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CO_2 capture by a *Clostridium beijerinckii* strain employing bicarbonate as an inorganic carbon source

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ABSTRACT

The increase in CO_2 emissions due to industrial progress is intensifying concerns about global warming. An alternative approach is to use CO_2 as a carbon source in biotechnological processes by microorganisms capable of capturing it autotrophically or mixotrophically, i.e. the simultaneous assimilation of organic and inorganic carbon. The *Clostridium beijerinckii*, strain Br21, isolated in our laboratory, is a butyric acid-producer and performs mixotrophic metabolism, which apparently occurs through three independent pathways. This work aims to investigate its ability to capture CO_2, added to the culture medium as bicarbonate, using glucose as a co-substrate. Fermentation kinetic assays were conducted, monitoring cell growth, organic acid production and substrate consumption for each condition. The addition of $NaHCO_3$ increased carbon recovery by 2.2 times compared to the glucose-alone condition. The incorporation of bicarbonate influenced the results: the addition of the salt solution after sterilization resulted in a 19.67% higher carbon recovery. These data suggest that the higher carbon recovery in the conditions with bicarbonate demonstrates the Br21 strain's ability to use inorganic carbon, with the $KHCO_3$ solution increasing its availability and favoring mixotrophic metabolism.

Keywords: HCO_3^-. CO_2 capture. Mixotrophic metabolism. Experimental design. *Clostridium beijerinckii*.

1 INTRODUCTION

Population increase, urbanization, and technological advances have increased the need for energy and the production of goods[1]. As a result, the increase in CO_2 emissions has raised environmental concerns[2], since this gas is the main contributor to global warming. New technologies are needed to sequester excess atmospheric CO_2 in order to achieve global carbon neutrality. One of the most sustainable approaches is the use of microorganisms in biotechnological processes, using CO_2 as a carbon source. Some microorganisms perform mixotrophic metabolism, they can assimilate organic and inorganic carbon concomitantly, making them attractive for industrial applications[4]. Their metabolism involves converting CO_2 into organic compounds through three main steps: CO_2 incorporation, enzyme-catalyzed reduction and regeneration of reducing equivalents[5].

The Wood-Ljungdahl (WL) metabolic pathway is a widely known route for CO_2 and CO assimilation in anaerobic organisms, such as *Clostridium* genera. These bacteria can use CO_2 to form acetyl-CoA through enzymatic reactions[6]. In *C. beijerinckii* ATCC 35702, despite the absence of acetyl-CoA synthase, the WL pathway is still active, suggesting significant variations in the metabolic pathway. Other routes have also been explored, including the oxidation of CO by enzyme carbonic anhydrase and the assimilation of C-1 into acetyl-CoA, through the reverse mechanism of the enzyme pyruvate-ferredoxin oxidoreductase (rPFOR)[7].

In this context, the strain *C. beijerinckii* Br21, isolated in our laboratory, has been studied for its production of hydrogen and butyric acid from carbohydrates[8]. In our studies, yields above the theoretical ones were noted, which suggests other carbon assimilation routes. Corroborated by the fact that this strain has genes related to WL metabolism, except for acetyl-CoA synthase, it is believed that it has the potential to utilize C-1. Therefore, here we aim to investigate the ability of this microorganism to assimilate CO_2 in the form of bicarbonate in aqueous solution, using glucose as a co-substrate. This investigation was carried out using kinetic tests that aimed to monitor and quantify the flow of carbon to the fermentation products, organic acids.

2 MATERIAL & METHODS

The fermentation assays with *C. beijerinckii* were carried out in Reinforced Clostridial Medium (RCM) containing glucose 5 g L^{-1}, peptone 10 g L^{-1}, meat extract 10 g L^{-1}, yeast extract 3 g L^{-1}, NaCl 5 g L^{-1}, soluble starch 1 g L^{-1}, cysteine 0.5 g L^{-1}, sodium acetate 3 g L^{-1} and agar 0.5 g L^{-1}, and 10 g L^{-1} of glucose (pH at 7.2). Inorganic carbon was introduced as sodium and potassium bicarbonate salts. The fermentation was carried out in 100mL penicillin flasks and, as it is an anaerobic microorganism, the air contained was exchanged for pure nitrogen. The flasks were then sterilized in an autoclave at 120 ºC and 1 kgf/cm^2 for 20 minutes.

Aliquots of the culture medium were collected during fermentation intervals to determine cell concentration, pH and substrate and product concentration. The cell concentration was quantified by reading the optical density at 600nm on a spectrophotometer (BEL Engineering - UV-M51). The concentrations of the substrate and fermentation products were obtained using HPLC (High Perfomance Liquid Chromatography), on an Aminex HPX-87H column, at a controlled temperature of 60°C, under isocratic conditions. The mobile phase used was H_2SO_4 0.005mol L^{-1} and UV-Vis and RID detectors were used.

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3 RESULTS & DISCUSSION

In order to increase the availability of dissolved C-1, sodium and potassium bicarbonate were compared, as well as the way these salts were added to the culture medium. The solubility in water at 25 °C of $NaHCO_3$ and $KHCO_3$ were 111 g L^{-1} and 390 g L^{-1}, respectively[9]. Therefore, $KHCO_3$ should increase the availability of inorganic carbon and favor mixotrophic metabolism. In addition, the sterilization temperature could affect the salt concentration. So, two sterilization approaches were tested: first the bicarbonate solution was sterilized by filtration and then added to the sterile medium, and second the bicarbonate was sterilized by temperature within the medium. Figure 1 shows the *C. beijerinckii* Br21 growth and pH during fermentation under heterotrophic and mixotrofic conditions, as well as after the two sterilization approaches.

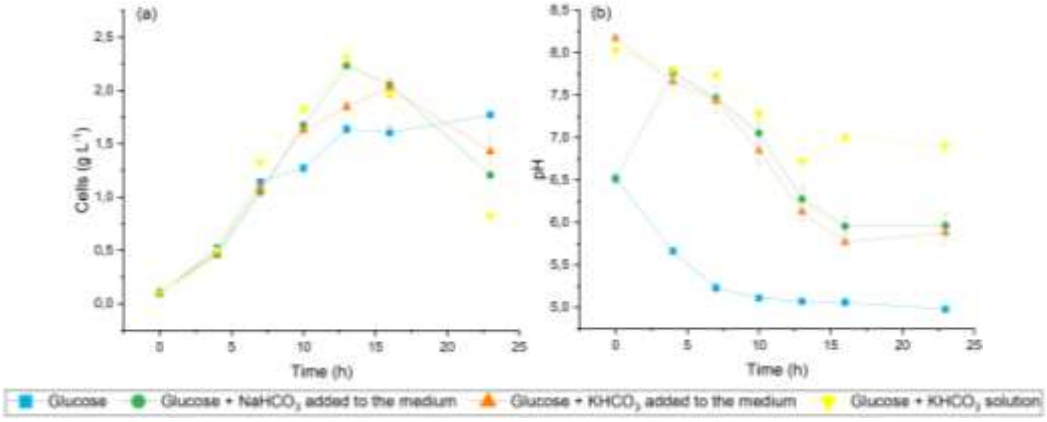

Figure 1 Kinetic assays with *C. beijerinckii* Br21 in RCM, with 10 g L^{-1} initial glucose, where (a) cell concentration and (b) pH. Condition A (glucose 10 g L^{-1}) is represented by the blue line, Condition B (glucose 10 g L^{-1} + $NaHCO_3$ 100 mmol L^{-1}) by the green line, Condition C (glucose 10 g L^{-1} + $KHCO_3$ 100 mmol L^{-1}) by the orange line, and Condition D (glucose 10 g L^{-1} + filtered $KHCO_3$ 100 mmol L^{-1} solution) by the yellow line.

Cell growth was similar between the conditions with bicarbonate (B-D). Nevertheless, the maximum cell concentration achieved in condition A was 23.3% lower than the maximum obtained in the experiment as a whole, which was achieved with the $KHCO_3$ solution. An important difference was the pH curve. In the first 5 hours, the medium with $NaHCO_3$ had an increased pH due to the presence of the basic salt. However, the curves for conditions C and D began at a pH close to 8.0-8.2, around 1.5 units of difference. This variation is possibly related to the dissociation equilibrium of these salts. $KHCO_3$, more soluble, has a higher concentration of HCO_3^- ions in the medium, contributing to a basic medium from the beginning of the assay. In the other hand, $NaHCO_3$ is less dissociated and does not increase the pH at the start of the test. However, as microbial activity takes place, the $NaHCO_3$ dissociation equilibrium (1) is shifted towards the formation of dissociated ions due to the use of HCO_3^- to consume the H^+ generated in the medium (2), which leads to an increase in pH[10].

$$NaHCO_3 \leftrightarrow Na^+ + HCO_3^- \quad (1)$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2 \quad (2)$$

Due to these variations in initial pH, it is challenging to compare the results. Despite this, it is possible to make comparisons mainly between the conditions that had the same starting point. To better visualize the metabolites produced, a carbon recovery graph was drawn up (Figure 2). The addition of $NaHCO_3$ provided a 2.2-fold higher carbon recovery compared to the glucose-alone condition, suggesting the utilization of inorganic carbon from bicarbonate by *C. beijerinckii* Br21.

Comparing the two $KHCO_3$ sterilization conditions, the assay with the salt solution added after sterilizing the medium showed a 19.67% higher total carbon recovery compared to the assay with the sterilized bicarbonate added to the medium. In this case, the carbon was mainly directed to acetic acid (16.39%) and butyric acid (4.46%) compared to condition C (8.72% and 2.95%, respectively). Therefore, the way in which bicarbonate is incorporated into the medium is important, probably because it is in a higher concentration at the beginning of the test.

The initial pH has changed by varying $NaHCO_3$ to $KHCO_3$; however, in the first few hours of fermentation with $NaHCO_3$, it is not clear at what point, the pH had already equaled those of conditions C and D. Considering this, and given that $KHCO_3$ is 3.5 times more soluble than $NaHCO_3$, the employment of $KHCO_3$ is an effective strategy for increasing the concentration of C-1 in the medium.

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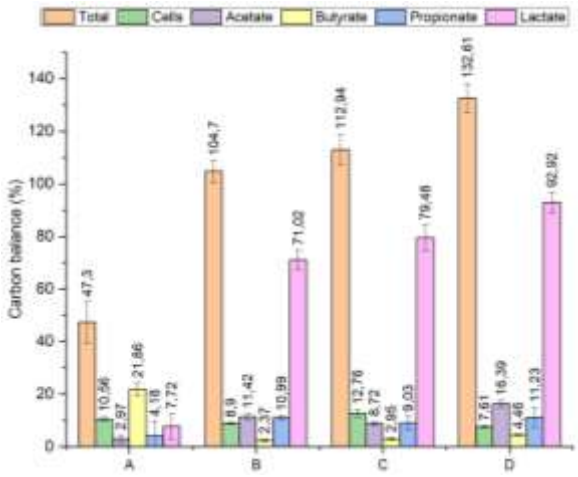

Figure 2 Carbon balance (%) for the fermentations with: glucose (A); glucose + $NaHCO_3$ in the medium (B); glucose + $KHCO_3$ in the medium (C); and glucose + $KHCO_3$ solution (D). It was assumed that the organic carbon consumed came from glucose and the acids that were consumed during fermentation.

In addition, it was observed that lactic acid was the main product obtained under conditions B, C and D. This suggests that the bicarbonate addition increases the activity of rPFOR as a mechanism for C-1 capture, resulting in pyruvate, which is easily converted to lactate by the enzyme lactate dehydrogenase (LDH).

4 CONCLUSION

The higher carbon recovery in the assays with bicarbonate compared to the control indicates the ability of *C. beijerinckii* Br21 to use inorganic carbon. The addition of $KHCO_3$ increases the availability of inorganic carbon in the medium, favoring the mixotrophic metabolism of *C. beijerinckii* Br21, especially when the bicarbonate solution was added after sterilization. It was also observed that lactic acid was the main product under the conditions tested with bicarbonate, suggesting the activity of rPFOR and lactate dehydrogenase. To confirm the capture and utilization of CO_2 in the form of HCO_3^- by *C. beijerinckii* Br21, transcriptomic and qRT-PCR analyses are planned, as well as quantification of the total concentration of inorganic carbon, which is currently underway.

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