Research Article

Impact of Yeast Strain Selection on Ethanol Yield from Low Concentration $KMnO_4$ Pretreated Rice Straw: Process Design and Utility Cost Analysis

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Abstract
This study evaluates the impact of yeast strain selection on ethanol yield from $KMnO_4$-pretreated rice straw, integrating process design and utility cost analysis. $KMnO_4$—a cost-effective, widely available, and less toxic alternative to acid pretreatments—is applied at a 1.36% concentration. Fermentation of a 49 mg/mL sugar solution using four yeast strains identified *Pichia kudriavzevii* TISTR 5147 (PK 5147) as the most efficient, achieving a 93.59% ethanol conversion—significantly outperforming *Saccharomyces cerevisiae* (20.95%), *Kluyveromyces marxianus* TISTR 5116 (5.96%), and *K. marxianus* TISTR 5616 (7.51%). Aspen Plus® simulations reveal that although PK 5147 requires 20–24% more distillation energy, its utility cost per ton of ethanol is substantially lower—22 times lower than TISTR 5116 and 13 times less than *S. cerevisiae*. Higher ethanol concentrations reduced purification energy, and solvent recycling further optimized process costs. Additional savings are achieved through the integration of high-temperature solvent and water recycling within the process design. The wide range of ethanol yields observed (5.96–93.59%) highlights the critical role of software-based cost estimation in evaluating experimental results during early-stage process design.

Keywords: Bioethanol, Biorefinery, Consolidated bioprocessing, Fermentation efficiency, Lignocellulosic biomass, Process cost analysis

1 Introduction

The production of bioethanol from renewable sources offers a promising means of mitigating fossil fuel dependence and environmental impact. Among various feedstocks, rice straw—a widely available agricultural residue—has gained significant attention due to its abundance and potential for valorization. Converting rice straw into bioethanol not only addresses agricultural waste management but also supports circular economy practices. It contributes to reducing greenhouse gas emissions, thereby promoting a cleaner energy landscape [1]. Over the past decade, rice straw has emerged as a focal point for research on ethanol production from fermentable sugars [2], [3]. However, efficient ethanol production from lignocellulosic biomass requires optimizing feedstock preparation, pretreatment, enzymatic hydrolysis, fermentation, and product separation. Feedstock preparation—including drying, size reduction, and conditioning—enhances surface area and uniformity, which improves overall process efficiency [4], [5]. Pretreatment is a critical step that disrupts the recalcitrant lignocellulosic structure—primarily composed of cellulose, hemicellulose, and lignin—to improve enzymatic accessibility. In this study, $KMnO_4$ pretreatment is employed to oxidize and partially degrade hemicellulose and lignin, exposing the cellulose fraction for subsequent enzymatic action. During enzymatic hydrolysis, cellulase enzymes convert cellulose $(C_6H_{10}O_5)_n$ into glucose monomers via the reaction:

$$\left(C_6H_{12}O_6\right)_n + nH_2O \xrightarrow{cellulase} nC_6H_{12}O_6$$

The resulting glucose is then fermented by yeast into ethanol and carbon dioxide:

$$C_6H_{12}O_6 + nH_2O \xrightarrow{yeast} 2C_2H_5OH + 2CO_2$$

This process forms the foundation for cellulosic ethanol production from rice straw [6]. Advancements in each stage, particularly in pretreatment and strain selection for fermentation, are critical for improving yield, lowering costs, and enhancing process sustainability.

Several studies have investigated pretreatment methods to improve rice straw conversion into bioethanol. Ningthoujam *et al*., [7] employed 4% NaOH in the pretreatment process to improve the structural accessibility of the biomass for enzymatic hydrolysis, resulting in an optimal reducing sugar concentration of 0.62 g/L. Subsequent fermentation produced a significant improvement over the 3.67% yield from untreated straw. Lee *et al*., [8] investigated the effects of temperature and acid concentration on rice straw pretreatment. Optimal pretreatment conditions were found to be 1% H_2SO_4 at 160 °C, yielding 259 mg/g reducing sugar after enzymatic hydrolysis. Mohammadi *et al*., [9] employed an ionic liquid, 1-H-3-methylmorpholinium chloride, to optimize pretreatment, significantly boosting hydrolysis yield to 70.1% and ethanol production to 64% (from 21.9%) of the theoretical maximum. This approach demonstrated cost-effectiveness owing to its simple synthesis and low required concentration. Mutrakulcharoen *et al*., [10] optimized potassium permanganate pretreatment of rice straw to enhance enzymatic saccharification. The optimized conditions significantly increased sugar yield, demonstrating the potential for improved biofuel and value-added product production. Prasad *et al*., [11] investigated the enzymatic saccharification of microwave-assisted, alkali- and acid-pretreated rice straw using various fungal strains. They found that *T. reesei* yielded the highest fermentable sugar concentration (55.6 g/L) from alkali-pretreated rice straw. Subsequent fermentation with *P. stipitis* produced the highest ethanol concentration (25.3 g/L).

Different yeast strains exhibit varying ethanol yields and inhibitor tolerances, significantly impacting overall process efficiency. Tadesse *et al*., [12] isolated yeasts from different sources in Ethiopian forests, with 67% producing ethanol. *Saccharomyces cerevisiae* 9Li2 yielded up to 99.5 g/L ethanol from 200 g/L glucose in 48 h. Kirdponpattara *et al*., [13] proposed a cell immobilization technique using *Pichia stipitis* TISTR5806 on water hyacinth and silk cocoon, reporting the highest ethanol concentration of 13.3 g/L with water hyacinth, and stable ethanol production (8.2–10.4 g/L) over five repeated batches due to its high porosity and surface area. A study showed that fermentation of dewaxed and alkali-pretreated tobacco residue using yeast resulted in a 34% increase in ethanol yield compared to standard pretreated biomass, underscoring the impact of feedstock conditioning on fermentation efficiency [14]. Sharma *et al*., [15] introduced a strategy combining sequential substrate addition with concurrent saccharification and co-fermentation (mf-SSCF), employing *S. cerevisiae* to efficiently produce high-concentration

ethanol (68.72 g/L) from dilute-acid-pretreated rice straw at a lower production cost. This process involves multiple feedings of pretreated straw without detoxification, solid-liquid separation, or sterilization, resulting in high ethanol yield (0.42 g/g) and productivity (0.95 g/L/h). Nandal *et al*., [16] evaluated the fermentation efficiency of four yeast strains on alkali-pretreated rice straw hydrolysates, synthetic sugars, and inhibitor-containing media, with *P. stipitis* NCIM3497 achieving the highest ethanol yield (57.30%) within 24 h. Kumar *et al*., [17] optimized alkali pretreatment of rice straw using 2% NaOH, achieving 55.34% delignification and 53.30% cellulose enrichment. Subsequent enzymatic hydrolysis by *Aspergillus niger* resulted in 80.51% cellulose conversion, and fermentation with *S. cerevisiae* showed higher conversion efficiency (70.34%) compared to *Zymomonas mobilis* (39.18%). Abdel-Salam *et al*., [18] applied recombinant technology by cloning the avicelase gene from *B. subtilis* into *E. coli*, optimizing its expression, and successfully producing bioethanol from rice straw with a yield of 5.26% (v/v) and 86% efficiency.

Downstream processing, which involves separating and purifying ethanol, is energy-intensive and represents another area for efficiency improvements. Developing separation techniques or integrating bioethanol production with other bioproduct recovery processes can enhance overall process economic viability. For instance, Ranganathan [19] conducted a techno-economic analysis of ethanol production from rice straw, focusing on minimizing utility costs. Their study identified a scenario as the most cost-effective, demonstrating the potential for significant utility savings. Botshekan *et al*., [20] proposed a type of dividing-wall column and pervaporation as energy-efficient alternatives to conventional separation units. Their Aspen Plus® simulation and techno-economic analysis achieved significant reductions in energy consumption (up to 67%) and capital costs (up to 19%), resulting in a lower ethanol production cost.

Despite notable advancements, challenges remain in optimizing pretreatment methods, selecting efficient yeast strains, and ensuring process sustainability. To improve saccharification efficiency, this study employs $KMnO_4$ pretreatment on rice straw—a method offering several advantages such as low cost, wide availability, and reduced toxicity compared to conventional acid-based approaches. The novelty of this work lies in extending prior research on mild-temperature $KMnO_4$ pretreatment [10] by incorporating a separate hydrolysis and fermentation (SHF) strategy to enhance bioethanol production. A key contribution of this study is the integration of experimental data from multiple yeast strains into an Aspen Plus® simulation model to assess ethanol yield and utility requirements. This simulation framework allows the evaluation of different fermentation conditions and provides insight into their impact on process efficiency. By focusing on cost-centric optimization, the study identifies the most economically viable yeast strain and suitable fermentation conditions. This contribution offers valuable guidance for early-stage design decisions and supports the development of sustainable bioethanol production from $KMnO_4$-pretreated rice straw.

2 Materials and Methods

2.1 *Sample preparation and pretreatment*

The optimal pretreatment conditions using $KMnO_4$ were previously established, and the following outlines the sample preparation and pretreatment steps [10]. Rice straw, sourced from Phra Nakhon Si Ayutthaya province, Thailand, was dried in a hot air oven (60 °C) to reduce moisture content. The dried straw was mechanically ground with a food processor and subsequently passed through a 20-mesh sieve to obtain a consistent particle size. $KMnO_4$ pretreatment was employed to enhance the enzymatic saccharification of rice straw. Optimal pretreatment conditions were determined in prior work using a Box-Behnken Design (BBD) to systematically investigate the effects of temperature, time, and $KMnO_4$ concentration on sugar release. This rigorous optimization identified a $KMnO_4$ concentration of 1.36% (w/v) as the optimal condition. Pretreatment conducted at 84 °C for 360 minutes with a biomass-to-solvent ratio of 1:10 led to a substantial improvement in sugar release, thereby enhancing the efficiency of fermentation and ethanol production. Following pretreatment, the biomass was separated from the solvent through filtration with Whatman No. 1 filter paper and thoroughly rinsed with deionized water until a neutral pH was obtained. The rinsed biomass was subsequently dried in a hot air oven at 60°C until a stable weight was achieved. Both the dried pretreated biomass and the untreated samples underwent enzymatic hydrolysis for comparative analysis. The composition of lignocellulosic biomass

before and after pretreatment is analyzed using high performance liquid chromatography (HPLC), following protocols established by the National Renewable Energy Laboratory (NREL) [21]. The analysis involved sample preparation according to NREL standard biomass analytical procedures (LAP), which include biomass drying, grinding, and extraction using a suitable solvent system. The fermentable monosaccharides (glucose, arabinose, and xylose) are measured using high-performance liquid chromatography (HPLC) equipped with a CTO-10AS VP system (Shimadzu, Kyoto, Japan) and an Aminex HPX-87 H column (Bio-Rad Laboratories, Inc., California, USA). The analysis is carried out at 65 °C and 549 kPa, utilizing 0.005 M sulfuric acid as the mobile phase at a flow rate of 0.5 mL/min. Standards of analytical-grade monosaccharides with known concentrations, obtained from Sigma Aldrich, are used for calibration [22], [23].

2.2 *Enzymatic hydrolysis and analysis of reducing sugar*

Enzymatic saccharification of pretreated rice straw is performed in 2 mL centrifuge tubes using citrate buffer (50 mM, pH 4.8). Cellic CTec2, a commercial enzyme cocktail containing cellulases, β-glucosidases, and hemicellulases (Sigma-Aldrich, density 1.15 g/mL), is added to the pretreated rice straw at a concentration of 1.4 μL/g biomass. The mixture is then incubated in a shaking incubator at 50 °C and 150 rpm for 72 h [24]. After incubation, the liquid hydrolysate is centrifuged to separate the solid residue. The supernatant is collected and analyzed for reducing sugar content using a modified DNS method (Miller 1959). For the DNS assay, 50 μL of hydrolysate is mixed with DNS reagent and incubated at 95 °C for 5 minutes. The mixture is then cooled, diluted with water, and analyzed using a UV/Vis spectrophotometer at 540 nm. The reducing sugar content is quantified based on a standard glucose curve.

2.3 *Bioethanol fermentation*

2.3.1 Fermentation using different yeast strains

In this study, batch fermentation is conducted to produce bioethanol from hydrolysate derived from rice straw lignocellulose biomass. Four yeast strains obtained from The Thailand Institute of Scientific and Technological Research are employed: *Kluyveromyces marxianus* TISTR 5116 (KM 5116), *Kluyveromyces marxianus* TISTR 5616 (KM 5616), *Pichia kudriavzevii* TISTR 5147 (PK 5147), and *S. cerevisiae*. Each fermentation experiment uses the selected yeast culture comprising 19 mL of liquid hydrolysate and 1 mL of yeast inoculum. Cell concentration was determined spectrophotometrically at 660 nm. A specific optical density (OD) of 1 was used to ensure consistent inoculation. Glucose (1% w/v) and yeast extract (1% w/v) are added to facilitate yeast acclimatization as carbon and nitrogen sources [25]. The sample pH is adjusted to 5.0, and then incubated at 30 °C for 60 hours in a shaking incubator (100 rpm). After fermentation, the yeast cultures are centrifuged to separate the liquid supernatant (8000 rpm, 10 min). Ethanol concentration in the supernatant is determined using gas chromatography with a flame ionization detector (GC-FID). A GC-2010 system (Shimadzu, Japan) with a 30 m × 0.25 mm × 0.25 μm DB-wax column is used for the analysis. Ethanol samples are injected in split mode (1:20) with a 1 mL injector volume [22]. Ethanol content is quantified based on an absolute ethanol standard curve (99.8%), with three measurements performed for accuracy. Ethanol yield, sugar yield, and ethanol conversion were calculated using standard methods as described in [26]. The ethanol conversion is calculated as the ratio of ethanol produced to the initial reducing sugar content in the hydrolysate, providing a clear measure of the fermentation efficiency in converting sugars to ethanol.

2.3.2 Statistical analysis

All experiments are conducted in triplicate, and the results are presented as mean ± standard deviation (SD). Differences in ethanol yield and conversion efficiency among the four yeast strains (*K. marxianus* TISTR 5116, *K. marxianus* TISTR 5616, *P. kudriavzevii* TISTR 5147, and *S. cerevisiae*) are evaluated using one-way analysis of variance (ANOVA). Please note that, although statistical variations are taken into account through triplicate experiments and ANOVA, only the average values are used in the process simulation to represent typical fermentation performance. This simplification is necessary to enable effective scale-up and techno-economic analysis in Aspen Plus®.

2.4 *Process flowsheet development and description*

2.4.1 Basis for simulation

The simulation is conducted using Aspen Plus® V11 (www.aspentech.com), based on 50 t/h of milled dry rice straw. The simulated rice straw composition comprised 39.42% hemicellulose, 36.15% cellulose, 11.33% acid-insoluble lignin, 9.53% ash, 2.26% acid-soluble lignin, and a minor fraction attributed to crude protein (1.3%) [22]. The simulation follows the SHF (Separate Hydrolysis and Fermentation) process, with pretreatment using 1.36% $KMnO_4$. The model utilized the NREL database for chemical compounds and structural components in lignocellulosic biomass, specifically in the Aspen Plus® simulation DW1102A [27]. NRTL equations of state are employed for modeling vapor-liquid equilibrium. Note that the parameters used in the Aspen Plus® simulations were carefully selected to align with our experimental data. Regarding the process conditions, we employed the DSTWU model to design column conditions, optimizing parameters such as the number of stages (as detailed in Table 4). While these parameters may require further adjustment during validation with real-world process data, they provide a solid foundation for early-stage process design.

2.4.2 Process description

In the pretreatment phase, milled dry rice straw is introduced into a semi-batch reactor (Pretreatment) where it is treated with 1.36% $KMnO_4$ heated to 84 °C in a heat exchanger and heater (HTEX-1 and HEATER-1). This process facilitates the breakdown of the hemicellulose, thereby enhancing the susceptibility of cellulose to enzymatic hydrolysis. Subsequently, the pretreated biomass undergoes separation from the liquid phase using filtration (FILTER-1) and is washed with recycled water sourced from the separation section using filtration (FILTER-2) before proceeding to the hydrolysis and fermentation stages. The high-temperature pretreatment solvent is recycled to preheat the incoming liquid stream at the heat exchanger (HTEX-1). Multiple pretreatment reactors are employed to ensure uninterrupted process operation during the loading and removal of rice straw. The pretreated biomass is then fed into the batch reactor for hydrolysis (HYDROLYS), which is operated at 50 °C. Make-up water is heated to 50 °C (HEATER-2) and then added to the batch hydrolysis reactor along with cellulase enzymes. This creates a solid loading of 20 wt% biomass, which allows the cellulase enzymes to catalyze the hydrolysis of cellulose into glucose. Recycled water is avoided from this point onward to prevent impurities that may affect the subsequent fermentation. After hydrolysis, the liquid fraction of the product stream is separated from the solid fraction (FILTER-3), sterilized, cooled to 30 °C (CHILLER-2), and fed into the fermenter (FERMENTER). A fresh yeast inoculum is added to the fermenter for each fermentation cycle, where the yeast converts glucose into ethanol and CO_2. The process design in this study involves inoculating a new yeast culture at the start of every fermentation cycle rather than reusing yeast strains across multiple cycles. This strategy eliminates the need to monitor cell viability and ethanol conversion efficiency over repeated cycles, as each cycle begins with a freshly prepared inoculum.

The CO_2 is released from the fermenter, while the solid phase is separated from the liquid product stream (FILTER-4). The product separation section features two distillation columns, each designed to achieve a final product specification of 95% ethanol. Both columns are designed to ensure a 95% recovery of ethanol in the distillate. In the first distillation column (BEER-COLUMN), the distillate stream contains varying ethanol percentages for each strain due to different fermentation yields. The conditions of the second column (RECT-COLUMN) are adjusted for each strain to obtain the desired 95% ethanol specification. The bottom streams from both columns are either recycled or purged to optimize overall process efficiency and product quality. The process flow diagram is demonstrated in Figure 1. It should be noted that undesirable compounds from fermentation may affect the purification process and warrant further study. However, the process design includes a filtration step prior to purification, which effectively removes solid impurities that could impact purification efficiency. Impurities in the liquid phase are expected to have minimal impact on distillation. Given the relatively low volatility of ethanol compared to potential contaminants, these compounds can be largely disregarded in this study, as their effect on final product purity is negligible. The process flowsheet is developed with a similar structure for all strains, with differences only in the reaction settings in the fermenter and in the conditions of the distillation columns.

Table 1: Aspen Plus® block specifications for biomass-to-ethanol conversion simulation.

	Unit Operation	Block Type	Specification
Pre-treatment	Heat exchanger	HEATX	$T_{in,\ feed}$ = 20 °C
	Heater	HEATER	T_{out} = 84 °C
	Pretreatment reactor	RSTOICH	T = 84 °C, Time = 6 h Rxn: Hemicellulose → Xylose
	Washing and Solids	MIXER	Recycled process water
	Recovery Unit	SEP	Split fraction: 99% liquid
Hydrolysis	Hydrolysis reactor	RSTOICH	T = 50 °C, Time = 72 h Rxn: Cellulose → Glucose
	Heater	HEATER	T_{out} = 50 °C
	Hydrolysis filter	SEP	Split fraction: 95% liquid
Fermentation	Fermenter	RSTOICH	T = 30 °C, Time = 60 h Rxn: Glucose→ $2CO_2$ + 2 EtOH
		HEATER	$T_{out,\ hot}$ = 90 °C, $T_{out,\ cold}$ = 30 °C
		MIXER	Hydrolysate + Inoculum
	Fermentation filter	SEP	Split fraction: 95% liquid
Separation	Beer column	RADFRAC	LK: EtOH,
	Rectification Column	RADFRAC	HK: water, Kettle reboiler
	Water Recycling Unit	MIXER	Bottom product
		FSPLIT	Split fraction: 85% liquid

2.5 *Model construction*

All reactors are modeled using RSTOICH blocks with conversions based on previous studies for pretreatment and hydrolysis [22], and the results of current work for fermentation. Following pretreatment, the washing step is simulated using MIXER and SEP blocks. The fermenter model incorporates HEATER and MIXER blocks to represent the sterilization system, encompassing both sterilization and inoculation. The model acknowledges the presence of CO_2 generated during fermentation, which can be removed during the actual process. For simulation purposes, the SEP block removes CO_2 along with other solids. The separation section leveraged MIXER and FSPLIT blocks to depict the water recycling and purge system. While the RadFrac block served as the primary model for the distillation columns, achieving the desired final product specification of 95% ethanol necessitated a more complex internal configuration incorporating both DSTWU and RadFrac blocks. Table 1 summarizes the Aspen Plus® block specifications used in this simulation.

2.6 *Utility cost analysis*

The total energy consumption for each scenario is calculated using Aspen Plus® and further analyzed with the Aspen Process Economic Analyzer (APEA). The utilities considered include medium-pressure steam, refrigerated water, and cooling water. Utility costs are estimated using the methodology outlined by [28]. It is assumed that steam is produced in a natural gas boiler, while electricity for cooling and refrigeration water production is sourced from a coal-based power plant. Aspen Plus® simulations are conducted utilizing the DSTWU column model for initial distillation column design and the RadFrac model for more rigorous column simulations.

3 Results and Discussion

3.1 *Compositional analysis of rice straw*

To build on the compositional analysis described earlier, HPLC is employed following standard protocols established by the NREL [27]. The rice straw is subjected to preprocessing steps to enhance its suitability for pretreatment. These steps include drying, size reduction, and cleaning to reduce moisture content, increase surface area, and remove impurities, respectively. This pretreatment enhances the effectiveness of the following enzymatic hydrolysis and fermentation processes. Table 2 summarizes the composition of rice straw in both its raw and potassium permanganate-pretreated forms.

Table 2: Effect of $KMnO_4$ pretreatment on rice straw composition.

Sample	Cellulose (%)	Hemi-cellulose (%)	Lignin (AIL) (%)	Lignin (ASL) (%)	Ash (%)
Raw	36.15	39.42	11.33	2.26	9.53
Pretreated	42.65	14.70	10.87	2.00	7.13

The substantial decrease in hemicellulose content from 39.42% to 14.7%, coupled with a corresponding increase in cellulose content from 36.15% to 42.65% following $KMnO_4$ pretreatment, is

noteworthy. This compositional shift is anticipated to enhance cellulose accessibility for cellulases, thereby potentially improving subsequent saccharification yields. The hemicellulose reaction mechanism proposed in Table 1 is supported by experimental observations, which indicate limited lignin degradation. This is consistent with the presence of residual lignin in the liquid phase post-pretreatment, subsequently removed by washing. Experimental data was utilized to scale up the hydrolysis process in the simulation.

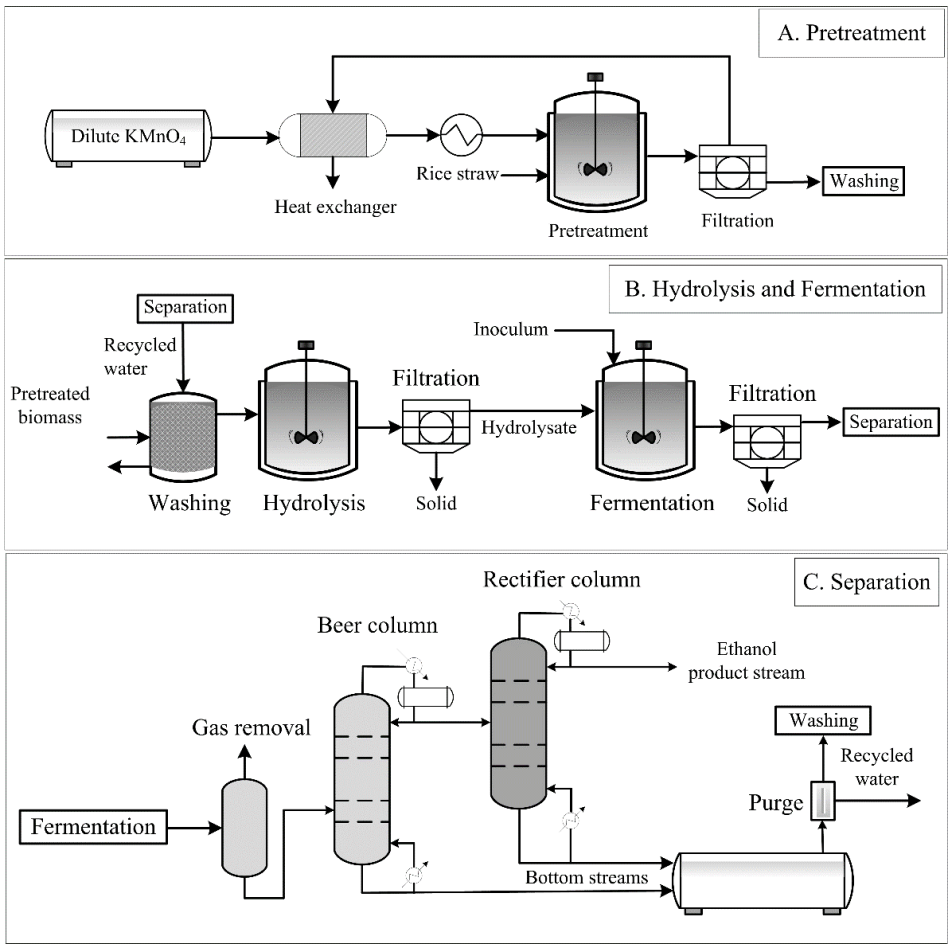

Figure 1: Process flow diagram for ethanol production from rice straw using the different proposed yeast strains.

Further studies should investigate the impact of $KMnO_4$ on waste disposal and identify strategies to enhance its benefits, including the development of regulatory systems for wastewater treatment [29]–[31]. However, using $KMnO_4$ at a low concentration (1.36%) in this study is expected to have minimal environmental impact, though data remains limited. Some research suggests that $KMnO_4$ can slightly reduce the chemical oxygen demand in water samples and shows promise in wastewater treatment for pollutant removal and sludge recycling. As a green oxidant, $KMnO_4$ offers potential environmental benefits, including wastewater decontamination and risk mitigation [29], [30]. The compositional data presented herein served as the input for the Aspen Plus® process simulations detailed in the basis for simulation subsection.

3.2 *Experimental ethanol yields*

Table 3 presents a comparison of bioethanol yield and conversion rates achieved during the fermentation of rice straw biomass using various yeast strains. The initial sugar concentration of 49 mg/mL used for

process design aligns with previous work [22]. The conversion rate is expressed as a percentage and is based on the initial concentration of reducing sugars present [25]. The data presented in the table highlight the significant influence of yeast strain selection on the efficiency of bioethanol production from lignocellulosic biomass. *P. kudriavzevii* TISTR 5147 emerged as the most efficient strain, achieving a remarkable bioethanol yield and conversion rate of 93.59%. This value is substantially higher compared to the yields obtained with *S. cerevisiae* (20.95%), *K. marxianus* TISTR 5616 (7.51%), and *K. marxianus* TISTR 5116 (5.96%). The non-conventional yeast *P. kudriavzevii* presents a high potential for enhancing ethanol fermentation, as previously reported in [32]. While different strains of *P. kudriavzevii* may exhibit varying characteristics, this particular strain demonstrated superior ethanol tolerance and production capabilities compared to *S. cerevisiae* strains. It could grow and produce ethanol efficiently at a wide temperature range (10–40°C) and high ethanol concentrations (up to 20% v/v). Additionally, its growth and substrate consumption kinetics were well-predicted by the Gompertz model, while ethanol production followed the Luedeking-Piret model. These findings suggest a strong correlation between the specific yeast strain and its ability to convert reducing sugars into ethanol during rice straw fermentation. The superior performance of *P. kudriavzevii* TISTR 5147 warrants further investigation into its potential for large-scale, optimized bioethanol production from this abundant agricultural residue. The observed lower yield of *S. cerevisiae* in this study may be attributed to several factors, particularly the formation of inhibitory compounds produced throughout the rice straw pretreatment and hydrolysis stages. Additionally, the specific strain of *S. cerevisiae* used in this study may have inherent limitations in terms of tolerance to these inhibitory substances. Future studies should focus on identifying and characterizing specific inhibitors produced during pretreatment and understanding their effects on the fermentation performance of different yeast strains. A comprehensive analysis of how each strain tolerates or adapts to these inhibitors could provide valuable insights into optimizing strain selection.

The substantial variation in ethanol conversion efficiency among the tested yeast strains (5.96–93.59%) has significant implications for downstream process design, particularly with respect to distillation column configuration and operating conditions. To evaluate these impacts, subsequent Aspen Plus® simulations are conducted, leveraging the compositional data presented in Table 2. The results of these simulations, presented in the following subsection, provide insights into the economic consequences of different ethanol conversion rates and inform strategies for enhancing bioethanol production, including the potential for strain improvement.

Table 3: Comparison of ethanol yield from different yeast fermentation experiments.

Yeast strain	Ethanol Concentration (%w/v)	Ethanol Conversion (%)
KM 5116	0.15 ± 0.06	5.96
KM 5616	0.19 ± 0.09	7.51
PK 5147	2.34 ± 0.49	93.59
S.cerevisiae	0.52 ± 0.22	20.95

3.3 *Process simulation*

The feedstock requirements are consistent across all scenarios with the same pretreatment. Each scenario processes 50 t/h (50,000 kg/h) of rice straw, maintaining a constant mass balance around the pretreatment section. This ensures identical biomass quantities entering the hydrolysis and fermentation stages, leading to consistent enzyme usage. Fermentation conditions are standardized to a pH of 5.5–6.0, a temperature of 30–32 °C, and an initial substrate concentration determined experimentally. The Aspen Plus® simulation is validated by calibrating key process parameters based on experimentally determined ethanol yields and conversion efficiencies for each yeast strain. These values are directly incorporated into the stoichiometric reactions within the fermentation block. Simulation outputs are subsequently compared with experimental results, and input parameters are adjusted as necessary to ensure consistency with laboratory-scale observations [33]. Although the simulation is conducted under steady-state assumptions, it is designed to reflect experimental performance under controlled conditions. Potential uncertainties in input parameters—such as biomass composition, conversion efficiency, and energy requirements—may influence the accuracy of utility and cost estimations. To mitigate these effects, averaged experimental values are employed, and a sensitivity analysis is carried out to evaluate the impact of price fluctuations and process variability. Future work may incorporate uncertainty propagation or stochastic modeling to

further enhance the reliability of the economic assessment.

Compared to conventional acid and alkali pretreatment methods, $KMnO_4$ offers distinct advantages. Acid pretreatments (e.g., dilute H_2SO_4) effectively solubilize hemicellulose but often produce fermentation inhibitors such as furfural and HMF, necessitating detoxification. Alkali pretreatments (e.g., NaOH) are efficient in lignin removal but involve high chemical usage and generate strongly alkaline wastewater. In contrast, $KMnO_4$ pretreatment at low concentration (1.36%) and mild temperature (84 °C) effectively disrupts hemicellulose and enhances cellulose accessibility without generating significant inhibitors. Compositional analysis confirms this, showing a reduction in hemicellulose from 39.42% to 14.7% and an increase in cellulose from 36.15% to 42.65%. Although the sugar release may be slightly lower than with optimized alkali systems, the minimal need for neutralization or detoxification simplifies downstream processing. Additionally, $KMnO_4$ is cost-effective at low dosages and is considered a green oxidant, offering potential benefits for wastewater treatment and overall environmental sustainability [8], [24], [27]. The use of low-concentration $KMnO_4$ also simplifies the washing process, making pH adjustment during fermentation more manageable. While dynamic sensitivity analysis could provide further insight into strain-specific performance under varying substrate concentrations, such modeling requires extensive experimental data and is beyond the current scope. Therefore, a steady-state simulation approach is adopted in Aspen Plus®, based on available experimental inputs. Ethanol production varies significantly across yeast strains, ranging from 507 to 13,575 tons per year (Figure 2), with PK 5147 yielding the highest output (Table 3). This variation directly influences the production rate of 95% ethanol in the separation stage. Although higher ethanol concentrations demand more energy for distillation, they ultimately reduce the cost per ton of ethanol due to greater overall yield [33].

3.4 *Distillation configurations*

Given the substantial variation in ethanol conversion efficiencies among the tested yeast strains (5.96–93.59%), it is anticipated that optimal distillation column configurations would vary accordingly. To explore this, as previously mentioned, Aspen Plus® simulations are conducted using the DSTWU column model for preliminary distillation column design and the RadFrac model for rigorous simulation. These models are utilized to determine the required number of stages and operating conditions.

Table 4 presents a comparison of distillation configurations and production rates for the different yeast strains. As illustrated, the required number of stages for both the beer column and rectifier column varies significantly depending on the feed ethanol concentration.

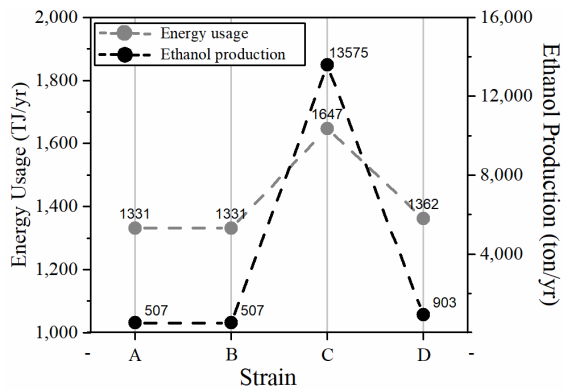

Figure 2: Annual energy consumption and production comparison of different yeast strains; A: KM 5116, B: KM 5616, C: PK 5147, and D: *S. cerevisiae*.

For instance, strains with lower ethanol concentrations (KM 5116 and KM 5616) necessitate a greater number of stages compared to those with higher ethanol concentrations (PK 5147).

Table 4: Comparison of distillation configurations and production rates for different yeast strains.

Yeast Strain	Number of Stages		Total Utilities	Production Rate
	1st column	2nd column	(GJ/h)	(kg/h)
KM 5116	30	35	168	64
KM 5616	30	35	168	65
PK 5147	15	15	208	1,714
S. cerevisiae	20	35	172	114

As illustrated in Table 4, the selection of yeast strain significantly impacts both energy consumption during distillation and subsequent ethanol production rates. A notable deviation from the conventional high-energy pretreatment approach is achieved by employing a milder temperature of 84 °C. This milder condition facilitates the isolation of the effects of yeast selection on the subsequent distillation step. A positive correlation is observed between energy

consumption during distillation and the resulting ethanol production rate. Notably, *P. kudriavzevii* TISTR 5147 emerged as the strain exhibiting the highest energy consumption for distillation (208 GJ/h) (refer to Table 6 for detailed utility costs). This strain also achieved the greatest ethanol production rate (1,714 kg/h) (see Figure 2 for yearly energy usage and production rates). *P. kudriavzevii* TISTR 5147 consumed approximately 23.8% more energy for distillation compared to *K. marxianus* TISTR 5116. However, despite this increased energy demand, *P. kudriavzevii* TISTR 5147 achieved a significantly higher ethanol production rate, exceeding that of *K. marxianus* TISTR 5116 by a remarkable 25 times. When compared to the traditional strain, such as *S. cerevisiae*, PK 5147 is found to require 21% more total utility but produced ethanol at a rate 15 times higher. These findings highlight a crucial trade-off inherent in yeast selection for bioethanol production. While certain strains like *P. kudriavzevii* TISTR 5147 offer the potential for significantly enhanced ethanol output, this benefit may be offset by increased energy consumption during distillation. Consequently, the optimal selection of a yeast strain necessitates a comprehensive evaluation that considers both production efficiency, as measured by ethanol yield, and energy usage throughout the entire process.

3.5 *Material and utility cost*

As detailed in Table 5, the cost of rice straw is set at 42 USD/ton, reflecting the lowest feedstock price reported in the literature, specifically from Thailand, based on market value assessments. The price of $KMnO_4$ is set at 3 USD/kg (99% purity), which will be used to prepare a 1.84% solution for the pretreatment stage. This study considers only the material costs of rice straw and $KMnO_4$. It is important to note that the costs of enzymes, fermentation media, and culture preparation are not included in this analysis, as these costs can vary significantly depending on the specifics of each production facility and may substantially affect overall expenses.

Utility costs are estimated using Aspen Process Economic Analyzer (APEA), following the methodology by Ulrich and Vasudevan [28], with assumptions that electricity is supplied from coal-based power plants and steam is generated by natural gas boilers. Although PK 5147 exhibits the highest overall energy consumption, its ethanol productivity is substantially greater than that of other strains, resulting in lower utility cost per ton of ethanol produced. The significantly higher output volume effectively distributes energy usage, making PK 5147 the most cost-effective option in terms of utility cost. While electricity and steam prices may vary under different scenarios, the high productivity of PK 5147 is expected to maintain its economic advantage under moderate fluctuations.

Table 5: Raw material costs for $KMnO_4$ pretreatment of rice straw.

Material Cost	Cost	Pre-treatment	
	(USD/kg)	ton/yr	MUSD/yr
Rice straw	0.042	396,000	16.63
$KMnO_4$	0.060	71,280	4.28
Total			20.90

MUSD = Million US dollars

Table 6: Utility costs per ton of product for different yeast strains.

Type	Utility Costs (USD/Ton)			
	KM 5116	KM 5616	PK 5147	*S. cerevisiae*
Electricity	0.4069	0.4069	0.0155	0.2284
Cooling Water	0.5582	0.5585	0.0260	0.3248
Refrigerant - Freon 12	1.56×10^{-8}	1.56×10^{-8}	5.673×10^{-3}	1.754×10^{-8}
Steam @100PSI	10.528	10.530	0.46630	6.0477
Total	11.49	11.50	0.508	6.600

This seemingly contradictory result can be attributed to the significantly lower electricity and steam requirements per ton of ethanol produced by PK 5147 compared to other strains, particularly KM 5116. Notably, the cost analysis in Table 6 shows that the electricity cost for PK 5147 is more than 25 times lower in USD per ton than that of KM 5116, and the steam cost is over 22 times lower in USD per ton. This superior efficiency in utilizing specific utilities results in a significant cost advantage, emphasizing the importance of economic considerations alongside traditional energy consumption metrics in selecting optimal yeast strains for ethanol production (see Figure 2 for a visual comparison of energy consumption and production rates). To compare our results with existing literature, we refer to [33]. This study focuses on ethanol production from weeping love grass, targeting a 90% ethanol concentration. While using different feedstocks, pretreatment methods, and yeast strains, our work targets a higher 95% ethanol concentration and achieves a lower utility cost range of 6.4–7.7 MUSD/year compared to the estimated costs of 9.0–11.9 MUSD/year reported in the work. However, direct comparisons are challenging

due to these variations in process parameters. Figure 3 presents a sensitivity analysis of ethanol production to a ±30% change in raw material price, ranging from -30% to +30% in 10% increments. Note that climate conditions can significantly impact the performance of distillation and cooling systems, particularly when adapting the process to different geographic regions. This study focused on a baseline analysis using default ambient temperature settings in Aspen Process Economic Analyzer. Lignin, a major byproduct, offers substantial potential as a feedstock for diverse applications, including adsorbents, carbon materials, thermosets, hydrogels, thermoplastics, and nanoparticles. However, challenges in downstream processing and product separation hinder its broader commercialization. Large-scale implementation requires substantial investment in infrastructure for biomass storage, transportation, pretreatment, hydrolysis, fermentation, and distillation. These components must be designed to handle high volumes and variable feedstock qualities, while also considering equipment durability and maintenance. Regulatory uncertainties and market factors further emphasize the need for supportive policies and cross-sectoral collaboration to facilitate scalability.

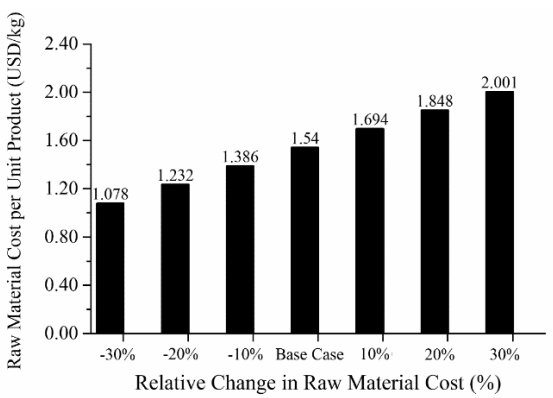

Figure 3: Sensitivity of ethanol production to raw material price changes.

4 Conclusions

This study investigated four yeast strains for converting rice straw pretreated with a low concentration of $KMnO_4$ (1.84%) into bioethanol. PK 5147 emerged as the most productive strain, achieving a remarkable ethanol conversion rate of 93.59%, significantly outperforming all other contenders. However, PK 5147 exhibited a slightly higher energy consumption during distillation (20–24%). Interestingly, the Aspen Plus® cost analysis highlights utility costs as a critical factor. PK 5147 demonstrated notably lower electricity and steam requirements, leading to a substantial cost advantage. The electricity cost for PK 5147 is more than 25 times lower per ton of bioethanol compared to KM 5116, with steam costs following a similar trend, showing a reduction exceeding 22 times per ton. The wide range of ethanol yields (5.96–93.59%) emphasizes the importance of using software-based utility cost estimation to evaluate and compare experimental results. It is crucial to recognize that actual production costs, including capital costs, could vary significantly due to differences in process design and implementation across different facilities. Further optimization of yeast strain selection and considerations for process scaling are critical to improving overall efficiency and economic viability.

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Author Contributions

S.A.: methodology, investigation, writing - review & editing. M.S.: supervision, resources. A.T.: conceptualization, methodology, software, writing — original draft, writing – review and editing. P.T.: methodology, investigation. S.T.: conceptualization, writing - original draft. T.P.: investigation, data curation. P.S.: supervision. K.K.: conceptualization, writing — original draft. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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