Sustainable Glycolic Acid Production: The Role of Corn Steep Solids

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ABSTRACT

The increasing demand for glycolic acid (GA) in various industrial sectors has led to the development of a process focused on microbial bioproduction as a sustainable solution. Recent advances in biotechnology show that GA bioproduction is a safer and more environmentally friendly alternative to classical chemical processes. In this context, the main innovation of this research is based on optimizing the biological conversion of ethylene glycol to GA, using corn steep solids as the primary nitrogen source for the metabolism of *Gluconobacter oxydans* bacteria.

Keywords: Glycolic acid. Corn steep solids. *Gluconobacter oxydans*. Ethylene glycol.

1 INTRODUCTION

The main route to the production of glycolic acid (GA) is from fossil resources through formaldehyde carbonylation at high pressure and temperature (DAPSENS et al., 2014; LACHAUX et al., 2019; SUN et al., 2009). The demand for this organic acid has increased exponentially in the industry, especially in the cosmetic sector, as this molecule is particularly valued for its exfoliating properties (RAKA; BRAHMBHATT, 2019)

This scenario, coupled with the need to develop economically and environmentally sustainable solutions, has led to a greater focus on microbial production pathways. Recent advances in GA production biotechnology show that biotransformation, i.e., microbial bioproduction of GA, is a viable way to achieve cleaner and more ecological GA production compared to chemical production (CABULONG et al., 2021; HUA; DU; XU, 2019; LEE; CHOI; WOO, 2019; ZHANG et al., 2020). *Gluconobacter oxydans* is one of the well-known biotechnological models to study GA production (DAI et al., 2018; HUA; DU; XU, 2019; HUA; ZHOU; XU, 2018). Its unique ability to incompletely oxidize substrates, since glycolysis is absent due to the lack of the enzyme phosphofructokinase, has led to significant efforts to understand the GA synthesis process in *G. oxydans* (DEPPENMEIER; HOFFMEISTER; PRUST, 2002; MATSUTANI et al., 2011). *G. oxydans* obtains energy through the oxidation of sugars via the Entner-Doudoroff pathway and the pentose phosphate pathway, with initial phosphorylation occurring at the cytosolic level followed by oxidation (MISHRA; JAIN; KUMAR, 2008).

The biochemical production of glycolic acid from ethylene glycol (EG) represents a sustainable alternative to obtaining this organic acid, as it does not require the use of traditional petrochemical routes. While ethylene glycol is traditionally obtained from ethylene in the petrochemical industry, it can also be obtained from bioethanol (VAN UYTVANCK et al., 2014), glycerol (a by-product of the biodiesel industry) (KANDASAMY; SAMUDRALA; BHATTACHARYA, 2019), and other lignocellulosic biomass (VAN UYTVANCK et al., 2014), making the glycolic acid production process even more environmentally friendly. The inclusion of different lignocellulosic biomasses, such as sugarcane or corn bagasse in bioethanol production is crucial to integrate the EG to GA bioconversion process into the context of biorefineries, as bioethanol can be used as a building block in EG production.

In addition, carbon and nitrogen are essential nutrients in the metabolism of microorganisms. Corn Steep Solids (CSS) are rich in soluble carbon and nitrogen nutrients and are often a by-product of the corn industry. Due to its nutritional composition, CSS can be used as a raw material in biotechnology and studies can be carried out to evaluate its effect on the production of building blocks such as GA. This work first showed the effect of adding CSS on the composition of the growth medium of *G. oxydans* compared to other nitrogen sources. Then, an optimization was conducted using experimental design, and experiments were carried out in a bioreactor using CSS as the primary nitrogen source.

Finally, the core of this research lies in exploring the concept of biotransformation, understood as the process by which organic compounds are converted into other compounds, based on the low or, in some cases, high specificity of the enzymatic apparatus of the microbial cell. Microbial biotransformations, or microbial biotechnology, are becoming increasingly important and are being used extensively for the production of mass-produced products, leading to increases in productivity.

2 MATERIAL & METHODS

2.1. Microorganism activation and storage conditions.

Gluconobacter oxydans CCT 0552 strain was obtained from the Tropical Culture Collection (TCC). The bacterium was aseptically transferred using an inoculation loop in a vertical laminar flow hood (Grupo Veco BioProtector, BioSeg12) to: i) another plate containing sorbitol 25 g/L, yeast extract 5 g/L, peptone 3 g/L, and agar 15 g/L; and ii) an Erlenmeyer flask

containing liquid medium with the same composition mentioned, except for agar. After the medium became turbid, 10% of the content was inoculated into a second growth flask at 28 °C and 200 rpm (shaker New Brunswick Scientific, Innova 44). Agar plates and growth flasks were prepared in triplicate. After 16 hours, all content in the flasks was aseptically centrifuged at 10,000 rpm for 10 minutes at 10 °C for cell recovery, using a centrifuge (Thermo Scientific Sorvall Lynx 4000). The pellet cells were resuspended in 75 mL of the mentioned liquid culture medium with 20% glycerol and stored, with the addition of 2 mL of resuspended cells in sterile 2 mL cryogenic tubes, kept at -20 °C until use.

2.2. Choice of nitrogen source for cell growth medium.

Sorbitol was chosen based on previous research (TENÓRIO, 2023) as a carbon source that promotes the cellular growth of *G. oxydans*. Subsequently, different nitrogen sources were varied to evaluate the bacterial cell growth behavior, including yeast extract (YE), peptone (P), ammonium sulfate (AS), Corn Steep Solids (CSS), casein (CA), and urea (U), each with or without the addition of PEP. The experiments were conducted in 500 mL Erlenmeyer flasks with an operational volume of 200 mL and 10% (v/v) pre-inoculum, incubated for 16 hours at 28 °C and 200 rpm. Finally, DCW was measured by spectrophotometry at 600 nm.

2.3. Optimization of cell growth medium.

The growth medium was optimized using a Central Composite Rotatable Design (Statistica software, Statsoft Inc®, USA) with a p-value of 0.05. Sorbitol (20-100 g/L) and CSS (5-25 g/L) were defined as factors, and DCW (g/L) as the response variable. The analysis included three repetitions at the central points and four tests at the axial conditions, totaling eleven tests in flasks at 28 ºC, 200 rpm, for 16 hours

2.4. Bioproduction of glycolic acid.

Experiments were conducted in batch and extended feeding mode. Biotransformation experiments were carried out in stirred-tank bioreactors (Eppendorf, BioFlo120) with an operational volume of 4 L, containing a medium composed of 0.5 g/L $MgSO_4$, 1 g/L KH_2PO_4, 2 g/L K_2HPO_4, 5 g/L $(NH_4)_2SO_4$, 5 g/L corn steep solids, 1 g/L sorbitol, and 20 g/L ethylene glycol (EG) as the substrate. The pH was maintained between 5.5 and 6.5 by automatic addition of 9.0 M NaOH. The temperature was adjusted to 28 °C, and the aeration rate was controlled at 2.5 vvm.

2.5. High Performance Liquid Chromatography (HPLC) analysis.

The concentrations of GA and EG were measured using an HPLC system (Waters, Alliance 2690) equipped with a refractive index detector and a Hi-plex H column from Agilent, heated to 60°C. The mobile phase was 5 mM H_2SO_4, with a flow rate of 0.6 mL/min.

3 PARTIAL RESULTS & DISCUSSION

3.1. Optimization of growth culture medium for *Gluconobacter oxydans*.

The impact on cell growth, as depicted in Figure 1, was assessed by examining a range of nitrogen sources such as Corn Steep Solids (CSS), yeast extract (YE), peptone (P), ammonium sulphate (AS), casein (CA), and urea (U), with and without the addition of peptone.

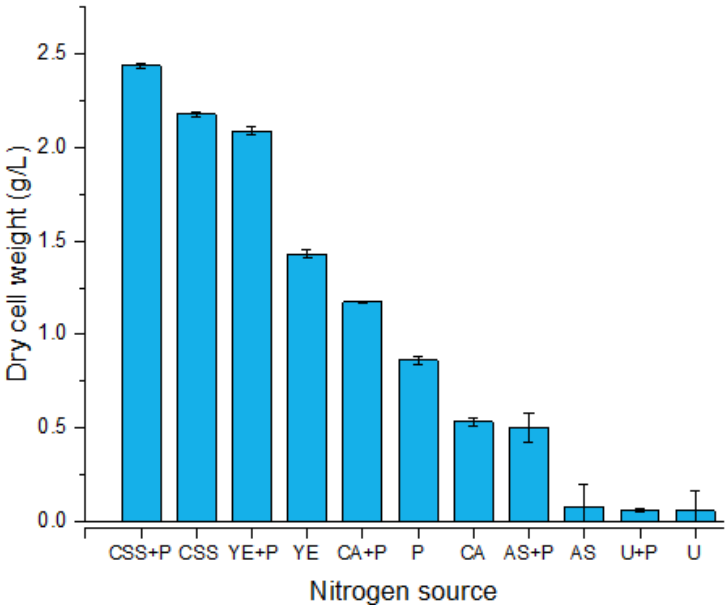

Figure 1. Evaluation of *Gluconobacter oxydans* growth under different nitrogen sources at 28 ºC, 200 rpm and 16 h. CSS: corn steep solids, P: peptone, YE: yeast extract, CA: casein, AS: ammonium sulphate, U: urea.

CSS emerged as the nitrogen source that facilitated the highest cell growth estimate (2.2 g/L), followed by YE (1.4 g/L), P (0.9 g/L), and CA (0.5 g/L). The presence of CSS led to a notable 36.4% increase in cell growth compared to YE, highlighting its sustainability and eco-friendliness. Conversely, the utilization of AS and U, with or without P, resulted in negligible growth. The addition of P (3 g/L) resulted in the highest cell concentration when paired with another nitrogen source. Notably, differences between nitrogen sources, particularly CSS+P and YE+P, had the most significant impact on cell growth (2.4 g/L and 2.08 g/L, respectively). These findings are corroborated by Moghadami et al. (2019), who emphasized that organic nitrogen sources like CSS, YE, and CA, combined with lower peptone concentrations, notably increase bacterial concentration (MOGHADAMI; FOOLADI; HOSSEINI, 2019). Our study's results demonstrated that organic nitrogen sources such as CSS and YE, with or without peptone, fostered rapid growth and high cell yields in *G. oxydans*

3.2. Expected results

Once the growth medium has been optimized regarding the concentration of corn steep solids (CSS) using the Central Composite Rotatable Design (CCRD), we intend to conduct ethylene glycol to glycolic acid biotransformation experiments in different bioreactor configurations, using CSS as the primary nitrogen source. We aim to compare these results with previous research, where yeast extract and other nitrogen sources different from CSS were used.

4 CONCLUSION

The use of CSS as a nitrogen source for the growth of *Gluconobacter oxydans*, outperforming conventional nitrogen sources such as yeast extract by up to 36%, highlights the transformative potential of this industrial byproduct. Furthermore, the inclusion of CSS as a nitrogen source in the bioconversion of ethylene glycol to glycolic acid (GA) with *G. oxydans* not only demonstrates its versatility but also underscores its role in sustainable biorefinery practices. This synergy between ethylene glycol and CSS, both originating from the biorefinery context, emphasizes their central role in future efforts to produce essential building blocks such as glycolic acid.

Despite advances in GA production through biotransformation, further research and developments are still required to improve production levels, focusing on biotransformation and biocatalysis pathways. One of the challenges in increasing GA production is the low biomass yield, often caused by a decrease in pH in the culture medium. However, the bottlenecks associated with GA production by naturally occurring microorganisms can be overcome with new approaches to optimize the process described in this proposal. This research outlines strategies for producing GA using naturally occurring *G. oxydans*, utilizing corn steep solids as a primary nitrogen source, and exploring various bioreactor configurations

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