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# Catalysts CONNECT

VOLUME-38

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## From the Managing Director's Desk



**Munish Madan**  
MANAGING DIRECTOR

As The Catalysts Group completes 23 years of its journey, it is both a moment of pride and reflection. Over the past two decades, our mission has remained consistent – to help fermentation-based industries unlock higher productivity, stronger process efficiency and sustainable profitability through biotechnology.

Today, Catalysts Group stands as India's most trusted biotech solutions partner for the distilling, brewing and sugar industries. Our work is driven by a simple philosophy: to add measurable profits to our customers' businesses by improving operational performance and eliminating process inefficiencies.

Over the last 23 years, we have had the privilege of partnering with more than 1,053 clients across 25+ countries. Through advanced biotech-driven solutions, our technologies and process interventions have helped deliver more than ₹14,080 crore in measurable benefits to our customers – a number that reflects not just innovation, but trust built over time.

Our strength lies in combining biotechnology with deep process understanding. Catalysts Group offers a comprehensive portfolio of enzymes, yeast, antimicrobials and specialty additives designed to improve fermentation performance and plant efficiency across diverse industrial applications.

However, our role goes far beyond supplying products. We work closely with our customers to diagnose process challenges, optimize plant operations and unlock hidden efficiencies. Our teams regularly provide on-site technical support, analytical diagnostics and continuous performance monitoring to ensure that every solution translates into real operational gains.

The theme of this edition of our company magazine focuses on GMO yeast – one of the most exciting developments shaping the future of industrial fermentation. Advanced yeast technologies are enabling plants to operate with greater resilience, improved yields and enhanced process stability, even under increasingly demanding production environments.

As fermentation industries evolve globally, the ability to harness advanced microbial technologies will become a defining competitive advantage. GMO yeast, improved enzyme systems and precision fermentation tools are opening new possibilities for higher productivity and smarter resource utilisation.

For us at Catalysts Group, innovation has always been guided by one clear objective: to maximize value for our customers. Every solution we develop and every process improvement we implement is ultimately measured by how effectively it helps our partners improve productivity, reduce costs and strengthen their operational resilience.

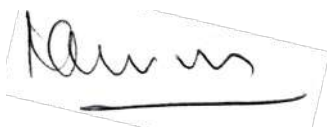
Looking ahead, the opportunities for biotechnology in industrial fermentation are immense. As the world accelerates its transition toward sustainable fuels, circular bioeconomies and more efficient manufacturing systems, biotechnology will continue to play a pivotal role.

Our commitment remains unwavering: to combine science, process expertise and customer partnership to help industries operate smarter and more profitably.

As we celebrate 23 years of The Catalysts Group, I extend my sincere gratitude to our customers, partners and our dedicated team whose passion and expertise continue to drive our progress.

The journey ahead is filled with exciting possibilities – and we look forward to continuing to innovate, collaborate and create lasting value for the industries we serve.

Warm regards,

A handwritten signature in black ink, appearing to read 'Munish Madan', is enclosed within a white rectangular box with a thin black border. The signature is written in a cursive, flowing style.

Munish Madan



## The challenge and promise of Engineering yeast to ferment Pentose sugars



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Native yeast excels at hexose fermentation but lacks efficient pathways for these pentoses, prompting extensive metabolic engineering efforts. Engineering yeast like *Saccharomyces cerevisiae* to ferment C5 sugars such as xylose and arabinose addresses a key bottleneck in biofuel production from lignocellulosic biomass but offers the major promise of enabling cost-effective and sustainable second-generation biofuel production from abundant lignocellulosic biomass.

### Stability and productivity remain major challenges

**Natural Inability:** *Saccharomyces cerevisiae* is considered to be a potential cell factory and has been used to produce various fuels and chemicals, but it cannot metabolize xylose, which has greatly limited the utilization of lignocellulose materials. Therefore, numerous studies have attempted to develop xylose fermenting strains in past decades. The simple introduction of the xylose metabolic pathway does not enable yeast to rapidly utilize xylose, and several limitations still need to be addressed.

**Metabolic and Redox Imbalance:** Introducing heterologous pathways (from other organisms) for

C5 metabolism often disrupts the yeast's internal redox balance, leading to the accumulation of byproducts (like xylitol) and lower ethanol yields. **Carbon Catabolite Repression (CCR):** In mixed-sugar environments (containing both C6 glucose and C5 sugars), glucose is preferentially consumed first, and its presence represses the uptake and metabolism of C5 sugars. This results in inefficient utilization of all available carbon sources. Glucose repression hinders C5 uptake when hexoses are present, slowing co-fermentation (Wagner and Gasch, 2023).

**Inhibitor Sensitivity:** The pretreatment of lignocellulosic biomass to release C5/C6 sugars also generates toxic byproducts (e.g., acetic acid, furfural, phenolic compounds). Engineered yeast strains often exhibit reduced robustness or tolerance to these inhibitors compared to native strains, hampering industrial application.

### Engineered Yeasts Serve as Outstanding Chassis for Biochemical Production

**Consolidated Bioprocessing (CBP):** Engineered yeasts are key to achieving the highly efficient CBP concept, where enzyme production, hydrolysis, and fermentation occur in a single step, streamlining

the process and reducing infrastructure costs.

Omics analyses have also revealed new xylose metabolism and regulation mechanisms. Some of the engineered strains have shown good fermentation performance in cellulosic hydrolysates and are ready for industrial application. Currently, the engineered strains are mainly designed for ethanol production. The budding yeast *Saccharomyces cerevisiae* is frequently used in industry due to its genetic tractability and unique metabolic capabilities (Wagner and Gasch, 2023). *S. cerevisiae* has been engineered to produce novel compounds from diverse sugars found in lignocellulosic biomass, including pentose sugars, like xylose, not recognized by the organism (Hou et al., 2017). Developing microorganisms that co-utilize glucose and xylose to produce other biofuels and chemicals will expand the application of lignocellulosic biomass.

As an example of the challenges *S. cerevisiae* engineering efforts, enabling anaerobic xylose fermentation as a model system and showcasing the regulatory interplay's controlling growth, metabolism, and stress defence. Enabling xylose fermentation in *S. cerevisiae* requires the introduction of several key metabolic enzymes but also regulatory rewiring of three signalling pathways at the intersection of the growth and stress defence responses: the RAS/PKA, Snf1, and high osmolarity glycerol (HOG) pathways (Durchschlag et al., 1998). The current studies reviewed here suggest the modulation of global signalling pathways should be adopted into biorefinery microbial engineering pipelines to increase efficient product yields.

**Economic Viability:** Efficient fermentation of both C5 and C6 sugars is critical for the economic feasibility of industrial second-generation ethanol production. The ability to co-ferment all sugars significantly increase the overall process yield and efficiency, reducing operational costs (e.g., less tank cleaning, reduced need for inoculation yeast).

**Versatile Cell Factories:** Beyond ethanol,

engineered yeasts capable of C5 fermentation can serve as “microbial cell factories” for producing a wide range of high-value chemicals, such as advanced biofuels (“higher alcohols”), organic acids (lactic acid), and microbial lipids, contributing to a circular bioeconomy.

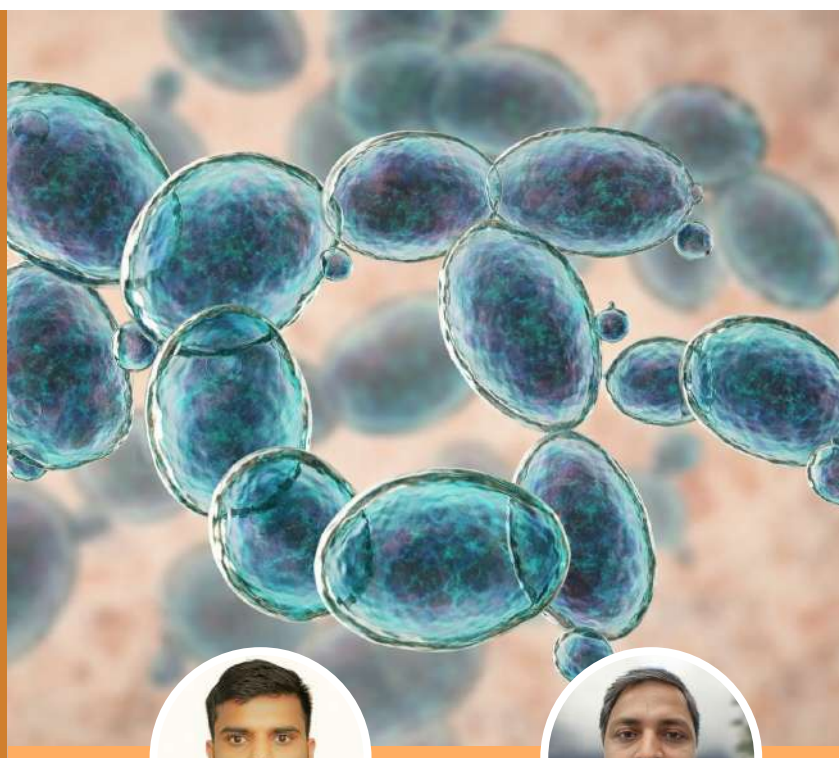
**Innovative Strategies:** Ongoing research using advanced genetic engineering tools like CRISPR-Cas9, inverse metabolic engineering, and directed evolution is continually improving strain performance, leading to more robust strains with enhanced C5 consumption kinetics and higher ethanol titers.

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# Market Scenario in GMO Yeast



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## Introduction

The global biotechnology industry has witnessed a remarkable shift over the past two decades, moving from nascent genetic manipulation to the routine industrial application of genetically engineered microbial strains. Yeast, predominantly *Saccharomyces cerevisiae*, has been at the core of this transformation owing to its versatile fermentation capabilities, genetic tractability, and broad industrial applicability. While conventional yeast strains have long been used in food and beverage fermentations, the advent of genetically modified organisms (GMOs) has unlocked new potential for tailored performance in bioethanol production, high value biochemicals, and precision fermentation products.

The market interest in GM yeast has grown significantly alongside the demand for renewable fuels, sustainable chemical feedstocks, and efficient bioprocessing systems. Bioethanol production depends on yeast strains optimized for high sugar conversion, resistance to inhibitors, and

performance under industrial stress conditions. As governments worldwide adopt low-carbon fuel standards and expand mandates for ethanol blending in transportation fuels, the demand for specialized yeast strains has increased. Numerous market analyses predict substantial revenue growth in the bioethanol yeast segment, with compound annual growth rates (CAGRs) exceeding double digits in many forecasts (Grand View Research, 2024; Precedence Research, 2025). This growth mirrors broader biotechnological advances, including CRISPR/Cas-based editing and systems biology approaches that enable precision in strain design.

Beyond biofuels, GM yeasts are gaining traction in food fermentation, pharmaceutical biosynthesis, and industrial enzyme production. These diverse applications further cement the role of the yeast biotechnology sector in next-generation industrial processes.

## Market Definition and Scope

### What Constitutes “GM Yeast” in Today’s Market

In commercial and regulatory contexts, “genetically modified yeast” refers to yeast strains whose genomes have been intentionally altered through molecular methods to enhance desirable traits. These may include:

- Enhanced substrate utilization (e.g., pentose fermentation)
- Increased tolerance to ethanol, temperature, and inhibitors
- Improved metabolic pathways for biochemical production
- Reduced formation of by-products that detract from yield

Such modifications are typically achieved using technologies such as CRISPR–Cas9, homologous recombination, random mutagenesis, adaptive evolution, and synthetic biology platforms that allow multiplex editing and metabolic pathway reconstruction. These engineered yeast strains are distinct from wild-type or conventionally bred strains because of their targeted precision modifications that confer operational advantages in industrial applications.

## Market Scope and Segmentation

The GM yeast market spans multiple industry segments, including bioethanol production for fuel and industrial applications, food and beverage fermentations requiring specialized fermentation profiles, pharmaceuticals and fine chemicals for biocatalytic synthesis, and biotech research tools that support synthetic biology platforms. Among these, bioethanol production represents the most economically significant segment, driven by its large-scale production volumes and strong regulatory mandates promoting the integration of renewable fuels.

## Global Bioethanol Yeast Market: Drivers and Forecasts

### Market Size and Growth Trajectory

Multiple market intelligence sources project strong

growth in the bioethanol yeast sector, a major sub-segment of the broader GM yeast market. According to a comprehensive industry report, the global bioethanol yeast market was valued at approximately USD 20.57 billion in 2024 and is expected to grow to approximately USD 43.63 billion by 2030, reflecting a CAGR of approximately 12.8% from 2025 to 2030 (Grand View Research, 2024). Similarly, alternative projections estimate that the market could expand from approximately USD 23.24 billion in 2025 to approximately USD 76.37 billion by 2035, sustaining double-digit growth over a decade (Precedence Research, 2025).

This anticipated expansion is linked to several factors. First, global energy markets are increasingly prioritizing the adoption of renewable fuels to mitigate climate change. Ethanol blending mandates, such as the E10/E20 standards in North America, Europe, and Asia Pacific, directly stimulate ethanol production volumes and, by extension, the demand for specialized yeast strains. Second, advancements in lignocellulosic feedstock processing highlight the need for robust genetically engineered yeast capable of fermenting pentose sugars that conventional strains cannot efficiently metabolize. Third, increasing institutional and private investments in fermentation biotechnology accelerates both R&D and the commercialization of next-generation yeast solutions.

## Drivers of Growth

The bioethanol yeast market is being shaped by several interrelated drivers. Renewable fuel policies, including mandatory ethanol blending mandates implemented in more than 70 countries, ensure a steady and predictable demand for ethanol production, thereby supporting sustained growth of the yeast market. At the same time, technological breakthroughs, particularly the development of engineered yeast strains with improved stress tolerance and higher fermentation efficiency are enhancing production economics and operational reliability. The diversification of feedstocks, ranging from traditional sources such as corn and sugarcane to lignocellulosic residues,

is further increasing the need for versatile and adaptable yeast platforms. In parallel, strategic collaborations between biotechnology companies and ethanol producers are accelerating the adoption of advanced strains and facilitating their successful scale-up across commercial operations.

### Challenges and Market Restraints

Despite strong growth potential, the bioethanol yeast market faces several challenges that influence its overall dynamics. Volatility in feedstock prices, particularly for corn and sugarcane, can adversely affect fermentation economics and reduce profitability for ethanol producers. In addition, regulatory complexity remains a significant hurdle, as approval frameworks for genetically modified yeast differ widely across regions, slowing adoption and increasing compliance burdens. Public perception also plays a role, with persistent dependence toward genetically modified organisms in certain markets limiting acceptance and market penetration.

### Regional Market Dynamics

North America remains a dominant player in the bioethanol yeast market, accounting for nearly 40% of global production and investment. In the United States, the Renewable Fuel Standard and related policies strongly incentivize domestic ethanol production, thereby driving demand for advanced genetically modified yeast strains, while Canada also supports biofuel initiatives, albeit at a comparatively smaller scale. The Asia-Pacific region, meanwhile, is positioned for rapid growth due to the expansion of biofuel programs across China, India, and Southeast Asian countries, where rising ethanol blending targets and improved feedstock availability significantly enhance market potential. In Latin America, Brazil's mature sugarcane-based ethanol industry continues to benefit from a well-established yeast fermentation infrastructure; although large-scale deployment of cellulosic ethanol is still in its early stages, ongoing modernization efforts are sustaining demand for next-generation yeast solutions.

### Competitive Landscape

Major global biotechnology and fermentation companies exert a significant influence on the GM

yeast market. These include:

- Lallemand Biofuels & Distilled Spirits
- Novonosis
- Lesaffre Group

With increasing patent activity focusing on stress-tolerant and lignocellulose-adapted strains, competitive differentiation is linked to intellectual property and collaborative alliances.

### Regulatory and Public Policy Considerations

Market evolution depends heavily on the regulatory frameworks governing GMO organisms. While some countries have streamlined approval pathways for ethanol-related GM yeast imports and use, others maintain stringent biosafety measures that can slow down commercialization. For example, India's Genetic Engineering Appraisal Committee approved the import of engineered yeast for ethanol production under controlled conditions in 2025, highlighting the regulatory engagement with industrial biotechnology. In the Indian scenario, government regulations governing the use of genetically modified (GM) yeast in the ethanol industry are evolving and remain comparatively cautious. India's regulatory oversight is primarily guided by bodies such as the Genetic Engineering Appraisal Committee (GEAC) under the Ministry of Environment, Forest and Climate Change, which evaluates the environmental and biosafety risks associated with GM organisms. While India strongly promotes ethanol production through initiatives like the Ethanol Blended Petrol (EBP) Programme, the commercial use of GM yeast in industrial fermentation is subject to stringent approval processes, limited field-scale precedents, and strict containment requirements. As a result, adoption has been slower compared to countries with more streamlined regulatory pathways. However, increasing emphasis on energy security, reduced crude oil imports, and utilization of diverse feedstocks is encouraging policy-level discussions on enabling controlled and safe use of GM fermentation organisms.

#### Key regulatory pointers in the Indian context:

- Approval of GM yeast falls under GEAC, with mandatory biosafety and environmental risk assessments.
- Strict contained-use guidelines are required for

industrial fermentation applications.

- Limited clarity and timelines in approval processes can delay commercial adoption.
- Alignment with national programs such as EBP and bioenergy missions could support future regulatory easing.
- Growing focus on sustainability and feedstock efficiency may drive a more enabling regulatory framework for GM yeast in the coming years.

## Conclusion

The market scenario for genetically modified yeast reflects a dynamic intersection of technological innovation, economic demand, and policy support. As bioethanol production and other industrial fermentation applications expand, engineered yeast strains designed for efficiency, robustness, and versatility have become indispensable. With strong growth projections and evolving applications beyond fuel, the GM yeast market is poised for sustained expansion, shaping the future of industrial biotechnology and renewable resource utilization.

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# Role of Genetically Modified Yeast in Improving Ethanol Yield and Fermentation Kinetics



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## Introduction

Bioethanol produced through microbial fermentation is one of the most important renewable liquid fuels currently in use, offering a practical means of reducing dependence on fossil resources. First generation bioethanol is derived largely from sugar- and starch-based crops, such as sugarcane, corn, and wheat, whereas second-generation bioethanol is produced from lignocellulosic materials, including agricultural residues, grasses, and woody biomass, following pretreatment and enzymatic hydrolysis. Among fermentative microorganisms, *Saccharomyces*

*cerevisiae* dominates industrial ethanol production because of its robustness, rapid fermentation capacity, and high ethanol tolerance, particularly during the fermentation of glucose and sucrose. Despite these features, wild-type *S. cerevisiae* has inherent metabolic limitations. It cannot naturally metabolise pentose sugars abundant in lignocellulosic hydrolysates and diverts a significant portion of carbon toward by-products, such as glycerol and organic acids, to maintain redox balance under stress. These factors restrict both the ethanol yield and fermentation rate under industrial conditions. Recent advances in yeast

strain development have focused on overcoming these bottlenecks through genetic modifications. Modern industrial strains often outperform conventional laboratory strains by approximately 5% in terms of ethanol yield, while exhibiting greater resistance to inhibitors, osmotic stress, and ethanol toxicity. A new generation of “speciality” ethanol yeasts has emerged, which are designed to grow rapidly in high-sugar environments, ferment a broader spectrum of substrates, and withstand harsh process conditions. Parallel research has explored non *Saccharomyces* yeasts with advantageous physiological properties. For example, *Kluyveromyces marxianus* is thermotolerant and Crabtree-negative, allowing for efficient fermentation at elevated temperatures, which is particularly useful in high-temperature simultaneous saccharification and fermentation (SHF) processes. Meanwhile, *Scheffersomyces stipitis*, a natural xylose-fermenting yeast, has been engineered and hybridised with *S. cerevisiae* to enhance pentose utilisation.

Therefore, genetic modifications introduced into yeast over the past decade that have demonstrably improved ethanol yield and fermentation kinetics of yeast. Strategies applied to both *S. cerevisiae* and alternative yeast species are discussed across major feedstocks, including sugarcane, corn, and lignocellulosic biomass. Quantitative outcomes are summarised where available, and key engineering approaches, such as gene deletion, heterologous pathway insertion, and adaptive evolution, are examined in relation to industrial performance. Recent studies published between 2015 and 2025 are emphasised, along with examples of commercially deployed, engineered yeasts. Finally, the prospects and remaining challenges in the large-scale application of genetically modified yeast for cost-effective bioethanol production are considered.

### Yeast Strains and Genetic Engineering Approaches

The studies reviewed in this article primarily employed genetically modified strains of *Saccharomyces cerevisiae*, along with selected

non-conventional yeasts, such as *Kluyveromyces marxianus* and *Scheffersomyces stipitis*. Genetic modifications were achieved using well established molecular biology techniques, including homologous recombination, CRISPR-Cas9 mediated genome editing, and plasmid-based heterologous gene expression. Target genes can be selected based on their involvement in central carbon metabolism, redox balance, stress response, and sugar transport. To improve the ethanol yield, genes associated with glycerol synthesis (GPD1 and GPD2) can be partially or completely disrupted to reduce carbon diversion away from ethanol. In parallel, regulatory genes controlling glycolytic flux and fermentation efficiency are either overexpressed or fine-tuned to enhance metabolic throughput. For lignocellulosic applications, yeast strains have been engineered to express heterologous xylose-utilising pathways, including xylose isomerase or the xylose reductase-xylitol dehydrogenase system, enabling efficient pentose fermentation. Additionally, adaptive yeast evolution can be introduced to enhance the performance of yeast.

### Enhancement of Ethanol Yield

Across multiple studies, engineered *S. cerevisiae* strains have achieved ethanol yields ranging from 0.48 to 0.51 g/g sugar, approaching the theoretical maximum. These improvements were largely attributed to reduced glycerol formation and improved redox balance resulting from targeted gene deletions and pathway optimisations. In molasses-based fermentations, yield improvements of 5–10% have been frequently reported, whereas more complex multi-gene modifications have produced yield gains exceeding 15%. Importantly, these increases were achieved without compromising cell viability or fermentation robustness, indicating that metabolic redirection was successfully balanced with cellular stress management.

### Improvement in Fermentation Kinetics

In addition to yield enhancement, the engineered yeast strains displayed markedly improved

fermentation kinetics. Reduced lag phases and faster sugar uptake rates were observed, particularly under high-gravity conditions. Several strains completed fermentation cycles 10–20% faster than conventional industrial yeasts, resulting in higher plant throughput and reduced operational times. Engineered strains expressing stress-response regulators or adaptive evolution-derived traits exhibit superior ethanol tolerance, maintaining high productivity even at ethanol concentrations exceeding 12–14% (v/v). These characteristics are particularly advantageous for industrial-scale operations, where prolonged exposure to ethanol and osmotic stress often limits the fermentation efficiency.

## Conclusion


Genetically modified yeast strains can fundamentally reshape the landscape of industrial ethanol fermentation. Targeted genetic interventions can significantly enhance ethanol yield, accelerated fermentation kinetics, and expanded substrate utilisation to include lignocellulosic sugars in the fermentation process. These improvements can directly translate into higher productivity, reduced processing time, and improved economic viability of bioethanol production. Although regulatory, stability, and scalability challenges persist, ongoing advances in genome editing technologies and metabolic engineering continue to enhance yeast performance for industrial applications. The convergence of genetic engineering, systems biology, and process optimisation is expected to yield next-generation yeast strains capable of

meeting the demands of sustainable and large-scale biofuel production. Genetically modified yeasts are expected to remain a cornerstone of future bioethanol production technologies.

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# Metabolic Engineering of Yeast for High-Efficiency Ethanol Production: Types of Genetic Engineering Strategies



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## Introduction

Bioethanol has established itself as one of the most widely produced renewable fuels, playing a critical role in reducing dependence on fossil resources and mitigating greenhouse gas emissions. Industrial ethanol production is primarily based on microbial fermentation of carbohydrate-rich feedstocks such as sugarcane molasses, corn starch, and increasingly, lignocellulosic biomass. Among fermentative microorganisms, *Saccharomyces cerevisiae* has long been the organism of choice due to its high ethanol productivity, and compatibility with large-scale industrial operations.

Despite these advantages, conventional yeast strains are not inherently optimized for maximal ethanol yield under industrial conditions. Carbon loss through glycerol formation, organic acid production, and

biomass accumulation limits theoretical ethanol yields. Additionally, industrial fermentations are frequently challenged by fluctuating substrate quality, temperature variations, inhibitory compounds, and microbial contamination. These factors collectively necessitate robust yeast strains with superior metabolic efficiency and stress resistance.

Metabolic engineering offers a rational framework to redesign yeast metabolism through targeted genetic modifications. By manipulating metabolic fluxes, cofactor balances, transport systems, and regulatory circuits, it is possible to channel a greater proportion of substrate carbon toward ethanol while minimizing by-product formation. Over the past two decades, advances in molecular biology, systems biology, and genome editing technologies have significantly expanded the scope of yeast metabolic engineering.

### Genetic Engineering Strategies for High-Efficiency Ethanol Production

Genetic engineering of yeast has become a cornerstone strategy for enhancing ethanol production efficiency, robustness, and yield in industrial fermentation processes. Conventional *Saccharomyces cerevisiae* strains often face limitations such as low tolerance to high ethanol concentrations, inhibitors from lignocellulosic hydrolysates, suboptimal sugar utilization, and reduced productivity under industrial stress conditions. To overcome these constraints, diverse genetic engineering approaches have been developed, ranging from classical mutagenesis and targeted gene overexpression to advanced genome editing and systems-level metabolic rewiring. These strategies aim to optimize carbon flux toward ethanol, improve stress tolerance, expand substrate utilization, and enhance overall fermentation performance. The following sections outline the major types of genetic engineering techniques applied to yeast for ethanol production enhancement, highlighting their principles, applications, and industrial relevance.

### Genome-Scale Metabolic Modelling

Genome-scale metabolic models (GEMs) have become indispensable tools in yeast metabolic engineering. These models enable the simulation of metabolic flux distributions and the prediction of genetic modification targets that maximize ethanol production. Constraint-based modelling approaches, such as flux balance analysis, provide insights into trade-offs between growth and product formation.

### CRISPR-Cas-Based Genome Editing

CRISPR technology is a modern genetic engineering tool that allows scientists to precisely edit the DNA of living organisms. The term CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, which, along with CRISPR-associated (Cas) proteins, forms a natural defence system found in bacteria and archaea. In nature, this system protects microbes from viral infections by recognizing and cutting foreign genetic material. Scientists have adapted this mechanism into a programmable genome-editing tool by using a short guide RNA (gRNA) to direct the Cas enzyme, most commonly Cas9, to a specific DNA sequence, where it creates a targeted cut.

Once the DNA is cut, the cell's natural repair machinery is activated, allowing researchers to delete, insert, or modify specific genes. Because CRISPR is highly accurate, fast, cost-effective, and easy to design compared to earlier gene-editing methods, it has become widely used in research and industry. In biotechnology applications such as yeast-based ethanol production, CRISPR technology enables precise metabolic engineering to improve ethanol yield, stress tolerance, and substrate utilization, making it a powerful tool for developing advanced industrial microbial strains. CRISPR-Cas-based genome editing has emerged as a transformative tool for precise and efficient genetic modification of yeast strains used in ethanol production. This technology enables targeted gene knockouts, insertions, and substitutions with high accuracy, allowing researchers to directly manipulate metabolic

pathways involved in sugar uptake, glycolysis, and ethanol biosynthesis. In *Saccharomyces cerevisiae*, CRISPR-Cas systems have been widely applied to eliminate competing pathways that divert carbon flux away from ethanol, enhance expression of key enzymes such as pyruvate decarboxylase and alcohol dehydrogenase, and improve tolerance to industrial stresses including high ethanol concentrations, osmotic pressure, and inhibitory compounds present in lignocellulosic hydrolysates. Beyond single-gene edits, CRISPR-Cas technology supports multiplex genome editing, enabling simultaneous modification of multiple genes in a single engineering step. This capability is particularly valuable for constructing robust industrial yeast strains with improved co-fermentation of mixed sugars (glucose, xylose, and arabinose) and enhanced redox balance. Additionally, CRISPR-based regulatory tools, such as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), allow fine-tuning of gene expression without altering DNA sequences, offering reversible and controllable metabolic optimization. Together, these advancements make CRISPR-Cas-based genome editing a powerful and scalable approach for developing next-generation yeast strains for high-efficiency ethanol production.

### Gene Overexpression

Overexpression of key glycolytic and fermentative genes significantly enhances metabolic flux toward ethanol production by accelerating sugar uptake and conversion efficiency in yeast. Target genes such as PDC1, ADH1, HXK2, PFK1, along with sugar transporter genes of the HXT family, help eliminate metabolic bottlenecks and improve substrate utilization. From an industrial standpoint, this genetic strategy leads to faster fermentation rates, reduced processing time, and higher ethanol productivity, making it particularly effective for both batch and continuous fermentation systems.

### Gene knockout and deletion

Gene knockout and deletion strategies are widely employed to minimize carbon loss toward unwanted by-products and redirect metabolic

flux toward ethanol formation. These genetic modifications are commonly achieved through homologous recombination and the Cre-loxP system, enabling precise and stable removal of specific genes. From an industrial perspective, such knockouts result in higher ethanol yields and reduced formation of inhibitory by-products, simplifying downstream processing and improving overall process economics.

A prominent example is the incorporation of xylose-utilization pathways through heterologous expression of XYL1, XYL2, and XYL3, coupled with enhancement of the pentose phosphate pathway to support efficient redox balance and carbon flow. These complex genetic modifications are typically implemented using homologous recombination and Cre-loxP-based marker recycling systems, allowing sequential integration of multiple genes without excessive genetic burden. Industrially, pathway-engineered yeast strains enable efficient fermentation of lignocellulosic hydrolysates containing mixed C5 and C6 sugars, thereby improving feedstock flexibility and supporting the economic viability of second-generation bioethanol production.

### Adaptive Laboratory Evolution (ALE)

Adaptive Laboratory Evolution (ALE) is a strain improvement approach that involves prolonged cultivation of yeast under defined selective pressures, such as high ethanol concentrations or the presence of fermentation inhibitors, to naturally select robust and high-performing phenotypes. Beneficial genetic adaptations acquired during ALE are later identified through genome sequencing, without relying on direct recombinant DNA techniques. From an industrial perspective, ALE-derived strains exhibit enhanced ethanol tolerance and inhibitor resistance, leading to more stable and efficient fermentations, while also offering simplified regulatory acceptance in certain regions due to the non-transgenic nature of the modifications.

## Random mutagenesis

Random mutagenesis is a classical strain improvement method used to enhance ethanol production in yeast by inducing genetic variability without targeting specific genes. This approach typically employs physical mutagens (such as UV irradiation) or chemical mutagens (such as ethyl methanesulfonate or nitrosoguanidine) to generate large mutant libraries, which are subsequently screened for desirable traits including higher ethanol yield, improved fermentation rate, and increased tolerance to ethanol and process-related stresses. Industrially, random mutagenesis has been widely adopted because it is cost-effective, scalable, and often faces fewer regulatory constraints compared to recombinant DNA technologies, making it a practical strategy for developing robust yeast strains for commercial ethanol production.

## Challenges and Future Perspectives

Despite significant advancements in genetic engineering of yeast for ethanol production, several challenges continue to limit full-scale industrial implementation. Yeast metabolism is governed by highly interconnected and tightly regulated networks, and modifications to one pathway often result in unintended effects on growth, redox balance, stress response, or by-product formation. Such metabolic trade-offs can reduce overall fermentation efficiency or strain robustness, particularly under high-gravity and lignocellulosic fermentation conditions. In addition, engineered traits that perform well at laboratory scale may not always translate effectively to industrial environments, where fluctuations in temperature, pH, substrate composition, and inhibitor levels impose additional stress on microbial systems.

Looking ahead, ensuring the long-term genetic stability of engineered yeast strains under continuous and large-scale fermentation conditions remains a critical challenge. Genetic drift, loss of inserted traits, or selective pressure-driven reversions can compromise process consistency and productivity over time. Future strategies are expected to integrate systems biology, genome-scale metabolic modelling, and adaptive evolution with advanced genome-editing tools to design more predictable and stable strains. Coupled with improved regulatory frameworks and real-time process monitoring, these approaches will support the development of next-generation yeast platforms capable of delivering high ethanol yields with enhanced robustness and industrial reliability.

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# Molecular and metabolic basis of ethanol production in Industrial yeast



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## Introduction

The yeast *Saccharomyces cerevisiae* is the most studied and well-known used eukaryote in the ethanol, brewing, baking and many more industries. It has been used for alcohol the production for thousands of years. One of the most important characteristics of this yeast is its efficient ethanol production using glucose as a carbon source (Parapouli et al., 2020a). However, they prefer its growth and development in the presence of oxygen, but in absence of oxygen it produces ethanol by converting pyruvic acid via acetaldehyde with the help of pyruvate decarboxylase and alcohol dehydrogenase enzyme, hence maintains the balance of nicotinamide adenine dinucleotide (Hydrogen) (NADH/NAD<sup>+</sup>) inside the cell. Because of its low cost and efficient ethanol production capability, it is the first choice in the wine and brewery industries (Mohd Azhar et al., 2017). Currently, more than 100 billion liters of ethanol are produced annually worldwide. Since the 1980s, the production of bioethanol has increased due to the growing popularity of ethanol as an alternative fuel. Moreover, Global ethanol output increased to 25.68 billion gallons in 2015 from 13.12 billion gallons in 2007. The largest producer of ethanol is United States with the production of nearly 15

billion gallons. Furthermore, the United States and Brazil alone produce close to 85% of the ethanol produced worldwide (Mohd Azhar et al., 2017). Ethyl alcohol (C<sub>2</sub>H<sub>5</sub>OH) is known as bioethanol, and this can be directly blended with gasoline to obtain “gasohol” (Staniszewski et al., 2007). Bioethanol has greater flammability limitations, higher temperatures of vaporization, higher octane numbers (108), and wider flame speeds. In contrast to gasoline fuel, bioethanol is less hazardous, readily biodegradable, and produces fewer airborne pollutants (John et al., 2011; Pejín et al., 2009). However, to produce first, second and third generation bioethanol a variety of feedstocks are used. The feedstocks rich in sucrose (sugar beet, sugar cane, fruits etc.) and starch (corn, rice, wheat, potato, cassava, etc.) are used in first generation ethanol production. Conversely, lignocellulosic biomass (rice straw, sugarcane bagasse, wheat straw, paddy straw, etc.) is used to make second generation bioethanol. Third generation bioethanol is produced from algal biomass, which includes both macro- and microalgae (Nigam & Singh, 2011). Some yeast strains have been shown to be good producers of ethanol from various sugars, such as *Pichia stipites* (NRRL-Y-7124), *S. cerevisiae* (RL-11), and *Kluyveromyces fragilis* (Kf1) (Mussatto et al.,

2012). Industrial ethanol production is hampered because of harsh conditions and presence of inhibitors. Additionally, *S. cerevisiae* is unable to compete with the contaminations caused by wild type yeast during fermentation process. The yeast cannot survive during fermentation due to extreme conditions such as fluctuations in temperature, osmotic stress, contamination by bacteria, and an increase in ethanol concentration. This challenge is overcome by using a stress tolerant yeast in the fermentation process (Basso et al., 2008a). Therefore, high temperature ethanol fermentation is a useful technology because it selects the microorganisms that are thermotolerant and eliminates the need for cooling expenses (Tofighi et al., 2014). Furthermore, using a thermotolerant yeast for high-temperature fermentation lowers the possibility of bacterial contamination during the process and boosts fermentation efficiency. It also promotes long-lasting life spans during harvest, good fermentation capacity, high rates of sugar-to-ethanol conversion, and low glycerol output (Fonseca et al., 2008). *S. cerevisiae* is widely used in the ethanol industry because of its long-lasting life spans during the harvest, good fermentation capacity, elevated sugar-to-ethanol conversion rates, low output of glycerol, low foam levels, tolerance to high concentrations of substrate and ethanol, resistance to acidity and high temperatures, genetic stability, flocculence, good fermentation efficiency, high productivity, elevated cell growth speeds, elevated ethanol output, and substrate consumption speeds (Parapouli et al., 2020). Moreover, it is a non-pathogenic strain and is susceptible to transformation techniques and isolation of mutants, among others (Basso et al., 2008b). Therefore, to maximize the yeast's capacity to produce ethanol and minimize the amounts of secondary metabolites produced, the fermentation conditions (substrate concentration, pH, temperature, and aeration) and yeast specificity should be carefully considered (Da Silva Fernandes et al., 2022). This article elucidates the role of yeasts in bioethanol fermentation and presents an overview of the parameters affecting yeast metabolism during the fermentation process. We will provide a concise summary of genetic techniques utilized in metabolic engineering and evolutionary engineering strategies designed to

enhance first- and second-generation bioethanol production in *Saccharomyces cerevisiae*.

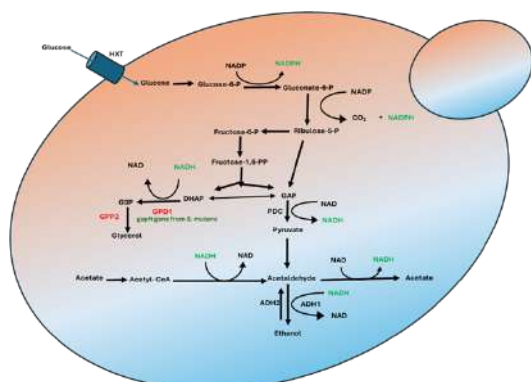
### Yeast genetics

In fermentation industry, yeast is the key player involved in fermentation process. Yeast can easily catabolize six-carbon molecules such as glucose into two carbon molecules such as ethanol with a final oxidation product carbon dioxide. The process begins with glucose uptake and catabolism through glycolysis, yielding two molecules of pyruvate, ATP, and NADH. Glycolytic flux is a major determinant of ethanol productivity. The key enzymes and genes involved in glycolysis starts from transportation of glucose units in the yeast cell with the help of hexose transporters which is encoded by gene HXT. In this pathway next catabolism step is phosphorylation of glucose with hexokinase I and II encoded by genes HXK1 and HXK2 respectively. Next, phosphofructokinase commits glucose to glycolysis which is encoded by PFK1 and PFK2 gene. The final step in glycolysis pathway is pyruvate which is further converted to acetaldehyde. Under anaerobic conditions, pyruvate is diverted to ethanol with the help of pyruvate decarboxylase genes (PDC1, PDC5, PDC6) producing acetaldehyde and CO<sub>2</sub>. In the final step, acetaldehyde is converted to ethanol with the help of alcohol dehydrogenase enzyme encoded by gene ADH1. This metabolic switch, as well as fermentative ethanol production, is critically dependent on the enzyme Alcohol Dehydrogenase (EC 1.1.1.1), encoded by the ADH1 locus. ADH1 catalyzes the reduction of acetaldehyde to ethanol during glucose fermentation, but it can also operate in the reverse direction—converting ethanol back into acetaldehyde—although with markedly lower catalytic efficiency (Mohd Azhar et al., 2017).

In *Saccharomyces cerevisiae*, two major genes encode alcohol dehydrogenase (ADH). Among these, ADH1 is expressed constitutively, whereas ADH2 expression is induced when intracellular glucose concentrations decline. The primary substrate of ADH2 is ethanol, enabling its oxidation under low-glucose conditions. Regulation of ADH2 expression is mediated by transcription factors, and genome sequencing combined with transcriptomic analyses has elucidated the structural features

and DNA-binding elements of these regulators (Parapouli et al., 2020a).

**Figure 1. Yeast genetic pathway for ethanol production**



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# Case Study: Revolutionizing Sugar Recovery Through Microbial Control Enzymes

## Introduction: Tackling Chronic Purity Losses in Sugar Processing

Daily Crushing	:	12000 TCD sugarcane
Mixed Juice Generation	:	12000 KL (Brix-14.52, Pol-11.76, Purity-80.99)
Syrup Diversion to Distillery	:	30% (3600 KL)
Ethanol and RS	:	14 %v/v and 0.29 %w/v
Retention Time	:	50 Hrs (Feeding-25 Hrs, retention-25 Hrs)
Distillery Production	:	240 KLPD

In the competitive Indian sugar industry, mills face relentless pressure from fluctuating cane quality, microbial contamination, and process inefficiencies. A leading integrated sugar-distillery operation struggled with persistent purity drops averaging 1.1-1.22%, translating to 14 MT daily sugar losses worth ₹4.2 lakh (MSP ₹31/kg) alone. Other by products Volatile acids (VA up to 7,940 ppm), lactic acid (1,405 ppm), and residual sugars (0.52% w/v) exacerbated fermentation losses, while distillery yields lagged lower ethanol yield, more retention time with poor spent wash recycling.

This case study documents a 51-day trial (Dec 2025-Jan 2026) where The Catalysts Group deployed Bactosafe I and II—proprietary enzyme formulations for microbial control. Dosed alternately at just 6 ppm on cane crush quantity (usages help in restricting microbial resistance and uncontrolled proliferation), these enzymes eliminated microbial degradation root causes. Combined with disciplined monitoring (pH/Brix/POL/purity in PJ/MJ, VA/lactic in MJ/syrup/SW) and CIP sanitization, the intervention slashed purity drops by 90%, delivered ₹4.78 lakh daily net profit, and unlocked scalable gains up to ₹2.01 lakh/day.



### Mill Condition Before Intervention: A Process Under Siege

Parameter	Historical Control	Recent Control	Impact
Purity Drop	1.22%	1.12%	0.1% reduction. 15 MT sugar loss prevented 1.4 Cr/Monthly Saving
Volatile Acid	7,940 ppm	1,779 ppm	6,161 ppm
Lactic Acid	1,405 ppm	628 ppm	777 ppm
Fermentation RS	0.52 %w/v	0.29 %w/v	0.23% indication stress reduction, Yeast boost

- **Resource Savings:** 16% process water; 11% SW recycling (52 m<sup>3</sup> reused vs 40 m<sup>3</sup>)
- **Batch Metrics:** RS 0.40%; cycle time -1 hr/batch; net SW ~60%

### Quantified Benefits: Direct ROI + Compounding Gains

Benefit Category	Daily Gain	Monthly (30 days)
Sugar Recovery	₹4.70 lakh	₹1.41 Cr
Net ROI (after cost)	₹4.78 lakh	₹1.43 Cr
Distillery Ethanol (30% diversion)	₹1.06 lakh	₹31.7 lakh
Syrup Process (70%)	₹95,591	₹28.7 lakh
<b>Total @ 12k TCD</b>	<b>₹2.01 lakh</b>	<b>₹6.04 Cr</b>

**Compounding Effects:** 2.3% distillation energy savings, ETP volume reduction, consistent gravity drops to 1.000, and high-purity MJ for seamless syrup handling.

### Breakthrough Success: A Template for Industry Transformation

This trial represents Catalysts Group's technical mastery in enzyme-driven microbial control, achieving:

**10% purity drop elimination** at minimal 6 ppm dosing  
**12.6% ethanol yield surge** as proven ancillary benefit

**Scalable blueprint:** Works across variable crushing (5k-20k TCD)

The operation's discipline—real-time analytics + CIP—amplified enzyme impact, setting a gold standard for integrated sugar-distillery optimization. This success validates Catalysts' value proposition amid India's ethanol blending mandates and cane price volatility.

### Conclusion & Results: Ready-to-Replicate Efficiency

The intervention converted a loss-making process into a high-margin operation, proving that targeted

enzymes address root-cause microbial threats more effectively than traditional chemicals. Total projected savings: ₹6 Cr/month at scale.

**Key Takeaway:** With Bactosafe I/II at 6 ppm + monitoring + CIP, any sugar mill can capture 1%+ sugar recovery while boosting distillery yields—delivering ₹2 Cr+ monthly competitive advantage.

### Final Results Snapshot:

10% Purity Drop Reduction  
4.78L Daily Net Profit  
239 KLPD Ethanol (+12.6%)  
16% Water Savings  
Scalable to ₹6 Cr/Month

This case study demonstrates The Catalysts Group's ability to deliver measurable, bankable results through biotechnology precision.

# PartnerLynx: Helping Ethanol Plants Run Smarter and Faster



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Industry Technology Specialist,  
Novonesis



**Pandurang Shinde**  
Technical Sales Manager,  
Novonesis

In ethanol production, even small delays or minor process deviations can lead to losses in yield, corn oil recovery, energy efficiency, and overall output. To stay competitive, ethanol producers need faster decisions, real time visibility, and stronger control across the entire process.

**PartnerLynx®** from Novonesis brings plant data, laboratory results, and expert support together in one secure digital system. It helps improve efficiency, increase production, and reduce losses - without adding new equipment or major capital investment.

## PartnerLynx® - A Secure Digital Connection

It creates a secure, encrypted digital connection between the plant and Novonesis experts. Data is shared safely and only when the plant chooses to do so.

The system is easy to install, requires minimal IT effort, which remains fully under producer's control. Instead of relying on site visits or exchanging reports, experts can access real time data and provide faster, more effective recommendations.

## Full Process Visibility in One Platform

It connects to key systems such as the DCS/PLC, plant historian, and lab historian. This allows production data - from raw material handling through fermentation and distillation - to be viewed together in one place. When information is connected across the entire process, issues become easier to identify and resolve. The main requirements at the plant level are an OPC UA license to connect the DCS with PartnerLynx® and a working internet connection.

## Bringing Lab Data into Operations

PartnerLynx® integrates data from existing lab instruments, including HPLC, GC, and NIR. These instruments

measure sugars, glycerol, organic acids, ethanol, impurities, moisture, starch, and other key parameters, generating valuable data every day. But often, this information stays inside the lab and is not fully linked to plant operations.

By combining lab results with live plant data, PartnerLynx® supports early detection of fermentation issues, identification of residual sugars before yield is lost, monitoring of ethanol purity, improved corn oil recovery, reduced losses in CO<sub>2</sub> and DDGS, and early identification of negative trends.

### Improving Fermentation and Yield

Fermentation is the heart of ethanol production. PartnerLynx® continuously tracks temperature, pH, fermentation time, CO<sub>2</sub> production, and residual sugars. When fermentation slows or yeast stress begins, issues can be detected early.

#### Timely intervention helps:

- Shorten fermentation cycles,
- Increase ethanol yield,
- Improve yeast performance,
- Reduce contamination risk.

Even small savings per batch can translate into meaningful gains in annual throughput.

### Reducing Losses Without New Equipment

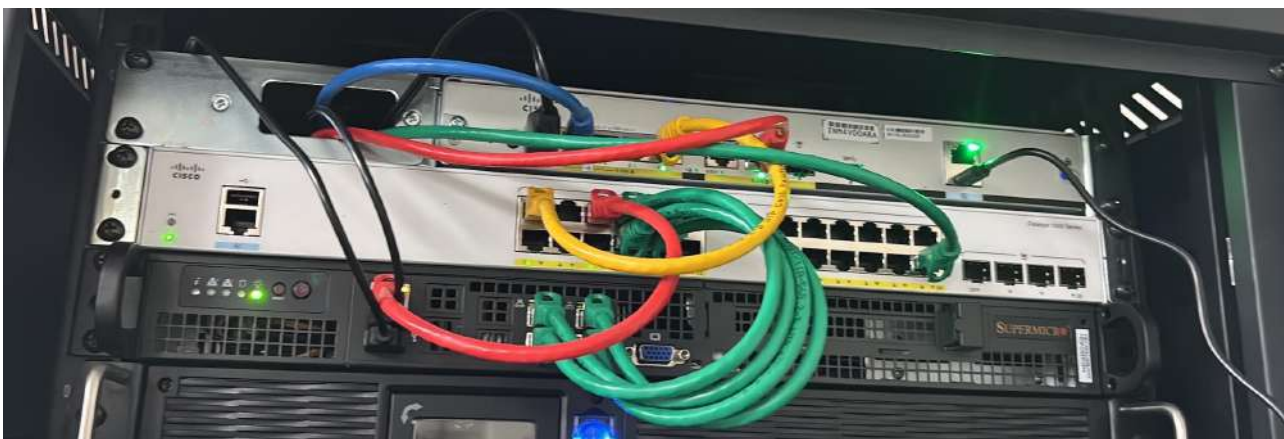
Losses can occur across starch conversion, fermentation, energy use, corn oil separation, and equipment performance. By monitoring the full process, PartnerLynx® helps teams move from guesswork to data based decisions. Reducing cycle times, improving stability, optimizing enzyme and yeast performance, and identifying bottlenecks allow higher throughput using existing assets - without heavy capital investment.

### PartnerLynx® - not just a digital tool, but a smarter way of working

By combining biotechnology, plant data, and lab results in one connected system, ethanol plants can move from reactive problem-solving to proactive performance improvement.

#### The result is simple:

- More insight.
- Less loss.
- Higher production.
- Better profitability.



# Troubleshooting Checklist for Industrial Ethanol Production



**Dr. Sibabrata Mukherjee**  
Leaf Technical Advisor



**Chloe Marteel**  
Marketing and Communication Manager



**Romain Fromanger**  
Global Innovation Manager

Ensuring **smooth operations and complete fermentation** in ethanol production requires an effective troubleshooting strategy. To guarantee a successful approach in ethanol plants, there are several aspects to consider: knowing the plant's baseline, spotting critical changes, identifying the trouble source, having an effective problem-solving strategy, and knowing who to turn to for support in such happenings.

## Knowing your production trends to start troubleshooting

For effective troubleshooting in ethanol production, it is crucial to **establish a baseline for your ethanol process**. This involves documenting and analyzing key parameters such as temperature, tank levels, pressure, pH, fermentation time, and yield. By knowing your usual trends, you can **compare current production data with historical records** to identify changes. Regularly monitoring and recording the process parameters and trends over time allows you to spot any significant variations that can indicate potential problems.

In the case of trialing at industrial ethanol plants, it could be harder to track trends, as they are varying due to new product introductions. Having

a familiarity with your baseline data is all the more important to **correlate trials' outcomes with abnormal production changes** and rate the product impact versus the performance.

## Identifying critical changes to initiate your ethanol production troubleshooting

During ethanol production, certain changes can **indicate underlying issues**. These alterations might suggest contamination, nutrition deficiencies, or other unwanted parameter variations. By promptly recognizing these critical changes, you can **initiate troubleshooting measures before the problem escalates**. Identifying these critical variations is done through the **close monitoring of various parameters** such as the yeast cell counts, glycerol levels, dry matter, bacterial presence, temperature variations, and so on.

For troubleshooting in ethanol production plants, the most impactful time to identify changes is the first 24 hours. During this timeframe, corrective alterations may still be made to bring a positive impact on the rest of the process.

There are many variations that should raise the alarm in your troubleshooting:

- A lower-than-usual yeast cell count,
- Changes in the breakdown of sugars (DP4, DP3, DP2, and DP1) over time,
- Increased residual sugars at the end of the process,
- A higher than usual fermentation temperature,
- A mildly or radically different fermentation pH,
- The presence of unwanted coproducts,
- And a lower ethanol concentration at drop.

### Discerning the source of the problem

When troubleshooting your ethanol production, it is essential to **identify the root problem causing the observed changes**. It is important to start by thoroughly investigating potential sources that could be raw materials, equipment, operating procedures, or environmental factors. At this stage, **it is key to consider the whole process**, not only the fermentation part, as well as external factors.

- Examining the **quality and integrity of inputs**: raw materials, yeast, enzymes, etc.
- Checking the **condition and performance of all equipment**: fermenters, distillation columns, etc.
- The **human factor** should not be underestimated: Standard Operating Procedures (SOP) should be reviewed to ensure they are followed correctly
- **Environmental conditions and their impact** should also be analyzed: temperature, humidity, etc.

By systematically identifying the source of the problem, you will be able to address it effectively.

### Solving the problem

When it comes to troubleshooting in ethanol production, the first step to solving the problem is always to **formulate a hypothesis based on both the investigation and available data**. Test this hypothesis through controlled experiments and simulations to verify its validity. Once the cause of the issue has been confirmed, the action plan outlining the steps needed to resolve the problem can be implemented. An efficient action plan is comprised of the necessary **corrective measures to be implemented**, documentation of the changes made, and a close monitoring of their effects on the process.

Problem-solving protocols will have the plant back on its usual, or improved, production KPIs – yield, productivity, and ethanol titer. It is key to **regularly monitor process results** to assess the effectiveness of the solutions implemented.

It is important to update your troubleshooting strategies based on the lessons learned from each troubleshooting experience. Moreover, keeping track of the problem-solving protocols supports your proactiveness towards predictable events, like higher temperatures in the summer.

### Having a reliable partner for ethanol production troubleshooting

Partnering with a **reliable technical expert** can significantly enhance troubleshooting efforts in industrial ethanol production. A reliable partner, **experienced in ethanol production**, can offer a fresh perspective and an in-depth knowledge of industry best practices. While you are experts of your plant, process, and production running, having an extra set of eyes with extensive experience in the industry can make the difference. Relying on a partner can also **open access to advanced infrastructures and technologies** for testing.

**Troubleshooting in industrial ethanol production requires a systematic and detail-oriented approach. By knowing your production baseline, identifying critical changes, determining the root cause of problems, and implementing effective solutions, you can minimize impacts and maintain product quality. By following a comprehensive troubleshooting checklist and continuously improving your processes, you can optimize industrial ethanol production and achieve sustainable success.**

Moreover, partnering with a reliable technical expert adds valuable support and expertise to your troubleshooting efforts. Through our technical support offer, we are available to assist you in your troubleshooting and production optimization. Coupling your process knowledge to our teams' expertise on microorganisms, we can partner for your problem-solving with access to application tests and microbiology audits.



# SafSpirit™ M-1: Building Premium Malt Spirits from the Fermentation Stage



**Tom Ashton**  
Technical Sales Support  
Manager, APAC



**Denise Jones**  
Technical Services,  
USA



**Viren Malhotra**  
Business Development -  
Speciality Beverages



**Prakhar Tiwari**  
Technical Sales  
Support, India

In the evolving landscape of India's premium spirits industry, distillers are increasingly shifting focus from volume to value. As the market matures, the emphasis is no longer just on distillation and cask selection—but on the foundational stages that shape a spirit's long-term character. Among these, fermentation stands out as a critical, yet often underleveraged, driver of flavour.

At the heart of this process lies yeast.

Far from being a simple alcohol-producing agent, yeast defines a significant portion of a spirit's aromatic and structural identity. One strain that has gained strong relevance in malt whisky-style production is SafSpirit™ M-1—a yeast long associated with traditional Scotch whisky, and now increasingly adopted by Indian distillers aiming for consistency and maturation-friendly profiles.

## Why Yeast Choice Matters More Than Ever

In malt-based distillation, especially where enzyme additions may be limited by stylistic choices, yeast must do more than just ferment sugars. It must handle complex substrates, perform reliably under varying conditions, and most importantly, produce a balanced spectrum of flavour compounds. SafSpirit™ M-1 is designed specifically for these demands. It offers:

- High alcohol tolerance (up to approximately 15% ABV)

- Strong attenuation, including the ability to utilise more complex sugars
- Consistent fermentation kinetics across batches
- Robust performance under temperature and raw material variability

For Indian distilleries operating in warmer climates and often dealing with fluctuations in malt quality, this reliability translates directly into production stability and predictable outcomes.

**The Flavour Advantage: Designed for Maturation**  
What truly distinguishes M-1 is its ability to generate a maturation-friendly congener profile.

During fermentation, yeast produces a wide range of compounds—primarily esters, higher alcohols, and organic acids—that collectively shape the sensory profile of the spirit. These compounds are not static; they evolve significantly during cask ageing.

- **Esters** are highly volatile compounds produced by yeast as byproducts of metabolism and typically provide fruity and floral aromas. Examples include isoamyl acetate (banana), ethyl acetate (pear/solvent), and ethyl hexanoate (apple/aniseed).
- **Higher alcohols (Fusel Alcohols)** are produced by yeast metabolism from amino acids and sugars and often contribute warming, floral, or solvent-like notes. Key examples include isoamyl

alcohol (banana/pear and phenylethyl alcohol (rose/perfume)).

- **Organic acids** include lactic, acetic, butyric, and hexanoic acids, which can undergo important esterification reactions during maturation to enhance fruity and floral notes.

SafSpirit™ M-1 is known for producing these elements in a balanced and integrated manner, avoiding extremes. The result is a new-make spirit that is expressive but not aggressive—well-suited to interaction with oak over time.

### From New-Make to Matured Spirit

A key challenge in whisky production is ensuring that the character developed during fermentation translates effectively into the matured spirit. M-1 addresses this by creating a profile that evolves positively in cask.

In new make, distillers can typically expect:

- A clean cereal backbone reflecting the malt
- Subtle fruity notes, often in the orchard fruit spectrum
- Light floral tones
- Gentle warmth without harshness

As the spirit matures, these characteristics develop into:

- Richer fruit complexity, including dried and stewed fruit notes
- Enhanced aromatic depth through esterification
- Improved integration of alcohols, leading to a smoother mouthfeel
- Better synergy with oak-derived compounds such as vanillin, lactones, and spice

This makes M-1 particularly effective for ageing in ex-bourbon barrels, virgin oak, and wine casks, all of which are increasingly used by Indian producers to create differentiated profiles.

### Operational Considerations for Distillers

While yeast selection is critical, outcomes are influenced by how fermentation is managed. Some general best practices when working with strains like M-1 include:

- **Temperature control:** Lower fermentation temperatures tend to produce cleaner, more delicate profiles, while higher temperatures can drive heavier, fruit-forward character.
- **Consistent pitching rates:** Ensuring healthy yeast populations helps maintain predictable fermentation performance and avoids off-flavour development.
- **Cut point strategy:** The choice of distillation cuts can significantly influence how yeast-derived compounds are carried into the final spirit, allowing distillers to fine-tune style.

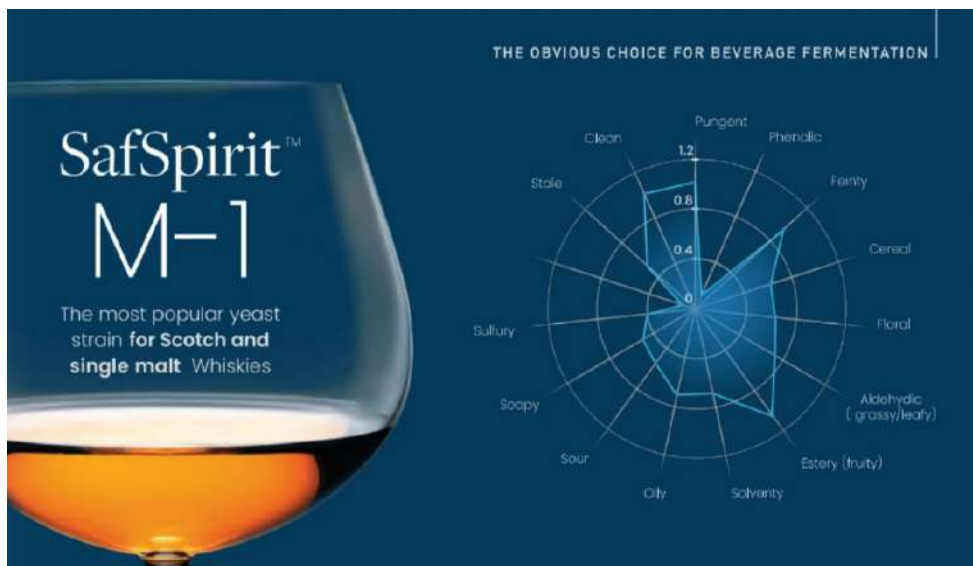
These should be treated as guiding principles, with adjustments made based on specific plant design and production goals.

### Conclusion: A Strategic Tool for Premiumisation

As Indian distillers continue to push towards globally competitive, high-quality spirits, the importance of fermentation cannot be overstated. Yeast selection is no longer a routine decision—it is a strategic one.

SafSpirit™ M-1 offers a compelling combination of process reliability and flavour precision, helping distillers create spirits that not only perform well in production but also mature with clarity and complexity.

In an industry where time, consistency, and differentiation are key, starting with the right yeast may be one of the most effective ways to build a premium spirit—from the very first stage.



# Optimizing Bioenergy from Filter Cake: A Case Study



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Ex-Director, National Sugar Institute  
& Managing Director, Greentech  
Consultants



**Dr. Meet Kamal**  
Professor, Deptt. of Chemistry, Christ  
Church College, Kanpur



**Herby Dikkumbura**  
Managing Director, Ceylon Sugar  
Industries & Holdings (Pvt.) Ltd.

One of the most significant environmental challenges faced globally today is the management of waste generated by various agro-processing industries, and the sugar industry is no exception. Increasing attention is therefore being given to minimizing waste generation and enhancing revenue through innovative utilization of by-products. With depleting natural resources and the rising demand for clean and green energy to replace conventional fossil-fuel-based systems, it has become essential to explore sustainable alternatives that maintain ecosystem balance while meeting societal energy needs. The adoption of bio-energy systems represents a rational approach toward achieving both economic and environmental sustainability while conserving fossil fuels. The recent energy crisis emerging due to Iran-Israel-America war has further underlined the need.

The conversion of a conventional sugar mill into a bio-energy-based processing unit can significantly

contribute to fossil fuel savings. Renewable electricity generated from bagasse, ethanol produced from cane juice or molasses, and biogas or compressed biogas (CBG) produced from organic residues can substitute fossil-based electricity and gasoline. A major advantage of bio-energy systems is their comparatively lower greenhouse gas (GHG) emissions compared to fossil-fuel-based energy systems.

The present study examines measures for enhancing potential of utilizing filter cake, a by-product of sugar factories, for the production of biogas and compressed biogas (CBG). Such utilization not only addresses environmental concerns associated with waste disposal but also enables value addition and resource recovery. Since the onset of industrialization, the extensive use of fossil fuels has significantly increased carbon emissions in the atmosphere. The rising carbon footprint has exceeded the natural assimilation capacity of the environment, leading to global

warming and climate change. The concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere has now reached critical levels, making it imperative to adopt measures that can mitigate further emissions.

The Indian sugar industry, one of the largest agro-based industries in the country, generates several solid by-products & wastes such as sugarcane trash, bagasse, filter cake, and bagasse fly ash. Traditionally, these materials were often considered waste, potentially posing environmental challenges. However, with appropriate valorization technologies, these by-products can be transformed into valuable resources. For instance, bagasse is widely used for cogeneration of electricity, while cane juice and molasses are utilized for the production of ethanol used in fuelblending programs. Despite these advancements, several additional opportunities exist for producing green energy from other by-products, among which biogas and CBG production is one of the most promising option.

In India, sugar factories crushed over 300 million tonnes of sugarcane during the 2024-25 crushing season. With filter cake generation estimated at about 3.5% of cane crushed, a substantial quantity of this by-product is produced annually. Filter cake typically contains 72–75% moisture, 8–10% ash, and about 20% volatile solids, with approximately 74–75% organic matter on a dry basis. Considering its high organic content and limited commercial utilization options, filter cake represents a suitable substrate for biogas production.

The fundamental principle behind biogas production is **anaerobic digestion**, a biological process in which microorganisms decompose organic matter in the absence of oxygen. The efficiency and quantity of gas production depend on several factors, including pH, temperature, organic matter concentration, and process conditions such as gas scrubbing efficiency. The gas produced mainly consists of methane and carbon dioxide and has fuel properties comparable to liquefied petroleum gas (LPG). After appropriate

purification and upgrading, the biogas can be converted into **bio-methane or compressed biogas (CBG)** suitable for use as a vehicular fuel or for other energy applications.

In addition to energy generation, the residue left after anaerobic digestion can be separated into solid and liquid fractions and used as organic fertilizer, thereby contributing to nutrient recycling and sustainable agriculture. However, there have been challenges in production of CBG using filter cake viz.

1. Sugar factories operate for few months only and hence if filter cake is used through out the year, its storage causes decay in quality lowering CBG generation potential
2. Handling of solid and liquid fractions
3. Disposal of CBG

Herein, studies carried out on use of “**EnzyProtect PM**”, a product by M/s Catalyst Biotechnologies as preservative for filter cake to optimize and maintain the CBG potential have been discussed.

## I. COMPRESSED BIO GAS (CBG) SOURCES IN INDIA

The graphical presentation at Fig. 1 gives an idea of total compressed bio-gas potential in the country which is estimated to be about 62 million metric tonnes per annum. Out of it, spent wash obtained from molasses based distilleries and filter cake (press mud) from sugar factories can contribute to the extent of about 2 million metric tonnes per annum.

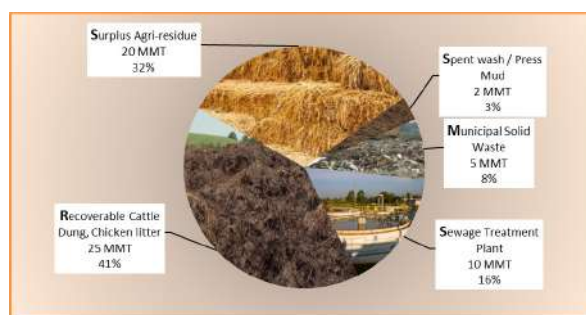


Fig 1 :Total CBG potential: 62 MMT

## II. BIOGAS AND COMPRESSED BIOGAS/BIO-CNG

Biogas is formed by anaerobic digestion using bacteria formed due to biodegradation of organic waste material. Biogas formed consists of methane, carbon dioxide and small amounts of H<sub>2</sub>S, N, CO and O<sub>2</sub>. Methane is considered the most important one with respect to use of biogas as fuel. It is pertinent to mention here that it is the methane content in the gas which determines its quality and characterizes it as biogas or compressed bio-gas/bio-CNG. While biogas is used for domestic purposes, compressed bio-gas/bio-CNG is employed as automotive fuel necessitating higher methane content and least of other impurities.

A general composition of biogas and compressed biogas/CBG is given in table 1.

**Table 1 :Characteristics of Biogas and Compressed-Biogas**

Sr. No.	Parameters	Biogas	Compressed Biogas
1.	Methane (v/v)	55-65 %	92-98 %
2.	CO <sub>2</sub> (v/v)	35-45 %	2-8 %
3.	H <sub>2</sub> S (ppm)	500-30,000	< 20 ppm
4.	Other Impurities	Present	Mostly removed, Not present
5.	Calorific Value (LCV)	19500 KJ/Kg	52000 KJ/Kg

## III. COMPOSITION OF FILTER CAKE:

The filter cake obtained from sugarcane based sugar factories usually consists of 72-75% moisture, 8-10% ash and 20 % of volatile solids along with 74-75% of organic matter on solids. However, it varies to certain extent depending upon the sugarcane quality and also on clarification process adopted by the sugar factory i.e. defecation, sulphitation and carbonation. General composition of filter cake from plantation white sugar factories adopting Double Sulphitation Process is as given in table 2.

**Table 2 :Characteristics of Filter Cake**

Sr. No.	Parameters	Average Values (% w/w)
1.	Moisture	72-75
2.	Ash	8-10
3.	Volatile Solids	18-22
4.	Organic matter on solids	72-76
5.	Sugar	1.5-2.5
6.	Wax	6-7
7.	C/N ratio	13-15

#### IV. PROCESS FOR COMPRESSED BIO-METHANE PRODUCTION

Typical process flow for production of compressed bio-gas/bio-methane/bio-CNG is shown in fig. 2. The producer gas is scrubbed to eliminate the carbon di-oxide and hydrogen sulphite which are formed along with raw gas. This process is necessary because higher concentrations of H<sub>2</sub>S gas can cause corrosion to some biogas plant parts, such as the combined heat and power units (CHP), biogas upgrading systems, and metal pipes and tanks, leading to high costs of maintenance [5]. During this process, the raw gas is absorbed then filtered and the methane content per unit volume of gas is increased which is required for bio-gas or bio-methane or as the case may be.

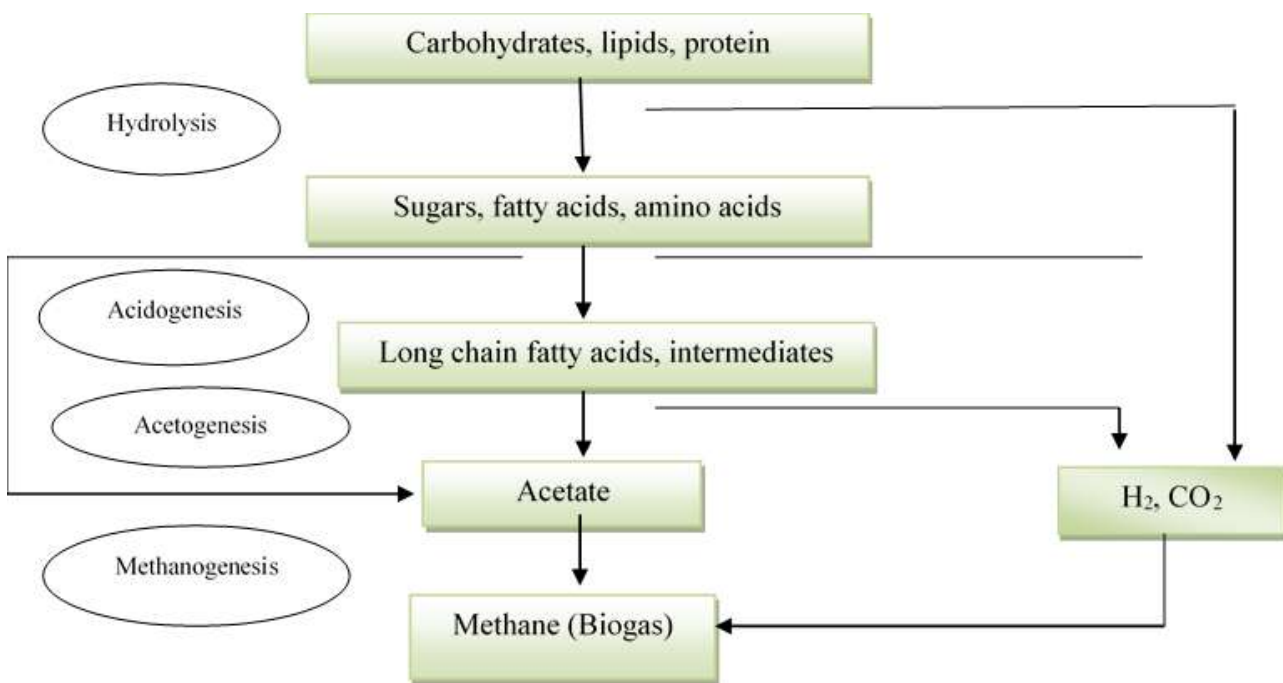


Fig 2 Diagrammatic Representation of Compressed Bio-Methane (CBG) Production

#### V. PRESS MUD PRESERVATION

Press mud from sugar industry is not preserved properly and therefore the carbohydrates and other organic components degrade with the passage of time to the extent that after few months it may be of no commercial use. In order to prevent such degradation, M/s Catalyst Biotechnologies developed a product “EnzyProtect PM”, which was tried on laboratory scale. The press mud was procured from a Sulphitation Sugar plant and “EnzyProtect PM” after dilution to 1% was applied @ 75 ppm. The contents were mixed and the covered to maintain temperature, exposure to direct sunlight and to minimize oxidation.

The treated and untreated press mud samples were analysed for various important parameters viz. TS, Ash, TVS, Lignin and TCVS content initially, after six months and one year. The observations are presented in the Table 3.

**Table 3: Analytical results on Press mud stabilization**

Time Period	TS%	VS%	Moisture%	Ash% on dry basis	TCVS% on dry basis	Lignin% on dry basis
December 2024	24.50	17.85	75.50	24.52	54.01	21.40
May 2025 (after 6 months)	24.65	17.80	75.35	24.74	53.96	21.70
November 2025 (after 12 months)	24.60	17.77	75.40	24.80	53.88	21.62

A perusal of the data contained in the table indicates no significant reduction in overall composition including TCVS content to the treated press mud sample over a period of one year.

## VI. CONCLUSION

Keeping in view the environmental concerns, the future is for green sustainable renewable energy. Using biomass can be an efficient way to reduce carbon emissions in the atmosphere and is also a good way to have a sustainable source of energy. A potential market for biogas/compressed biogas or compressed bio-methane or so called CBG is available in the India which needs to be harnessed.

With proper investment and planning, this untapped potential can be exploited which will also help in value addition for the sugar factories. The major issue of degradation of press mud quality impacting CBG production potential can be taken care using “EnzyProtect PM”, a press mud preservative by M/s Catalyst **Biotechnologies**.



# New Joinees



**Omkar Shindhe**  
Technical Solutions  
1-Oct-25



**Pathan Firoj Latif**  
R&D  
8-Oct-25



**Arnab Nandy**  
Business Development  
3-Nov-25



**Kanika Chandi**  
Marketing  
8-Dec-25



**Rahul Kumar**  
Accounts  
22-Dec-25



**Yashika Varun**  
Marketing  
17-Feb-26



**Deepak Singh**  
Administration  
2-Mar-26



**Shweta Srivastava**  
Quality Check  
5-Mar-26

# Events



23rd Anniversary Celebration



Team Retreat (HO and R&D)

# Events



Pune Office Inauguration



Lohri Celebration

**WEBINAR**

**Sulphite: The Hidden Challenge in Ethanol Fermentation**  
Root Causes, Process Impact, and Mitigation Strategies

9 January, 2026  
4:00 PM - 5:00 PM (IST)

**WEBINAR RECORDING**



Dr. K. V. T. S. Pavan Kumar  
Vice President - R&D, QA & QC



Webinar Q1

# Appreciation

## COASTAL BIOTECH PRIVATE LIMITED

**CIN : U24290OR2021PTC035710**

Head Office : 'Coastal One' Plot No. 1, Balaji Nagar, 3rd Floor, D.No. 8-1-5/4, Siripuram, Visakhapatnam - 530 003., A.P., India  
GSTIN : 21AAJCC3124H1Z6, Mobile : 92466 24660, e-mail : anandbammidi@yahoo.co.in

### Letter of Appreciation

We would like to sincerely thank "The Catalysts Group– South Team" for their excellent support in the Liquefaction and Fermentation processes right from the commissioning period.


We truly appreciate the timely technical assistance provided by your team, as committed. It has made a significant difference in ensuring our operations run smoothly.

We are equally pleased with your consistent on-time delivery of materials while maintaining high quality standards. Your team has demonstrated remarkable commitment, coordination, and professionalism throughout the process.

It has been a pleasure working with such a dedicated and responsible team. Your efforts have greatly contributed to our success, and we deeply value the support extended to us.

We look forward to continuing this association and wish the entire Catalysts Group team the very best in your future projects and continued success.

Best regards,

  
COASTAL BIOTECH PRIVATE LIMITED

Registered Office : Plot No. 144 and 146 and 147, Khata No. 118, Mouza Maringi, Garabandha PS, Garabandha, Gajapati, Paralakhemundi, Odisha, India - 761215, Mobile : 94925 81793

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