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CARBON SOURCE (GLUCOSE AND FRUCTOSE) INFLUENCE ON ETHANOL AND BUTANOL PRODUCTION THROUGH SYNGAS FERMENTATION BY *Clostridium carboxidivorans*

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ABSTRACT

An emerging technology to produce liquid biofuels is syngas fermentation. Syngas can be obtained from a wide variety of raw materials, including municipal waste. In this process, syngas is fermented by acetogenic bacteria capable of growing autotrophically in CO, CO_2 and H_2 and producing ethanol, butanol and other alcohols. The objective of this study was to evaluate the presence of the carbon source (glucose and fructose) and its absence in the production of ethanol and butanol through the fermentation of synthesis gas by *Clostridium carboxidivorans*. The results demonstrated that cell growth was favored by the presence of fructose and greater ethanol production occurred in the presence of glucose. However, ethanol was also produced without glucose or fructose and butanol was produced in higher amount in the absence of glucose.

Keywords: *Clostridium*. Syngas. Fermentation. Bioreactor. Biofuels.

1 iNTRODUCTION

Biofuels are produced from renewable biomass and can replace, partially or completely, fuels derived from petroleum and natural gas in combustion engines or in other types of energy generation. They have a low emission rate of polluting substances and are biodegradable, causing less impact on nature. The production of ethanol and butanol by acetogenic bacteria through gaseous fermentation represents a suitable strategy to use lignocellulosic raw materials such as agricultural residues or municipal solid waste. When using lignocellulosic materials, biomass gasification eliminates the pre-treatment step, reducing the cost of the process and enabling full use of the material, including lignin[1].

Anaerobic acetogenic bacteria, such as *Clostridium carboxidivorans*, are capable of fermenting gaseous substrates, as synthesis gas from gasification, mainly composed of CO, H_2 and CO_2. Through this process, acids, and alcohols (e.g. ethanol and butanol) can be produced via the Wood-Ljungdhal pathway[2]. A current problem with gaseous anaerobic fermentation is the low cell density[3]. To this end, several research have been carried out in search of solutions to overcome this issue, including the study of different culture media and bioreactor configurations[4].

The objective of this study was to evaluate the influence of the carbon source (glucose and fructose) and its absence on the production of ethanol and butanol, through the fermentation of synthesis gas by *Clostridium carboxidivorans.*

2 MATERIAL & METHODS

The microorganism used was Clostridium carboxidivorans DSM15243, purchased from DSMZ, Germany. The microorganism was activated and cultivated under anaerobic conditions in a 60 mL penicillin bottle containing 30 mL of TPYarg medium. The pre-inoculum was incubated at 37ºC and 150 rpm in 60 mL serum bottles containing 30 mL of TPYarg medium, and syngas (25% CO, 43.9% H_2, 10.02% CO_2, 10.05% N_2 and 11.01% CH_4). Three different culture media were used in this study, as shown in Table 1. The media were based on the optimization performed by Benevenuti et al. (2020), that resulted in greater cell growth of C. carboxidivorans with TPYGarg medium (Tryptone 12 g/L, gelatin peptone 12 g/L, yeast extract 7 g/L, glucose 1 g/L and L-arginine 1,2 g/L).

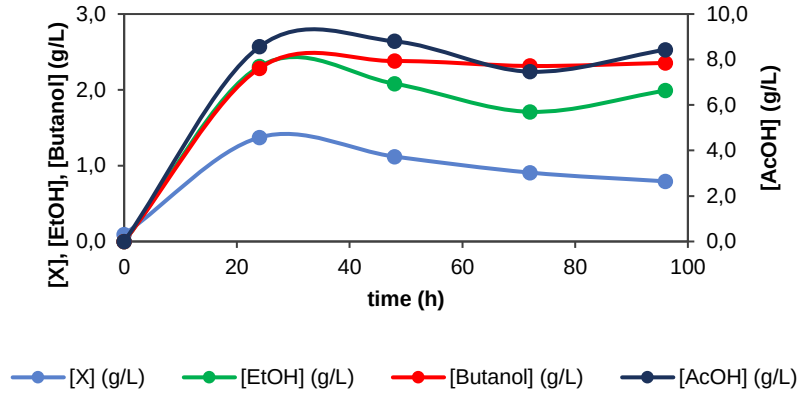
Syngas fermentation was carried out in a 750 mL bioreactor (INFORS HT Multifors), containing 450 mL of the studied culture medium and 50 mLof pre-inoculum. Before being inoculated, the nitrogen gas was purged, removing all oxygen present in the medium, ensuring anaerobiosis of the culture medium. The experiments were carried out at 37°C, 500 rpm for 96 hours, with syngas flow rate of 0,5 vvm. The samples were collected for analysis by high performance liquid chromatography (HPLC) from Shimadzu equipped with Aminex® HPX-87H and optical density at 600 nm (in a spectrophotometer model Shimadzu UV-1800).

Table 1 Culture media composition used for syngas fermentation.

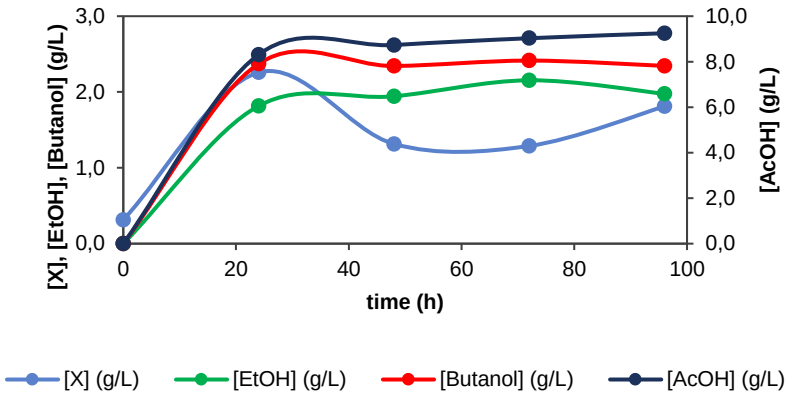
Medium	Tryptone (g/L)	Bacteriological peptone (g/L)	Yeast extract (g/L)	L-arginine (g/L)	Glucose (g/L)	Fructose (g/L)
TPYGarg	12.0	12.0	7.0	1.2	1.0	-
TPYFarg	12.0	12.0	7.0	1.2	-	1.0
TPYarg	12.0	12.0	7.0	1.2	-	-

3 RESULTS & DISCUSSION

Figure 1 shows that medium with fructose (TPYFarg) favored the highest cell growth (2.26 g/L), followed by the TPYarg medium (1.45 g/L) and the TPYGarg medium (1.37 g/L). Highest ethanol production occurred in the presence of glucose (TPYGarg) (2.31 g/L of ethanol). Butanol was also produced in this medium (2.28 g/L of butanol), but higher production was detected in the media without glucose (2,36 g/L of butanol for TPYarg and 2,37 g/L of butanol for TPYFarg). It was possible to observe that butanol production is associated with cell growth, with the highest production in the medium with the highest cell growth. The results presented are still preliminary and further experiments still need to be carried out to confirm them.



(a)



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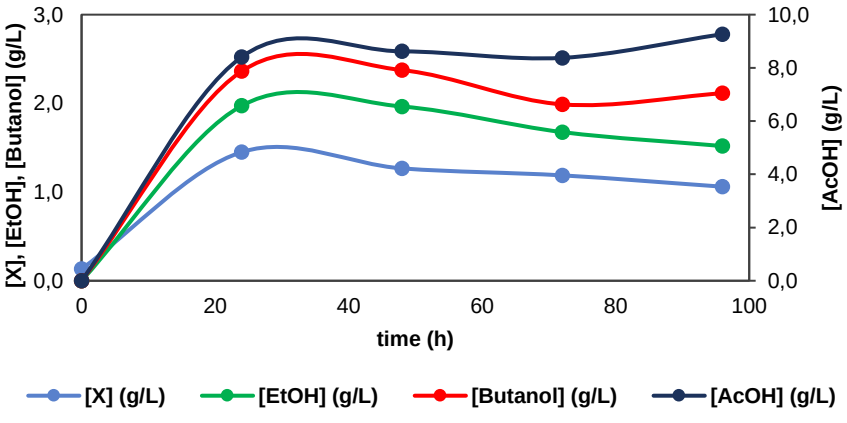

(c)

Figure 1 Cell growth profile, ethanol, butanol and acetic acid production in TPYGarg medium (a); TPYFarg medium (b) and TPYarg medium (c).

Figure 1 also highlights the high production of acetic acid for all media, much higher than that obtained by Benevenuti et al (2021)[5] (1.32 g/L). These differences in concentrations may be related to the lower agitation speed (300 rpm) and different culture medium (ATCC® 2713 medium) used by Benevenuti et al (2021).

4 CONCLUSION

Ethanol and butanol were produced in all carbon source conditions, as well as acetic acid. Despite the higher ethanol concentration in TPYGarg medium, butanol was produced in higher amount with fructose or in the absence of both carbon sources. The result demonstrates the possibility of producing biofuels from synthesis gas in a complex cultivation medium without glucose or fructose with acetogenic bacteria.

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