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Innovations in Methods and Technologies for the Production of Bioethanol from Sugarcane Molasses via Fermentation: A Systematic Review

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Abstract: Bioethanol is a potential renewable energy source in Indonesia, mainly due to the availability of sugarcane molasses as a by-product of the sugar industry. This study aims to examine methods and technologies for producing bioethanol from molasses for application in vocational education at SMKN 1 Anyer, which has distillation facilities and access to molasses from the PT PDSU sugar factory. The method used was a Systematic Literature Review (SLR) by analyzing 16 scientific articles indexed in Scopus. The results of the study showed seven categories of methods and technologies for producing bioethanol through molasses fermentation: co-fermentation, continuous fermentation, fermentation with statistical optimization, strain isolation, cell immobilization, fermentation with modeling, and fermentation pretreatment. Important aspects of fermentation methods and technologies that can be applied to practical procedures in vocational schools include the optimum conditions for molasses fermentation (temperature 30°C, pH 5.5, sugar content 30-35°Brix, duration 5-7 days), types of microorganisms or strains, and measurement of molasses and bioethanol yield. The research conclusion confirms that this SLR provides a scientific basis for the development of bioethanol practicum modules in vocational schools. The integration of scientific research with practical learning supports the transition to green energy through the utilization of local potential.

Keywords: bioethanol, molasses, fermentation.

Abstrak: *Bioetanol merupakan sumber energi terbarukan yang potensial di Indonesia, terutama karena ketersediaan molase tebu sebagai produk samping industri gula. Penelitian ini bertujuan mengkaji metode dan teknologi produksi bioetanol dari molase untuk diterapkan pada pembelajaran vokasi di SMKN 1 Anyer, yang memiliki fasilitas distilasi serta akses molase dari pabrik gula PT PDSU. Metode yang digunakan adalah Systematic Literature Review (SLR) dengan menganalisis 16 artikel ilmiah terindeks Scopus. Hasil kajian menunjukkan tujuh kategori metode dan teknologi produksi bioetanol melalui fermentasi molase: ko-fermentasi, fermentasi kontinyu, fermentasi dengan optimasi statistik, isolasi strain, imobilisasi sel, fermentasi dengan modeling dan praperlakuan fermentasi. Hal-hal penting dari metode dan teknologi fermentasi yang dapat diterapkan untuk prosedur praktikum di sekolah SMK meliputi kondisi optimum fermentasi molase tebu (Suhu 30°C, pH 5,5, kadar gula 30-35°Brix, waktu 5-7 hari), jenis mikroorganisme atau strain, serta pengukuran molase dan hasil bioetanol. Simpulan penelitian menegaskan bahwa SLR ini memberikan landasan ilmiah bagi pengembangan modul praktikum bioetanol di sekolah vokasi. Integrasi riset ilmiah dengan pembelajaran praktik mendukung transisi menuju energi hijau melalui pemanfaatan potensi lokal.*

Kata kunci: *bioetanol, molase, fermentasi.*

INTRODUCTION

SMKN 1 Anyer, through its Industrial Chemical Engineering program, is equipped with laboratory facilities and key equipment, including a distillation unit. This provides a significant opportunity to integrate Project-Based Learning (PjBL) focused on the utilization of waste from local industries. One of the local industries located near the school is PT Permata Dunia Sukses Utama (PDSU), a leading sugar factory in Cilegon, Banten. A byproduct of sugar production, molasses, can be utilized as a feedstock for bioethanol production. Molasses

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can be obtained from PT PDSU; this collaboration between the school and the industrial sector is relevant to vocational education and supports the fulfillment of renewable energy needs.

One potential source of renewable energy is bioethanol. Bioethanol is a liquid chemical (ethanol) derived from the fermentation of plant-based raw materials (carbohydrates); since its production involves biological processes, the resulting product is named bioethanol [1]. Among various biomass feedstocks, molasses (sugarcane juice) is one of the most widely utilized substrates for bioethanol production due to its abundant availability and high sugar content.

There are three steps to producing bioethanol: preparing the raw materials, conducting fermentation, and finally purification. Before processing, cassava, sorghum, sugarcane, or other materials used are first ground into a pulp. Enzymes are then added to facilitate the fermentation process. Fermentation is the process of converting the raw material into bioethanol [1].

Molasses is a byproduct of the sugar crystallization process in the sugarcane processing industry. This material contains high levels of sucrose, glucose, and fructose, as well as minerals such as calcium, potassium, and magnesium. This sugar content makes molasses an ideal substrate for fermentative microorganisms like *Saccharomyces cerevisiae* to produce ethanol. Additionally, using molasses as a bioethanol feedstock offers added value by reducing sugar industry waste while providing an economic solution for sugarcane-producing regions.

However, the use of molasses also faces several challenges. The presence of inhibitory compounds such as organic acids, furfural, and heavy metal ions can reduce microbial activity during fermentation. Therefore, various studies focus on the pretreatment or initial processing of molasses, for example through dilution, pH adjustment, or the addition of supplementary nutrients to improve fermentation efficiency.

The bioethanol fermentation process generally involves the conversion of sugar into ethanol and carbon dioxide by microorganisms, primarily yeast. The most commonly used microorganism is *Saccharomyces cerevisiae* because it has high tolerance for ethanol and high sugar concentrations.

In the context of vocational education at SMKN 1 Anyer, this literature review serves as a crucial foundation for developing research-based learning activities. With the availability of molasses feedstock from the PT PDSU sugar factory and the school's distillation laboratory facilities, the results of this scientific study can be directly implemented in the form of bioethanol fermentation laboratory experiments. Students not only understand the principles of biotechnology and process chemistry but also contribute to the development of renewable energy that supports the transition toward a green industry at local level.

In implementing learning through laboratory experiments, a standardized and ready-to-use experimental procedure is required to develop students' skills. However, in the initial stages when new experiments using new materials and equipment are conducted, a series of trial runs of the experimental procedure is necessary to produce the desired results. In practice, various challenges arise, including the lack of suitable laboratory procedures, the unknown factors influencing the molasses fermentation process, and the unknown characteristics of the molasses feedstock and available distillation equipment. Due to these challenges, a systematic literature review (SLR) of various scientific articles is required to answer the research questions: What are the fermentation methods and technologies used in the production of bioethanol from sugarcane molasses? And what are the key aspects of fermentation methods and technologies that can be applied to laboratory procedures in vocational high schools?

METHODS

The methodology used in this study was a Systematic Literature Review (SLR) of scientific articles indexed in Scopus, employing the PRISMA approach. The primary objective of this literature review was to analyze the methods and technologies used in the production of bioethanol from molasses. The review process followed the PICOS framework (Population, Intervention, Comparison, Outcomes, and Study design) to systematically evaluate the characteristics and results of each study.

Identification Process

The literature search was conducted using sources from Scopus. The keywords entered in the Scopus search were: ("bioethanol production" OR "ethanol fermentation" OR "bioethanol process") AND (molasses OR "sugarcane molasses" OR "blackstrap molasses") AND (fermentation OR "enzymatic hydrolysis" OR saccharification OR distillation).

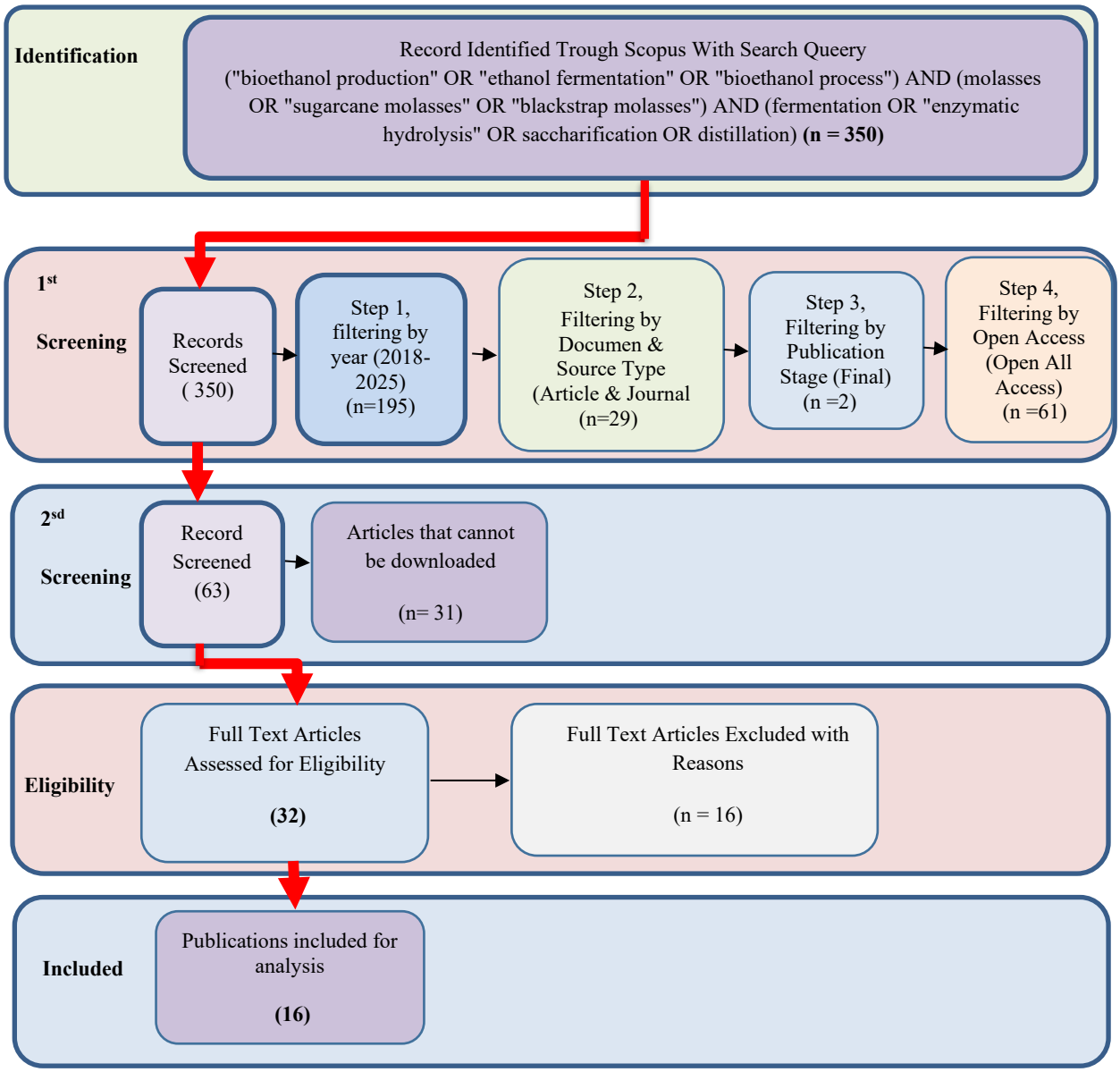

Figure 1. PRISMA flowchart for this study

Screening Process

Following the identification phase, the initial 350 articles were screened based on publication year (2018–2025), document type and source (articles and journals), publication status, and open-access availability. Articles that could not be downloaded or accessed in full text were excluded. Following this initial screening and quality check, 32 articles advanced to the eligibility assessment stage.

Eligibility Process

At this stage, the abstracts of the 32 articles were further evaluated to determine their relevance to the research focus. Articles that did not explicitly discuss methods and technologies in the bioethanol production process from sugarcane molasses were excluded. As a result, 16 articles were selected for in-depth analysis.

Data Synthesis Process

Data from the 16 eligible articles were collected and analyzed using the PICOS framework (Population, Intervention, Comparison, Outcome, and Study design). After analysis, a data table summarizing the article content was created and analyzed based on the research questions. The synthesis focused on the methods and technologies in the bioethanol production process from sugarcane molasses and key aspects of fermentation methods and technologies.

Process Results

The PRISMA flow diagram summarizes the steps of identification, screening, and eligibility assessment, culminating in the final selection of 16 articles for this systematic review.

RESULTS AND DISCUSSION

Results

This section presents the findings and discussion from a literature review of 16 articles analyzed using the PICOS framework (Population, Intervention, Comparison, Outcome, and Study design). Based on the results of the systematic literature review, a summary of the data and research contexts from the 16 articles was obtained and is presented in Table 1.

This study was designed to assess innovations in methods and technologies for bioethanol production from sugarcane molasses through fermentation. The primary focus is to analyze how various fermentation methods and technologies for sugarcane molasses influence bioethanol production outcomes, particularly as the results of this study may impact the implementation of practical procedures in vocational high schools (SMK). This study specifically aims to answer the key questions: What are the fermentation methods and technologies used in the bioethanol production process from sugarcane molasses? And what are the key aspects of fermentation methods and technologies that can be applied to practical procedures in vocational high schools?

Table 1. Data summary and research context

No	Ref	Population/Problem	Intervention	Comparison	Outcome	Study Design
1	[2]	Low molasses fermentation efficiency	Co-fermentation of *S. cerevisiae* and *W. anomalus*	Single fermentation	Ethanol yield increased to 35.5 g/L	Experimental
2	[3]	Low bioethanol productivity	IAABR continuous reactor	Batch process	Productivity reached 4.63 g/L·h	Experimental
3	[4]	Inefficient high gravity fermentation	Plackett–Burman & Taguchi optimization	Non-optimized process	Efficiency reached 83.82%	Experimental
4	[5]	Low industrial yeast efficiency	Wild yeast isolation	Industrial yeast	Ethanol production up to 14%	Experimental
5	[6]	Yeast tolerance limitation	*Candida tropicalis* isolation	*S. cerevisiae*	Higher tolerance to stress	Experimental
6	[7]	High raw material cost	Corn cob and molasses integration	Single substrate	Ethanol 93.34 g/L	Experimental
7	[8]	Low bagasse efficiency	SSF of bagasse and molasses	Single substrate	Ethanol 94.2 g/L	Experimental
8	[9]	Inhibition by Ca^{2+} ions	Ca^{2+} inhibition study	No inhibitor	Reduced sugar production	Experimental
9	[10]	High concentration molasses limitation	Engineered tolerant strain	Wild strain	Ethanol 111.65 g/L	Experimental
10	[11]	Inaccurate fermentation model	Genetic Programming model	Conventional model	Higher prediction accuracy	Experimental & Modeling
11	[12]	Fermentation optimization	Monod & Andrews model	Model comparison	Andrews model more accurate	Modeling
12	[13]	Benzoic acid inhibition	Stress tolerance testing	No acid condition	Reduced ethanol production	Experimental
13	[14]	Low ethanol production	Selenium supplementation	No supplementation	Ethanol increased to 55 g/L	Experimental
14	[15]	Extreme condition fermentation	Marine yeast fermentation	Conventional yeast	Improved ethanol yield	Experimental
15	[16]	Fermentation contamination	Electron beam disinfection	Control	Faster fermentation	Experimental
16	[17]	Wort contamination	Electron irradiation	No irradiation	Improved efficiency	Experimental

Based on the analysis of the collected data, significant findings were identified regarding the annual number of publications, the methodologies employed, and the types of sugarcane molasses fermentation methods and technologies studied in bioethanol production. The period from 2018 to 2025 generally shows an upward trend from 2018 to 2019; however, research activities declined from 2019 to 2021, likely due to the COVID-19 pandemic affecting nearly all countries and reducing global research intensity. A significant increase occurred post-pandemic, specifically from 2021 to 2024. This surge in 2024 indicates that a growing number of studies are examining renewable energy in response to the demands of rapidly advancing technologies and sustainable development.

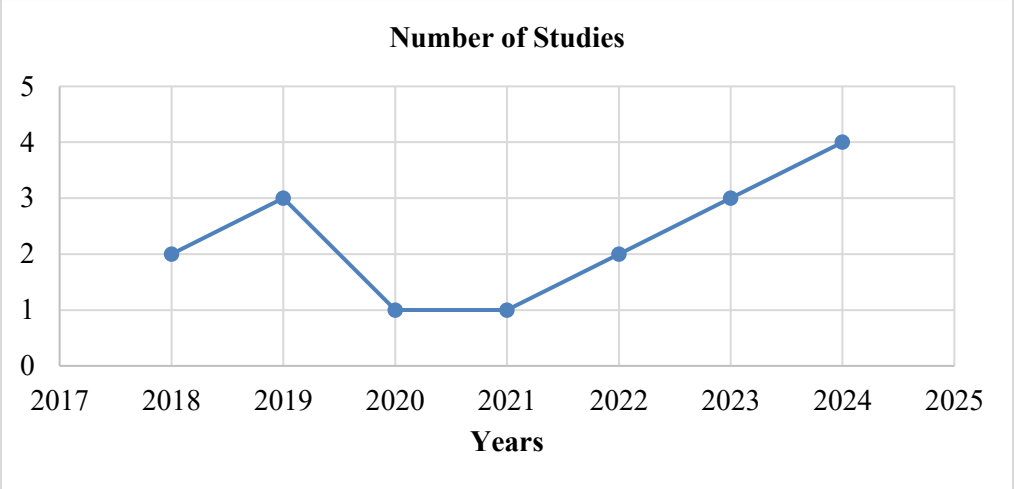

Figure 2. Number of studies each year

A systematic review of the 16 articles shows that innovations in methods and technologies for the production of sugarcane molasses-based bioethanol can be classified into seven fermentation categories, as illustrated in Figure 3. These categories include: strain improvement and isolation of tolerant strains (5 studies); co-fermentation / mixed culture (2 studies); continuous fermentation & simultaneous saccharification and fermentation with native microorganisms / simultaneous saccharification and fermentation (SSFNM/SSF) (2 studies); high gravity fermentation & statistical optimization (2 studies); modeling & fermentation kinetics (2 studies); pretreatment / disinfection (2 studies); and cell immobilization (1 study).

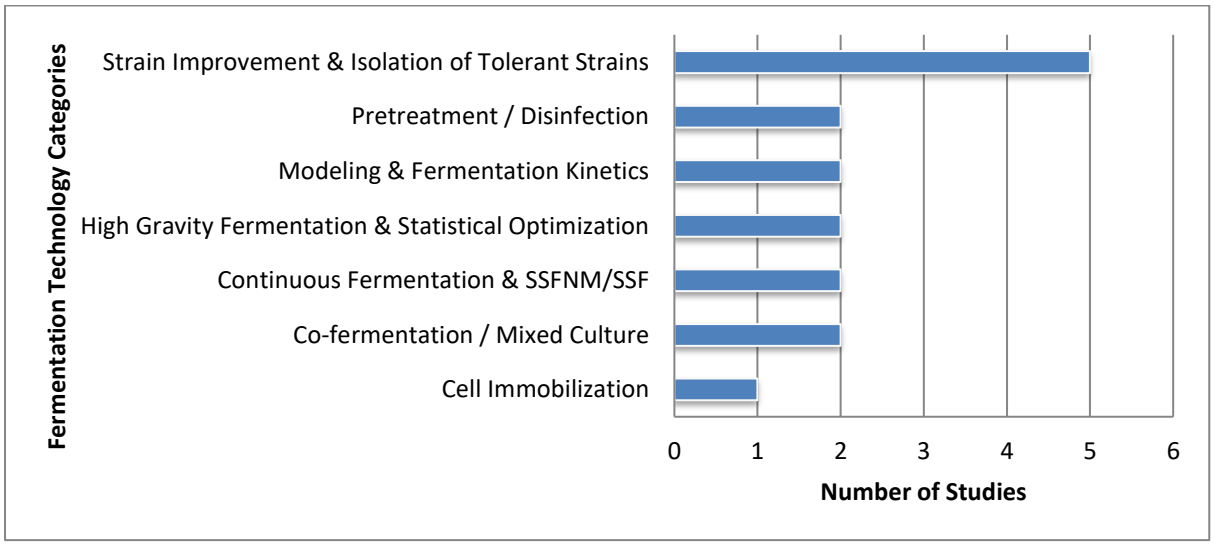

Figure 3. Graph showing the number of studies by fermentation technology category.

Based on the analysis results, there has been an increase in interest in bioethanol production research, particularly regarding fermentation methods and technologies. This is reflected in the growing number of publications between 2021 and 2024. This trend underscores the importance of sugarcane molasses fermentation methods and technologies in bioethanol production. Research innovations in various countries have been shown to be significantly influenced by the methods and technologies employed. Various studies highlight the fermentation process as the primary stage in bioethanol production, which is inherently influenced by the type of microorganisms, pretreatment, fermentation methods, and overall operational parameters. To provide a clear categorization without replicating the PICOS analysis, the 16 selected studies are classified into 7 distinct fermentation technology categories, as detailed in Table 2.

Table 2. Distribution of research methodologies

Fermentation Technology Category	Description / Focus	Ref	Article / Method	Findings / Results
Co-fermentation / Mixed Culture	Using two or more strains to increase ethanol	[2]	S. cerevisiae TA2 + W. anomalus HCJ2F-19 (RSM-CCD)	Ethanol increased by 29–53%
	Lignocellulose + molasses co-fermentation	[8]	SSF High Solid Loading	Ethanol 94.2 g/L
Continuous Fermentation	Continuous bioreactor system	[3]	IAABR reactor	Productivity 3.4× higher
	SSF native microbes	[7]	Molasses + corn cob	Ethanol 93.34 g/L
High Gravity Optimization	High gravity fermentation	[4]	Statistical optimization	Efficiency 83.82%
	Selenium supplementation	[14]	RSM optimization	Ethanol 55 g/L
Strain Improvement	Genetic engineering	[10]	ARTP + ALE	Ethanol 111.65 g/L
	Multi-stress yeast	[5]	Isolation	Ethanol 14% v/v
	New yeast strain	[6]	Candida tropicalis	Tolerant to 41°C
	Marine yeast	[15]	Seawater fermentation	93.5 g/L
	Benzoic acid stress	[13]	RNA-Seq	Reduced ethanol
Cell Immobilization	Cell reuse	[16]	$MnFe_2O_4$ immobilization	42.10 g/L
Modeling	Temperature prediction	[11]	Genetic Programming	Optimal temperature
	Kinetic model	[12]	Monod vs. Andrews	Andrews model performed better
Pretreatment / Disinfection	e-beam sterilization	[17]	15 kGy irradiation	Faster fermentation
	Ca^{2+} inhibition	[9]	Invertase kinetics	Competitive inhibition

Microbial performance is the most critical factor in the conversion of sugar to ethanol. The type and resilience of yeast strains were studied to determine their tolerance levels to temperature, osmotic pressure, pH, and sugar concentration. The most commonly used yeast strain is *Saccharomyces cerevisiae* (SC); however, some studies have employed combinations of strains, such as SC with other yeast types, lactic acid bacteria strains, ions, and immobilized yeast cells. There are also studies that do not use SC but instead use other types of microorganisms, such as *Meyerozyma caribbica* and *Candida tropicalis*. Combining two or more microorganisms can enhance fermentation efficiency.

Molasses derived from sugarcane has a high sugar content as well as many interfering components. Sugar concentration is expressed in °Brix. In a study, fermentation using a combination of *S. cerevisiae* TA2 (high ethanol tolerance) and *W. anomalus* HCJ2F-19 (low ethanol tolerance) significantly increased bioethanol efficiency and productivity from sugarcane molasses compared to single-strain fermentation (*S. cerevisiae* alone), resulting in a significant increase in bioethanol production of 35.5 g/L [2]. Another study comparing potential yeast strains *Saccharomyces cerevisiae*, *Meyerozyma caribbica* MJTm3, and *Wickerhamomyces anomalus* showed that *Meyerozyma caribbica* MJTm3 is a superior strain with high tolerance to ethanol, temperature, pH, and high sugar levels, achieving a 38% increase in fermentation efficiency after condition optimization [5].

Challenges in molasses-based bioethanol production are often hindered by the limited tolerance of *Saccharomyces cerevisiae* to temperature stress, acidic pH, and high sugar concentrations. Kasmiarti et al. conducted research focused on identifying new yeast strains that are more tolerant to the extreme conditions of the bioethanol industry. Three superior isolates were identified: *Candida tropicalis* strain L2 (K5), *C. tropicalis* strain MYA-3404 (K15), and *C. tropicalis* strain Y277 (K20). All three isolates showed consistent growth at 41°C, pH 3.5, and a sugar concentration of 35°Brix, whereas the control *S. cerevisiae* only grew optimally under normal conditions (30°C, neutral pH). The results indicate that *C. tropicalis* is capable of adapting to extreme fermentation conditions and has the potential to replace or complement conventional industrial strains in molasses fermentation [6].

The fermentation process is influenced by temperature, pH, time, pretreatment, and gravity. Using the same method, namely CCD-based RSM, for the co-fermentation of *Saccharomyces cerevisiae* TA2 and *Wickerhamomyces anomalus* HCJ2F-19 under optimal conditions of 30°C, 30°Brix sugar content, and pH 5.5, fermentation increased by 29–53% [2].

Application of a two-stage sequential optimization strategy: the Plackett-Burman Design to identify significant factors (urea, wheat bran, soy flour, and inoculum size) and the Taguchi Design to optimize fermentation conditions (temperature, agitation speed, fermentation time, and agitation time) [4].

Yeast immobilization is defined as the physical confinement of intact cells within a specific spatial region while preserving biological activity (Jaime Moreno). The immobilization of *Candida tropicalis* (an isolate from coconut) on $MnFe_2O_4$ coated with chitosan has proven to be an effective strategy. This immobilized yeast strain increased bioethanol production from molasses (50% sugar) compared to free yeast [16].

The use of a laboratory-scale Integrated Aerobic–Anaerobic Baffled Reactor (IAABR) for continuous fermentation without a pH control system can be operated effectively and stably for the continuous production of bioethanol from sugarcane molasses. A productivity of 4.63 g/L·h indicates significant potential for efficiency improvements compared to industrial batch systems [3].

Research using the Simultaneous Saccharification and Fermentation with Native Microorganisms (SSFNM) approach successfully developed a cost-effective and environmentally friendly process for bioethanol production using corn cobs and sugarcane molasses, utilizing the native microorganisms present in the feedstock, thus eliminating the need for added enzymes or commercial yeast [7].

Modern fermentation technologies such as continuous fermentation, immobilized cells, IAABR, and SSF have been shown to increase bioethanol productivity compared to traditional batch fermentation. The findings indicate that cell stability and reactor residence time significantly contribute to increased production. Additionally, the use of experimental design and statistical optimization (RSM, Taguchi) consistently improves final ethanol yield. The integration of molasses with sugarcane bagasse (SCB) that has been alkali-pretreated in the SSF (Simultaneous Saccharification and Fermentation) process with high solids loading is an effective method for increasing cellulosic bioethanol production and optimizing the sequential utilization of biomass resources [8].

Nutrient factors such as urea, wheat bran, soy flour, and inoculum size significantly influence fermentation efficiency. Using Plackett-Burman Design optimization, efficiency reached 83.82%, approaching normal fermentation conditions [4]. Selenium (Se) supplementation during sugar beet molasses fermentation using *Saccharomyces cerevisiae* significantly increased bioethanol production without significantly affecting yeast growth rates. An optimal selenium concentration of 15 μg and a substrate concentration of 25°Bx resulted in nearly a twofold increase in ethanol concentration (55 g/L compared to 29 g/L in the control) [14]. This confirms that selenium plays a crucial role in enhancing the efficiency of sugar-to-ethanol conversion.

Research on inhibition identification confirms that the molasses fermentation process for bioethanol is affected by substrate inhibition, where very high sugar concentrations inhibit yeast growth [12]. Microbial contamination in the wort (fermentation medium) causes significant losses in global ethanol production. The industry currently relies heavily on chemicals and antibiotics to control contamination [17].

Potassium (K^+) and calcium (Ca^{2+}) ions present in high-concentration sugarcane molasses are the primary limiting factors inhibiting ethanol production by *Saccharomyces cerevisiae*. Through ARTP (Atmospheric and Room Temperature Plasma) physical mutagenesis and adaptive evolution, an SC strain was engineered and successfully developed into a highly resilient SC strain (NGTM-F1) capable of overcoming K^+ and Ca^{2+} ion inhibition, yielding 111.65 g/L of ethanol [10]. The presence of Ca^{2+} ions in the sucrose hydrolysis system using the invertase enzyme inhibits enzyme activity and reduces the production of reducing sugars (fermentable sugars). The higher the Ca^{2+} ion concentration, the lower the production of reducing sugars [9].

Sugarcane molasses often contains benzoic acid compounds that have a significant toxic effect on *Saccharomyces cerevisiae* yeast cells during ethanol fermentation, causing oxidative stress and cellular damage, thereby substantially reducing ethanol production and fermentation efficiency [13].

Discussions

Most studies focused on selecting yeast strains tolerant to extreme molasses conditions such as osmotic pressure, high sugar levels, inhibitory ions, and high ethanol concentrations. Strains such as *Meyerozyma caribbica*, *Candida tropicalis*, as well as yeast derived from mutagenesis and adaptive evolution, have been shown to provide significantly higher ethanol conversion rates compared to standard industrial yeast. This reinforces the finding that cellular resilience is key to improving yield.

Sugarcane molasses as a feedstock holds both potential and challenges. Its high sucrose content supports ethanol production, but the presence of Ca^{2+}, K^+, and benzoic acid has been shown to reduce fermentation activity. SLR studies indicate that Ca^{2+} inhibition of invertase and oxidative stress caused by benzoic acid are major contributors to reduced fermentation rates. Thus, molasses pretreatment and nutrient supplementation are strategic steps to improve fermentation performance.

The patterns of findings across all articles suggest a trend that production efficiency can be enhanced through the optimization of biology, processes, and technological engineering. Consistent similarities are observed in factors such as pH, temperature, sugar concentration, and strain characteristics that influence ethanol yield. However, variations among studies emerge regarding the formulation of supplemental nutrients, yeast type, reactor design (batch vs. continuous), and the presence of substrate pretreatment. These differences indicate that while the general principles of fermentation are the same, each study tailors its optimization to the characteristics of the molasses feedstock and the selected technology.

Integrating Methodological Findings with Vocational Education

Based on the findings of the systematic literature review, it can be concluded that the most promising technologies for implementation in practical learning at Vocational High Schools (SMKs) are simple co-fermentation, optimization of fermentation conditions, and the utilization of locally available raw materials. These three approaches offer several advantages, including ease of implementation, cost efficiency, and compatibility with school laboratory facilities. Furthermore, these technologies are capable of representing the fundamental scientific principles of bioethanol production, such as microbial interactions, the influence of process variables, and the conversion of substrates into products. Therefore, their implementation is not only practical but also supports contextual and application-oriented experimental learning aligned with the needs of vocational education.

Among the various technologies reviewed, simple co-fermentation emerges as the most promising approach for implementation in SMK practical activities. This is because the method can clearly demonstrate improvements in fermentation efficiency without requiring complex equipment or procedures. By comparing single and mixed fermentation processes, students can directly observe differences in outcomes, thereby strengthening their empirical understanding of bioprocess concepts. Moreover, the integration of this method into learning activities has the potential to enhance students' critical thinking skills, analytical abilities, and work readiness in the field of industrial biotechnology. Therefore, the development of co-fermentation-based practical modules can serve as an innovative strategy to improve the quality of vocational education in alignment with the demands of industry and the labor market.

The dominance of experiment-based quantitative methodologies in molasses fermentation research is closely linked to vocational education, particularly in Industrial Chemical Engineering programs at vocational high schools. Vocational education is required to prepare students to understand and operate fermentation and distillation processes using a scientific approach in line with industry needs. Therefore, the results of this SLR study can be adapted into Grade 11 learning modules that emphasize the learning outcomes (LO) for Industrial Microbiology and Distillation.

Relevance to the LO for Industrial Microbiology

Quantitative methods, which dominate fermentation research, can serve as a foundation for developing microbiology curriculum materials, particularly regarding the following competencies:

- Identification, isolation, and characterization of industrial microorganisms;
- Basic to advanced fermentation techniques;
- Microbial growth conditions (pH, temperature, nutrients, stress);
- Process optimization through experimentation.

Through SLR findings, students can be introduced to the isolation of industrial yeast [5], [6], the impact of pH, temperature, and osmotic pressure on yeast performance, batch and continuous fermentation in line with bioethanol fermenter practices, and inhibitory factors such as Ca^{2+}, K^{+}, and benzoic acid. This integration makes microbiology learning more contextual, research-based, and relevant to the needs of the chemical industry.

Connection to the Distillation Curriculum

Most articles provide numerical data related to ethanol production, making them highly relevant to the distillation learning outcomes for 11th-grade students, including:

- Principles of mixture separation;
- Simple and fractional distillation;
- Calculation of alcohol concentration from fermentation;
- The relationship between fermentation quality and distillation efficiency.

Students can be trained to measure the ethanol content of fermentation products, analyze the relationship between ethanol concentration and distillation energy requirements, and understand that optimal fermentation can reduce distillation costs [4]. These findings make distillation instruction more practical and directly linked to bioethanol industry practices.

Integration of SLR into Learning Modules

Research methodology outcomes can be developed into bioprocess-based Project-Based Learning (PjBL) modules for 11th-grade students, including:

- Isolation of local yeast (*Saccharomyces cerevisiae*);
- Laboratory-scale molasses fermentation (batch 1–2 L) utilizing the available distillation unit facilities;
- Measuring molasses concentration using a refractometer in °Brix units;
- Measuring bioethanol yield using a pycnometer or alcoholometer;

- Optimizing fermentation process conditions (pH 5.5, temperature 30°C, sugarcane molasses with a sugar concentration of 30–35°Brix);
- Quantitative data analysis, including calculating fermentation efficiency;
- Discussing fermentation results and their direct implications for the subsequent distillation process.

With this approach, students not only understand the core theory but also actively apply the scientific method as practiced in modern bioethanol research.

CONCLUSIONS

A synthesis of the 16 selected articles indicates a clear shift in molasses fermentation research from conventional methods toward more integrated, efficient, cost-effective, and sustainability-oriented approaches. The innovations in fermentation methods and technologies for sugarcane molasses in bioethanol production encompass seven distinct categories: co-fermentation, continuous fermentation & SSFNM/SSF, high-gravity fermentation & statistical optimization, strain improvement & isolation of tolerant strains, cell immobilization, fermentation modeling & kinetics, and pretreatment/disinfection.

Key aspects of these fermentation methods and technologies that are highly applicable to practical laboratory procedures at vocational high schools (SMKs) include the establishment of optimal conditions for sugarcane molasses fermentation (temperature of 30°C, pH of 5.5, sugar content of 30–35°Brix, and a duration of 5–7 days), the selection of appropriate microorganisms or robust strains, and the precise calculation and measurement of molasses and bioethanol yields.

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REFERENCES

[1] N. Huda, *Proses Pembuatan Bioethanol*. Kemendikbud, 2017.

[2] E. Hawaz *et al.*, "Bioethanol production from sugarcane molasses by co-fermentation of Saccharomyces cerevisiae isolate TA2 and Wickerhamomyces anomalus isolate HCJ2F-19," *Ann. Microbiol.*, vol. 74, no. 1, p. 13, 2024, doi: https://doi.org/10.1186/s13213-024-01757-8.

[3] Margono, M. Kaavessina, M. A. K. M. Zahari, and A. Hisyam, "Continuous Bioethanol Production Using Uncontrolled Process in a Laboratory Scale of Integrated Aerobic–Anaerobic Baffled Reactor," *Period. Polytech. Chem. Eng.*, vol. 64, no. 2, pp. 172–178, 2020, doi: https://doi.org/10.3311/PPch.14653.

[4] H. E. Mohammed, M. I. Abo-Alkasem, E. M. Ammar, T. E. Mazeed, and A. M. Abu Taleb, "Enhancement of bioethanol production by Saccharomyces cerevisiae under high gravity fermentation using sequential optimization strategy," *Egypt. J. Chem.*, vol. 64, no. 12, pp. 7017–7028, 2021, doi: https://doi.org/10.21608/ejchem.2021.78360.3904.

[5] E. Hawaz *et al.*, "Isolation and characterization of bioethanol producing wild yeasts from bio-wastes and co-products of sugar factories," *Ann. Microbiol.*, vol. 72, no. 1, p. 39, 2022, doi: https://doi.org/10.1186/s13213-022-01695-3.

[6] G. Kasmiarti, D. Oktiarni, P. L. Hariani, N. Novia, and H. Hermansyah, "Isolation of Novel Yeast from Coconut (Cocos nucifera L.) Water and Phenotypic Examination as the Potential Parameters in Bioethanol Production," *Fermentation*, vol. 8, no. 6. p. 283, 2022. doi: https://doi.org/10.3390/fermentation8060283.

[7] S. Chongkhong, "Integration of Ensiled Corncob to Diluted Molasses as Carbon and Microbial Sources for Ethanol Production," *Trends Sci.*, vol. 21, no. 1, p. 7015, Oct. 2023, doi: https://doi.org/10.48048/tis.2023.7015.

[8] M. Fan, S. Zhang, G. Ye, H. Zhang, and J. Xie, "Integrating sugarcane molasses into sequential cellulosic biofuel production based on SSF process of high solid loading," *Biotechnol. Biofuels*, vol. 11, no. 1, p. 329, 2018, doi: https://doi.org/10.1186/s13068-018-1328-0.

[9] Hargono, B. Jos, Abdullah, and T. Riyanto, "Inhibition Effect of Ca2+ Ions on Sucrose Hydrolysis Using Invertase," *Bull. Chem. React. Eng. Catal.*, vol. 14, no. 3, pp. 646–653, Dec. 2019, doi: https://doi.org/10.9767/bcrec.14.3.4437.646-653.

[10] W.-Y. Wang, B.-P. Wang, H.-S. Su, M.-M. Wei, Y.-T. Wei, and F.-X. Niu, "Key role of K+ and Ca2+ in high-yield ethanol production by S. Cerevisiae from concentrated sugarcane molasses," *Microb. Cell Fact.*, vol. 23, no. 1, p. 123, 2024, doi: https://doi.org/10.1186/s12934-024-02401-5.

[11] C. Nolasco-Hipolito *et al.*, "Modeling the Effect of Temperature on Ethanol Fermentation Using Saccharomyces Cerevisiae CSI-1 by Genetic Programming: Modeling the Effect of Temperature on Ethanol Fermentation," *J. Appl.*

Res. Technol., vol. 21, no. 5, pp. 753–763, Oct. 2023, doi: https://doi.org/10.22201/icat.24486736e.2023.21.5.1997.

[12] H. Zentou, Z. Zainal Abidin, R. Yunus, D. R. Awang Biak, M. Zouanti, and A. Hassani, "Modelling of Molasses Fermentation for Bioethanol Production: A Comparative Investigation of Monod and Andrews Models Accuracy Assessment," *Biomolecules*, vol. 9, no. 8. p. 308, 2019. doi: https://doi.org/10.3390/biom9080308.

[13] L. Xiu-Feng, X. Yu-Lei, and Z. Xue-Mei, "Response mechanism of Saccharomyces cerevisiae under benzoic acid stress in ethanol fermentation," *Sci. Rep.*, vol. 14, no. 1, p. 28757, 2024, doi: https://doi.org/10.1038/s41598-024-80484-1.

[14] S. Faramarzi, Y. Anzabi, and H. Jafarizadeh-Malmiri, "Selenium supplementation during fermentation with sugar beet molasses and Saccharomyces cerevisiae to increase bioethanol production," *Green Process. Synth.*, vol. 8, no. 1, pp. 622–628, 2019, doi: https://doi.org/10.1515/gps-2019-0032.

[15] A. S. Zaky, D. Greetham, G. A. Tucker, and C. Du, "The establishment of a marine focused biorefinery for bioethanol production using seawater and a novel marine yeast strain," *Sci. Rep.*, vol. 8, no. 1, p. 12127, 2018, doi: https://doi.org/10.1038/s41598-018-30660-x.

[16] G. Kasmiarti, H. Hermansyah, Novia, and P. L. Hariani, "The Use of Molasses in Producing Bioethanol Catalyzed by Candida tropicalis (Isolated from Cocos nucifera. L) Immobilized MnFe2O4 Coated-Chitosan," *Molekul*, vol. 19, no. 1, pp. 180–188, Mar. 2024, doi: https://doi.org/10.20884/1.jm.2024.19.1.10765.

[17] R. P. Calegari *et al.*, "Wort disinfection treatment with electron beam for bioethanol production," *Sci. Agric.*, vol. 80, p. e20210260, 2023, doi: https://doi.org/10.1590/1678-992X-2021-0260.