**Potential effect of crude glycerol components on the lactic acid fermentation by *Lactobacillus sp.***

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**Highlights**

* Three samples of Colombian crude glycerol were characterized.
* The effect of crude glycerol components on volumetric productivity of lactic acid fermentation was evaluated.

**1. Introduction**

In recent years, biofuels has been widely used and their production has grown exponentially. Crude glycerol – CG- is the main by-product obtained from the production of biodiesel from vegetable oils, in quantities of 10-40% by weight. In 2017 the production of biodiesel in Colombia was 459,807 tons and it is estimated that by 2020 its production will be six times higher than the market demand. As a consequence, CG has a great potential to be used as a raw material in other processes due to its low commercial price. However, one of the main challenges is the variability of its composition, since it depends on biodiesel production parameters. Despite the importance of CG chemical composition, few references have been found on its characterization [1,2].

On the other hand, our previous work showed that crude glycerol used as a substrate in lactic acid fermentation allows higher productivities than glycerol USP. Therefore, it is vital to understand the effect of CG components on reactions catalyzed by microorganisms [3].

In this sense, this research explains the effect of CG components on the volumetric productivity of lactic acid. To fulfill this scope, the following stages are being developed: a) chemical characterization and identification of CG compounds of three samples, b) design of experiment of mixtures to evaluate the productivity of Lactobacillus sp. in a fermentation medium.

**2. Methods**

In general, three methods were used for the chemical characterization of CG: the periodic iodometric acid method, liquid chromatography (HPLC), and gas chromatography [1]. The density of CG was determined by measuring volume and weight at room temperature. To determine the pH, CG was measured by a digital pH meter at room temperature. The viscosity of the CG was obtained using a viscometer, and the ash content was determined by a high temperature furnace. The soap and alkalinity content in CG were determined following the methodology recommended in AOCS Cc 17-95. The water content was determined by volumetric Karl Fischer titration. All measurements were made in triplicates [2].

Also, aerobic fermentations were performed with *Lactobacillus* strains (ATCC 7469 and 393) in a medium with CG as a carbon source, which contained nutrients and and trace elements that favored lactic acid production [4].

Fermentations were carried out with each sample of CG, with glycerol USP, and synthetic CG composed of the main CG components. In this way, the influence of the compounds identified as potential substrates in the fermentative production process of lactic acid was evaluated. Considering the CG characterization, different media were formulated. A synthetic glycerol medium was constructed in which three minority components found in the samples were involved, and the fermentation process was developed as described. The simplex network design, Simplex-Centroid Design - DCS, was chosen because it was desired to consider the points inside the region, because in the case of glycerol it is possible to obtain mixtures containing all the components. Finally, the metabolites were identified and quantified by HPLC, each test was done triplicated.

**3. Results and discussion**

All CG samples showed low FFA contents, it indicates they are derived from the acidification of the soap that existed in it. Also, the soap contents were relatively high. The composition of the samples varied significantly. The strain *Lactobacillus rhamnosus* ATCC 7469 in an axenic culture, achieved a satisfactory assimilation of CG. Reaching conversions up to 90%, after a stage of adaptation. For the evaluated conditions, it was possible to identify that the optimal volumetric productivity is around 10 hours of fermentation.

**4. Conclusions**

Despite the variation in the proportion of their components, all CG samples were shown to contain glycerol, soap, methanol, FAMEs, water, glycerides, FFAs, and ash. It is necessary to characterize the composition of CG before considering any value-added conversion.

 The CG components influence the response variables of the fermentations, which improves or inhibits the growth and the conversion capacity of the microorganism. These effects depend directly on the biodiesel production process and its conditions, the raw materials used and the purification processes.

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