PROPIONIC ACID PRODUCTION FROM SUGARCANE BAGASSE HYDROLYSATE BY *Propionibacterium acidipropionici*

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ABSTRACT

Propionic acid is a carboxylic acid widely used in the chemical, pharmaceutical, and food industries, predominantly obtained through petrochemical routes. However, there is potential to produce it more sustainably using fermentative processes with renewable feedstocks. Sugarcane bagasse, the primary residue of the Brazilian agroindustry, is rich in carbohydrates and represents an excellent option for fermentative acid production. This study aimed to evaluate propionic acid production in pulsed fed-batch fermentation using the bacterium Propionibacterium acidipropionici and alkaline organosolv pretreated sugarcane bagasse hydrolysate as a carbon source. During fermentation, *P. acidipropionici* exhibited a preference for glucose assimilation, with a glucose to cellular biomass conversion factor of 0.21 g/g. The maximum specific growth rate (μx = 0.09 h^{-1}) was observed prior to the first feeding pulse, decreasing to 0.07 to 0.01 h^{-1} after 18 hours of fermentation and subsequent feeding pulses. At the end of fermentation, the bacterial strain achieved a final concentration of propionic acid of 8.76 g/L, with a significant increase in production following the first pulse, along with succinic acid and acetic acid as by-products, with acetic acid being the most predominant. These results underscore the promising potential of this microorganism for industrial applications in fermentation processes utilizing biomass hydrolysates.

Keywords: Biomass hydrolysate. Fed-batch fermentation. Sugarcane bagasse. Biorefinery. Lignocellulose

1 INTRODUCTION

Propionic acid is a three-carbon carboxylic acid with wide-ranging industrial applications. It can be used in pharmaceuticals, herbicides, cosmetics, food preservatives, and as a chemical intermediate in polymer production. Due to its versatility, the global market demand for this organic acid reached 470,000 tons in 2019 and is projected to increase to 550,000 tons by 2026 [1]. Additionally, it is classified as one of the 30 most promising chemicals to be produced from renewable sources by the United States Department of Energy [2]. Currently, its commercial production is primarily through petrochemical routes. However, the demand for microbial production of propionic acid has been increasing alongside the growing demand for more eco-friendly products [3]. Several microorganisms can produce propionic acid via fermentation processes, including bacteria belongs to the genera *Propionibacterium*, *Veillonella*, *Selenomonas*, *Clostridium* and *Fusobacterium.* Among them, *Propionibacterium acidipropionici* stands out as a promising acid-producer, exhibiting higher conversion yields compared to other strains. In addition, these bacteria can utilize several carbon sources, such as glucose, xylose, sucrose, glycerol, and lactate, demonstrating potential to be employed in biotechnological processes that seek to valorize waste materials [4].

However, commercial microbial production of propionic acid remains challenging. The reported fermentation parameters, such as productivity and yield, are still below the levels required for industrial scale-up. Several factors contribute to this low efficiency and high cost, including the low specific growth rate of the bacteria, the formation of by-products like acetic and succinic acids, and the inhibitory effects of propionic acid itself, which exhibits antimicrobial activity [5]. Therefore, to make microbial production of propionic acid economically viable, it is essential to develop fermentation strategies that aim for high titers and productivity, as well as to explore the use of alternative substrate sources. Lignocellulosic biomass is a promising feedstock for the production of sustainable biofuels and building block chemicals. Sugarcane bagasse is the largest residue of the Brazilian agroindustry. According to the Institute of Applied Economic Research (IPEA), Brazil is the world's largest producer and exporter of sugarcane. It is estimated that each year, 5 to 12 million tons of this material remain, which corresponds to approximately 30% of the crushed cane [6]. With a cellulose concentration of approximately 40%, which can be hydrolyzed into glucose, sugarcane bagasse becomes an excellent option for propionic acid production through microbial fermentation processes. Therefore, this study aims to evaluate a pulsed fed-batch fermentation strategy for propionic acid production by *P. acidipropionici* using sugarcane bagasse hydrolysate as carbon source.

2 MATERIAL & METHODS

The bacterial strain used in this study was the *Propionibacterium acidipropionici* ATCC 4875 (American Type Culture Collection). Cells were cultured anaerobically in serum bottles containing Reinforced Clostridial Medium (RCM) at 32 ºC, without agitation. After growth, the cells were stored at - 80 ºC in glycerol 25% until used in the cultivations. For pre-inoculum and inoculum, the standard medium used was composed of (per liter): 10 g of yeast extract, 5 g of tryptic soy broth, 0.25 g of K_2HPO_4, 0.05 g of $MnSO_4.H_2O$, and 3 g (pre-inoculum) or 15 g (inoculum) of glucose. Nitrogen gas was sparged in the serum bottles for 30 min before bacteria inoculation to ensure anaerobic conditions.

Sugarcane bagasse hydrolysate was prepared by glycerol organosolv pretreatment and enzymatic saccharification [7]. The pretreatment was conducted at 210 ºC for 30 min, with 30% of solids and 70% of glycerol. The solubilized liquid fraction (liquor)

was separated for another study application, while the solid fraction was used in the following enzymatic hydrolysis step with the enzymes Cellic Ctec2. The assays were carried out in Erlenmeyer flasks containing solid loading of 10% in 50 mM sodium citrate buffer, pH 4.8, and 15 FPU/g biomass (Filter Paper Activity) of enzyme per gram of biomass. Flasks were incubated in an orbital shaker at 50 °C and 150 rpm for 72 hours. Samples were taken for high-performance liquid chromatography (HPLC) analysis.

Fermentations were conducted in a pulsed fed-batch mode using Bioflo 115 bioreactors (New Brunswick) with a total working volume of 0.75 L. For the pre-inoculum, an aliquot of the stock strain (- 80 ºC) was transferred into the serum bottles and incubated at 32 ºC, without agitation. After 24h, 7 mL of this culture was transferred to the inoculum, which was incubated at same conditions for 24h. Cells were centrifuged at 3000 g for 15 min, washed with sterile 0.1% peptone water and then used to inoculate the bioreactors. The fermentative process was maintained at a temperature of 32 ºC, agitation of 150 rpm, and pH 7.0 controlled by automated addition of NaOH 4 M. The initial volume was 0.3 L of medium composed of (per liter): 10 g yeast extract, 0.25 g K_2HPO_4, 3 g ammonium diphosphate, and 30 g of total sugars from sugarcane bagasse hydrolysate. Two pulses containing sugarcane bagasse hydrolysate supplemented with yeast extract were used in the feeding to readjust the sugar concentration to approximately 30 g/L. Anaerobic conditions were achieved by sparging pure nitrogen for 30 min in the headspace of serum bottles immediately following sterilization or within the media in the bioreactors. Samples were collected, centrifuged and the supernatants filtered through 0.22 µm PVDF syringe filter for further HPLC analysis. The metabolite compounds were quantified using a Shimadzu Prominence LC-20A system equipped with a Refractive Index Detector and an Aminex HPX-87H column. Cell growth was measured by the gravimetric method. The experiments were performed in duplicate.

3 RESULTS & DISCUSSION

The main components of the raw sugarcane bagasse used in this project consist in 47.7% cellulose, 30.2% hemicellulose, 22.9% lignin, 1.4% ash and 1.8% extractives. This biomass was subjected to the organosolv pretreatment with glycerol, and only the resultant solid fraction was reserved for saccharification and subsequent propionic acid production. After enzymatic hydrolysis, it was obtained a hydrolysate containing 59.41 g/L glucose, 9.55 g/L xylose and 1.13 g/L acetic acid, with a yield of glucose per gram biomass of 0.47 g/g.

Figure 1 shows the fermentative performance of the *P. acidipropionici* strain for propionic acid production using the sugarcane bagasse hydrolysate. From the hydrolysate obtained in the saccharification step, a fermentative medium was formulated containing initially around 30 g/L of total sugars (22.16 g/L glucose, 3.92 g/L xylose and 0.18 g/L arabinose). It is possible to note that *P. acidipropionici* was not able to completely consume the available carbon sources present in the hydrolysate during the period of cultivation. Glucose was preferentially assimilated, whereas xylose and arabinose content remained stable over time. The conversion factor of glucose into cell biomass was 0.21 g/g. The maximum specific growth rate was observed before the first feeding (μ_x = 0,09 h^{-1}). After 18h of fermentation, the specific growth rate dropped, achieving 0.07 to 0.01 h^{-1} after feeding pulses 1 and 2.

Propionic acid production by the bacterial strain reached a final concentration of 8.76 g/L at the end of the fermentation, with a significant increase in the acid production after 20 h (first pulse). In addition to propionic acid, the strain also produced succinic acid and acetic acid, with acetic acid being the main by-product. In the Table 1 is presented the kinetic parameters of the fermentation process. Regarding propionic acid production, the conversion factor of glucose into the organic acid ($Y_{PA/S}$) and the volumetric productivity were of 0.34 g/g and 0.07 g/L/h, respectively.

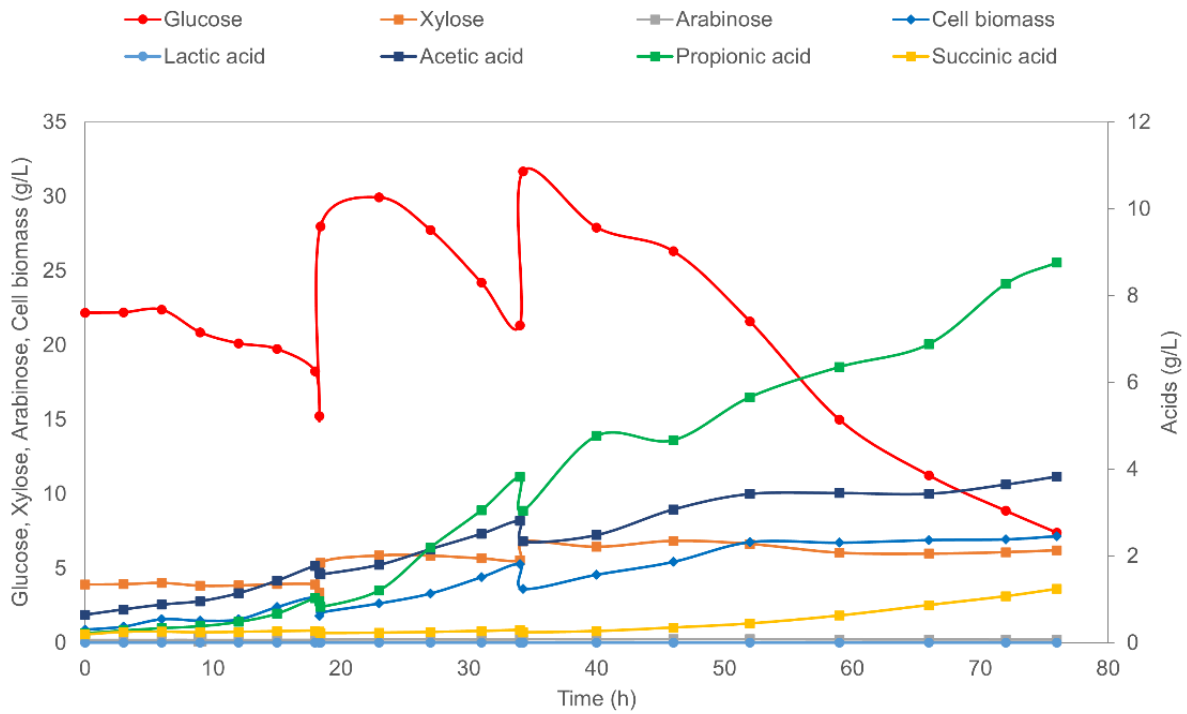

Figure 1 Pulsed fed-batch fermentation of sugarcane bagasse hydrolysate by *P. acidipropionici* in bioreactor. The fermentative assay was carried out in anaerobiosis at 32 ºC, 150 rpm, and pH 6.8 ± 0.2, for 76h, with pulses feeding at 18 and 34h.

Propionic acid yields from 0.30 to 0.7 have been reported in the literature depending on the substrate (such as glucose, lactate, glycerol, and molasses) and the fermentation operation mode employed [4,8]. For instance, using molasses as substrate, a propionic acid concentration of 27 g/L, with a productivity of 0.38 g/L/h and a yield of 78% was achieved in high cell density and fed-batch fermentation with the *P. acidipropionici* strain ATCC 4875. Notably, higher productivity and yields were attained with a derived mutant of this strain, indicating that chemical mutagenesis and evolutionary engineering are effective and relevant strategies for strain improvement [8]. Exploring immobilized cells and repeat-batch and fed-batch fermentations, high yield and productivity was also observed previously using sugarcane bagasse hydrolysate [9].

Table 1 Kinetic parameters of the pulsed fed-batch fermentation by *P. acidipropionici* ATCC 4875.

Parameters	Values
Initial sugar content (g/L)	26.27*
Glucose (g/L)	22.16 ± 1.26
Xylose (g/L)	3.92 ± 0.22
Arabinose (g/L)	0.18 ± 0.00
Cell biomass (g/L)	7.16 ± 0.58
μ_{max} (h^{-1})	0.09**
$Y_{X/S}$ (g/g)	0.21
Propionic acid (g/L)	8.76 ± 0.29
$Y_{P/S}$ (g/g)	0.34
Q_P (g/L/h)	0.07
Yield (%)	61.8***
Succinic acid (g/L)	1.24 ± 0.22
Acetic acid (g/L)	3.83 ± 0.49

* Total sugars from sugarcane bagasse hydrolysate at 0h.
** Value related to specific growth rate before first pulse.
***The value 0.55 g/g corresponds to the stoichiometric conversion of glucose into propionic acid.

4 CONCLUSION

This study demonstrated the feasibility of employing the bacterial strain *P. acidipropionici* for propionic acid production from sugarcane bagasse hydrolysate. Under the tested conditions, a propionic acid concentration of 8.76 g/L was obtained. Although this value is lower compared to those reported in the literature, these results revealed the potential of sugarcane bagasse hydrolysate as a sustainable substrate for propionic acid production. Several approaches can be explored to enhance the organic acid production by this strain. Further research could focus on optimizing fermentation media and feeding strategies, using high cell densities in the fermentation process as well as utilizing genetic engineering strains or mutants more tolerant to the acid.

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