STUDY OF SYNTHETIC MEDIATORS FOR APPLICATION IN ELECTROFERMENTATION OF *Clostridium beijerinckii*

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ABSTRACT

In electro-fermentation, the application of an electric potential difference enables microbial electrochemical control, thereby altering the production rate of value-added compounds. For indirect transfer to occur, the presence of a natural or synthetic mediator is necessary. The bacterium *Clostridium beijerinckii* utilizes the Wood-Ljungdahl pathway, allowing it to fix carbon dioxide. Thus, the objective of this study was to select a synthetic mediator for the electro-fermentation of *Clostridium beijerinckii*, with a focus on product formation, cellular growth, and substrate consumption. To achieve this, fermentative assays were conducted under anaerobic conditions, using glucose as the substrate, along with bicarbonate and the mediators methyl viologen and neutral red. In the presence of these mediators, there was a reduction in cellular growth and a decrease in the production of lactic and acetic acids. However, there was an increase in butyric acid production in the presence of methyl viologen, and partial glucose consumption in the presence of Neutral Red. Consequently, methyl viologen demonstrated greater advantages for future stages of electro-fermentation.

Keywords: Methyl Viologen Neutral Red, Electro-Fermentation.

1 INTRODUCTION

With the increasing emission of greenhouse gases, it is expected that global temperatures will rise by 1 to 3.7°C [1]. Acetogenic organisms, such as *Clostridium beijerinckii*, have the ability to fix carbon dioxide and carbon monoxide into acetyl-CoA via the Wood-Ljungdahl pathway [2.] In electro-fermentation (EF), electrodes serve as sources or sinks of electrons for microbial electrochemical control. Altering the reduction-oxidation potential and the NADH/NAD+ ratio enhances glucose consumption rates, specific bacterial growth rates, and the production of value-added products, thereby reducing the fermentation time for acetone-butanol-ethanol (ABE) production [3]. Electron transfer within and outside the cell can occur directly or indirectly through natural or artificial redox mediators. Thus, this project aims to select an appropriate mediator, prioritizing cellular growth, substrate consumption, and compound production for electro-fermentation in future stages.

2 MATERIAL & METHODS

The *Clostridium beijerinckii* Br21 strain was isolated from a vinasse reservoir. All experiments were conducted in penicillin-type flasks with a capacity of 100 ml. Nitrogen gas was bubbled into all culture media to remove any dissolved oxygen. Initially, the microorganism was activated in RCM medium. After 24 hours, a pre-inoculum was prepared in RCM medium with half the concentrations used during activation and 16,7mM of glucose. Additionally,10 mM of bicarbonate, 0.5 mM of Neutral Red, and 0.05 mM of methyl viologen were added in specific combinations. These parameters were maintained throughout the assay, which began after 18 hours, using an inoculum sufficient to achieve a DO level of 0.2. Samples were collected at the first time point after 4 hours and subsequently every 3 hours during the experiment. Measurements included pH, DO, and quantification of metabolites and glucose using high-performance liquid chromatography (HPLC). All conditions were performed in triplicate.

3 RESULTS & DISCUSSION

During the fermentative assay, cellular growth was monitored by pH variation because the Neutral Red mediator exhibited a red color, hindering proper absorbance measurement at 600 nm. A decrease in cellular growth was observed in the presence of both mediators compared to conditions with glucose and/or bicarbonate alone. Additionally, the stationary phase was reached more quickly in the presence of mediators, indicating a reduction in fermentation time (Figure 1).

The consumption and production were calculated for each of the metabolites. The production of butyric acid significantly increased in the presence of mediators, particularly considering the decrease in cellular growth, as indicated by pH. This increase was particularly notable in the presence of methyl viologen. Conditions containing bicarbonate led to an increase in the production of lactic acid, which was also favored in the presence of mediators. In conditions without bicarbonate, lactic acid was consumed or produced minimally. Acetic acid was metabolized in most conditions, with higher consumption observed in the presence of methyl viologen. The presence of bicarbonate reduced the consumption of acetic acid. Finally, propionic acid was favored under conditions involving both mediator and bicarbonate, being metabolized or produced minimally under other conditions (Figure 2).

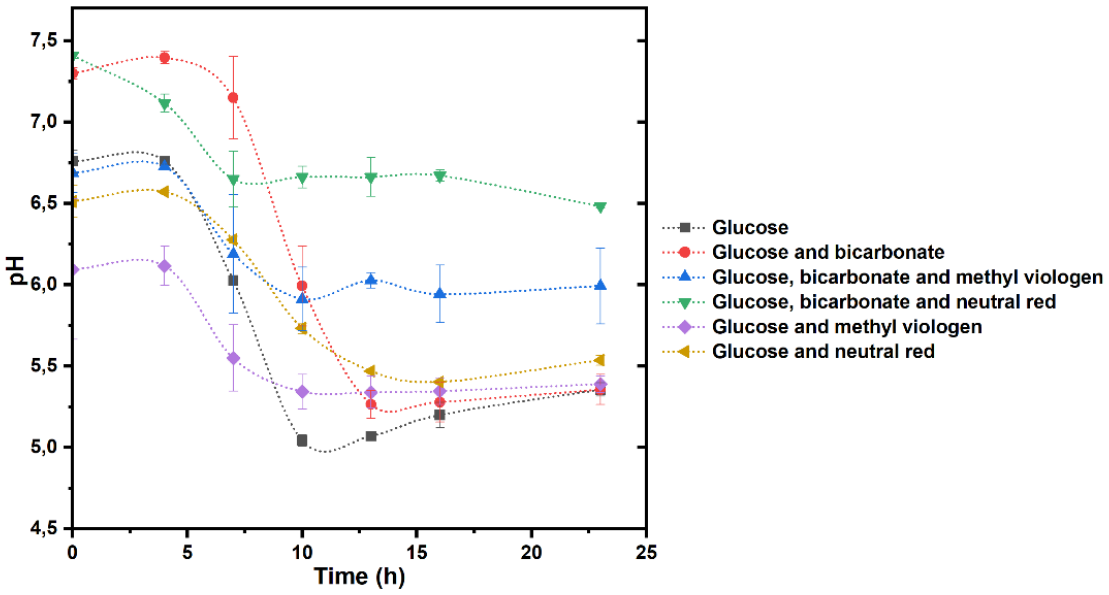

Figure 1. pH variation during the 23-hour fermentative assay under different conditions**.**

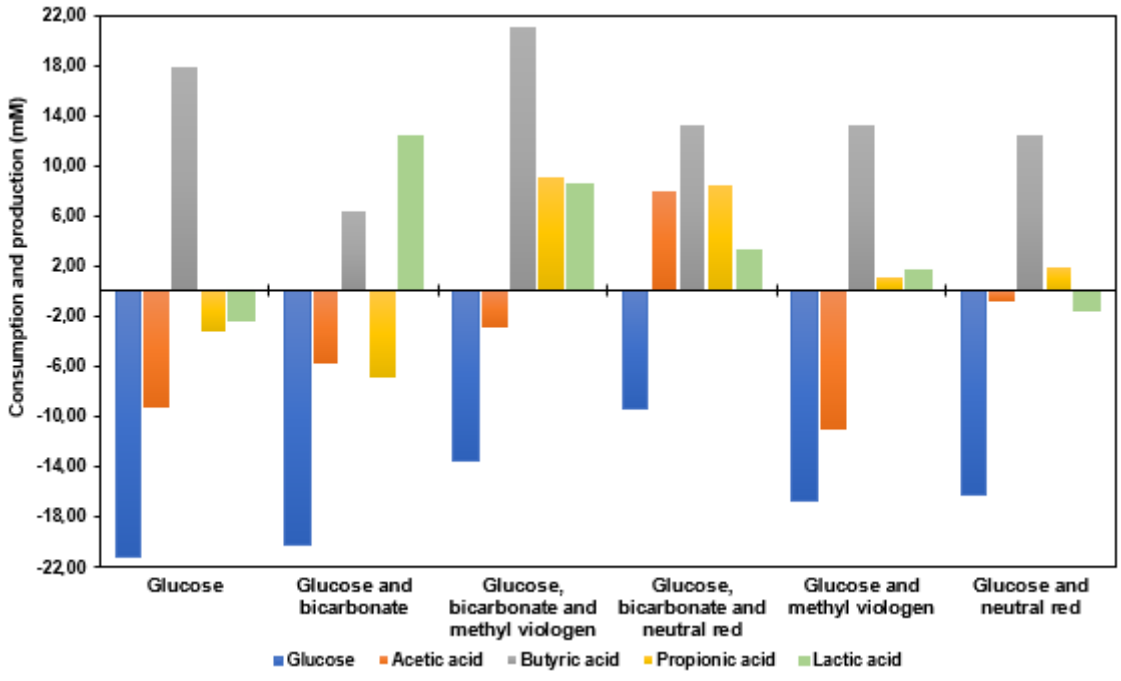

Figure 2. Production and consumption of products and substrate under all conditions during a 23-hour fermentative assay

4. CONCLUSION

During the fermentative assay, a decrease in solution pH was observed due to the production of acids. Conditions with mediators showed slower growth, likely due to mediator toxicity and a quicker onset of the stationary phase. Glucose consumption was nearly total in almost all conditions, except for those containing bicarbonate and Neutral Red. Butyric acid production increased, particularly when compared to the decrease in cell growth, in the presence of the methyl viologen mediator. Acetic and lactic acids had reduced production in the presence of mediators, with lactic acid production being higher under bicarbonate conditions. Therefore, considering the necessity of a mediator for electro-fermentation, methyl viologen is the most suitable for application in future steps.

REFERENCES

[1] ANDERSON, T.R., HAWKINS, E. AND JONES, P.D., 2016. CO2, the greenhouse effect and global warming: from the pioneering work of Arrhenius and Callendar to today's Earth System Models. *Endeavour*, *40*(3), pp.178-187.

[2] RAGSDALE, SW, 2008. Enzimologia da via Wood-Ljungdahl da acetogênese. *Anais da Academia de Ciências de Nova York* , *1125* (1), 129-136.

[3] MOSCOVIZ, R., TOLEDO-ALARCÓN, J., TRABLY, E., & BERNET, N., 2016. Electro-fermentation: how to drive fermentation using electrochemical systems. *Trends in biotechnology*, *34*(11), 856-865.

[4] HE, A.Y., YIN, C.Y., XU, H., KONG, X.P., XUE, J.W., ZHU, J., JIANG, M. AND WU, H., 2016. Enhanced butanol production in a microbial electrolysis cell by *Clostridium beijerinckii* IB4. *Bioprocess and biosystems engineering*, *39*, pp.245-254.

[5] GONG, Z., YU, H., ZHANG, J., LI, F. AND SONG, H., 2020. Microbial electro-fermentation for synthesis of chemicals and biofuels driven by bi-directional extracellular electron transfer. *Synthetic and Systems Biotechnology*, *5*(4), pp.304-313.

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