VOLATILE FATTY ACID PRODUCTION VIA CONSOLIDATED BIOPROCESSING: EXPLOITING LIGNOCELLULOSIC MATERIALS WITH *CLOSTRIDIUM* STRAINS

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ABSTRACT

Volatile fatty acids (VFAs) are precursors of great interest to several industrial sectors and can be produced by strains of the *Clostridium* genus through the optimization of fermentation processes. However, the industrial production of these acids through biotechnological routes is still a challenge that requires efficient and low-cost solutions. The current paradigm shift towards environmentally and economically sustainable methods, such as the conversion of lignocellulosic materials (LMs) into fuels or chemicals with high added value, is efficiently represented by the consolidated bioprocessing (CBP) strategy. This innovative strategy consolidates enzyme production, biomass hydrolysis, and sugar fermentation into a single unitary operation. However, the industrial production of VFAs from LMs with high yield and productivity by CBP is still a challenge. Studies that establish solid and economically viable strategies for implementing these Clostridial strains and using LMs in CBP are essential. In this context, this study aims to evaluate the current state of the art on the use of LMs as substrate by strains of the genus *Clostridium* and in the development of integrated fermentation processes to produce VFAs within the scope of CBP. The study has the potential to contribute to the advancement of sustainable production of biofuels and chemical products with high added value, and the development of more efficient and economical industrial processes.

Keywords: Bioprocessing. Circular bioeconomy. Lignocellulosic materials. Biorefinery.

1 INTRODUCTION

The primary challenge in producing cellulosic biofuels and high-value-added chemicals is converting lignocellulosic materials (LM) into products that are both cost-effective in downstream processing and significant productivity[1]. In recent years, several research have focused on the solubilization of carbohydrate components found in non-food LM, such as agro-industrial and forestry residues[2–4]. These studies explore various technologies, including the biological and non-biological conversion of different raw materials, such as straw, sugarcane bagasse, and corn cobs[5]. The most common biological strategies are simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF), both utilizing fungal-derived cellulases added to the process as biocatalysts. Currently, there is a trend towards generating products via biotechnological routes where few unit operations are needed: consolidated biosaccharification (CBS), and consolidated bioprocessing (CBP), which are integrated processes. It is essential to emphasize that CBP represents an innovative approach, integrating all biologically catalyzed steps (e.g., enzyme production, biomass hydrolysis, and sugar fermentation) in a single operation, that is, occurs in one reactor[6]. Furthermore, CBP has the potential to lower both capital and operational costs associated with the biosynthesis of high-value products from LM. Nevertheless, multiple factors are required to properly understand the utilization of LM in CBP[7]. These include the biophysical and kinetic role of microbial cells in cell-enzyme-substrate complexes, the conversion of solubilized products to sustain microbial growth, bioenergetic and metabolic control in enzyme production, microorganism ability to degrade plant cell walls and strategies for optimizing carbohydrate solubilization[5,7,8]. The effects of industrial and scale-up conditions on microbial activity and the dynamics of microbial co-cultures on the fermentation of LM are also relevant[5].

Clostridium species are notable for their ability to produce volatile fatty acids (VFAs) at high concentrations, yields, and productivity from various substrates (e.g., glucose, xylose, lactose, and glycerol)[9]. Additionally, these microorganisms exhibit resilience to a wide array of environmental conditions, such as elevated temperatures, low pH levels, and anaerobic environments, rendering them suitable for application in CBP[6]. The *in situ* production of enzymes and enzyme complexes by these microorganisms positions CBP as an emerging methodology for the synthesis of biofuels and fine chemicals[10]. Nonetheless, the identification and development of microorganisms capable of optimally performing all these functions remain a significant challenge in research and development[3].

Short-chain VFAs are essential raw materials for a range of industrial applications[11]. VFAs have a wide range of applications in the chemical industry (e.g., as a fiber additive, polymer monomer, and flame retardant), pharmaceutical industry (e.g., anti-anxiety and inflammation suppression), perfumery (e.g., bactericide, synthetic perfume, and extractor), and animal feed (e.g., promoting water and sodium absorption, maintaining gastrointestinal microecological balance, and promoting epithelial proliferation)[12]. The recurring challenge is to improve industrial production of VFAs from LM, to attain commercially acceptable titration and productivity levels. Several publications have described the fermentative production of VFAs from LM, such as rice straw[13,14], hemp[15], switchgrass[16] and sugarcane bagasse[17,18]. However, to our knowledge, there are insufficient reports on the optimized production of VFAs by *Clostridium* strains using untreated LM as substrate in CBP. In this context, behind a literature review, this study evidences different strains for CBP using *Clostridium* strains and offers a systematic comparative analysis between them.

2 LITERATURE SURVEY

Literature survey of *Clostridium* strains producing VFAs: To identify *Clostridium* strains capable of producing VFAs, a comprehensive systematic review was conducted in the Scopus and Web of Science databases. This review included selection criteria using booleans ("*Clostridium*" AND "consolidated bioprocessing" AND "lignocellulosic materials"), covering studies published between 2010 and 2022. Therefore, 20 related documents and 12 patents were selected. The inclusion criteria were based on the use of LM and bacteria from the *Clostridium* genus in CBP. Studies that involved synthetic media or genetically modified microorganisms were excluded. The search considered the title, the abstract, and the keywords of the published works.

3 THE POTENTIAL OF CBP FOR THE PRODUCTION OF VFA

It is commonly known that *Clostridium* strains possess two essential characteristics for biotechnological applications: their biphasic metabolism and their effectiveness in breaking down LM. In the first acidogenic phase of acetone-butanol-ethanol (ABE) fermentation, when the production of primary products, including lactate, acetate, and butyrate[3]. There are *Clostridium* strains that exclusively carry out acidogenesis and do not complete the full ABE fermentation process. These strains are referred to as acidogenic *Clostridium*. During metabolic transitions, these acids are used in the production of solvents to produce butanol, acetone, and ethanol. This metabolic shift suggests an adaptation process that is not completely understood. It is linked to changes in extracellular pH and the commencement of sporulation[19]. These bacteria capacity to withstand a variety of environmental factors, such as high temperatures, low pH, and oxygen deprivation, is essential for their viability in CBP applications[3]. Therefore, the unique resistance and adaptation characteristics of *Clostridium* are extremely relevant for optimizing the efficacy and sustainability of CBP processes. Figure 1 displays a detailed diagram that establishes the correlation between LM (brown) and the *Clostridium* genus strains (blue) employed in CBP strategies, as reported in selected scientific publications during the 2010 to 2022 interval.

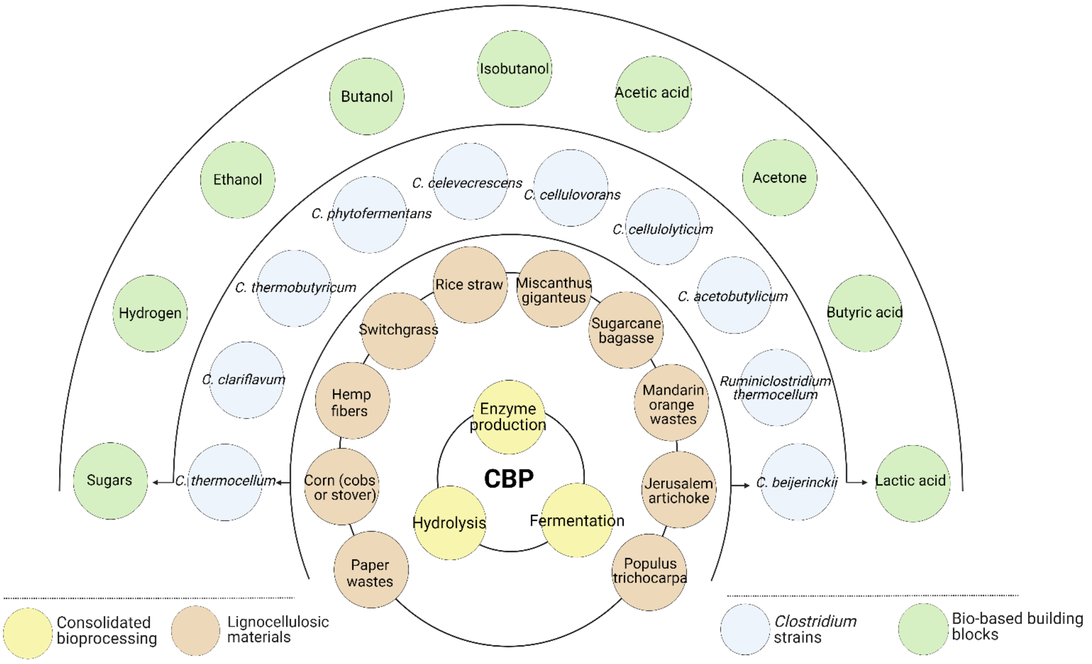

Figure 1 Diagram of the lignocellulose materials (brown) and the *Clostridium* genus strains (blue) employed in CBP strategies, as reported in selected scientific publications during the 2010 to 2022 interval

Over the past decade, *C. thermocellum* has emerged as a pivotal microorganism in CBP[13]. Its ability to collaborate with other bacterial species in co-cultures enables the synergistic exploitation of complex substrates (e.g., rice straw and corn cobs), resulting in the effective synthesis of VFAs. The versatility of *C. thermocellum* in biomass conversion positions it as an essential component in optimizing CBP operations for energy sustainability and the production of high-value chemicals[6]. Other *Clostridium* genus strains - e.g., *C. cellulovorans*, *C. thermobutyricum, C. acetobutylicum, C. thermoaceticum*, *C. beijerinckii*, *C. clariflavum*, and *C. phytofermentans* -, are also gaining prominence in CBP[20,21]. These strains have demonstrated significant efficiency in processing LM, thereby expanding the available options and diversifying the methodologies applied in advancing CBP processes. They demonstrated remarkable efficiency in processing LM by contributing to expand available options and diversifying the methodologies applied in advancing CBP. It is noteworthy that the strategy of employing multifaceted microorganisms in an integrated process aligns seamlessly with the biorefinery concept. This approach not only enhances the efficiency of ethanol production from LM but also facilitates the integrated production of various high-value products such as VFAs[22,23]. Short-chain VFAs, such as butyric acid and acetic acid, are commonly produced by various bacterial genera, (e.g., Acetobacter, *Thermoanaerobacter*, *Butyribacterium*, *Butyrivibrio*, and *Clostridium* spp.)[24,25]. Among these, the genus *Clostridium* stands out for industrial applications due to its high efficiency in fermenting these compounds, particularly in the production of butyrate. The most extensively studied strains for large-scale industrial production of butyric acid include *C. butyricum*, *C. tyrobutyricum*, and *C. thermobutyricum*. In particular, *C. tyrobutyricum* is considered the most promising microorganism for the bioproduction of butyric acid, reaching the highest titer recorded to date (86.9 g/L)[26]. This strain is capable of producing and tolerating high concentrations of butyric acid with relative purity, although it has a more limited capacity to metabolize a wide range of substrates compared to *C. butyricum* and *C. thermobutyricum*[13]. Nevertheless, challenges remain, such as optimizing growth conditions and broadening the range of microorganisms with lignocellulolytic properties. Despite these challenges, the utilization of native microorganisms for CBP represents a viable and sustainable option for large-scale production.

4 CONCLUSION

In conclusion, with the systematic analysis, it was possible to highlight the growing role of *Clostridium* strains in developing CBP, particularly in producing biofuels such as ethanol and butanol. There is still a gap in the literature on producing VFAs from lignocellulosic materials through CBP by *Clostridium* strains. The efficiency of *C. thermocellum* and other strains, such as *C. cellulovorans* and *C. thermobutyricum*, demonstrates the feasibility of harnessing complex agricultural substrates (pre-treated or not) for the sustainable production of biofuels and valuable chemicals. Acidogenic clostridial strains have the potential to enable new strategies in the production of VFA in CBP processes Diversifying strategies and continuously optimizing these processes suggest a promising future for industrial biotechnology, with significant implications for energy sustainability and innovation in green chemistry.

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