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The Biochemical Basis of Ethanol Fermentation and Its Industrial Applications

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Abstract This study explored the application of ethanol fermentation in industry, especially in biofuel production and waste disposal. The study highlights several key discoveries in the field of ethanol fermentation. It was demonstrated that the aldehyde: ferredoxin oxidoreductase (AOR) enzyme is critical for ethanol formation in acetogenic bacteria, and inactivation of the bi-functional aldehyde/alcohol dehydrogenase (AdhE) significantly enhances ethanol production. Additionally, the metabolic pathways and regulatory mechanisms of ethanol-H_2 co-production in anaerobic bacteria were elucidated, revealing the importance of FeFe-hydrogenases and pyruvate ferredoxin oxidoreductase (PFOR) in this process. Thermodynamic analyses identified bottlenecks in the ethanol production pathway from cellobiose in *Clostridium thermocellum*, suggesting potential genetic interventions to improve ethanol yield. Furthermore, metabolic engineering of *Geobacillus thermoglucosidasius* successfully diverted carbon flux towards ethanol production, achieving high yields under thermophilic conditions. The conservation and regulation of ethanol fermentation pathways in land plants were also examined, showing that while ethanol production is conserved, its regulation varies across plant species. The findings of this study underscore the versatility and industrial potential of ethanol fermentation. By understanding and manipulating the biochemical pathways involved, it is possible to enhance ethanol production for biofuel applications and improve waste treatment processes. These insights pave the way for future research and development in metabolic engineering and anaerobic biotechnology.

Keywords Ethanol fermentation; Biofuel production; Metabolic engineering; Anaerobic biotechnology; Acetogenic bacteria; Thermophilic conditions; Regulatory mechanisms

1 Introduction

Ethanol fermentation is a biochemical process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy, producing ethanol and carbon dioxide as by-products. This process is primarily carried out by microorganisms like yeast and certain bacteria. *Saccharomyces cerevisiae*, commonly known as baker's yeast, is the most widely used organism for ethanol production due to its high efficiency in converting sugars to ethanol (Chandrakant and Bisaria, 1998; Madhavan et al., 2012; Nakanishi et al., 2017). The fermentation process can utilize various feedstocks, including lignocellulosic biomass, which is abundant and renewable, making it a promising substrate for sustainable ethanol production (Chandrakant and Bisaria, 1998; Hahn-hägerdal et al., 2007; Madhavan et al., 2012).

Ethanol fermentation holds significant industrial and economic importance. It is a cornerstone of the biofuel industry, providing a renewable alternative to fossil fuels. The production of ethanol from biomass not only helps in reducing greenhouse gas emissions but also promotes energy security and rural development by utilizing agricultural residues and other waste materials (Kim et al., 2012; Nakanishi et al., 2017; Cortivo et al., 2020). Additionally, ethanol is a valuable chemical feedstock and is used in the production of various industrial chemicals, pharmaceuticals, and beverages (Bai et al., 2008; Maicas, 2021). The development of efficient fermentation technologies and engineered microbial strains has further enhanced the economic viability of ethanol production, making it a key player in the global bioeconomy (Hahn-hägerdal et al., 2007; Crespo et al., 2012; Zhang et al., 2016).

This study aims to provide a comprehensive overview of the biochemical basis of ethanol fermentation and its industrial applications. The specific objectives are to discuss the key steps involved in the uptake and metabolism of various sugars by fermenting organisms, with a focus on both hexose and pentose sugars, highlight the challenges and bottlenecks in the fermentation process, particularly in the context of lignocellulosic biomass conversion, review the recent advancements in metabolic engineering and fermentation strategies that have been developed to improve ethanol yield and productivity, and explore the industrial applications of ethanol fermentation, including its role in biofuel production and other sectors.

2 Historical Background

2.1 Early developments in ethanol production

The history of ethanol production is deeply intertwined with the development of human civilization. Alcoholic fermentation is considered one of the oldest biotechnological processes, predating recorded history. The use of yeast, particularly *Saccharomyces cerevisiae*, has been central to this process. This yeast has been utilized for millennia in the production of alcoholic beverages such as beer and wine, highlighting its cultural and social significance (Carmona-Gutierrez et al., 2012). The fundamental biochemical process involves the conversion of carbohydrates, such as starch or sugar, into alcohol or acid, a method that has been refined over thousands of years to produce consistent and high-quality alcoholic beverages (Maicas, 2021).

2.2 Evolution of fermentation technologies

Over time, the technologies and methodologies surrounding fermentation have evolved significantly. Initially, fermentation was a natural process with little control over the variables involved. However, as understanding of the biochemical processes improved, so did the ability to manipulate and optimize these processes. The introduction of specific yeast strains, such as *Saccharomyces cerevisiae*, and the control of fermentation conditions like temperature and sugar content, have allowed for more predictable and efficient production of ethanol (Maicas, 2021). These advancements have not only improved the quality of alcoholic beverages but have also paved the way for the use of fermentation in other industrial applications, such as biofuel production (Carmona-Gutierrez et al., 2012).

2.3 Transition to industrial-scale production

The transition from small-scale, artisanal production to industrial-scale ethanol production marked a significant milestone in the history of fermentation. This shift was driven by the need for large quantities of ethanol, both for alcoholic beverages and as a biofuel. Industrial-scale production requires the optimization of fermentation performance to ensure economic feasibility. This includes addressing challenges such as yeast stress due to high osmotic pressure, pH changes, and the accumulation of fermentation products, which can hinder yeast growth and survival. By improving the tolerance of yeast cells to these stresses, the efficiency of the fermentation process can be significantly enhanced, making large-scale ethanol production more viable and sustainable (Carmona-Gutierrez et al., 2012).

3 Biochemical Pathways of Ethanol Fermentation

3.1 Glycolysis: the initial step

Glycolysis is the first step in the ethanol fermentation process, where glucose is broken down into pyruvate. This pathway, known as the Embden-Meyerhof-Parnas (EMP) pathway, involves a series of enzymatic reactions that convert glucose into two molecules of pyruvate, generating a net gain of two ATP molecules and two NADH molecules. In some hyperthermophilic Archaea, such as *Pyrococcus furiosus*, modifications to the glycolytic pathway have been observed, where the typical intermediate 1,3-bisphosphoglycerate is absent, altering the energy yield and redox balance (Straub et al., 2020).

3.2 Pyruvate decarboxylation to acetaldehyde

The decarboxylation of pyruvate to acetaldehyde is catalyzed by the enzyme pyruvate decarboxylase (PDC). This reaction releases one molecule of CO_2 and converts pyruvate into acetaldehyde. PDC is a key enzyme in this process and has been studied in various organisms, including thermoacidophilic Archaea, where it exhibits thermostable and oxygen-stable properties, making it suitable for industrial applications (Alharbi et al., 2022). In plants, PDC also plays a crucial role in energy production under anaerobic conditions (Bui et al., 2019) (Figure 1).

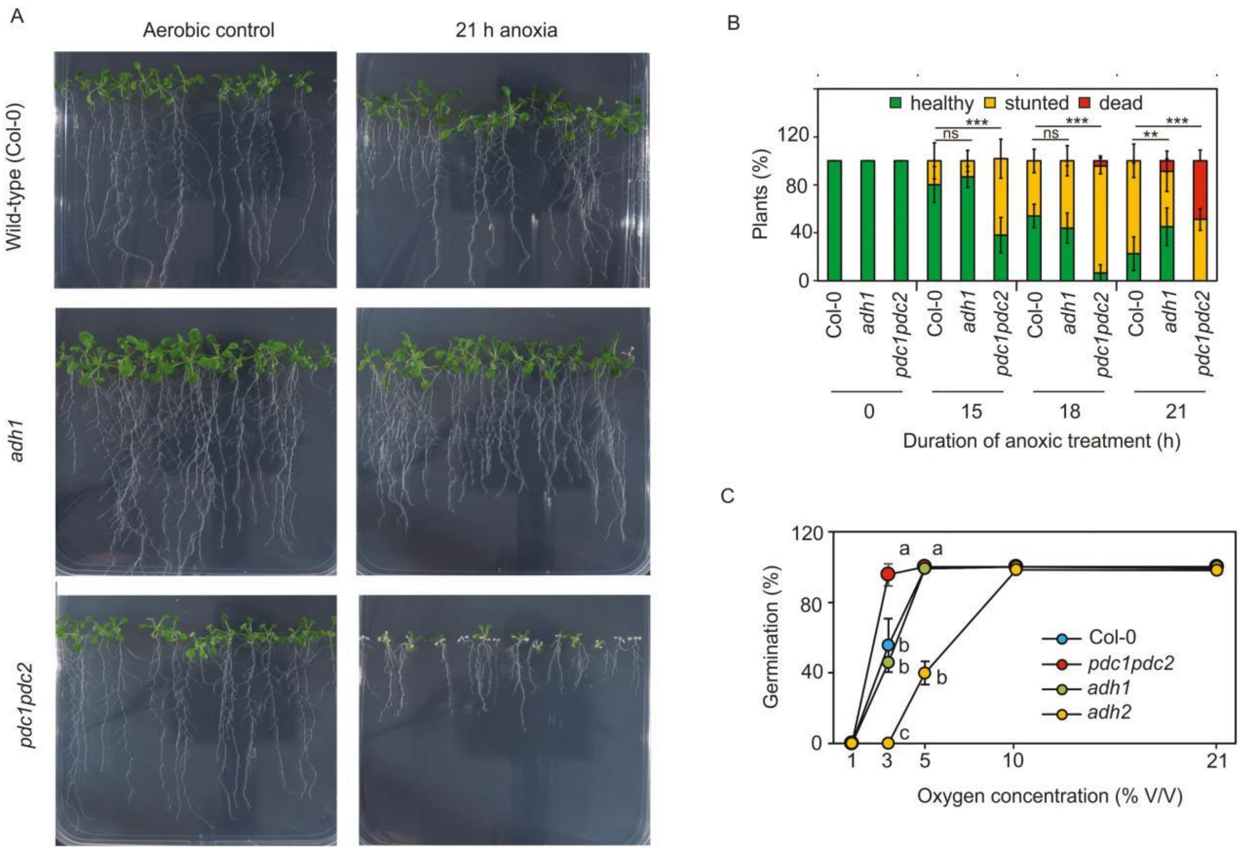

Figure 1 Contribution of hypoxia-inducible ADH and PDC genes to low oxygen conditions (Adopted from Bui et al., 2019)

Image caption: (A) Phenotypic difference of plants treated with 21 h with anoxia. (B) Progressive scoring as arbitrary units of wild type (Col-0) plants and adh1 or pdc1pdc2 knock-out mutants subjected to an anoxic treatment of a variable duration. Comparison among different Arabidopsis genotypes was evaluated by the proportion of observations in categories applying a $\chi 2$ test. (C) Germination percentage of wild-type and adh1, adh2, and pdc1pdc2 seeds under variable oxic atmospheres after seven days. Statistical significance of the differences observed between genotypes at each time point was assessed by 1-way ANOVA followed by Holm-Sidak post-hoc test (n=5) (Adopted from Bui et al., 2019)

3.3 Conversion of acetaldehyde to ethanol

The final step in ethanol fermentation involves the reduction of acetaldehyde to ethanol, a reaction catalyzed by alcohol dehydrogenase (ADH). This step also regenerates NAD+ from NADH, which is essential for the continuation of glycolysis. The bifunctional enzyme AdhE, found in bacteria, catalyzes both the conversion of acetyl-CoA to acetaldehyde and the subsequent reduction to ethanol, highlighting its importance in bacterial ethanol fermentation (Pony et al., 2020).

3.4 Role of enzymes in the fermentation process

Enzymes play a critical role in the ethanol fermentation process. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) are the primary enzymes involved. In some organisms, such as *Thermoanaerobacterium saccharolyticum*, both pyruvate kinase (PYK) and pyruvate phosphate dikinase (PPDK) are involved in the conversion of phosphoenolpyruvate to pyruvate, indicating the complexity and redundancy of the metabolic pathways (Cui et al., 2020). Additionally, the filamentation of AdhE in bacteria is essential for its enzymatic activity and regulation, which is crucial for efficient ethanol production (Pony et al., 2020).

3.5 Energy yield and byproducts

The energy yield of ethanol fermentation is relatively low compared to aerobic respiration, with a net gain of only two ATP molecules per glucose molecule. However, the process is advantageous under anaerobic conditions where oxygen is limited. Byproducts of ethanol fermentation include CO_2, which is released during the decarboxylation of pyruvate, and other metabolites such as acetaldehyde. In some engineered strains, efforts have been made to reduce ethanol byproducts and increase the yield of other valuable chemicals, such as 2,3-butanediol, by manipulating the carbon flux at key metabolic branching points (Ishii et al., 2018).

By understanding these biochemical pathways and the role of specific enzymes, researchers can optimize ethanol fermentation processes for industrial applications, improving efficiency and yield (Straub et al., 2020; Wang et al., 2020; Alharbi et al., 2022).

4 Microbial Organisms in Ethanol Fermentation

4.1 Yeasts: saccharomyces cerevisiae and others

Yeasts, particularly *Saccharomyces cerevisiae*, are the most commonly used microorganisms in ethanol fermentation due to their high ethanol tolerance and efficient fermentation capabilities. *S. cerevisiae* has been extensively studied and genetically modified to enhance its ethanol production, especially in the context of consolidated bioprocessing (CBP) where it is engineered to express cellulases for direct fermentation of cellulose to ethanol. Additionally, other yeast species have been explored for their potential in ethanol production, often focusing on improving their fermentation efficiency and stress tolerance through genetic modifications (Liu et al., 2018).

4.2 Bacteria: zymomonas mobilis and others

Zymomonas mobilis is a highly efficient ethanologenic bacterium that has garnered significant attention for its potential in industrial ethanol production. Unlike *S. cerevisiae*, *Z. mobilis* metabolizes sugars through the Entner-Doudoroff pathway, which results in less ATP and biomass production, thereby channeling more sugar towards ethanol production. This bacterium can be engineered for pentose metabolism without cofactor imbalance, making it a robust candidate for cellulosic ethanol production (Xia et al., 2019). *Z. mobilis* also exhibits several advantageous traits such as high ethanol yield, rapid fermentation rates, and the ability to tolerate high concentrations of ethanol and other inhibitory compounds (Todhanakasem et al., 2020; Fuchino et al., 2021). Other bacteria, such as Lactobacillus amylovorus, have been shown to improve ethanol yields in mixed cultures with yeasts by cross-feeding metabolites like acetaldehyde (Lino et al., 2020).

4.3 Genetic modifications and strain improvement

Genetic engineering plays a crucial role in enhancing the ethanol production capabilities of both yeasts and bacteria. For instance, *S. cerevisiae* has been genetically modified to express recombinant cellulases, enabling it to directly ferment cellulose to ethanol (Liu et al., 2018). Similarly, *Z. mobilis* has been subjected to various genetic modifications to improve its ethanol yield, stress tolerance, and substrate utilization. Advanced genetic tools, including mutation techniques, genome editing, and metabolic engineering, have been employed to optimize *Z. mobilis* strains for industrial applications (Todhanakasem et al., 2020). Additionally, the availability of genome sequence information for multiple *Z. mobilis* strains has facilitated targeted genetic modifications (Xia et al., 2019).

4.4 Comparison of different microbial systems

When comparing different microbial systems for ethanol fermentation, several factors need to be considered, including ethanol yield, fermentation rate, substrate range, and tolerance to inhibitory compounds. *S. cerevisiae* is well-known for its robustness and high ethanol tolerance, making it a preferred choice for many industrial applications. However, *Z. mobilis* offers several advantages, such as higher ethanol yields and faster fermentation rates due to its unique metabolic pathways (Xia et al., 2019; Todhanakasem et al., 2020). Moreover, *Z. mobilis* produces fewer by-products and has a lower biomass yield, which can be beneficial for industrial processes (Szambelan et al., 2023). The choice of microbial system often depends on the specific requirements of the fermentation process, including the type of feedstock and the desired product characteristics. Genetic modifications and strain improvements continue to enhance the performance of both yeasts and bacteria, making them increasingly competitive for various industrial applications (Liu et al., 2018; Li et al., 2020a; Todhanakasem et al., 2020).

5 Industrial Applications of Ethanol Fermentation

5.1 Biofuel production

Ethanol fermentation plays a crucial role in the production of biofuels, which are essential for reducing reliance on fossil fuels and mitigating climate change. Biofuels derived from ethanol fermentation can be categorized into first-generation and second-generation biofuels.

First-generation biofuels are produced from food crops such as sugar cane, sugar beet, and corn (Huang, 2024). These crops are rich in sucrose and starch, which are fermented by yeast, primarily *Saccharomyces cerevisiae*, to produce ethanol. This ethanol can then be used as a renewable fuel source. The production of first-generation biofuels is well-established and provides a significant portion of the global biofuel supply (Tse et al., 2021a; Vinotha et al., 2023).

Second-generation biofuels are produced from lignocellulosic biomass, which includes agricultural residues, wood chips, and other non-food plant materials. These biofuels are more sustainable as they do not compete with food supply. The production process involves pretreatment to break down the complex carbohydrates into fermentable sugars, followed by fermentation using genetically engineered microorganisms capable of converting a wide range of sugars into ethanol. This approach helps in utilizing waste materials and reducing greenhouse gas emissions (Robak and Balcerek, 2018; Nanda et al., 2023).

5.2 Alcoholic beverage industry

Ethanol fermentation is the cornerstone of the alcoholic beverage industry. Yeast fermentation of sugars derived from grains, fruits, and other plant materials produces ethanol, which is the primary alcohol in beverages such as beer, wine, and spirits. The process not only produces ethanol but also contributes to the flavor and aroma of the beverages through the production of various secondary metabolites (Tse et al., 2021a).

5.3 Pharmaceutical and chemical industries

In the pharmaceutical and chemical industries, ethanol fermentation is used to produce a variety of compounds. Ethanol itself is a valuable solvent and disinfectant. Additionally, the fermentation process can yield other valuable chemicals such as organic acids, glycerol, and acetone. These compounds are used in the manufacture of pharmaceuticals, cosmetics, and industrial chemicals, highlighting the versatility of ethanol fermentation beyond biofuel production (Tse et al., 2021a; Shanmugam et al., 2023) (Figure 2).

5.4 Emerging applications

Emerging applications of ethanol fermentation include the production of high-value biochemicals and biofuels through advanced microbial and chemical processes. For instance, ethanol can be used as an electron donor in microbial fermentation to produce medium-chain fatty acids, which have applications in the food, pharmaceutical, and chemical industries (Sarkar et al., 2021). Additionally, integrated processes combining fermentation with other chemical treatments can enhance the yield and quality of biofuels, such as biodiesel from microalgae (Rahman et al., 2019a; 2019b). These innovative approaches are expanding the potential of ethanol fermentation in various industrial sectors.

6 Technological Advances in Ethanol Fermentation

6.1 Process optimization

Process optimization in ethanol fermentation has seen significant advancements, particularly in the context of high gravity (HG) and very high gravity (VHG) conditions. These methods have been developed to achieve higher ethanol concentrations, exceeding 15% v/v, by using saccharine and starchy substrates. Researchers have also explored unconventional and cost-effective substrates, as well as nitrogen supplements, to enhance the fermentation process. Additionally, the selection of industrial strains, flocculating yeasts, and the construction of novel strains with osmotolerance and high ethanol yield capabilities have been prioritized. Process control aspects such as redox potential and dissolved CO_2 profiling, along with optimization and modeling strategies, have further contributed to the cost-effectiveness and efficiency of ethanol production (Puligundla et al., 2019).

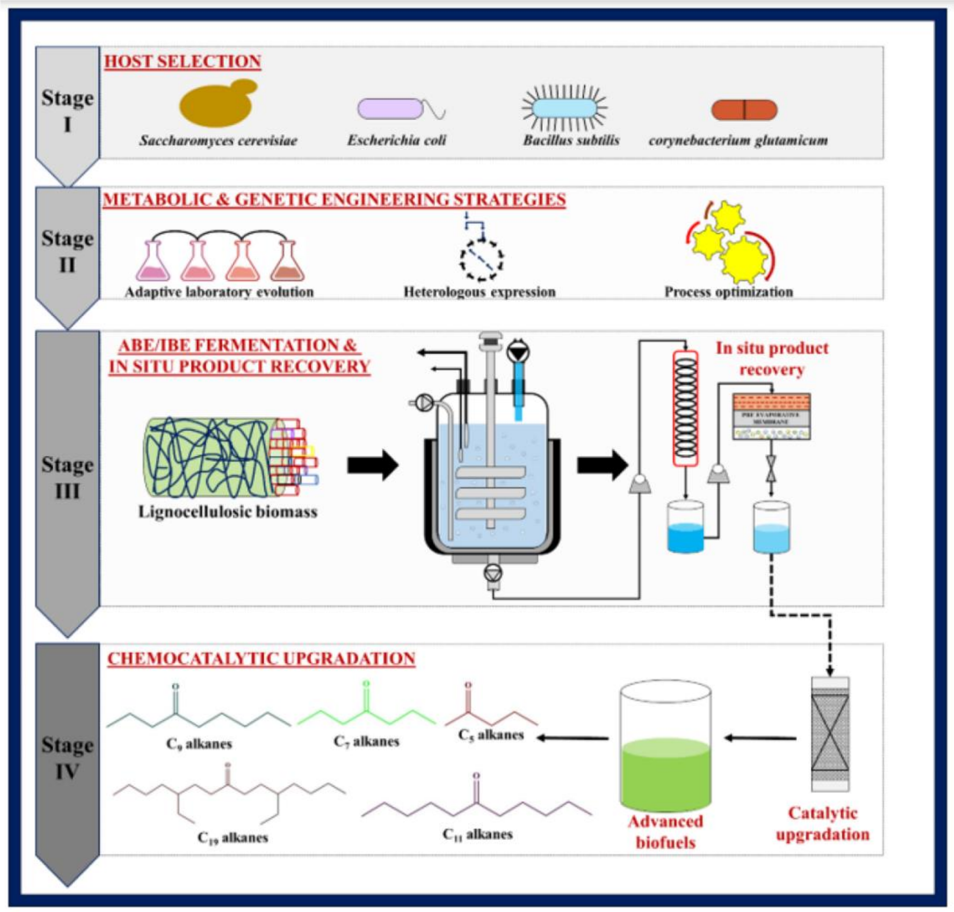

Figure 2 Integrated chemo and bio-catalytic approaches for advanced biofuel production from ABE fermentation (Adopted from Shanmugam et al., 2023)

6.2 Fermentation technology innovations

Innovations in fermentation technology have focused on consolidated bioprocessing (CBP) and simultaneous saccharification and fermentation (SSF). CBP technologies, particularly for raw starch, have been developed using amylolytic *Saccharomyces cerevisiae* strains. These advancements have led to improved enzyme combinations, alternative feedstocks, and novel host strains, although further research is needed for industrial-scale application (Cripwell et al., 2020). SSF has also been applied to lignocellulosic biomass, addressing challenges such as the recalcitrance of biomass and the need for efficient pretreatment technologies. The integration of saccharification and fermentation processes has been refined to enhance ethanol yields and reduce energy consumption (Liu et al., 2019).

6.3 Integration with other biotechnologies

The integration of ethanol fermentation with other biotechnologies has opened new avenues for sustainable production. Gas fermentation using carbon-fixing microorganisms has been commercialized, offering a scalable solution for converting waste gases to ethanol. This process leverages genetic tool development and metabolic engineering to expand the product spectrum and improve strain development for acetogens and other nonmodel organisms (Fackler et al., 2021). Additionally, the use of sequential fermentations, where acetogenic bacteria convert C1 compounds into acetate and ethanol, followed by further conversion into higher-value products by aerobic organisms, has shown promise in creating a sustainable biotechnology framework (Stark et al., 2022).

6.4 Scale-up challenges and solutions

Scaling up ethanol fermentation processes from laboratory to industrial scales presents several challenges, including maintaining efficiency and yield. Single-step ethanol production from raw cassava starch has been successfully scaled up from 5-L laboratory fermenters to 3000-L industrial fermenters, achieving high yields and productivities comparable to commercial industries. This process involves the combination of raw starch hydrolysis and fermentation, with careful optimization of conditions and technical procedures to ensure scalability (Krajang et al., 2021). Additionally, the use of pervaporation (PV) technology for the simultaneous production and extraction of bio-chemicals has been explored, although large-scale implementation faces constraints such as cost, specificity, fouling, and energy consumption. Innovations in PV-fermentation configurations and anti-biofouling membranes offer potential solutions to these challenges (Serna-Vázquez et al., 2021).

7 Economic and Environmental Impact

7.1 Cost-efficiency in ethanol production

Ethanol production's cost-efficiency is influenced by various factors, including feedstock type, process technology, and scale of production. Studies have shown that first-generation ethanol production from sugarcane bagasse and other lignocellulosic biomass can be optimized to achieve high ethanol yields at relatively low costs. For instance, the use of simultaneous saccharification and fermentation (SSF) processes has demonstrated significant cost reductions, achieving up to 92% of theoretical ethanol yield in batch processes and 88% in fed-batch processes with reduced enzyme loads (Guilherme et al., 2019). Additionally, integrating products from enzyme-assisted aqueous extraction processing (EAEP) of soybeans into corn-based ethanol fermentation can enhance economic returns despite slightly higher production costs, due to the increased quantities of ethanol and valuable by-products produced (Rosentrater and Zhang, 2021).

7.2 Environmental benefits and concerns

Ethanol production from renewable biomass offers substantial environmental benefits, including reduced reliance on fossil fuels and lower greenhouse gas emissions. For example, the production of ethanol from sugarcane bagasse is considered an environmentally friendly process that reduces the need for oil (Guilherme et al., 2019). However, the environmental impact varies depending on the production method and feedstock. Life-cycle assessments (LCA) have shown that ethanol production via biochemical routes can result in higher CO_2 emissions compared to thermochemical routes, although it achieves higher energy efficiency (García-Velásquez and Cardona, 2019). Moreover, the co-production of ethanol and hydrogen using genetically engineered Escherichia coli has been shown to improve the environmental sustainability of lignocellulosic biorefineries by reducing the total production cost and environmental impact (Lopez-Hidalgo et al., 2021).

7.3 Policy and regulatory aspects

Policy and regulatory frameworks play a crucial role in shaping the ethanol production industry. Government incentives, subsidies, and mandates for renewable fuel usage can significantly impact the economic viability of ethanol production. For instance, policies promoting the use of renewable electricity to meet process energy needs can improve ethanol yields and lower production costs, thereby enhancing the economic feasibility of ethanol production (Petersen et al., 2021). Additionally, regulatory measures aimed at reducing carbon emissions and promoting sustainable agricultural practices can further support the growth of the ethanol industry by encouraging the use of second and third-generation feedstocks, which do not compete with food crops (Tse et al., 2021b).

7.4 Global market trends

The global market for ethanol is influenced by various factors, including technological advancements, feedstock availability, and policy changes. The demand for bioethanol is expected to grow as countries seek to reduce their carbon footprints and transition to renewable energy sources. Technological innovations, such as the development of more efficient pretreatment and fermentation processes, are critical for enhancing the economic and environmental performance of ethanol production (Liu et al., 2019). Furthermore, the integration of biorefinery products and the co-production of ethanol with other valuable chemicals can open new market opportunities and improve the overall profitability of ethanol production (Lopez-Hidalgo et al., 2021; Rosentrater and Zhang, 2021). The global market trends indicate a shift towards more sustainable and cost-effective production methods, with a focus on utilizing non-food biomass and improving process efficiencies to meet the increasing demand for bioethanol (Saeed et al., 2018).

8 Future Perspectives

8.1 Innovations in fermentation technology

The future of ethanol fermentation technology is poised for significant advancements, particularly in the realm of gas fermentation. Gas fermentation using carbon-fixing microorganisms has shown promise as an economically viable and scalable solution for converting waste gases into ethanol. This technology not only offers flexibility in feedstock and product but also contributes to a circular carbon economy by capturing and converting greenhouse gases (Fackler et al., 2021). Additionally, the development of consolidated bioprocessing (CBP) technologies for raw starch to ethanol conversion has made strides, although further research is needed to scale these technologies to an industrial level (Cripwell et al., 2020). High gravity (HG) and very high gravity (VHG) ethanol fermentation technologies have also seen substantial progress, achieving ethanol concentrations in excess of 15% v/v, which could further enhance the economic and environmental benefits of ethanol production (Puligundla et al., 2019).

8.2 Potential of synthetic biology

Synthetic biology holds immense potential for revolutionizing ethanol fermentation. Recent advancements in genetic and metabolic engineering have enabled the development of acetogenic bacteria that can convert C1 gases like CO_2 and CO into ethanol and other valuable products (Bourgade et al., 2021). These synthetic biology approaches are crucial for overcoming the genetic and metabolic challenges associated with these microorganisms. Moreover, the engineering of non-methylotrophic model organisms to utilize methanol via the introduction of C1 utilization pathways represents another promising avenue for biomanufacturing (Zhu et al., 2019). The integration of synthetic biology with fermentation processes could lead to more efficient and sustainable production methods, expanding the range of feedstocks and products.

8.3 Challenges and opportunities in sustainable production

Despite the promising advancements, several challenges remain in achieving sustainable ethanol production. The recalcitrance of lignocellulosic biomass to degradation and the need for efficient pretreatment technologies are significant hurdles (Liu et al., 2019). Additionally, the separation and extraction of bio-chemicals from fermentation broths pose technical challenges, although pervaporation (PV) technology has shown potential in addressing these issues (Serna-Vázquez et al., 2021). The valorization of diverse feedstocks, including algae and lignocellulosic biomass, presents both opportunities and challenges for sustainable alcohol production (Shenbagamuthuraman et al., 2021). Furthermore, the coupling of ethanol-type fermentation with other biotechnologies, such as anaerobic acidogenesis, could enhance bioenergy recovery and improve the overall sustainability of the process (Li et al., 2020b). Addressing these challenges through innovative technologies and integrated approaches will be key to realizing the full potential of ethanol fermentation in industrial applications.

9 Concluding Remarks

Ethanol fermentation is a critical biochemical process with significant industrial applications, particularly in the production of biofuels. Key advancements have been made in the metabolic engineering of microorganisms to enhance ethanol production. For instance, the engineering of *Clostridium autoethanogenum* has demonstrated the importance of specific enzymes like aldehyde: ferredoxin oxidoreductase (AOR) in ethanol formation, leading to enhanced ethanol production when certain genes are inactivated. Additionally, the conversion of biomass into ethanol has seen substantial progress, with various microorganisms being engineered to ferment complex carbohydrates, such as xylose, which are not fermentable by traditional brewers' yeast. The development of integrated biological processes has also addressed major issues like high enzyme loading and slow fermentation rates, significantly improving ethanol productivity. Furthermore, the role of yeast in industrial fermentation processes extends beyond ethanol production to the formation of various aroma compounds, which are crucial for product quality.

The advancements in ethanol fermentation have profound implications for the biofuel industry. The ability to engineer microorganisms to selectively produce ethanol from various biomass resources, including lignocellulosic materials, opens up new avenues for sustainable fuel production. The integration of novel biological processes that enhance enzyme recycling and yeast cell reuse can lead to more cost-effective and efficient ethanol production systems. Moreover, understanding the factors that affect yeast ethanol tolerance and fermentation efficiency can further optimize industrial fermentation processes, making them more robust and scalable. The production of secondary metabolites by yeast during fermentation also adds value to the process by improving the sensory qualities of the final product, which is particularly important in the food and beverage industries.

Future research in ethanol fermentation should focus on further optimizing the genetic engineering of microorganisms to enhance their ethanol production capabilities. This includes exploring new metabolic pathways and regulatory mechanisms that can increase yield and efficiency. Additionally, the development of more integrated and sustainable fermentation processes that minimize waste and maximize resource utilization will be crucial. The exploration of electro-fermentation and its potential to control fermentation environments and reduce the need for external electron donors is another promising area. Finally, a deeper understanding of the ecological and physiological roles of fermentation by-products, such as aroma compounds, can lead to innovative applications in various industries. By addressing these areas, the ethanol fermentation industry can continue to evolve and contribute to a more sustainable and efficient bioeconomy.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Alharbi F., Knura T., Siebers B., and Ma K., 2022, Thermostable and O_2-insensitive pyruvate decarboxylases from thermoacidophilic archaea catalyzing the production of acetaldehyde, Biology, 11(8): 1247.
https://doi.org/10.3390/biology11081247

Bai F., Anderson W., and Moo-young M., 2008, Ethanol fermentation technologies from sugar and starch feedstocks, Biotechnology advances, 26(1): 89-105.
https://doi.org/10.1016/j.biotechadv.2007.09.002

Bourgade B., Minton N., and Islam M., 2021, Genetic and metabolic engineering challenges of C1-gas fermenting acetogenic chassis organisms, FEMS Microbiology Reviews, 45(2): fuab008.
https://doi.org/10.1093/femsre/fuab008

Bui L., Novi G., Lombardi L., Iannuzzi C., Rossi J., Santaniello A., Mensuali A., Corbineau F., Giuntoli B., Perata P., Zaffagnini M., and Licausi F., 2019, Conservation of ethanol fermentation and its regulation in land plants, Journal of Experimental Botany, 70: 1815-1827.
https://doi.org/10.1093/jxb/erz052

Carmona-Gutierrez D., Sommer C., Andryushkova A., Kroemer G., and Madeo F., 2012, A higher spirit: avoiding yeast suicide during alcoholic fermentation, Cell Death and Differentiation, 19: 913-914.
https://doi.org/10.1038/cdd.2012.31

Chandrakant P., and Bisaria V., 1998, Simultaneous bioconversion of cellulose and hemicellulose to ethanol, Critical Reviews in Biotechnology, 18(4): 295-331.
https://doi.org/10.1080/0738-859891224185

Cortivo P., Hickert L., Rosa C., and Ayub M., 2020, Conversion of fermentable sugars from hydrolysates of soybean and oat hulls into ethanol and xylitol by Spathaspora hagerdaliae UFMG-CM-Y303, Industrial Crops and Products, 146: 112218.
https://doi.org/10.1016/j.indcrop.2020.112218

Crespo C., Badshah M., Alvarez M., and Mattiasson B., 2012, Ethanol production by continuous fermentation of D-(+)-cellobiose, D-(+)-xylose and sugarcane bagasse hydrolysate using the thermoanaerobe Caloramator boliviensis, Bioresource technology, 103(1): 186-191.
https://doi.org/10.1016/j.biortech.2011.10.020

Cripwell R., Favaro L., Viljoen-Bloom M., and Zyl W., 2020, Consolidated bioprocessing of raw starch to ethanol by *Saccharomyces cerevisiae*: Achievements and challenges, Biotechnology Advances, 42: 107579.
https://doi.org/10.1016/j.biotechadv.2020.107579

Cui J., Maloney M., Olson D., and Lynd L., 2020, Conversion of phosphoenolpyruvate to pyruvate in *Thermoanaerobacterium saccharolyticum*, Metabolic Engineering Communications, 10: e00122.
https://doi.org/10.1016/j.mec.2020.e00122

Fackler N., Heijstra B., Rasor B., Brown H., Martin J., Ni Z., Shebek K., Rosin R., Simpson S., Tyo K., Giannone R., Hettich R., Tschaplinski T., Leang C., Brown S., Jewett M., and Köpke M., 2021, Stepping on the gas to a circular economy: accelerating development of carbon-negative chemical production from gas fermentation, Annual Review of Chemical and Biomolecular Engineering, 12(1): 439-470.
https://doi.org/10.1146/annurev-chembioeng-120120-021122

Fuchino K., Chan H., Hwang L., and Bruheim P., 2021, The ethanologenic bacterium Zymomonas mobilis divides asymmetrically and exhibits heterogeneity in DNA content, Applied and Environmental Microbiology, 87(6): e02441-20.
https://doi.org/10.1128/AEM.02441-20

García-Velásquez C., and Cardona C., 2019, Comparison of the biochemical and thermochemical routes for bioenergy production: A techno-economic (TEA), energetic and environmental assessment, Energy, 172: 232-242.
https://doi.org/10.1016/j.energy.2019.01.073

Guilherme A., Dantas P., Padilha C., Santos E., and Macêdo G., 2019, Ethanol production from sugarcane bagasse: Use of different fermentation strategies to enhance an environmental-friendly process, Journal of Environmental Management, 234: 44-51.
https://doi.org/10.1016/j.jenvman.2018.12.102

Hahn-hägerdal B., Karhumaa K., Fonseca C., Spencer-Martins I., and Gorwa-Grauslund M., 2007, Towards industrial pentose-fermenting yeast strains, Applied Microbiology and Biotechnology, 74: 937-953.
https://doi.org/10.1007/s00253-006-0827-2

Huang W.Z., 2024, The dual role of agricultural products as food and fuel: energy conversion and utilization, Journal of Energy Bioscience, 15(1): 32-47.
https://doi.org/10.5376/jeb.2024.15.0005

Ishii J., Morita K., Ida K., Kato H., Kinoshita S., Hataya S., Shimizu H., Kondo A., and Matsuda F., 2018, A pyruvate carbon flux tugging strategy for increasing 2,3-butanediol production and reducing ethanol subgeneration in the yeast *Saccharomyces cerevisiae*, Biotechnology for Biofuels, 11: 1-21.
https://doi.org/10.1186/s13068-018-1176-y

Kim S., Ha S., Wei N., Oh E., and Jin Y., 2012, Simultaneous co-fermentation of mixed sugars: a promising strategy for producing cellulosic ethanol, Trends in Biotechnology, 30(5): 274-282.
https://doi.org/10.1016/j.tibtech.2012.01.005

Krajang M., Malairuang K., Sukna J., Rattanapradit K., and Chamsart S., 2021, Single-step ethanol production from raw cassava starch using a combination of raw starch hydrolysis and fermentation, scale-up from 5-L laboratory and 200-L pilot plant to 3000-L industrial fermenters, Biotechnology for Biofuels, 14: 1-15.
https://doi.org/10.1186/s13068-021-01903-3

Li R., Jin M., Du J., Li M., Chen S., and Yang S., 2020a, The Magnesium Concentration in Yeast Extracts Is a Major Determinant Affecting Ethanol Fermentation Performance of *Zymomonas mobilis*, Frontiers in Bioengineering and Biotechnology, 8: 957.
https://doi.org/10.3389/fbioe.2020.00957

Li Z., Gu J., Ding J., Ren N., and Xing D., 2020b, Molecular mechanism of ethanol-H2 co-production fermentation in anaerobic acidogenesis: Challenges and perspectives, Biotechnology advances, 46: 107679.
https://doi.org/10.1016/j.biotechadv.2020.107679

Lino F., Bajić D., Vila J., Sánchez Á., and Sommer M., 2020, Complex yeast-bacteria interactions affect the yield of industrial ethanol fermentation, Nature Communications, 12(1): 1498.
https://doi.org/10.1038/s41467-021-21844-7

Liu C., Xiao Y., Xia X., Zhao X., Peng L., Srinophakun P., and Bai F., 2019, Cellulosic ethanol production: Progress, challenges and strategies for solutions, Biotechnology Advances, 37(3): 491-504.
https://doi.org/10.1016/j.biotechadv.2019.03.002

Liu H., Sun J., Chang J., and Shukla P., 2018, Engineering microbes for direct fermentation of cellulose to bioethanol, Critical Reviews in Biotechnology, 38: 1089-1105.
https://doi.org/10.1080/07388551.2018.1452891

Lopez-Hidalgo A., Magaña G., Rodríguez F., Léon-Rodríguez A., and Sánchez A., 2021, Co-production of ethanol-hydrogen by genetically engineered Escherichia coli in sustainable biorefineries for lignocellulosic ethanol production, Chemical Engineering Journal, 406: 126829.
https://doi.org/10.1016/j.cej.2020.126829

Madhavan A., Srivastava A., Kondo A., and Bisaria V., 2012, Bioconversion of lignocellulose-derived sugars to ethanol by engineered *Saccharomyces cerevisiae*, Critical Reviews in Biotechnology, 32: 22-48.
https://doi.org/10.3109/07388551.2010.539551

Maicas S., 2021, Advances in Wine Fermentation. Fermentation, 7(3): 187.
https://doi.org/10.3390/fermentation7030187

Nakanishi S., Soares L., Biazi L., Nascimento V., Costa A., Rocha G., and Ienczak J., 2017, Fermentation strategy for second generation ethanol production from sugarcane bagasse hydrolyzate by *Spathaspora passalidarum* and *Scheffersomyces stipitis*, Biotechnology and Bioengineering, 114(10): 2211-2221.
https://doi.org/10.1002/bit.26357

Nanda S., Pattnaik F., Patra B., Kang K., and Dalai A., 2023, A review of liquid and gaseous biofuels from advanced microbial fermentation processes, Fermentation, 9(9): 813.
https://doi.org/10.3390/fermentation9090813

Petersen A., Okoro O., Chireshe F., Moonsamy T., and Görgens J., 2021, Systematic cost evaluations of biological and thermochemical processes for ethanol production from biomass residues and industrial off-gases, Energy Conversion and Management, 243: 114398.
https://doi.org/10.1016/j.enconman.2021.114398

Pony P., Rapisarda C., Terradot L., Marza E., and Fronzes R., 2020, Filamentation of the bacterial bi-functional alcohol/aldehyde dehydrogenase AdhE is essential for substrate channeling and enzymatic regulation, Nature Communications, 11(1): 1426.
https://doi.org/10.1038/s41467-020-15214-y

Puligundla P., Šmogrovičová D., Mok C., and Obulam V., 2019, A review of recent advances in high gravity ethanol fermentation, Renewable Energy, 133: 1366-1379.
https://doi.org/10.1016/j.renene.2018.06.062

Rahman Q., Zhang B., Wang L., and Shahbazi A., 2019a, A combined pretreatment, fermentation and ethanol-assisted liquefaction process for production of biofuel from Chlorella sp., Fuel, 257: 116026.
https://doi.org/10.1016/j.fuel.2019.116026

Rahman Q., Zhang B., Wang L., Joseph G., and Shahbazi A., 2019b, A combined fermentation and ethanol-assisted liquefaction process to produce biofuel from Nannochloropsis sp., Fuel, 238: 159-165.
https://doi.org/10.1016/j.fuel.2018.10.116

Robak K., and Balcerek M., 2018, Review of second generation bioethanol production from residual biomass, Food Technology and Biotechnology, 56(2): 174-187.
https://doi.org/10.17113/ftb.56.02.18.5428

Rosentrater K., and Zhang W., 2021, Techno-economic analysis of integrating soybean biorefinery products into corn-based ethanol fermentation operations, Fermentation, 7: 82.
https://doi.org/10.3390/fermentation7020082

Saeed M., Ma H., Yue S., Wang Q., and Tu M., 2018, Concise review on ethanol production from food waste: development and sustainability, Environmental Science and Pollution Research, 25: 28851-28863.
https://doi.org/10.1007/s11356-018-2972-4

Sarkar O., Rova U., Christakopoulos P., and Matsakas L., 2021, Ethanol addition promotes elongation of short-chain fatty acids to medium-chain fatty acids using brewery spent grains as substrate, Journal of Environmental Chemical Engineering, 9: 105990.
https://doi.org/10.1016/j.jece.2021.105990

Serna-Vázquez J., Ahmad M., and Castro-Muñoz R., 2021, Simultaneous production and extraction of bio-chemicals produced from fermentations via pervaporation, Separation and Purification Technology, 279: 119653.
https://doi.org/10.1016/j.seppur.2021.119653

Shanmugam S., Hari A., Pugazhendhi A., and Kikas T., 2023, Integrated catalytic upgrading of biomass-derived alcohols for advanced biofuel production, Energies, 16(13): 4998.
https://doi.org/10.3390/en16134998

Shenbagamuthuraman V., Patel A., Khanna S., Banerjee E., Parekh S., Karthick C., Ashok B., Velvizhi G., Nanthagopal K., and Ong H., 2021, State of art of valorising of diverse potential feedstocks for the production of alcohols and ethers: Current changes and perspectives, Chemosphere, 286(Pt1): 131587.
https://doi.org/10.1016/j.chemosphere.2021.131587

Stark C., Münßinger S., Rosenau F., Eikmanns B., and Schwentner A., 2022, The potential of sequential fermentations in converting C1 substrates to higher-value products, Frontiers in Microbiology, 13: 907577.
https://doi.org/10.3389/fmicb.2022.907577

Straub C., Schut G., Otten J., Keller L., Adams M., and Kelly R., 2020, Modification of the glycolytic pathway in *Pyrococcus furiosus* and the implications for metabolic engineering, Extremophiles, 24: 511-518.
https://doi.org/10.1007/s00792-020-01172-2

Szambelan K., Szwengiel A., Nowak J., Frankowski J., and Jeleń H., 2023, Bioethanol production from sorghum grain with *Zymomonas mobilis*: Increasing the yield and quality of raw distillates, Journal of the Science of Food and Agriculture, 103(12): 6080-6094.
https://doi.org/10.1002/jsfa.12688

Todhanakasem T., Wu B., and Simeon S., 2020, Perspectives and new directions for bioprocess optimization using *Zymomonas mobilis* in the ethanol production, World Journal of Microbiology and Biotechnology, 36(8): 112.
https://doi.org/10.1007/s11274-020-02885-4

Tse T., Wiens D., and Reaney M., 2021b, Production of bioethanol-a review of factors affecting ethanol yield, Fermentation, 7(4): 268.
https://doi.org/10.3390/fermentation7040268

Tse T., Wiens D., Chicilo F., Purdy S., and Reaney M., 2021a., Value-added products from ethanol fermentation-a review, Fermentation, 7(4): 267.
https://doi.org/10.3390/fermentation7040267

Vinotha T., Umamaheswari N., Pandiyan J., Al-Ghanim K., Nicoletti M., and Govindarajan M., 2023, Biofuel production from mango and orange peel and tapioca shells by fermentation using consortium of Bacteria: agricultural and food waste valorization, Fermentation, 9(7): 678.
https://doi.org/10.3390/fermentation9070678

Wang Q., Sha C., Wang H., Ma K., Wiegel J., Abomohra A., and Shao W., 2020, A novel bifunctional aldehyde/alcohol dehydrogenase mediating ethanol formation from acetyl-CoA in hyperthermophiles.
https://doi.org/10.21203/rs.3.rs-17223/v1

Xia J., Yang Y., Liu C., Yang S., and Bai F., 2019, Engineering *Zymomonas mobilis* for robust cellulosic ethanol production, Trends in Biotechnology, 37(9): 960-972.
https://doi.org/10.1016/j.tibtech.2019.02.002

Zhang B., Zhang J., Wang D., Han R., Ding R., Gao X., Sun L., and Hong J., 2016, Simultaneous fermentation of glucose and xylose at elevated temperatures co-produces ethanol and xylitol through overexpression of a xylose-specific transporter in engineered *Kluyveromyces marxianus*, Bioresource Technology, 216: 227-237.
https://doi.org/10.1016/j.biortech.2016.05.068

Zhu T., Zhao T., Bankefa O., and Li Y., 2019, Engineering unnatural methylotrophic cell factories for methanol-based biomanufacturing: Challenges and opportunities, Biotechnology Advances, 39: 107467.
https://doi.org/10.1016/j.biotechadv.2019.107467