STUDY OF CARBON CATABOLIC REPRESSION IN *Clostridium tyrobutyricum* STRAIN

Vitor C. Cordova[1], Maikon Kelbert[2], Débora de Oliveira[3] & Bruno A. M. Carciofi[3*]

[1] *Chemical Engineering/Technological Center/Department of Chemical Engineering and Food Engineering/Federal University of Santa Catarina, Florianópolis, Brazil.*
**vitorcascordova@gmail.com*
maikonkelbert@gmail.com
debora.oliveira@ufsc.br
bruno.carciofi@ufsc.br

ABSTRACT

The search for new sources of energy and biofuels has given rise to research into the use of bioprocesses and waste recovery. Lignocellulosic materials have shown potential for production and application as a substrate in biorefineries. As a result, fermentation processes have been increasingly studied to maximize production and make it economically viable. This study used *Clostridium tyrobutyricum* strain to produce volatile fatty acids, an important precursor in the production of biofuels. The catabolic repression of carbon was evaluated aiming to propose a destination of fractions containing pentoses and hexoses in future bioprocesses. It was shown that the medium pH acts as an important regulator in the volatile fatty acids production and cell growth. In addition, a certain delay in both growth and volatile fatty acids production was confirmed in media containing only xylose, while in media containing glucose and xylose, there was no consumption of pentose, showing the strain preference by glucose consumption.

Keywords: Lignocellulosic materials; Clostridium; Biorefinery.

1 INTRODUCTION

Volatile fatty acids (VFA), such as acetic acid (AA), lactic acid (LA), butyric acid (BA), are important materials for the food, pharmaceutical, chemical and biofuel industries [1]. The butyric acid is an important precursor for biopropane production, introducing a new way for bioenergy, but the main route production of this VFA is still the chemical synthesis [2]. As a sustainable alternative, fermentative bioprocesses to produce these precursors have shown positive and environmentally friendly results [3]. Several anaerobic microorganisms, especially from the Clostridium genus, are natural producers of VFAs [2]. Fermentation with Clostridium has attracted the interest of the industrial sector, being widely used in industrial plants to produce fuels such as butanol, through ABE fermentation (acetone-butanol-ethanol) [4]. In addition, Clostridium strains can also produce VFA of industrial interest from various substrates through acidogenic fermentation, including sugars from lignocellulosic materials (LM) [5].
Several strategies have emerged to apply the use of agro-industrial waste in the production of VFA from fermentative bioprocesses, using strains like *Clostridium tyrobutyricum*. Due to some recalcitrant structures, pretreatment processes are also required to facilitate hydrolysis of the material [6]. Glucose (Glu) and xylose (Xyl) are the major sugar produced by LM pretreatment and hydrolysis. However, Glu is preferentially consumed over Xyl by most of the bacteria strains. So-called carbon catabolic repression (CCR) significantly affects the efficiency of lignocellulosic biomass utilization [7]. The objective of the present study is evaluating the CCR in presence of Xyl and Glu for improve the VFA production, exploring strategies for using the two sugar fractions.

2 MATERIAL & METHODS

The *Clostridium tyrobutyricum* (ATCC 25755) cryopreserved were suspended in penicillin serum bottle containing 80 mL of RCM medium (Reinforced Clostridial Medium) [8]. The serum bottle was than purged with nitrogen gas before being closed and placed in an incubator at 37°C, 150 rpm for 72 hours. After, the medium was used as inoculum for subsequent tests.

The tests were carried out in 100 mL penicillin bottles, with 80 mL of volume. The flasks were closed with rubber stoppers, secured with aluminum seals, and purged with nitrogen for 3 min to maintain anaerobic conditions [9]. The composition of the culture medium is described below, according to the test carried out. All the flasks were kept at 37 °C and 150 rpm. Fermentation was carried out for 7 days and samples were taken every 12 hours. The levels of VFA and sugars were measured by HPLC [10].

Table1. Components of culture media

Culture media*		Trace Solution	
Component	Concentration ($g.L^{-1}$)	Component	Concentration ($g.L^{-1}$)
KH_2PO_4	0,5	$ZnCl_2$	0,07
K_2HPO_4	1	$MnCl_2.4H_2O$	0,10
Yield Extract	2	H_3BO_3	0,06

$CaCl_2.H_2O$	0,015	$CoCl_2.2H_2O$	0,20
$MgSO_4.7H_2O$	0,2	$CuCl_2.2H_2O$	0,50
$FeSO_4.7H_2O$	0,005	$NiCl_2.6H_2O$	0,025
$NaHCO_3$	6,25	$Na_2MoO_4.2H_2O$	0,035
Trace solution	2 ($mL.L^{-1}$)	HCl (37%)	0,90 ($mL.L^{-1}$)

*Three carbon sources are used in culture media: 30g.L^{-1} of Xyl, 30g.L^{-1} Glu and 30g.L^{-1} of Glu and Xyl (ratio 1:1).

3 RESULTS & DISCUSSION

Clostridium tyrobutyricum was able to grow in the presence of Glu and Xyl (Fig. 1 A, B and C). However, when xylose as used as only carbon source an increase of lag period of 1 day was observed (Fig. 1 B). This might happen due to inoculum been cultured in the RCM medium, which present glucose as carbon source, needing more time to adapt to the medium containing xylose [11]. Moreover, it is suggested the preferential consumption of Glu than Xyl by *Clostridium tyrobutyricum* strain. Another effect associated with the inoculum is the initial AA and AB concentration.

The Fig. 1 show the parameters collected in fermentation, like VFA production, sugar consumption, pH and optical density. Whit them is possible to correlate the data and the fermentation time. These results will be discussed below.

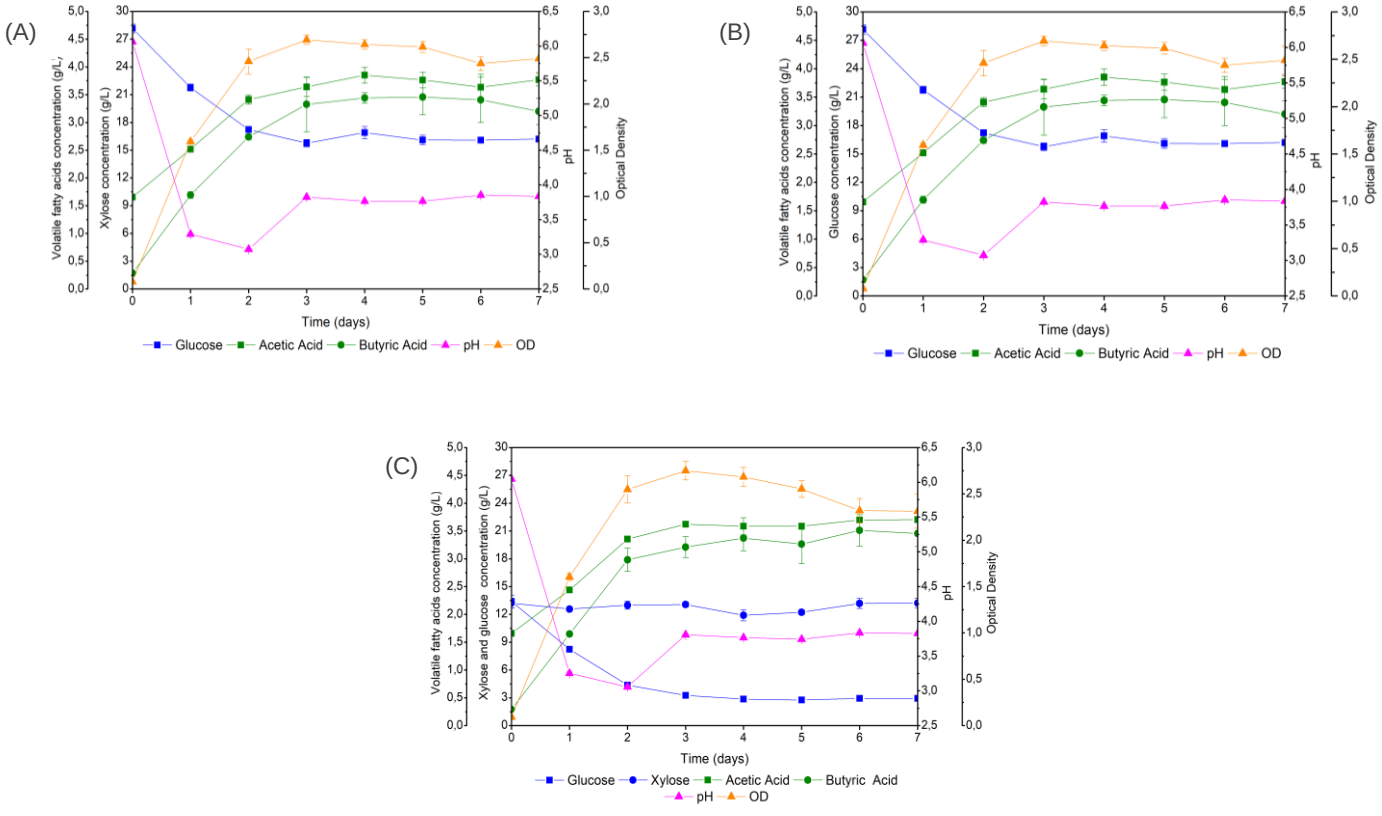

Figure 1. kinects parameters of fermentation with C. tyrobutyricum

The production of VFA period is associated with the cell growth, both were stabilized in 3 days for Fig A and C, and 4 days for Fig C. The major product was AA, this occurred may be associated with a decrease in the pH of the medium, which has already been reported in other studies, changing the predominance of VFA production for LA (not detected) and AA in this strain [12].

In the presence of glucose, xylose is not consumed (Fig 1.C), showing no significant variation in the tests. Xylose shows a certain decrease in cell growth and VFA production compared to media containing glucose, when used as the only carbon source (Fig 1.C), as expected [7].

4 CONCLUSION

This study evaluated the kinetic parameters associated with the use of three carbon sources to assess the catabolic repression of carbon in a *Clostridium tyrobutyricum* strain. The production of VFA was reported in all carbon sources, but a certain decrease in production was associated with the cultivation excluded with xylose, compared to those with glucose present, and the same occurred with cell growth. The pH of the medium was an important factor in the production of AA as the majority product compared to BA and LA (not detected).

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