Microbiological fermentation of glycerol to obtain alcohol in tryptose culture medium

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The energy consumption demand has grown every year, both to supply the population needs and the economy. It is known that fossil fuels are not renewable and require more from the environment for being highly pollutant. Thus, the search for renewable and clean sources of energy has increased continuously, which led to the growth of the biofuel such as biodiesel market. The production of biodiesel, however, generates a byproduct, the glycerol, through a transesterification process. The increase in this biofuel production generates an overload of glycerol in the market, which has impurities and for this reason cannot be directly used. The objective of this work is to carry out anaerobic fermentation of glycerol with the bacteria *Escherichia coli*, to obtain ethanol, a product with higher aggregated value. The highest percentual of ethanol obtained was 8.8%, employing 50 ml glycerin, 15 mL certified bacteria *E. coli* and 20 mL tryptose culture medium.

Keywords: Biofuel; *Escherichia coli*; Renewable Energy.

1. Introduction

Burning fossil fuels to generate energy leads to serious environmental problems, such as the pollutant gases emission and the pollution of water resources. For this reason, there is great interest in the development of clean energy sources, alternative to the fossil fuels[1]. One alternative which seeks to reduce the problems related to environmental conservation, are the biofuels, which take advantage of biomass resources, to produce safer and more reliable energy in order to preserve the environment[2].

Renewable energy such as biofuels are mainly focused on environmental preservation, avoiding processes that can harm the environment, or at least reducing environmental impact generated by the production of fuels and also by the extraction of raw material used in these energy generation processes, which bring consequences to the environment and biodiversity[3].

Meeting the necessary demand of energy to proceed with the economical growth and keeping the world population standard of life, requires a great volume of fuels and energy of different kinds[4]. Thus, the production of biofuel experiences a phase of fast growth, seeking for new technologies and differentiated processes that allow innovation in the energy area. Amongst these biofuels, is the biodiesel, produced from animal or vegetal renewable biomass[5].

To produce biodiesel, vegetal oil or animal fat is employed, which is a tri-ester derived from glycerin. The vegetal oil or the animal fat suffers transesterification through the action of a catalyst and in the presence of methanol or ethanol, forming three molecules of methyl or ethyl esters from fatty acids, which constitutes the biodiesel, releasing a molecule of glycerol[6]. Thus, the transesterification is the process through which the separation of glycerin and oil occurs, and its substitution by the alcohol in the chain[7]. This glycerol is the main by product of the biodiesel production, in which for each 90 m³ biodiesel produced through transesterification, about 10 m³ glycerol is generated. Due to all incentive to the production of biodiesel, it has been produced in large scale, which also generates some environmental concern, due to the excess of glycerol produced in the process, and this glycerol presents impurities such as water, salts, esters, alcohol and residual oil, for this reason cannot be disposed in the environment, besides representing an economical problem[8].

Taken into consideration all the problems faced by the exceeding glycerol in the market, the microbiological conversion of glycerol into products of higher economical value, appears as a viable option to reduce the problems resulting from this process of energy generation. Thus, the biofuel sector can be the best destination for this by product, which would complement the biodiesel route, reducing pollution and waste, and consequently bringing a suitable solution with the generation of renewable energy[9].

Glycerol is an excellent source of carbon, and it can be used by microorganisms to carry out anaerobic fermentation and convert it into products of higher economical value. Besides that, the use of glycerol in fermentation processes offers better yield response than others that use common sugar, as they show reduction in the carbon atoms[10].

Fermentation is a process that leads to a set of chemical reaction, enzyme controlled, where the degradation of organic molecules into simpler compounds occurs and the consequent release of energy. The bacteria *E. coli* is one of the microorganism able to produce alcohol, through the fermentation of glycerin by microbiological conversion. Suitable conditions, such as oxygen absence, pressure, temperature and ideal pH, enable a more effective response of the metabolic activity of the bacteria in relation to the fermentation[11].

This study aims to produce ethanol, through anaerobic fermentation of glycerol with bacteria *E. coli*, in tryptose culture medium.
2. Materials and Methods

2.1 Bacteria *E. coli* culture

Two different strains of *E. coli* were used, one type ATCC 25922 and the other sp.. The *E. coli* strains (ATCC 25922 and sp.) were cultivated in specific liquid medium for growth, which contained peptone, lactose, bovine bile, sodium chloride, dibasic potassium phosphate and monobasic potassium phosphate. The strains of bacteria were incubated in bacteriological oven for 48 hours, at 36°C, in neutral pH so that they could reproduce. Later on, they were used to prepare the bacterial concentrate which was used in the fermentation process.

Each 15 days, samples were replicated, aiming to keep the bacterial strains.

2.2 Fermentation process by the *E. coli* strains

The culture medium used was the tryptonate (formed by 18g tryptose Pa for 1000mL distilled water, composing an enzymatic hydrolysate of proteins). The pH value for the medium was approximately 7. Samples were inoculated in Erlenmeyer varying the amount and kind of bacteria *E. coli* and the amount of crude glycerin used were 50 mL for all tests. The inoculums in a semi-closed system received industrial nitrogen gas forming a system in anaerobiosis. The fermentation process was carried out in culture and bacteriology microprocessed oven Q316M/Quimis, for 72 hours at 36°C ± 0.5.

2.3 Experimental design

A 2² multivariate experimental design was employed with central point, for each medium studied. The variables were: concentration of culture medium and bacteria.

The generic experimental design carried out is presented in Table 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>Culture Medium (mL)</th>
<th>Bacteria <em>E. coli</em> (mL)</th>
<th>Response: Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

2.4 Distillation of the glycerol concentrate fermented by the bacteria *E. coli*

The concentrate fermented by the *E. coli* was distilled using a simple distiller for the separation of the sample alcohol. The separation occurs through differences between boiling points, in which the ideal temperature for alcohol distillation is at 70°C.

2.5 Ethanol content in the crude distilled

For this determination, the specific mass values at 20º C were used, according to the ASTM D-5501[12].

3. Results and discussion

In Table 2, the average values of ethanol produced from the fermentation of glycerin by *E. coli* certified with ATCC and tryptose culture medium.

<table>
<thead>
<tr>
<th>Test</th>
<th>Certified <em>E. coli</em> (mL)</th>
<th>Triptose medium (mL)</th>
<th>Ethanol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
<td>5,8 ± 2,1</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>20</td>
<td>7,5 ± 1,6</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
<td>6,7 ± 0,54</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>20</td>
<td>7,2 ± 0,01</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>6,9 ± 1,0</td>
</tr>
</tbody>
</table>

The highest volume obtained was 7,5ml ethanol, with the concentration at 15 mL certified *E. coli* and 20 mL tryptose culture medium (test 2). The lowest ethanol volume obtained was in the organized concentrate of 10 mL certified *E. coli* and 15 mL tryptose culture medium (test 1), obtaining 5,8 mL ethanol. This phenomenon can be seen in Figure 1.
In Figure 1 the response surfaces are presented, for the tryptose culture medium, resulting from the experimental design.

Figure 1 represents the response surface, following the model 2F1, with 59.51% significance, which considers the main effects (E. coli and culture medium) and their interactions, for certified E. coli and sp.

It can be observed that there is a tendency to occur higher ethanol production when there are higher amounts of medium and bacteria. However, values obtained at different concentrations under study do not present great alterations, ranging between 5.8 to 7.5 mL ethanol.

Ethanol average values produced from the glycerin fermentation by E. coli sp. and tryptose culture medium are found in Table 3.

Table 3 – Ethanol average from the E. coli sp. distillation and tryptose culture medium.

<table>
<thead>
<tr>
<th>Test</th>
<th>E. coli Sp. (mL)</th>
<th>Tryptose medium (mL)</th>
<th>Ethanol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
<td>6.5 ± 0.61</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>20</td>
<td>4.7 ± 0.80</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
<td>7.3 ± 1.84</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>20</td>
<td>6.9 ± 0.33</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>6.9 ± 0.86</td>
</tr>
</tbody>
</table>

The bacteria E. coli sp. associated to the tryptose culture medium obtained test 3 as the best result, using 15 mL bacteria and 10 mL substrate, with an average result of 7.3 mL ethanol. The lowest ethanol volume obtained was in test 2, which had the highest bacteria and substrate concentrations. This phenomenon can be visualized in Figure 2. It can also be observed that with the bacteria sp. the results were differentiated, and did not follow the same pattern of ethanol production as the certified bacteria, which reveals a differentiation in the generation of energy in the strains under study.

In Figure 2, the response surfaces of the tryptose culture medium are shown, resulting from the experimental design.
Figure 2 – Response surface obtained for the variables E. coli sp and tryptose culture medium.

Figure 2 represents the response surface following the model 2F1, with 59.51% significance, which considers the main effects (E. coli and culture medium) and their interactions, for certified E. coli and sp.

By analyzing the results obtained with the tryptose culture medium, it can be observed that the ethanol average values obtained at any proportion of bacteria and medium are closer and also due to the standard deviation of such measures being high, it is not possible to conclude which is the ideal condition for the production of the highest amount of ethanol.

Equations 1 and 2 represent the amount of ethanol which can be produced within the limits studied for the certified E. coli and sp, respectively.

\[
\text{Ethanol} = 4.37 + 0.16 \times [\text{vol. bacteria}] + 0.16 \times [\text{vol. medium}] - 0.0105 \times [\text{vol. bacteria}] \times [\text{vol. medium}] \quad \text{Equation (1)}
\]
\[
\text{Ethanol} = 7.735 + 0.0675 \times [\text{vol. bacteria}] - 0.025 \times [\text{vol. medium}] - 0.0105 \times [\text{vol. bacteria}] \times [\text{vol. medium}] \quad \text{Equation (2)}
\]

4. Conclusions

(1) Results demonstrated that the metabolic activity of Escherichia coli is influenced by the culture medium concentration and the bacteria concentration used;
(2) The highest volume of ethanol obtained with the tryptose culture medium was 7.5 mL, using the certified bacteria E. coli, at concentrations of 15 mL bacteria and 20 mL culture medium;
(3) In the tryptose culture medium both the certified E. coli and the E. coli sp. presented similar results, in this case, the use of bacteria sp. presents best economical advantage, as it does not represent high cost;
(4) The study demonstrated that it is possible to carry out the microbiological conversion of glycerol, with the bacteria E. coli in tryptose culture medium, which represents a new alternative in the biodiesel route.

References


