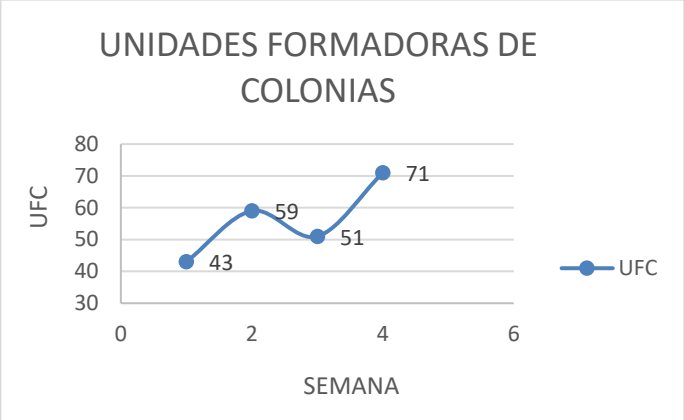


FIGURE 3
EQUIPMENT USED FOR CFU (COLONY FORMING UNITS) COUNTING



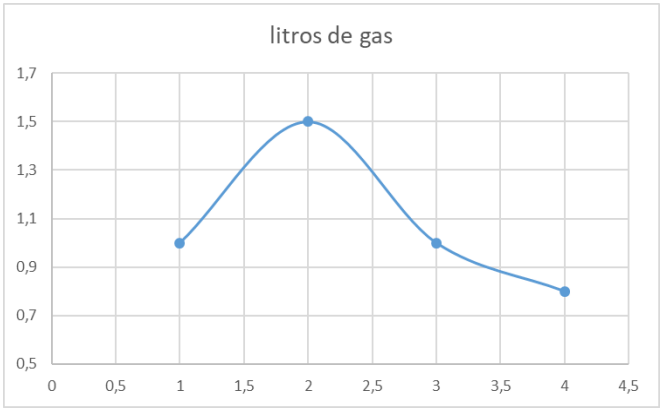
RESULTS

FIGURE 4
COLONY FORMING UNIT COUNTING RESULTS FOR WEEKS 1, 2, 3 AND 4



The previous chart shows the following data: In the first week of obtained a CFU count of 43, in the second week 59 CFU, in the third week 51 CFU, and in the last week 71 CFU. Growth described with the characteristics of the Differential Equation of the Verhulst Population Model.

FIGURE 5
RESULTS OF THE AMOUNT OF GAS OBTAINED IN THE BIODIGESTER PER WEEK, IN WEEKS 1, 2, 3 AND 4



This equation is called the Logistic Equation or Verhulst equation, which is described as follows:

$$\frac{dP}{dt} = rP(1 - \frac{P}{K}) \quad (1)$$

where:

r= Bacterial growth rate
K= Persistence capacity
t= time
P= Population (initial or known population)

The general solution of the above equation is:

$$P(t) = \frac{KP_0e^{rt}}{K+P_0(e^{rt}-1)} \quad (2)$$

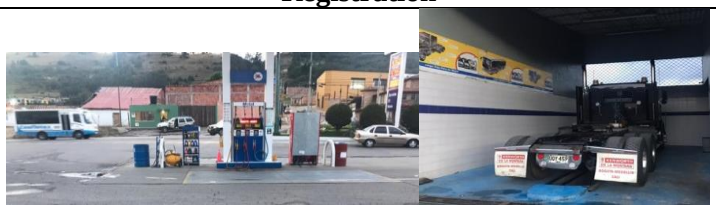
Characterization of Polluted Sludge

Hazardous waste generated at gas stations is due to products such as: lubricating oils: their composition is based on organic compounds and additives; the organic compounds are polynuclear aromatic compounds (PNA) that are highly carcinogenic because they have more than two benzene rings; used oils: they have high concentrations of chlorinated agents (trichloroethane, trichloroethylene, perchloroethylene) and heavy metals resulting from the wear of the lubricated machinery; filters: used to retain impurities from gasoline. (Ramirez, 2011)

> "The sludge generated in the washing of vehicles is considered a hazardous waste classified in category Y9, because it is the result of the mixture of petroleum hydrocarbons, mineral particles and water." (Collazos, 2017)" They also contain metals vital to living organisms such as zinc, copper, lead, mercury, heavy metals and vitamins such as cobalt; which start to become toxic as their concentration increases." (MURILLO, 2018)

The sludge characterization at the "MOBIL" ESD is given in Table No. 1:

TABLE 1
THE SLUDGE CHARACTERIZATION AT THE "MOBIL" ESD

Activity	Registration
Initially, the areas where this type of waste is generated are visualized. During this visit, areas were identified where oil changes, washes and fuel sales services are provided and where there are grease traps in the area.	
Next, the corresponding identification of the disposal place of this type of waste from the washing process, oil changes and grease traps was made.	

The following report belongs to laboratory results obtained in October 2019, performed on sludge samples from the inspection boxes, sent by the EDS "Mobil - Los Caciques":

TABLE 2
PHYSICAL-CHEMICAL ANALYSIS

PHYSICAL-CHEMICAL CHARACTERIZATION OF SLUDGE			
Flow (L/s)	0.14	pH	8.53
DQO (mg/L)	557	K (mg/Kg)	798.18
DBO5 (mg/L)	96	Ca (mg/Kg)	21741.38
SST (mg/L)	398	Ti (mg/Kg)	117.68
SS (mg/L)	1	Cr (mg/Kg)	37.09
Fats and Oils (mg/L)	14	Mn (mg/Kg)	262.69
Phenols (mg/L)	0.32	Fe (mg/Kg)	19501.24
Detergents (mg/L)	4.9	Cu (mg/Kg)	156.83
Hydrocarbons (mg/L)	6	Zn (mg/Kg)	3817.58
Phosphorus (mg/L)	8.63	N Total (mg/Kg)	14
Chlorides (mg/L)	7.54	Benzene (µg/L)	<2,5
Sulfates (mg/L)	204	Toluene (µg/L)	10.5
Acidity (mg/L)	18.22	Ethylbenzene (µg/L)	4.8
Alkalinity (mg/L)	79.8	p-Xylene (µg/L)	6.1
Calcium hardness (mg/L)	29.58	m-Xylene (µg/L)	13.7
Total hardness (mg/L)	43.4	o-Xylene (µg/L)	11.4
Color (UPC)	66.24		
Aromatic hydrocarbons (µg/L)	12.78		

Source: (Incitema, 2019)

DISCUSSION

According to the chart that represents the amount of gas obtained, it could be inferred that the first three weeks the level of gas remained balanced between 1 to 1.5 liters of gas, but in the last week there was a decrease in the amount of gas produced, because at the beginning the sample had more nutrients available for the development of bacteria, thermophilic and anaerobic microorganisms causing the production of gases in the biodigester, unlike the last week where the nutritional conditions are more limited for microorganisms.

The availability of nutrients allows the settlement of a microorganism in a given medium, time acts as a determinant factor for the growth of new microorganisms in a sample or the decrease of the same, thus the appearance of new microorganisms in a medium can define the growth and competition of the same.

Regarding the mathematical part of this project, an equation was sought to help estimate population growth taking into consideration the constant temperature of 21°C and variables such as gas production and the number of Colony Forming Units.

The logistic equation gave results with a margin of error of 3.6% concerning the estimation of the bacterial population. This margin of error was calculated week by week and an average was made between these errors, and the maximum value of the error (lagged value) was 4.5, which caused the arithmetic mean of the error to increase.

CONCLUSIONS

- It can be concluded that the CFU count shows a bacterial growth curve that reveals the type of growth phases of the colonies, an exponential growth phase and a death phase were determined, where from the first to the second week exponential growth of CFU was obtained, also from week 3 to 4 exponential growth was observed, unlike week 2 to 3 where a death phase and a decrease in CFU was obtained.
- The variation in bacterial population growth lies in the type of sample exposed in this research, where at the beginning the sample has an increase of CFU due to the fact that the nutrients found are very abundant and used by the microorganisms, during the second period of week 2-3 there is a decrease of colonies that may be due to the limitation of nutritional factors found in the medium, in addition to the appearance of new microorganisms that in this case are anaerobic and thermophilic.
- The microorganisms existing in the biodigester are well defined regarding their type and function, since most of them are present due to the combustion of the medium, thus leaving observations such as bacilli and their different forms of groupings throughout the medium, also taking into consideration the appearance of Gram-positive and Gram-negative bacteria, in addition to the notable presence of anaerobic microorganisms and gas-producing enzymes.
- The circular economy arises as a strategic solution in the energy conversion of hazardous waste generated in ESDs, providing economic, social and environmental benefits. Economically, diesel production is more cost-effective than the production of biogas and biofuels, although its process requires the use of microorganisms to recover complex fats, which is a limiting factor due to its low supply in the market. Likewise, the transesterification required to reduce the moisture content of the sludge decreases the efficiency of the process in complex fats.
- The economic factor in biogas production is significantly altered by the amount of waste to be treated, since the process is slow and requires the use of mechanical digesters. However, technological advances have made it possible to establish pretreatment methods that reduce the hydrolysis time in biodigesters, increasing economic and environmental benefits. Compared to diesel production, the microorganisms required for biogas production are more readily available and capable of supporting non-vegetable fats.
- Used lubricating oils are usually employed in the automotive industry as an input, completely wasting their energy capacity, without taking into consideration that the process of obtaining biofuels not only produces fuels, but also generates gas (syngas), providing two clean alternatives in the generation of energy. And although complex and costly processes are required to recover carcinogens and heavy metals from used oils, they could continue to supply the virtuous circle of the circular economy, providing economic, environmental and social balance.
- Public policies and national legislation have managed to increase the use of used lubricating oils through technical handbooks and environmental legislation in line with the increase in the generation of hazardous waste and the influence it has on people's quality of life, although there are still gaps in the economic retribution of those who make use of it, as well as support for investment projects in favor of research.

The use of hazardous waste corroborates the commitment to the SDGs, as it allows the implementation of the circular economy, giving value to what was considered waste and thus enabling the growth of sustainable cities and communities, promoting sustainable production and consumption.

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