I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM • XV SHEB • XV ENZITEC FLORIANÓPOLIS • 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIORREFINERY, BIOECONOMY AND CIRCULARITY

POTENTIAL INORGANIC CARBON CAPTURE BY *CLOSTRIDIUM BEIJERINCKII* Br21 UNDER MIXOTROFIC CONDITIONS

Jonatã Bortolucci[1*] & Valeria Reginatto[1*]

[1] *Departamento de Química; Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto; Universidade de São Paulo; Ribeirão Preto; Brasil.*
** Corresponding author's email address: jonata.bortolucci@usp.br, valeriars@ffclrp.usp.br.*

ABSTRACT

Biotechnological processes for the biological conversion of CO_2 to value-added products play an important role in biorefineries, aiding in biomass utilization and CO_2 emission mitigation. *Clostridium* species are promising biocatalysts due to their versatile metabolism. Our study explored the potential of *Clostridium beijerinckii* Br21, isolated in our laboratory, for CO_2 capture under mixotrophic conditions using glucose and HCO_3^- as organic and inorganic carbon sources, respectively. Fermentative assays were conducted in Reinforced Clostridial Medium (RCM) with 10 g L^{-1} glucose, supplemented with 10 g L^{-1} $KHCO_3$. Bioreactor agitation and incubation temperature were varied to increase CO_2 solubility. Results showed that HCO_3^- addition led to distinct variations in cellular growth and affected organic acid production patterns. Organic carbon loss, measured as the difference in initial and final non-purgeable organic carbon (NPOC) concentrations, was reduced with the addition of HCO_3^-, indicating possible CO_2 reassimilation. Organic carbon loss was further lowered when an assay with HCO_3^- supplementation was incubated at a lower temperature (25 °C), which probably has helped to increase CO_2 solubility. This study suggests that *C. beijerinckii* Br21 may perform mixotrophic metabolism by consuming glucose and capturing CO_2, resulting in improved carbon recovery as fermentation products.

Keywords: Mixotrophic metabolism. Wood-Ljungdahl pathway. Pyruvate-ferredoxin oxidoreductase. CO_2. HCO_3^-.

1 INTRODUCTION

The development of biotechnological processes for the biological conversion of CO_2 to value-added products represents an attractive possibility in biorefineries. In addition to promoting the full use of biomass, these processes can also help mitigate CO_2 emissions. Several living organisms, such as plants, algae, and cyanobacteria, assimilate inorganic carbon in the form of CO_2 and HCO_3^- [1].However, this capacity is not limited to photosynthetic organisms; it is also carried out by bacteria. The bacteria of the genus *Clostridium* stand out for their versatile metabolism, capable of generating different commercially valuable products, such as biofuels and an extensive platform of chemicals [2]. The vast majority of *Clostridium* are heterotrophic, but some species are known to be autotrophic, such as *Clostridium ljungdahlii* and *Clostridium autoethanogenum* [3]. Thus, the *Clostridium* genus has been studied as a promising biocatalyst in biological CO_2 capture processes.

Our research group has been exploring *Clostridium beijerinckii* Br21, a butyric acid producer isolated in our laboratory [4]. Its metabolism has been extensively studied, and an intriguing outcome has frequently been observed: the efficiencies in obtaining products, i.e., the carbon recovery in assays resulted in values above 100%. The hypothesis formulated for these observations was the capture of self-produced CO_2 by the microorganism, which has not been quantified and thus would cause inflation of carbon recovery values. This assumption was also raised and investigated by Sandoval-Espinola and collaborators [5], who suggested that *C. beijerinckii* ATCC 35702 possibly had the capability to perform mixotrophic metabolism, i.e., simultaneous consumption of glucose and CO_2. When searching for possible genetic markers responsible for C-1 assimilation, several genes related to the Wood-Ljungdahl metabolism were found in the ATCC 35702 strain. Other possibilities suggested by the authors [5] include a reversed mechanism of the pyruvate-ferredoxin oxidoreductase (PFOR) enzyme, and via the carbonic anhydrase enzyme. The same mechanisms could happen in *C. beijerinckii* Br21, as it also contains the same genetic markers reported for *C. beijerinckii* ATCC 35702.

Here, we investigated the potential inorganic carbon capture by *C. beijerinckii* Br21 under mixotrophic growth conditions. HCO_3^- was tested as inorganic carbon sorce. Furthermore, fermentative assays were conducted under conditions designed to increase the solubility of CO_2.

2 MATERIAL & METHODS

Fermentative assays were conducted using Reinforced Clostridial Medium (RCM) (5 g L^{-1} NaCl, 10 g L^{-1} meat extract, 3 g L^{-1} yeast extract, 10 g L^{-1} peptone, 0.5 g L^{-1} cysteine, 3 g L^{-1} sodium acetate, 1 g L^{-1} soluble starch, and 0.5 g L^{-1} agar), containing 10 g L^{-1} glucose as the organic carbon source. Fermentation was performed in 120-mL serum bottles containing 60 mL of medium. To ensure anaerobic conditions, flasks were sparged with nitrogen before autoclave sterilization. The inoculum was prepared from a culture stored at -80 °C, which was reactivated in RCM for 24 h. This culture was then used to inoculate fresh RCM under the conditions desired for each of the tests. After 18 h, these cultures were used as precultures for the fermentative assays which

were conducted at 37 °C and without agitation (unless otherwise noted). The initial optical density (OD_{600}) of the medium was set at 0.2. Fermentation monitoring was accomplished by periodic sampling for OD_{600} and pH measurements, as well as quantification of glucose and products using High Performance Liquid chromatography (HPLC). Non-purgeable organic carbon (NPOC) was also measured.

Inorganic carbon was supplied in the culture media with the addition of 10 g L^{-1} $KHCO_3$, and fermentative assays were conducted as described before. Additionally, the presence of $KHCO_3$ was combined with agitation at 110 rpm and incubation temperature at 25 °C, to increase CO_2 availability in the culture medium.

3 RESULTS & DISCUSSION

The addition of $KHCO_3$ as inorganic carbon to the assays resulted in OD_{600} variations (Figure 1a), ranging from 3.0 to 4.1. HCO_3^- addition decreased the final OD_{600}, but when combining it with agitation, the OD was similar to the control. However, coupling HCO_3^- and lower temperature (25 °C) slowed the growth (OD_{600} variation). pH ranged from 4.9 to 5.1 (Figure 1a). Glucose consumption averaged 8.0 g L^{-1} (Figure 1b), except at 25 °C, which was 4.9 g L^{-1}. HCO_3^- in combination with agitation hindered acetic acid consumption (Figure 1c), while in the other conditions there was a slight production and consumption. Butyric acid concentration was higher in the control (4.2 g L^{-1}) (Figure 1d), reduced by HCO_3^- (~3.7 g L^{-1}), and slightly lower with HCO_3^- at 25 °C (3.0 g L^{-1}). HCO_3^- in combination with agitation led to the lowest lactic acid concentration (1.8 g L^{-1}) (Figure 1e), likely shifting metabolism towards acetic acid synthesis. HCO_3^- prevented lactic acid consumption compared to the control, but maintained the same final concentration (2.6 g L^{-1}). The control showed the most accentuated propionic acid variation (0.5 g L^{-1} consumption) (Figure 1f), while others presented minor variations.

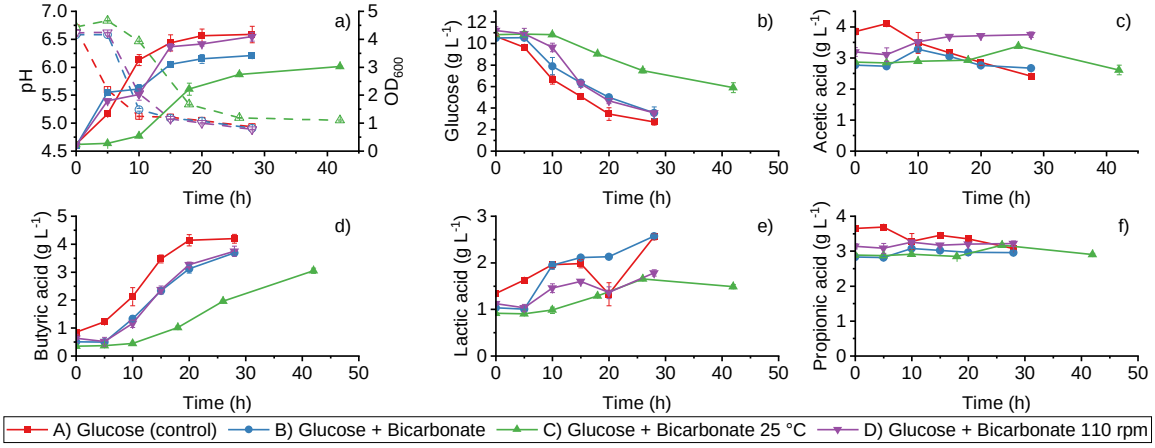

Figure 1 Growth experiment performed with *C. beijerinckii* Br21, with adition of 10 g L^{-1} $KHCO_3$. Altered parameters were agitation and incubation temperature. OD_{600} (filled symbols) and pH (empty symbols) (a), glucose (b), acetic (c), butyric (d), lactic (e), and propionic (f) acids. Error bars represent standard deviation calculated using a biological triplicate.

Besides glucose, the complex culture medium composition (RCM) contains other organic carbon sources available to the microorganism, such as yeast extract and peptone. To account for this observation, initial and final total carbon as NPOC of samples was quantified (Figure 2). With this analysis, we expected to observe different patterns in the variation of organic carbon, possibly indicating an increase in the final concentration, which could be due to the assimilation of CO_2 along the fermentation.

During the fermentation of glucose, organic carbon is used for cell growth, organic acids synthesis and is also lost in the form of CO_2. Considering that the main product synthesized by *C. beijerinckii* Br21 is butyric acid ($C_4H_8O_2$), the stoichiometry (Equation 1) indicates that 33 % of the carbon originating from glucose is converted to CO_2. This is an estimated value, as the global metabolism occurring in the bacteria is a complex network of reactions, and Equation 1 does not take into account the cell biomass.

$$C_6H_{12}O_6 \rightarrow 2\ C_4H_8O_2 + 2\ CO_2 + 2\ H_2 \quad (1)$$

The initial NPOC concentrations (Figure 2a) ranged from 22.25 to 24.81 g L^{-1}. This concentration includes glucose as a substrate (10 g L^{-1}) and the other organic carbon sources from the extracts in the culture medium. Additionally, organic acids are present at the beginning of fermentation, from the culture medium composition and inoculum (Figure 1). The final NPOC concentrations (Figure 2a) varied between 19.36 to 21.70 g L^{-1}. The organic carbon loss was then calculated as the difference between the initial and final values for each experimental condition, both in terms of concentration (Figure 2b) and percentage (Figure 2c).

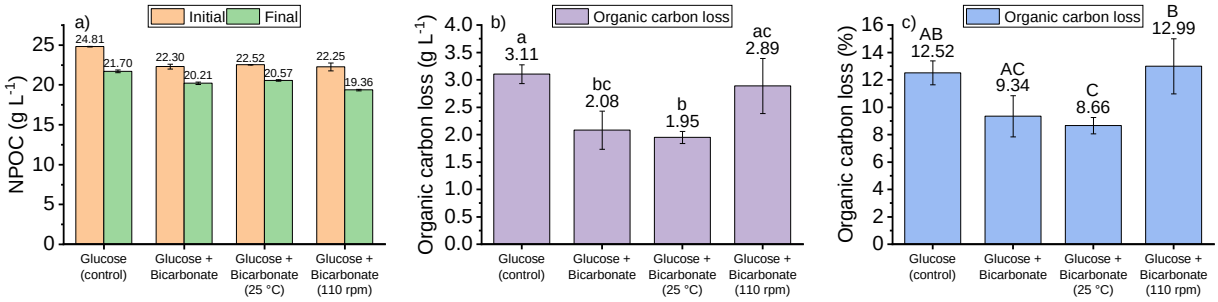

Figure 2 NPOC concentration in the growth experiment performed with *C. beijerinckii* Br21, with addition of 10 g L^{-1} $KHCO_3$. Altered parameters were agitation and incubation temperature. NPOC concentration (a), organic carbon loss in concentration (b) and percentage (c). In graphs (b) and (c), different letters above the bars indicate significant difference, as determined by Tukey's test ($p < 0.05$). Error bars represent standard deviation calculated using a biological triplicate.

Organic carbon loss in the control fermentation of our experiments was roughly half (12.52 %, Figure 2c) the amount expected by Equation 1, which potentially indicates the reassimilation of CO_2 produced by the bacteria during the fermentation, without the need to supply inorganic carbon in the culture medium. This effect was also observed in our previous work [4].

HCO_3^- addition significantly reduced the organic carbon loss (Figure 2b) compared to the control, 2.08 and 3.11 g L^{-1}, respectively. This suggests further inorganic carbon assimilation, as there was less organic carbon loss (Figure 2c) compared to the control, 9.34 and 12.52 %, respectively. The same pattern was observed for the assay with HCO_3^- at lower temperature: lower NPOC variation (1.95 g L^{-1}) and organic carbon loss (8.66 %) compared to the control. This might be associated to an increase in CO_2 solubility in the medium. Conversely, combining HCO_3^- with agitation seems to hinder inorganic carbon capture, as NPOC variation and organic carbon loss were similar to the control.

4 CONCLUSION

The results have suggested that *C. beijerinckii* Br21 possibly performs mixotrophic metabolism, by reassimilating its own produced CO_2 into organic products. Furthermore, the supplementation with HCO_3^-, as an extra source of inorganic carbon, combined with incubation temperature at 25 °C seems to stimulate mixotrophy. Lower cell growth and butyric acid synthesis, as well as changes in consumption/synthesis patterns of other organic acids were observed by the addition of HCO_3^-. In the next steps, the expression of genes related to CO_2 capture pathways will be measured.

REFERENCES

1 SALMERÓN, *et al*. 2022. J. CO_2 UTIL. 58. 101914.
2 JOSEPH, R.C, SANDOVAL, N.R. 2023. SYNTH SYS BIOTECHNOL. 8 (1). 148-156.
3 HEFFERNAN, *et al.* 2023. CHEM. ENG. J. 452 (1). 138950.
4 FONSECA, *et al.* 2016. INT. J. HYDROGEN ENERGY. 41. 21109-21120.
5 SANDOVAL-ESPINOLA, *et al.* 2017. SCI. REP. 7 (1). 1-13.

ACKNOWLEDGEMENTS

This work had financial support of FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil) – Research Grant 2022/04024-0 and 2023/00590-4.