Effect of the lactic acid as a substrate on the butyric acid production by *Clostridium beijerinckii 4A1* - preliminary data

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Abstract: *In recent years, interest in butanol and butyric acid as a potential substitute for traditional fuels has grown. Microbiological production of butanol and butyric acid from carbohydrates is not economically advantageous due to several process defects. The cost reduction potential of the product is related to expanding the substrates used and optimizing the process' economics. The metabolism of the strains Clostridia is in two stages - acidic and solvatogenic. In the beginning, carboxylic acids are formed, and afterward, these acids induce the preparation of solvents.*

The aim of this work is to study the effect of unusual substrate on butyric acid production by Clostridium beijerinckii 4A1. The experiments performed were based on a Reinforced Clostridial medium, using different concentrations of lactic acid as carbon source, at T = 37 °C, and anaerobic conditions.

Keywords*: BUTYRIC ACID, LASTIC ACID, UNUSUAL SUBSTRATE, CLOSTRIDIUM BEIJERINCKII*

1. Introduction

Nowadays, butyric acid (butanoic acid) is predominantly produced from petrochemical feedstocks via chemical synthesis. Butyric acid is widely used in chemical, food, and pharmaceutical industries. Its production from renewable, low-cost biomass has attracted large attention in recent years.

The butyric-acid fermenting bacteria were divided into two groups: those producing mostly butyric acid as a final product and those producing mostly butanol as a final product. The latter process—called acetone–butanol–ethanol (ABE) fermentation—was one of the oldest known industrial fermentations. [1; 2].

Traditional ABE fermentation methods employ mainly glucose as the substrate. The use of other substrates such as corn, or molasses has also been reported. However, with the rising price of these substrate materials, the feedstock cost has become a major factor determining the total economics of the ABE fermentation industry [3].

Low-price renewable feedstock and various biomass hydrolysates have been used as substrates in batch ABE fermentation [3 - 8].

Various studies have investigated and reported enhancing butyric acid production by fermentation of second and third generation substrates. An interesting substrate for butyrate and hydrogen production is a post-distillation slurry solution from alcoholic beverages. Millions of tons are discharged annually as a byproduct of alcohol manufacture. The slurry solution contains acetic acid, lactic acid, succinic acid, and other components including fiber and protein. The solution is with a very low pH [9].

Lactic acid has wide industry use, mainly like a polylactic acid (PLA), a biodegradable polymer used as environmentally friendly biodegradable plastics. Lactic acid is produced commercially either by chemical synthesis or by microbial fermentation. Approximately 90% of the total lactic acid produced worldwide is made by bacterial fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile. Lactic acid fermentation is a well-studied process that makes lactic acid available as a promising substrate for the production of butyric acid and butanol.

Many anaerobic microorganisms can produce butyric acid from sugars and other carbon sources. Most studied butyric acid producers are the facultative anaerobic bacteria such as *Clostridium*, *Enterobacter*, and *Escherichia* which convert glucose to hydrogen gas, carboxylic acids, and alcohols. [2].

Two physiological stages must be taken into account for *Clostridia*: one for the acidogenic phase, and one for the solventogenic phase. Under batch conditions, the fermentation process of solvent-producing *Clostridium* strains starts with the production of biomass, hydrogen, carbon dioxide, acetic acid, and butyric acid (acidogenesis). As the acid concentration increases (pH decreases), the cell metabolism shifts to solvent production (solventogenesis). The acidogenic cells – able to reproduce themselves – enter the solventogenesis state undergo a morphological change. During solventogenesis, the active cells become endospores unable to reproduce [10].

Lactic acid is not investigated as a suitable substrate for ABE fermentation.

In this study, we investigate the production of butyric acid with lactic acid as the only carbon source.

2. Materials and Methods

A newly isolated strain identified as *Clostridium beijerinckii* 4A1, isolated from chickpea fermentation was used. Reinforced Clostridial Medium (RCM) as a basic medium for the strain maintenance was used. RCM contains (g / l): glucose - 15; yeast extract - 13; peptone - 10; soluble starch - 1; sodium chloride - 5; sodium acetate - 3; cysteine hydrochloride - 0,5; pH = 6,8. The medium was sterilized for 20 minutes at 121ºC.

The inoculum was prepared by growing 1 ml cells' suspension in 10 ml sterile culture medium by incubation for 24 hours at 37°C and anaerobic conditions achieved with Anaerocult® A (Merck Milipore, Germany). Thus prepared inoculum was used in all subsequent experiments.

The effect of lactic acid as a substrate was assessed by adding four different concentrations (5; 10; 15; 20 g/l) to the referred starting media (RCM), keeping the constant concentrations of the other components in the media. The initial pH was adjusted to 6,8 and the medium is distributed in 300 ml Erlenmeyer flasks (200 ml in the flask) and sterilized.

The concentration of the substrate and the products (respectively lactic acid and butyric acid) was determined by HPLC. An Aminex HPX- 87H, 300x7,8 mm, column, and 0,01 N H2SO4 as mobile phase at a flow rate of 0,6 ml/min was used.

The biomass concentration was determined by optical density measurements at 620 nm with a spectrophotometer VWR UV-1600PC.

3. Results and Discussion

Experiments for butyric acid production by *Clostridium beijerinckii 4A1* were carried out with standard RCM where glucose was substituted by the lactic acid.

The results of the experiments with lactic acid as the substrate compared with the results obtained with glucose substrate are presented in Fig. 1.

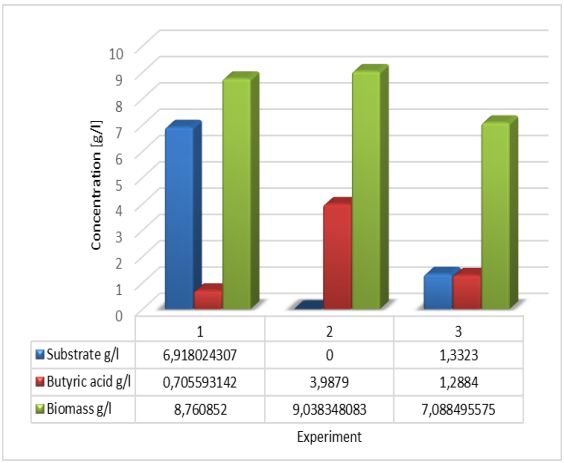

Fig. 1 *Concentration of Substrate, Product and Biomass after 72 hours. 1- substrate 15 g/l glucose, 2 and 3- substrate lactic acid, 5 and 10 g/l respectively.*

It can be seen (Fig. 1) that the accumulation of the target product varies depending on the type and concentration of the carbon source. The most complete consummation of the carbon source is observed in the experiments with 5 g / l lactic acid. In the experiments with a higher concentration of substrate, namely 15 g / l glucose and 10 g / l lactic acid, good microbial growth was observed, but significantly lower production of butyric acid. At a lactic acid concentration of 15 and 20 g / l, inhibition of the butyric acid production process was observed.

Fig.2 and Fig. 3 represent the growth of *Clostridium beijerinckii 4A1* strain, the production of butyric acid, and the depletion of carbon source during fermentation with glucose (15 g/l) and lactic acid (5 g/l).

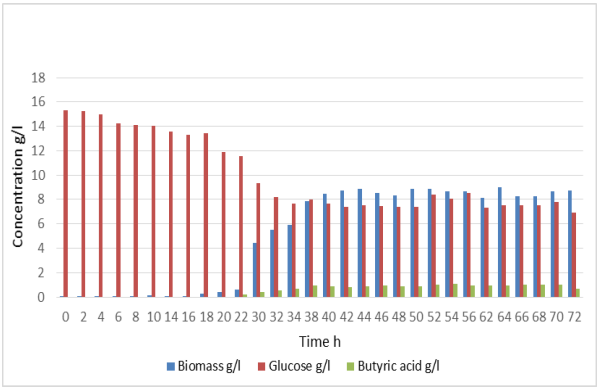

Fig. 2 *Time course of biomass and butyric acid production, and glucose depletion.*

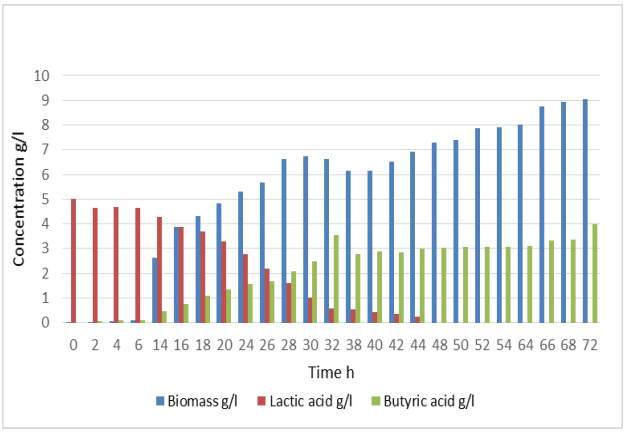

Fig. 3 *Time course of biomass and butyric acid production, and lactic acid depletion.*

As can be seen at both carbon sources, the biomass grows, the exponential phase starts at 25 hours at 15 g / l glucose, while at 5 g / l lactic acid as a substrate, the fermentation is significantly faster (about 14 hours). The stationary phase continues 30 and 45 hours in two processes.

4. Conclusions

The present study has shown that lactic acid can be successfully used as a carbon source for butyric acid production. A process with a low initial concentration of lactic acid as a carbon source is very promising, and a 100 % transformation of the substrate into a product is observed. The experiments with a higher concentration of the substrate, lead to slow growth of the bacterial strain *Clostridium beijerinckii 4A1* and a significant reduction in the butyric acid production. A concentration of lactic acid as a carbon source higher than 10 g / l inhibits the growth of microorganisms and butyric acid production.

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5. References

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