

# Analysis of the Application of Biological Agents in Obtaining Bioglycerin and Biodiesel from Recycled Edible Oils in the City of Arequipa

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**Abstract**– The purpose of this study was to obtain biodiesel and bioglycerin from recycled edible oils through biological agents. By means of controlled conditions in a bioreactor designed and manufactured by the research team of the Doctoral Thesis Project. The oily medium was conditioned with respect to pH, methanol concentration and temperature according to the technical requirements of Biological Agents (*Aspergillus niger*, lipase from *Aspergillus niger*, lipase acrylic resin from *Candida antarctica*). In the environmental conditions of the City of Arequipa, values higher than 80% of Biodiesel and 16.5% of Bioglycerin were obtained. Biodiesel is characterized with respect to its Physicochemical Properties, by means of ASTM Methods, regarding Flash Point, Density, Phase Separation, Cetane Number, Diesel Index and Viscosity; and for Bioglycerin, Infrared Spectroscopy with Fourier Transform, carried out in the Laboratory of Research and Services (LABINVSERV) of San Agustín National University Arequipa.

**Keywords**– Biological Agents; Recycled Edible Oils; Biodiesel; Bioglycerin.

## I. INTRODUCTION

Transesterification of a mixture of nonedible oils with methanol was achieved over lithium-impregnated waste tire-derived activated carbon (WTAC) as a new solid-based catalyst [1].

Ultrasound-assisted biodiesel synthesis using blended feedstock of inedible oils and commercial immobilized lipase from *Thermomyces lanuginosus* [2]. Thus, due to the large amount of demand for diesel fuel worldwide and the negative environmental and health impacts of its direct combustion, biodiesel production and consumption have increased globally as the best short-term substitute for mineral diesel. However, the use of edible and nonedible petroleum feedstocks for biodiesel production has led to several controversial issues, including feedstock availability and cost, greenhouse gas (GHG) emissions, land use changes (LUC) and fuel versus food/feed competition [3].

The usual transesterification of residual frying oil with ethanol does not give a final biodiesel concentration higher than 96.5%. The reaction conditions can be optimized by using ethanedioic acid as an activator of carbonylglycerol ester [4].

Environmental degradation and dwindling fossil fuel reserves are the main drivers for the search for biodiesel as an alternative fuel. *Jatropha curcas* oil has been well researched as a vegetable oil for biodiesel production using conventional processes that generate huge unpleasant wastes and consequently incur additional costs for the treatment and disposal of the wastes generated [5].

Calcium-based mixed oxides, CaO-NiO (calcium nickel) and CaO-Nd<sub>2</sub>O<sub>3</sub> (calcium neodymium), were synthesized by a coprecipitation process. *Jatropha curcas* crude oil (JCO) was used as feed stock for the synthesis of fatty acid methyl esters (FAMES) in the presence of CaO-NiO and CaO-Nd<sub>2</sub>O<sub>3</sub> mixed oxides. It was found that the mixed oxide solid bases represented high basicity and stability [6].

Although biodiesel is among the most studied biofuels for diesel engines, it is generally produced from edible oils, which gives way to controversy between the use of land as fuel and food. For this reason, residues such as olive pomace oil are considered alternative feedstocks for producing biodiesel that do not compete with the food industry [7].

The world's current energy situation is unsustainable due to the uneven geographic distribution of natural wealth, as well as environmental, geopolitical, and economic concerns. The ever-increasing energy consumption due to population growth, transportation and a luxurious lifestyle has motivated researchers to conduct research on biofuels as a sustainable alternative fuel for diesel engines. Renewability, cost-effectiveness, and reduction of pollutants in exhaust emissions are soon promoting biofuels as a suitable substitute for diesel fuel [8].

It is a good solution for biodiesel products by using waste edible oils (WEOs), such as used cooking oils and used frying oils, due to their low cost, disposal problems and possible contamination. Therefore, WEO biodiesels have been gradually produced and, therefore, have been applied to study their effects on engine performance and emissions [9].

Biodiesel (BD) is generally produced by alkali-catalyzed methanolysis of expensive edible oils. Although waste cooking oils (WCO) containing high free fatty acids (FFAs) are economical, they cannot be effectively processed by alkali catalysis due to soap formation, which reduces the yield of BD and complicates the downstream process. A process consisting of enzymatic hydrolysis followed by chemical esterification was developed to produce BD from WCO. The enzyme

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*Candida rugosa* lipase was used for the hydrolysis of WCO to fatty acids (FAs) [10].

Biodiesel is a nontoxic and biodegradable substitute for petroleum-based diesel. However, it is impractical to use refined edible oils to produce biodiesel due to their high cost and priority for food products, especially in China, while residual oils with high free fatty acids (FFAs) can be considered feedstocks [11].

Within the complete recycling of edible waste, oil has recently attracted considerable attention as a global social problem. The production of biodiesel fuel from used vegetable oil is considered an important step in reducing and recycling used oil. This technology employs used vegetable oil as a possible renewable alternative resource for fossil diesel fuel, and the transesterification reaction is the usual process to obtain biodiesel [12].

Thermal cracking of waste cooking oil (WCO) by pyrolysis was performed using a laboratory-scale fixed-bed reactor [13].

## II. MATERIALS AND METHODS

### A. Materials

The biological agents used to obtain bioglycerin and biodiesel from recycled edible oils were strains of *Aspergillus niger*, lipase from *Aspergillus niger* and lipase acrylic resin from *Candida antarctica* provided by Sigma–Aldrich (USA).

In conducting the doctoral research project, the design and construction of the Bioreactor, called Bioreactor for obtaining Bioglycerin and Biodiesel by Biological Agents from Recycled Edible Oils - Exp. N° 000368-2021/DIN - National Institute for the Defense of Competition and the Protection of Intellectual Property (INDECOPI) - Peru, has been developed. It has been conducted through Technological Services (Third Party Services), as shown in Fig. 1.

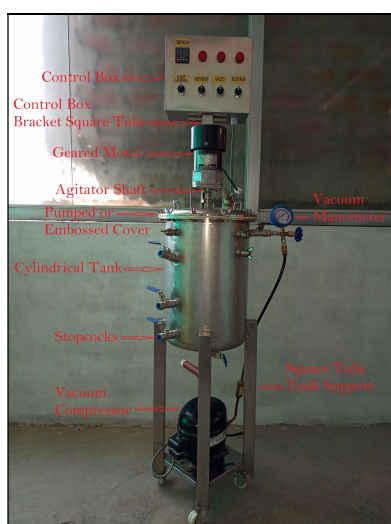


Fig. 1 Bioreactor designed and built.

### B. Methods

The application of the biological agents in the bioreactor was conducted in accordance with the research project, and the steps performed are described below.

Introduce into the Cylindrical Tank of the Bioreactor the content of the recycled edible oil to be processed, taking into account that the content must be above the heat resistance located in 2/3 parts of the total volume of the cylindrical tank (see Fig. 2a).



Fig. 2 Application Procedure in Edible Oils.

- a): Input of recycled edible oil; b): pH conditioning;
- c): Conditioning with methanol; d): Insertion of biological agents;
- e): Resistance ignition; f): addition of methanol.

Condition the pH value of the recycled edible oil by acetate buffer to values of 8.0 and 7.4, according to the technical requirements of the biological agents applied in the research (see Fig. 2b).

The recycled edible oil was conditioned by adding methanol to quantify a concentration of 20% of the total volume of recycled edible oil inside the cylindrical tank (see Fig. 2c).

Insert the Biological Agents (*Aspergillus niger*, Lipase from *Aspergillus niger*, Lipase Acrylic Resin from *Candida antarctica*) according to the planned experimentation, see Fig. 2d). According to TABLE I.

TABLE I  
BIOLOGICAL AGENTS APPLIED IN RESEARCH

Biological Agent	Quantity
<i>Aspergillus niger</i>	0.05%
Lipasa from <i>Aspergillus niger</i>	~200 U/g
Lipase Acrylic Resin from <i>Candida antarctica</i>	≥10,000 U/g

Source: Own elaboration.

By operating the electric resistor inside the cylindrical tank (see Fig. 2e), the temperature was set according to the technical requirements of the biological agents (*Aspergillus*

niger 37 °C, lipase from *Aspergillus niger* 40 °C, lipase acrylic resin from *Candida antarctica* 40 °C).

During the process, add successively, in a quantity of 5 times, to the recycled edible oil methanol to quantify a concentration of 20%, see Fig. 2f).

At the end of the time applied in the investigation, the operation of the bioreactor was stopped according to the programmed times and the technical requirements of the biological agents (*Aspergillus niger* 72 h, lipase from *Aspergillus niger* 8 h, lipase acrylic resin from *Candida antarctica* 8 h).

The biodiesel obtained was characterized with respect to its physicochemical properties, by means of ASTM methods, regarding flash point, density, phase separation, cetane number, diesel index and viscosity; for the bioglycerin, infrared spectroscopy with Fourier Transform was performed at the Research and Services Laboratory (LABINVSERV) of the San Agustín National University Arequipa.

The procedure for the different stages of the application of biological agents in recycled edible oils is shown below, see Fig. 3.

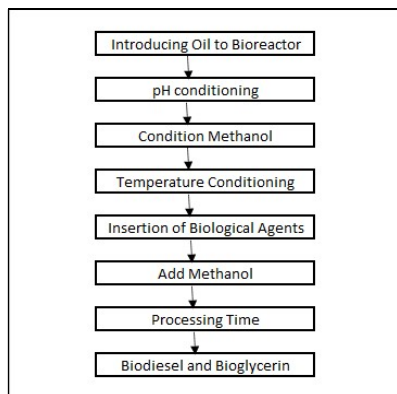


Fig. 3 Flowchart of the Application Procedure for Recycled Edible Oils.

### III. RESULTS AND DISCUSSION

#### A. Bioglycerin and Biodiesel obtained from Biological Agents

After the process of obtaining bioglycerin and biodiesel, Fig. 4, obtained by biological agents (*Aspergillus niger*, lipase from *Aspergillus niger*, lipase acrylic resin from *Candida antarctica*), and TABLE II is shown.



Fig. 4 Bioglycerin and biodiesel were obtained from Biological Agents.

TABLE II  
BIOGLYCERIN AND BIODIESEL OBTAINED FROM BIOLOGICAL AGENTS

Component	Unit	<i>Aspergillus niger</i>	Lipasa from <i>Aspergillus niger</i>	Lipase Acrylic Resin from <i>Candida antarctica</i>	Test Method
Bioglycerin	%	16.5	18.0	20	Volumetry
Biodiesel	%	83.5	82.0	80	Volumetry

Source: LABINVSERV – UNSA

According to the obtained results shown in TABLE II, it was observed that the intermediate values of Biodiesel (82%) occurred through the bioprocess with Lipase from *Aspergillus niger* and Bioglycerin (18%).

Through Lipase Acrylic Resin from *Candida antarctica*, a value of Biodiesel (80%) and Bioglycerin (20%) was observed.

In the microbiological cultivation of *Aspergillus niger*, Biodiesel (83.5%) and Bioglycerin (16.5%) were obtained.

#### B. Physicochemical Properties of Biodiesel Obtained by Biological Agents

The physicochemical properties of the biodiesel obtained by biological agents, according to TABLE III, are shown below.

TABLE III  
PHYSICOCHEMICAL PROPERTIES BIODIESEL OBTAINED BY BIOLOGICAL AGENTS

Biological Agent	Analysis	Unit	Result		Test Method
			Original Sample	Fraction Centrifuged	
<i>Aspergillus niger</i>	Flash point	°C	76.62	82.40	ASTM Method
		°F	169.92	180.32	
	Density 15 °C	g/mL	0.92557	0.88774	ASTM Method
		API	21.38	27.89	
	Phase Separation	%	10.10	-	Centrifugation Method
	Cetyl Cipher		37.69	35.14	ASTM Method
	Índice Diesel		40.69	38.14	ASTM Method
	Viscosity 40 °C	cSt	8.87	2.53	ASTM Method
	Flash point	°C	87.05	88.30	ASTM Method
		°F	188.69	190.94	
Density 15 °C	g/mL	0.9009	0.86472	ASTM Method	
	API	29.56	32.14		
Lipasa from <i>Aspergillus niger</i>	Phase Separation	%	10.50	-	Centrifugation Method
	Cetyl Cipher		41.36	51.42	ASTM Method
	Diesel Index		44.36	54.42	ASTM Method
	Viscosity 40 °C	cSt	2.87	2.19	ASTM Method
	Flash point	°C	76.06	78.00	ASTM Method

Resin from <i>Candida antarctica</i>	°F	169.88	172.40	
Density 15 °C	g/mL	0.880182	0.86031	ASTM Method
	API	29.26	32.98	
Phase Separation	%	8.3	-	Centrifugation Method
Cetyl Copher		44.19	52.76	ASTM Method
Diesel Index		17.19	55.76	ASTM Method
Viscosity 40 °C	cSt	2.83	2.08	ASTM Method

Source: LABINVSERV – UNSA

According to the biodiesel obtained by *Aspergillus niger*, the value obtained for the flash point of the centrifuged fraction of 82.40 °C was below that reported for sunflower biodiesel, with a value of 178.67 °C. In the case of density at 15 °C, a value of 0.88774 g/mL was reported, slightly higher than that of sunflower biodiesel with a density of 0.884 g/mL at 18 °C. Regarding the Cetane Number, a value of 35.14 has been reported, lower than that of Sunflower Biodiesel with a value of 45.55. In Viscosity at 40 °C, a value of 2.53 cSt was recorded, lower than that of Sunflower Biodiesel with a value of 5.03 cSt [14].

According to the biodiesel obtained by lipase from *Aspergillus niger*, the value obtained for the flash point of the centrifuged fraction of 88.30 °C was below that reported for sunflower biodiesel, with a value of 178.67 °C. In the case of density at 15 °C, a value of 0.86472 g/mL was reported, which was lower than that of sunflower biodiesel with a density of 0.884 g/mL at 18 °C. Regarding the Cetane Number, a value of 51.42 has been reported, higher than that of Sunflower Biodiesel with a value of 45.55. In viscosity at 40 °C, a value of 2.19 cSt was recorded, which was lower than that of sunflower biodiesel with a value of 5.03 cSt [14].

According to the biodiesel obtained by lipase acrylic resin from *Candida antarctica*, the value obtained for the flash point of the centrifuged fraction of 78.00 °C was below that reported for sunflower biodiesel, with a value of 178.67 °C. In the case of density at 15 °C, a value of 0.86031 g/mL was reported, which is lower than that of sunflower biodiesel with a density of 0.884 g/mL at 18 °C. Regarding the Cetane Number, a value of 52.76 has been reported, higher than that of Sunflower Biodiesel with a value of 45.55. In viscosity at 40 °C, a value of 2.08 cSt was recorded, which was lower than that of sunflower biodiesel with a value of 5.03 cSt [14].

### C. Infrared Spectroscopy Analysis of Bioglycerin Obtained by Biological Agents

The infrared spectroscopy analysis of the biodiesel obtained by biological agents is shown below. Fig. 5a), Fig. 5b), Fig. 5c).

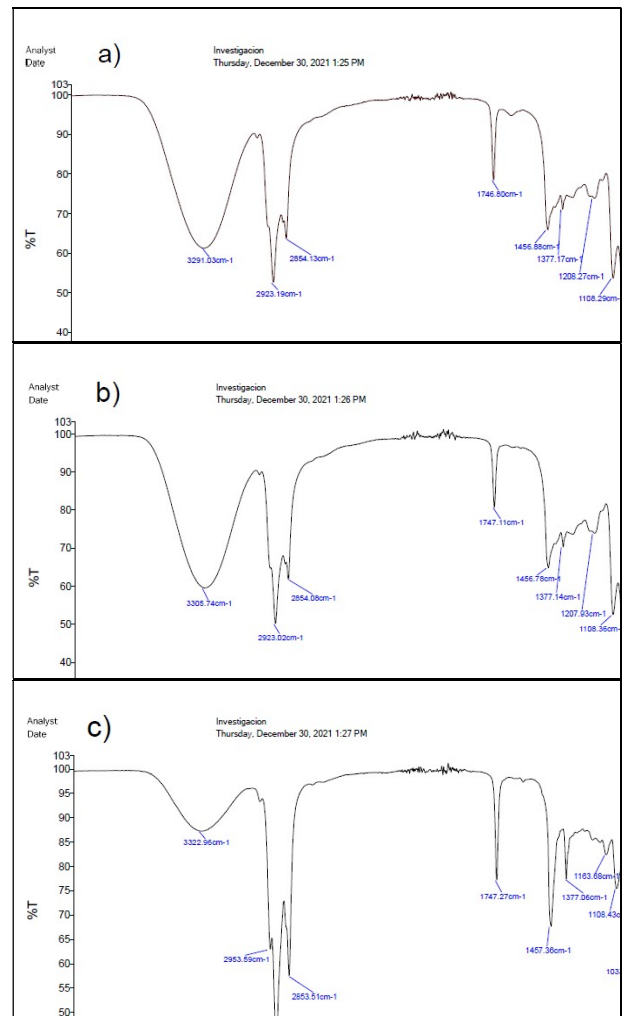


Fig. 5. Infrared Spectroscopy Analysis Graphs of Bioglycerin obtained. a): *Aspergillus niger*; b): Lipasa from *Aspergillus niger*; c) Lipase acrylic resin from *Candida antarctica*.

According to the bioglycerin obtained by *Aspergillus niger*, according to the results obtained from the spectrum of bioglycerin obtained by Fourier transform infrared spectroscopy (FTIR) in the region of 4000-400  $\text{cm}^{-1}$ , considerable similarity was manifested with respect to that reported [15], in which the FTIR spectrum of glycerol and its main absorption bands were observed. In the fingerprint region, bands are shown in the interval of 860 and 995  $\text{cm}^{-1}$ , which are assigned to vibrations of the C-C bond of the glycerol skeleton. Bands were also observed at 850 and 930  $\text{cm}^{-1}$ , corresponding to vibrations of the O-O and C-O groups, respectively [15].

According to the bioglycerin obtained by lipase from *Aspergillus niger*, according to the results obtained from the spectrum of bioglycerin obtained by Fourier transform infrared spectroscopy (FTIR) in the region of 4000-400  $\text{cm}^{-1}$ , considerable similarity was manifested with respect to that reported [15]. In the 1125-1100  $\text{cm}^{-1}$  range, vibration of the

CH-OH bond was observed, and in-plane deformation of the O-H bond occurred in the 1400 to 1200  $\text{cm}^{-1}$  range. The signals at 1029  $\text{cm}^{-1}$  were associated with the vibrations corresponding to the C-O bond of carbons C1 and C3 (extreme carbons), while the band at 1109  $\text{cm}^{-1}$  was associated with the C-O bond of carbon C2 (intermediate carbon). The bands at 3000-2800  $\text{cm}^{-1}$  and 3650-3200  $\text{cm}^{-1}$  corresponded to the tension of the C-H and O-H bonds [15].

According to the bioglycerin obtained by lipase acrylic resin from *Candida antarctica*, according to the results obtained from the spectrum of the bioglycerin obtained by Fourier transform infrared spectroscopy (FTIR) in the region of 4000-400  $\text{cm}^{-1}$ , there was considerable similarity with respect to the reported spectrum [15]. The FTIR spectrum of glycerol and its main absorption bands were observed. In the fingerprint region, bands were shown in the interval of 860 and 995  $\text{cm}^{-1}$ , which were assigned to vibrations of the C-C bond of the glycerol skeleton. Bands were also observed at 850 and 930  $\text{cm}^{-1}$ , corresponding to the vibrations of the O-O and C-O groups, respectively. The signals at 1029  $\text{cm}^{-1}$  were associated with the vibrations corresponding to the C-O bond of the C1 and C3 carbons (extreme carbons), while the band at 1109  $\text{cm}^{-1}$  was associated with the C-O bond of the C2 carbon (intermediate carbon). The bands at 3000-2800  $\text{cm}^{-1}$  and 3650-3200  $\text{cm}^{-1}$  corresponded to the tension of the C-H and O-H bonds [15].

#### IV. CONCLUSIONS

The design and manufacture of a bioreactor for the bioprocessing of recycled edible oils through a multidisciplinary collaborative team has allowed the development of an invention patent application with ownership of the San Agustín National University Arequipa, promoting innovation and the possibility of subsequent entrepreneurship based on a business model. The application of biological agents (*Aspergillus niger*, lipase from *Aspergillus niger* and lipase acrylic resin from *Candida antarctica*) allows the production of bioglycerin and biodiesel from recycled edible oils coming from the Food Industry of the City of Arequipa, proportions higher than 80% in Biodiesel and 16.5% in Bioglycerin. The characterization in Physicochemical Properties of Biodiesel and in Infrared Spectroscopy with Fourier Transform for Bioglycerin allows the final analysis of the obtaining process developed in the research, based on validated methodologies and conducted by the Research and Services Laboratory of the San Agustín National University Arequipa.

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