



METABOLIC DIVERSITY AND FERMENTATION YIELD PREDICTION IN SACCHAROMYCES CEREVISIAE USING BIOCHEMICAL INDICATORS

Dr. Kiran Krishna.V¹, Piyali Saha², Osama samy³

¹MS Orthopaedics -Doctor Vinayaka Mission University Cuddalore Pondicherry Main Road, Kirumampakkam Pondicherry -607403 ORCID ID: 0009-0004-0489-3878 Email ID: kirankrishna708@gmail.com

²Associate Professor, Meridian Nursing and Paramedical College, Varanasi, UP. Email: sahapiyali2016@gmail.com

³Doctor, Cairo. Email id: Osamasamy526@gmail.com

*Corresponding Author: Dr. Kiran Krishna.V

*Email: kirankrishna708@gmail.com

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Abstract

Experimental data obtained from sugarcane press-mud pretreatment and fermentation process were used to investigate biochemical diversity and fermentation performance in *Saccharomyces cerevisiae*. The variations in carbohydrate concentration, reducing sugars, ethanol production, fusel alcohols and acetic acid were analyzed for different pretreatment conditions. Descriptive statistics were used to describe biochemical relationships, and Pearson correlation analysis were used to identify potential fermentation performance indicators. Ethanol concentration of ethanol varied from 31.98 g/L to 63.59 g/L with the maximum being obtained at 130°C and pH of 4.8. The mean concentrations of carbohydrate and reducing sugar were 115.51 and 68.88 g/L, respectively. The pH of the pretreatment was strongly negatively correlated with reducing sugars ($r = -0.879$) and temperature was positively correlated with concentrations of fusel alcohol ($r = 0.785$). These results showed significant biochemical differences between the experimental conditions and may help to predict fermentation results. However, the confidence in the validity of reliable predictive models was limited by the few independent observations made. The study illustrates the need for integrated biochemical monitoring to increase the understanding of fermentation variability and pave the way for future optimization of sustainable bioethanol production based on data.

Keywords: *Saccharomyces cerevisiae*; Metabolic Diversity; Fermentation Yield; Biochemical Indicators; Bioethanol Production.

1. Introduction

Saccharomyces cerevisiae is one of the most widely studied microorganisms in industrial biotechnology due to its metabolic adaptability, excellent fermentative capacity and capacity to transform carbon sources rich substrates into valuable metabolites such as ethanol and other valuable metabolites. Growing use in bioethanol production, brewing, viniculture and valorization of biomass has motivated further research into the biochemical processes which determine the efficiency of fermentation. Although used extensively in industry, the fermentation performance is found to be great discrepancy in respect of the composition of the substrate, the environment and its physiology. The substrate utilization and product formation vary among *S. cerevisiae* populations, and this can be attributed to metabolic diversity of the cells. Under different operating conditions, variations in central carbon metabolism lead to different fermentation characteristics because of changes in the distribution of metabolic intermediates (Monnin et al., 2024). To identify biochemical indicators that can explain fermentation outcome and facilitate more efficient bioconversion processes, it is important to understand these differences.

Interactions between genetic traits, environment and biochemical regulatory mechanisms result in metabolic diversity in *S. cerevisiae*. Carbohydrate metabolism, stress resistance, secondary metabolite production, among other traits, varies greatly among different yeast strains and affects their use for industrial fermentation. Traditional fermentation systems have been investigated and it has been shown that different types of fermentation are associated with different metabolic profiles and fermentation properties of yeast populations (Li et al., 2024). The observations highlight the need to consider biochemical variability when assessing fermentation performance. In addition, metabolic engineering techniques have shown the possibilities to redirect the flow of carbon in cells to produce products of interest such as ethanol or other industrial chemicals. These methods show the correlation between metabolic control, substrate conversion efficiency and product formation, which provide a scientific basis for biochemical evaluation of fermentation systems (Yu et al., 2025). Physiochemical conditions under which the yeast metabolism takes place have a pronounced effect on its fermentation efficiency. All these factors together influence the growth rate, enzymatic activity and ethanol productivity. Response to specific fermentation environments could change the utilization and enhance the microbial performance when processing under challenging conditions. Studies indicated that the indigenous yeast strains differ significantly from commercial yeast strains in terms of how they react to physicochemical characteristics and microbial environments, which shows that environment adaptation is important in fermentation processes (Gao et al., 2022). This biochemical reaction to alteration in fermentation condition can also be a factor affecting the formation of organic acid and accumulation of secondary metabolites. Studies concerning *S. cerevisiae* have revealed the connections among fermentation metabolites, microbial composition and yeast activity during the fermentation process (Wenkang et al., 2024). This study agrees with using integrated biochemical indicators instead of ethanol production alone to assess fermentation systems.

Biochemical parameters that are especially significant are the concentration of carbohydrates and reducing sugar availability, which are key factors of substrate availability for fermentations. The amount of soluble carbohydrates released depends on hydrolysis and pretreatment processes, whereas the ethanol and secondary metabolites obtained depend on fermentation processes. But production of ethanol with carbohydrate alone is not sufficient to ensure more ethanol production as it also depends on the physiological condition of the microorganism and the presence of inhibitory compounds. Metabolic regulation can be affected by environmental stressors and so alter fermentation product formation. An integrated transcriptomic and metabolomic analysis has shown that external challenges can affect the fermentation properties and biochemical responses of *S. cerevisiae* (Que et al., 2024). Thus, extensive monitoring of fermentation biochemicals such as carbohydrates, reducing sugars, ethanol, fusel alcohol, and organic acids could help to determine the fermentation variability and possible limitations of the process.

In addition to the value of biochemical monitoring at the level of the fermentation experiment, it can also be used to determine the stability or production efficiency of a process over time. Fermentation performance may be different in other cultivation conditions so systematic characterization is needed to understand production consistency. During the assessment of engineered *S. cerevisiae* strains, the monitoring of fermentation performance over long operating periods has been highlighted as important to ensure the process reliability (De Mol et al., 2023). In addition, high-gravity fermentation methods and specific metabolomics studies have shown that it is feasible to engineer yeast metabolic properties to enhance ethanol fermentation (Yang et al., 2025). These improvements suggest that biochemical measurements can play a role in identifying optimal operating conditions and help to elucidate relationships between substrate availability, microbial activity and fermentation results.

In recent years, the field of computational biotechnology has gained relevance and the use of predictive modelling has been on the rise as an alternative method to traditional fermentation analysis. Physiological parameters and experimental data can be used in mathematical models to estimate the fermentation characteristics and the production of secondary metabolites. The potential of predicting secondary metabolism responses during wine fermentation using fermentation-related information has been demonstrated by modelling yeast physiological status (Moimenta et al., 2023). Likewise, combining yeast biodiversity with machine learning can lead to the discovery of intricate associations between yeast traits and metabolic capabilities (Watcharawipas et al., 2025). The use of machine learning to optimize the production of ethanol with changes to the regulatory components has also been explored to optimize ethanol production at different temperatures, demonstrating the increasing importance of computational solutions in fermentation biotechnology (Khamwachirap et al., 2023). Modeling frameworks that incorporate metabolic and process-related variables are also used in hybrid modelling, demonstrating the potential for additional insights into the behaviour of yeast fermenters in complex substrate conditions (Valencia-Velázquez et al., 2025).

Yet, there are notable research gaps regarding the use of easily measurable biochemical parameters combined with prediction of fermentation outcomes, especially with biomass based substrates. One of the drawbacks of many

investigations is the focus on strain engineering, specific metabolomic methods or specific process variables, but only relatively few investigations focus on bringing together conventional biochemical measurement methods to characterise the fermentation variability. Sugarcane press-mud is a meaningful substrate derived from biomass since the pretreatment of the press-mud can affect the availability of carbohydrates and ethanol production. Hence, biochemical variation among the different pretreatment conditions could be used to understand the relationships between the biochemical parameters, the fermentation products and the ethanol production. The present study aims to bridge this gap by using secondary analysis of experimental fermentation data for measurable biochemical parameters and their possible predictive significance. The research was guided by three objectives:

- 1.To characterize biochemical variation in *Saccharomyces cerevisiae* fermentation across different substrate pretreatment conditions and experimental settings.
- 2.To evaluate relationships between carbohydrate availability, reducing sugars, secondary metabolites, and observed ethanol fermentation outcomes.
- 3.To assess the potential of biochemical indicators and pretreatment variables for predicting ethanol fermentation performance.

2. Materials and Methods

2.1. Dataset Description and Experimental Characteristics

The study uses a experimental dataset, whereby biochemical characteristics and ethanol fermentation by *Saccharomyces cerevisiae* (Sanchez et al., 2020) are documented. The experimental measurements were taken from the sugarcane press-mud pre-treatment and fermentation processes. The data consisted of the pretreatment temperatures, pH conditions, carbohydrate concentrations, reducing sugars, ethanol concentrations, fusel alcohols, and acetic acid. Experimental data was retrieved from the provided Excel spreadsheets such as pretreatment screening and mass-balance data. Data from these measurements served as a quantitative basis to analyze biochemical variation and possible fermentation performance determinants during processing.

2.2. Data Preprocessing and Biochemical Variable Selection

Data Pre-processing included the review of the experimental worksheets, identification of analytical variables and the separation of the observations obtained prior to the pretreatment from the fermentation measurements. These parameters were chosen for study: temperature, pH, carbohydrate concentration, reducing sugar concentration, ethanol concentration, fusel alcohols and acetic acid. The units of measurement were standardised where necessary to degrees Celsius, grams per litre or milligrams per litre. Their recorded identifiers were used to match experimental conditions. Replicate values were separated from summary values to avoid repeats. Fermentation result variables were not included in candidate predictor sets if this would lead to introducing target leakage.

2.3. Characterization of Biochemical and Metabolic Variation

The biochemical variations have been characterized based on the available carbohydrate, reducing sugars, ethanol production and ferment products. Experimental conditions were categorized based on the pretreatment temperature and the pH. Concentrations differences between these conditions were described. To determine the linear relationships between selected biochemical indicators and ethanol concentration, Pearson correlation coefficients were computed. Multivariate variation was explored by considering the principal component analysis provided sufficient independent observations and complete measurements. These analyses were based on biochemical diversity measured without the assumption of unknown metabolic pathways or genetic differences.

2.4. Fermentation Yield Prediction and Model Development

The primary outcome for the dataset used for the pretreatment screening was ethanol concentration, for which the fermentation prediction was created. The possible explanatory variables were identified as temperature, pH, carbohydrate concentration, and reducing sugar. Ethanol yield measurements for the selected pretreatment groups were obtained using a separate mass-balance workbook. Linear regression, ridge regression and random forest regression were found to be potential modelling techniques. But with the few independent experimental conditions, careful consideration of overfitting needed to be taken. To perform a predictive evaluation, the model of this evaluation should be appropriate, and the number of models should be limited by the amount of samples.

2.5. Statistical Analysis and Model Evaluation

Descriptive comparisons and Pearson correlation coefficients under matched experimental conditions were performed. The original units of numerical measurements were used, and the common fermentation result, ethanol concentration, was used. Correlation coefficients measured the strength and direction of the linear relationships that were observed. The predictive models were formulated and given for testing with the R-squared, MAE and RMSE. Because of the small independent sample size, care should be taken when interpreting the results of the models. Inferential significance and predictive accuracy were not taken for granted with the lack of statistical calculations.

3. Results

3.1. Experimental Dataset Characteristics and Pretreatment Conditions

The pretreatment screening data set comprised 12 complete treated conditions, three temperatures with four pH levels. The pretreatments were from 2.0 to 4.8 in pH and 110 to 130°C in temperature. A balanced experimental design was created for each temperature (Figure 1) with four pH conditions. There was also an untreated control that was available

for comparison. The treatment structure allowed the systematic investigation of biochemical responses as a function of acidity and temperature. The twelve treated conditions are shown in Table 1 along with the measured ethanol concentration.

Table 1. Experimental Conditions and Ethanol Concentrations

Temperature (°C)	pH	Carbohydrates (g/L)	Reducing sugars (g/L)	Ethanol (g/L)
110	4.8	117.23	24.42	31.98
110	4.0	117.65	21.35	45.50
110	3.0	104.70	95.84	43.64
110	2.0	110.29	101.75	41.44
120	4.8	123.01	28.63	41.56
120	4.0	128.62	55.95	42.82
120	3.0	105.11	98.21	42.10
120	2.0	107.64	91.63	35.55
130	4.8	127.82	42.35	63.59
130	4.0	119.98	73.81	59.05
130	3.0	117.57	91.56	45.32
130	2.0	106.54	101.10	61.38

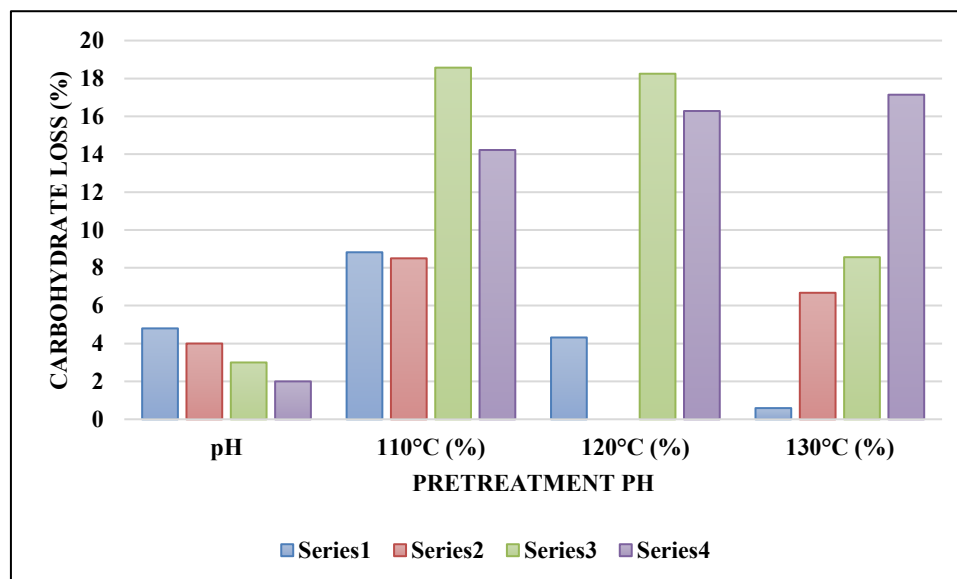


Figure 1. Effect of Pretreatment Conditions on Carbohydrate Loss

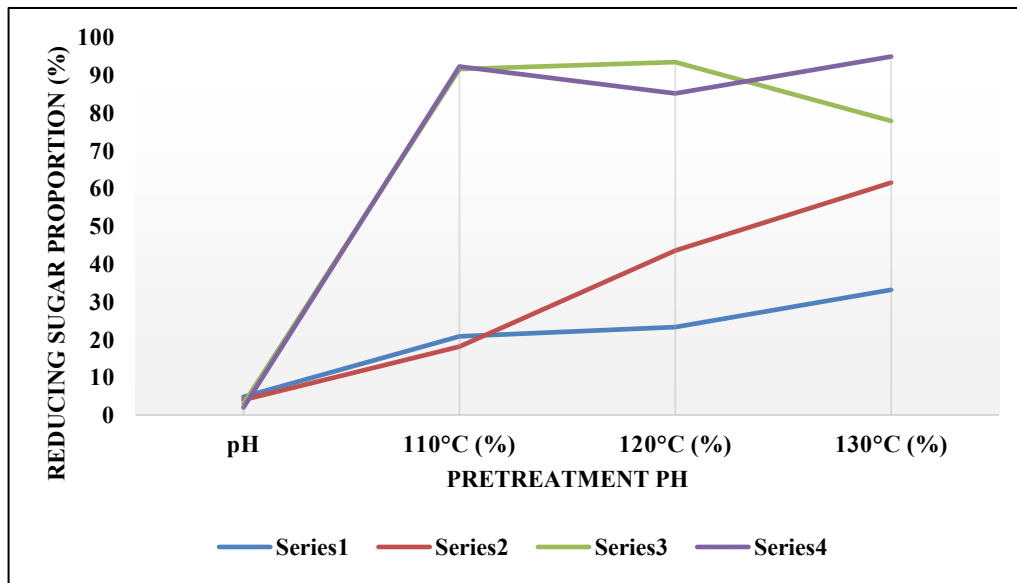
3.2. Biochemical Indicator Profiles and Variability

A significant variation was shown by biochemical measurements between the different pretreatments. The mean concentration of carbohydrates was 115.51 g/L, and the standard deviation was 8.56 g/L (Figure 2). The ranges of the concentrations of ethanol (31.98 to 63.59 g/L) and acetic acid (15.64 to 38.42 g/L) were also very wide among the experimental treatments, with averages of 46.16 g/L and 26.95 g/L, respectively. Ethanol concentrations ranged from 31.98 to 63.59 g/L with mean of 46.16 g/L and fusel alcohol concentrations ranged from 15.64 to 38.42 g/L with the mean of 26.95 g/L, while the concentrations of acetic acid ranged from 15.64 to 38.42 g/L and the mean was 26.95 g/L. Overall, there was greater dispersion in the level of the reducing sugars, which were on average 68.88 ± 32.32 g/L. These results showed that there was a variable biochemical response to pretreatment conditions, such as the decrease of available sugar and fermentation products as summarized in Table 2.

Table 2. Descriptive Statistics of Biochemical Indicators and Fermentation Products

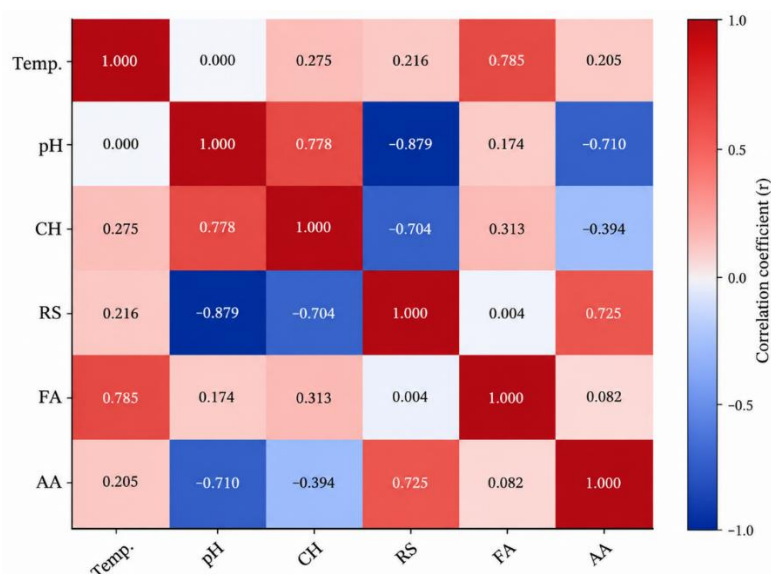
Indicator	N	Mean	SD	Minimum	Maximum
Carbohydrates (g/L)	12	115.51	8.56	104.70	128.62
Reducing sugars (g/L)	12	68.88	32.32	21.35	101.75
Ethanol (g/L)	12	46.16	9.97	31.98	63.59
Fusel alcohols (mg/L)	12	341.15	92.94	222.82	547.42
Acetic acid (mg/L)	12	281.91	68.44	173.00	417.43

SD = sample standard deviation. Statistics calculated from twelve treated conditions in the Results pretreatment worksheet.

**Figure 2. Reducing Sugar Proportion Across Pretreatment Conditions**

3.3. Metabolic Variation and Relationships Among Biochemical Indicators

Distinct relationships were observed between the pretreatment conditions, biochemical indicators and fermentation products by performing correlation analysis. The concentration of fusel alcohol was positively related to temperature ($r = 0.785$) and the concentration of reducing sugars was strongly negatively correlated with pH ($r = -0.879$). There was a negative correlation between carbohydrate concentration and reducing sugars ($r = -0.704$). A positive correlation ($r = 0.725$) was observed between reducing sugars and acetic acid. It was proposed that the biochemical composition was affected differently by these pretreatment conditions in the various fermentation samples. The observed correlations are shown in Figure 3.

**Figure 3. Pearson Correlation Matrix of Experimental and Biochemical Variables**

3.4. Fermentation Yield Prediction and Model Development

The ethanol production varied with the pretreatment temperatures, giving a first set of predictive relationships. Mean ethanol concentrations were 40.64, 40.51, and 57.33 g/L at 110°C, 120°C, and 130°C, respectively. The maximum ethanol production (63.59 g/L) was achieved at the highest temperature (130°C) and lowest pH (4.8) (Figure 4). The concentrations of carbohydrate and reducing sugar had relatively low positive correlation with ethanol concentration. Based on these observations, it was concluded that the fermentation results were influenced by the interactions between process parameters and not by the biochemical parameters alone. The results shown in Table 3 are summarized before the formal evaluation of the predictive models.

Table 3. Temperature-Specific Biochemical Characteristics and Fermentation Outcomes

Temperature (°C)	N	Mean reducing sugars (g/L)	Mean ethanol (g/L)	Maximum ethanol (g/L)
110	4	60.84	40.64	45.50
120	4	68.61	40.51	42.82
130	4	77.21	57.33	63.59

Values calculated from the twelve treated conditions. These are observed outcomes, not predictions from fitted machine learning models.

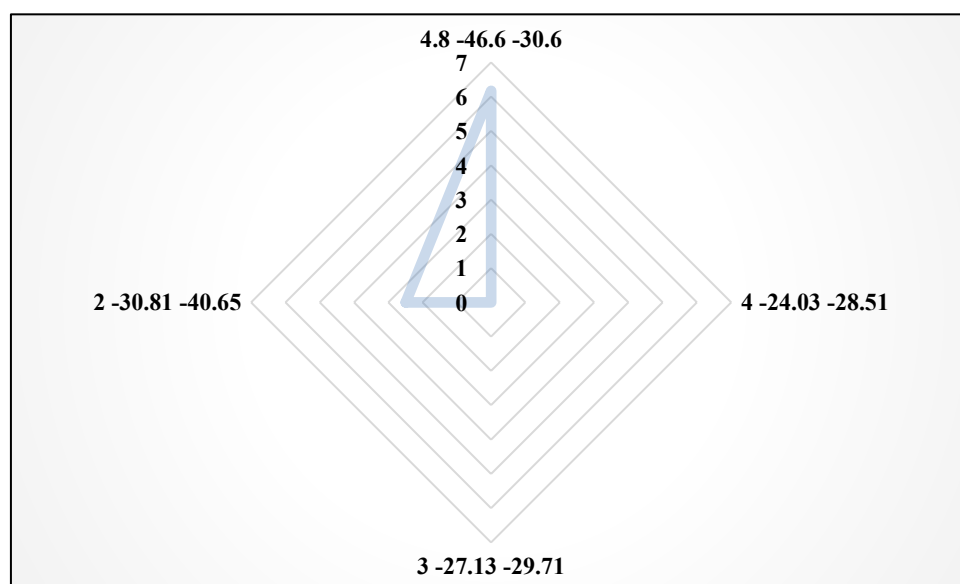


Figure 4. Relative Change in Ethanol Concentration Compared With Untreated Control

3.5. Identification of Key Biochemical Predictors and Model Interpretation

Differences in correlation between measured variables and ethanol concentration was found by exploratory analysis. The highest positive correlation with ethanol was with fusel alcohol ($r = 0.867$), which, however, is a concurrent fermentation product and is not the pre-fermentation parameter. This was followed by the acetic acid ($r = 0.309$) (Figure 5), which had a weaker positive correlation. Correlations of carbohydrate and reducing sugar were 0.221 and 0.107, respectively. Mass-balance calculations showed the best ethanol production to be for treatment M2 when performed individually. The results presented in Table 4 differentiated biochemical and non-biochemical variables for use in prospective fermentation prediction.

Table 4. Observed Ethanol Yields Under Selected Pretreatment Conditions

Treatment	Replicates	Mean yield (g/g)	SD (g/g)
M5	2	0.014109	0.004464
M3	2	0.014718	0.001048
M2	2	0.018035	0.000062

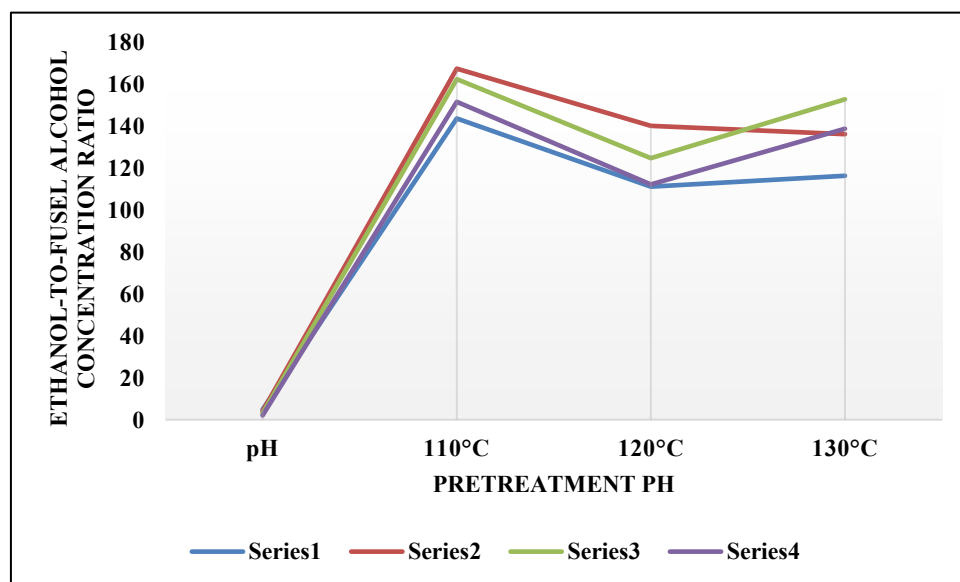


Figure 5. Ethanol-to-Fusel Alcohol Concentration Ratio

4. Discussion

Experimental measurement of sugarcane press-mud pretreatment and fermentation biochemical variation and biochemical performance of *Saccharomyces cerevisiae*. The results showed significant differences in the carbohydrate availability, reducing sugar level, ethanol production and secondary fermentation metabolites under the experimental conditions. The range of ethanol concentration achieved during fermentation was 31.98 to 63.59 g/L, showing a significant variation in fermentation results. Preliminary results indicated that maximum ethanol production occurred at 130°C and pH 4.8, indicating that pretreatment conditions played a role in the differences in ethanol production from the substrates and subsequent fermentation performance. However, the observed relationships are not necessarily a direct cause-and-effect relationship, as fermentation results can also be influenced by the nature of the substrate, inhibitory compounds and microbial physiological response.

The biochemical characterization showed that the relative variability of the reducing sugars was larger than that of the carbohydrate concentrations, suggesting that there were different amounts of readily fermentable substrates between the different pretreatment conditions. The reducing sugar concentration was the mean value of 68.88 g/L, and the mean carbohydrate concentration was 115.51 g/L. The differences indicate that the quality of substrates is related to both abundance of carbohydrates and availability of fermentable sugars. The negative correlation for the data between carbohydrate concentration and reducing sugars ($r = -0.704$) also suggested that increased total carbohydrate concentrations was not associated with increased reducing sugar availability. This correlation could be due to variation in hydrolysis efficiency, or to the difference in the amount of soluble carbohydrates and non-reducing carbohydrates. Therefore, biochemical properties of biomass based fermentation substrates should be studied at least in conjunction with both of these indicators.

Correlation analysis revealed that the pretreatment pH was negatively and highly correlated with reducing sugar concentration ($r = -0.879$), indicating that the reduction in pH is correlated with the increase in the concentration of reducing sugars in the experimental range of pH studied. The fusel alcohol concentration was found to be positively correlated with temperature of thermal pretreatment ($r = 0.785$), suggesting the difference of secondary fermentation products were influenced by the thermal pretreatment conditions. Furthermore, the correlation between reducing sugars and concentration of acetic acid ($r = 0.725$) indicated that the biochemical changes that take place during the processing of the substrates and fermentation reactions are rather complex. These results highlight the need to consider the metabolic variation that occurs during fermentation beyond just ethanol concentration. However, any correlation between pretreatment variables and fermentation products does not necessarily imply certain intracellular metabolic pathways or mechanisms of the biochemical changes observed.

These biochemical relationships have been corroborated by comparison with existing literature. Integrated biochemical monitoring is relevant as several studies have shown the correlation of some physicochemical properties, microbial activity, and volatile metabolite production during wine fermentation (Ge et al., 2024). The diversity of metabolites produced by fermentation has also been investigated in research, revealing differences in chemical composition between microbial fermentation systems, which is similar to the differences observed in the present dataset (Wang et al., 2024). The relationships between microbial diversity, phenolic compounds and fermentation related biochemical parameters also highlight the necessity of considering more than one biochemical parameter (Huang et al., 2022). Research on polyphenol and volatile compound changes during multidimensional yeast fermentation also highlights the intricate process of metabolite production and substrate utilization (Peng et al. 2025). In fact, different fermented rice wines have been explored in other studies, which are also found to show different properties, reinforcing the general potential of biochemical profiling in fermentation studies (Yao et al., 2023).

The ability of metabolic regulation and substrate characteristics to affect fermentation properties is further illustrated by comparison with recent studies. Opportunities to influence flavor metabolism through yeast have also been identified using multi-omics approach; however, the techniques used in these investigations were not biochemical measures that

were used in the present study (Chen et al., 2026). The metabolic diversity of sorghum has also been investigated and correlated with the fermentation quality through the characteristics of the sorghum substrate, which might affect the fermentation behavior in the downstream process (Yang et al., 2025). The significance of selection of strains for their tolerance to yeast stress has been highlighted for bioethanol production by investigations using predictive growth models (Canseco Grellet et al., 2026). The potential value of combining experimental measurements with computational analysis to optimize *S. cerevisiae* biomass production has also been explored by hybrid modelling approaches. Additionally, hybrid modelling approaches have been investigated, confirming the potential value of combining experimental measurements with computational analysis to optimize *S. cerevisiae* biomass production (Messaad et al., 2026). Other applications of genome-scale metabolic modelling and machine learning promise additional scope for predicting microbial production outcomes, but these require significantly more biological data than the data set currently available here (Nair & D'Souza, 2026).

The results also have implications for the overall understanding of biochemical responses related to fermentation. There has been some evidence of yeast supplementation in ruminants, with connections of *S. cerevisiae*, fermentation characteristics and biochemical outcomes, but the biological systems are very different from industrial ethanol fermentation systems (Ogbuewu & Mbajorgu, 2023). In addition, there have been studies that assessed the fermentation and biochemical parameters of the rumen when using yeast cultures in sheep, which showed the wider biological importance of yeast-related fermentation processes without directly demonstrating the ability to predict ethanol production (Wang et al., 2025). These comparisons highlight the need to understand the results of fermentation measurements in their experimental context.

While biochemical associations have been observed, there are a number of limitations to be noted. Only 12 screening conditions were analysed, limiting the power of the analysis and the reliability of complex predictive models. Furthermore, fermentation products, as measurements following the processing, could be a source of information leakage when used as predictors of measurements of ethanol at the same time. There was a limited number of yield observations in the separate mass-balance dataset, which makes validation of fermentation yield prediction difficult. The results are thus largely to provide biochemical variation and initial predictive relationships rather than predictive accuracy. Future studies should include larger independent fermentation data bases, standard yield measurement, other biochemical indicators and external model validation. These enhancements would facilitate more precise identification of fermentation determinants and boost the use of biochemical monitoring in sustainable bioethanol production.

5. Conclusion

The biochemical variation and fermentation performance of *Saccharomyces cerevisiae* based on the experimental data generated from pretreatment and fermentation of sugarcane press-mud. The results showed significant variation in the concentration of carbohydrates, reducing sugars, ethanol and in secondary fermentation metabolites depending on the experimental conditions. The concentrations of ethanol were between 31.98 and 63.59 g/L, with the highest concentration at pH 4.8 and the temperature of 130°C. Correlation analysis revealed good negative correlation between the pH of the feed before treatment and reducing sugars and good positive correlation between concentration of fusel alcohol and the temperature of feed before treatment. These results indicated that pretreatment conditions had an impact on biochemical properties and fermentation results. The differences in the way carbohydrates were used and metabolites formed also highlighted the need to look at several biochemical parameters instead of just ethanol concentration. The relationships that were identified seemed to have a potential for the variables relevant to the prediction of fermentation, however, this development and validation of reliable predictive models were limited because of the small number of independent experimental observations. Hence, the results of this study should not be presented as definitive proof of biochemical associations, but as preliminary evidence. Future experiments with more data, more metabolic measurements, and more rigorous machine learning methods would help to enhance the yield estimation of fermentation. Overall, the study can be used as a base for biochemical monitoring methods, optimization of fermentation processes and for the better use of biomass substrates for sustainable bioethanol production.

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