OPTIMIZING HYDROLYSIS OF *Kappaphycus alvarezii* FOR FERMENTABLE SUGARS: A POTENTIAL FEEDSTOCK FOR BIOETHANOL PRODUCTION

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ABSTRACT

This study explores the potential of *Kappaphycus alvarezii* (KA) as a bioethanol feedstock due to its advantageous properties, such as high growth rates, significant carbohydrate content, and lack of lignin. Hydrolysis conditions were optimized using a central composite design, achieving a peak sugar yield of 41.938 g/L at 126°C, 3% sulfuric acid, and 15 minutes. HPLC analysis identified glucose and galactose as the primary fermentable sugars, with acetic acid as the only manageable byproduct. Integrating a DecisionTreeRegressor model provided high predictive accuracy (R^2 = 0.975), enhancing the understanding of hydrolysis process dynamics. Future studies should focus on identifying yeast strains capable of efficiently fermenting galactose and employing machine learning algorithms to optimize scaling processes. These findings underscore KA's viability as a sustainable biofuel source, contributing to economically viable biofuel solutions and supporting energy security and environmental sustainability.

Keywords: *Kappaphycus alvarezzi*. Bioethanol. Hydrolysis optimization. Algal biomass. Machine learning modeling.

1 INTRODUCTION

Pursuing alternative and sustainable energy sources is critical due to the environmental and economic challenges of dependence on fossil fuels. Bioethanol, a renewable energy source derived from biomass, can help mitigate greenhouse gas emissions and reduce reliance on non-renewable resources[1]. This study aims to maximize the production of fermentable sugars from *Kappaphycus alvarezii* (KA), a red macroalga, through an efficient and sustainable process while minimizing byproduct generation. KA contains carrageenan, which, upon hydrolysis, yields sugars suitable for ethanol production by industrial yeasts[2].

KA also offers several advantages over traditional biomass sources like corn and sugarcane[3]. Algae do not require arable land[4], thereby avoiding competition with food crops. They grow rapidly and have a high carbohydrate content, which can be easily converted into fermentable sugars. Additionally, algae are low in or lack lignin[5], a complex polymer that complicates the breakdown of cellulose in terrestrial plants, facilitating more efficient conversion into biofuels. Algae also have the capability to sequester carbon, aiding in climate change mitigation[1]. Previous studies have demonstrated the potential of KA for bioethanol production. Hargreaves et al. reported up to 105 liters of ethanol per ton of dry biomass[6]. Khambhaty et al. and Meinita et al. achieved reducing sugar concentrations of 70 g/L[7] and 22.4 g/L[8] under different pretreatment conditions, respectively. Recent research by Zaenab et al. showed enzymatic hydrolysis yielding 12.27 g/L of reducing sugars[9].

This study further incorporates machine learning (ML) techniques to optimize hydrolysis, aiming to enhance the pretreatment of *Kappaphycus alvarezii* biomass to release fermentable sugars. ML algorithms, such as DecisionTreeRegressor, predict process outcomes[10], allowing real-time monitoring and adjustments. This approach enhances process efficiency, reduces costs, and accelerates research. By optimizing the hydrolysis of KA, the study aims to maximize the availability of sugars for future fermentations and bioethanol production, contributing to sustainability and energy security.

2 MATERIAL & METHODS

The *Kappaphycus alvarezii* biomass used in this study was provided through a collaborative initiative involving Epagri/Cedap, the Federal University of Santa Catarina (UFSC)[11], and the Federal University of Paraná (UFPR). This biomass, cultivated in the marine waters of the southern region of Florianópolis, Santa Catarina/BR, underwent preparatory procedures upon acquisition. It was rinsed with fresh water to remove marine salts and impurities, followed by drying at 60°C for 48 hours until constant weight. The dried material was then finely ground to a granulometry of ≤0.42 mm and stored in airtight containers to prevent moisture uptake and contamination. The centesimal composition analysis, conducted according to National Renewable Energy Laboratory (NREL) protocols, confirmed carbohydrates as the predominant component, complemented by significant amounts of proteins and minerals with minimal lipid content.

For the hydrolysis experiments, a central composite design (CCD) was employed to evaluate the effects of sulfuric acid concentration (2% to 4%), reaction temperature (100°C to 120°C, with an additional star point at 126°C), and reaction time (10 to 20 minutes). The experiments were conducted using 3.3 g of dry weight (DW) biomass with 30 mL dilution in 150 mL Erlenmeyer flasks, with the autoclave set to the desired conditions. After hydrolysis, the autoclave was cooled down for 30 minutes to ensure uniform temperature reduction. Samples were adjusted to pH 7 using calcium carbonate and filtered to remove unreacted biomass. The filtrate was analyzed using a high-performance liquid chromatography (HPLC) system equipped with an Aminex HPX-87H column, maintained at 65°C with a 5 mM sulfuric acid mobile phase at a flow rate of 0.6 mL/min, identifying sugars and byproducts as acetic acid. All statistical analyses, including experiment design, response surface analysis, and model training, were performed using Python's scientific libraries (NumPy, SciPy, statsmodel, and scikit-learn).

In addition to experimental analytics, a machine learning (ML) model was developed using Python to predict the outcomes of hydrolysis. The data collected from various experimental runs were used to train the DecisionTreeRegressor algorithm. This model predicted total sugar yield based on time, temperature, and acid concentration input parameters. The model's predictive accuracy facilitated a comprehensive understanding of the hydrolysis process, and it was evaluated using the mean absolute error (MAE) and the root-mean-square error (RMSE) between predicted and actual sugar concentrations. Combining traditional methods with ML improves process efficiency, adaptability, and scalability for industrial applications, optimizing bioethanol production from algal biomass and reducing costs sustainably.

3 RESULTS AND DISCUSSION

This study investigated the hydrolysis of Kappaphycus alvarezii (KA) under various conditions to determine the most influential parameters for maximizing fermentable sugar yield. The central composite design (CCD) approach facilitated a thorough examination of three key variables: sulfuric acid concentration, reaction temperature, and time. A total of 11 experiments were conducted: 8 at +1 and -1 levels, two central points at level 0, and one star point, as shown in Table 1. The conditions of Experiment 11, which involved a higher temperature of 126°C, a mid-range sulfuric acid concentration of 3%, and a reaction time of 15 minutes, resulted in the highest total sugar concentration, 41.938 g/L. This suggests that elevated temperatures facilitate a more efficient breakdown of carrageenan, a significant component of KA's hemicellulose, into fermentable sugars.

The centesimal composition analysis indicated that carbohydrates constituted 38.49% ± 1.46, proteins 19.90% ± 0.78, ashes 32.42% ± 0.12, and total solids 90.61% ± 0.04, with minimal lipid content completing the composition of the macroalgal biomass. This analysis underscores the algae's suitability for bioethanol production.

Table 1 Experimental conditions and outcomes for hydrolysis of *Kappaphycus alvarezii*.

Experiment assay	**Independent variables**			**Dependent variables**	
	Temperature (ºC)	Time (min)	H_2SO_4 Concentration (%)	Acetic acid (g/L)	Total Sugars (g/L)
1	100º (-1)	10 (-1)	2% (-1)	0,0000	1,596
2	100º (-1)	20 (+1)	2% (-1)	0,0000	2,876
3	100º (-1)	10 (-1)	4% (+1)	0,0000	5,530
4	100º (-1)	20 (+1)	4% (+1)	1,5320	6,006
5	120º (+1)	10 (-1)	2% (-1)	1,5020	12,316
6	120º (+1)	20 (+1)	2% (-1)	4,3040	37,974
7	120º (+1)	10 (-1)	4% (+1)	1,8740	23,996
8	120º (+1)	20 (+1)	4% (+1)	11,9280	37,896
9	110º (0)	15 (0)	3% (0)	0,7600	22,070
10	110º (0)	15 (0)	3% (0)	0,7580	18,222
11	126 (+1.41)	15 (0)	3% (0)	8,6240	41,938

The HPLC used to analyze the hydrolysates revealed that glucose and galactose are the primary fermentable sugars. The average glucose concentration across all experiments was 2.6 ± 1.9 g/L, while the average galactose concentration was 14.86 ± 13.6 g/L. The sugar ratios highlight the potential for industrial yeast strains that ferment glucose and galactose to produce ethanol.

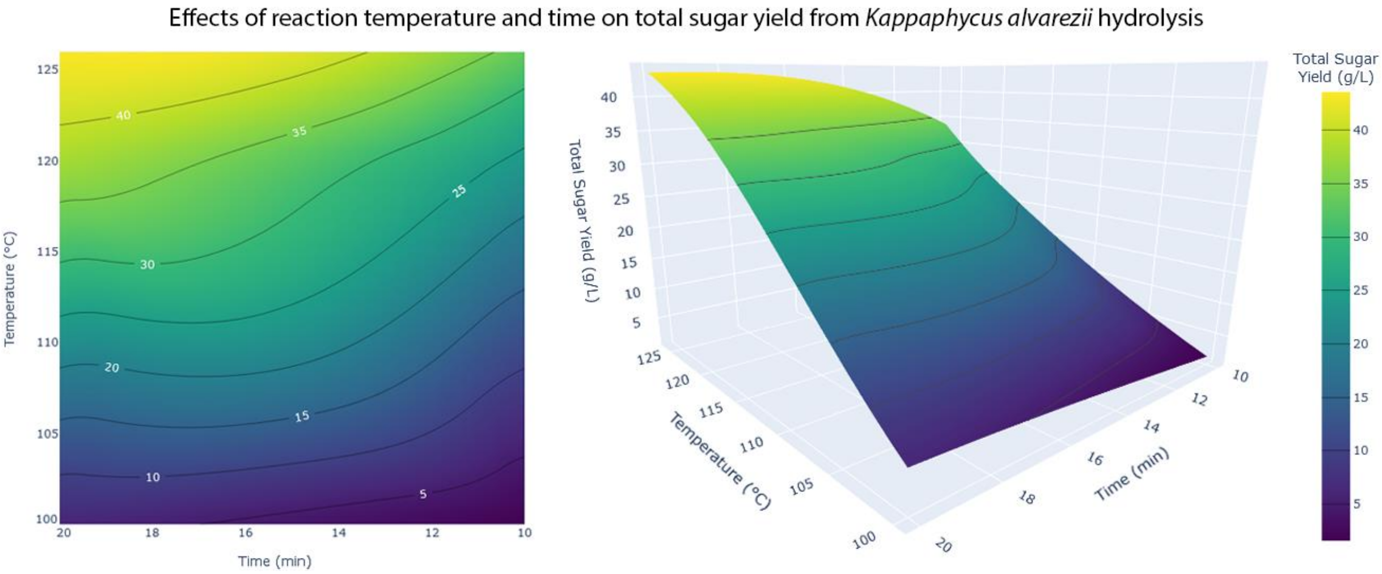

Figure 1 Surface response plots illustrating the effects of reaction temperature and time on the total sugar yield (g/L) from the hydrolysis of *Kappaphycus alvarezii*. The 3D plot (left) and contour plot (right) both indicate that higher temperatures and optimal reaction times significantly increase the total sugar yield, with the highest sugar concentration at 126°C and 15 minutes.

The only byproduct identified by the analysis was acetic acid, which aligns with other observations[6]. Although acetic acid is a weak acid that can be easily removed, its presence must be carefully considered. Acetic acid can influence the fermentation process and impact the overall efficiency of ethanol production. Therefore, while its removal is manageable, it introduces an additional step that must be optimized to ensure the maximum yield of fermentable sugars.

The analysis of variance (ANOVA) indicated significant effects of temperature and acid concentration on the yield of sugars ($p < 0.05$), corroborating the experimental observations where higher temperatures and mid-range acid concentrations optimized the hydrolysis process. These findings align with previous studies that highlight the susceptibility of carrageenan to thermal and acidic breakdown[6], which enhances the release of fermentable sugars.

The surface response graphs (Figure 1) further illustrate the effect of varying temperature and time on total sugar yield, showing that optimal conditions lie within a specific range of these parameters. The Pearson correlations analysis revealed a strong positive correlation between temperature and total sugar yield (0.873), indicating that higher temperatures generally promote greater sugar release. Additionally, a moderate positive correlation between temperature and acetic acid (0.68) suggests that elevated temperatures also increase acetic acid production, as well as sulfuric acid, which showed a weak positive correlation with acetic acid (0.27), indicating that higher acid concentrations slightly increase the production of the weak acid.

A DecisionTreeRegressor model was employed to enhance the optimization process further. This model was constructed with an ensemble of decision trees to predict total sugars released under various conditions, achieving an R^2 of 0.975, indicating high accuracy. The mean squared error (MSE) was 5.48, and the root mean square error (RMSE) was 2.34 g/L, demonstrating the model's robustness. These metrics suggest the model can reliably predict sugar yields, allowing real-time adjustments in hydrolysis conditions to maximize efficiency and yield. The key hyperparameters included a maximum tree depth of 5, a minimum of 2 samples required to split an internal node, and a random state 42 for reproducibility. This model's predictive capability is particularly valuable in scaling up bioethanol production, as it enables continuous optimization and reduces operational costs by minimizing the trial-and-error approach typically associated with process development.

Overall, this study highlights the significant potential of KA as a feedstock for bioethanol production, especially given its high galactose content and the efficiency of industrial yeasts in fermenting galactose. The integration of machine learning models enhances the precision and scalability of the process, making bioethanol production from KA more feasible and economically viable. This contributes to broader goals of sustainability and energy security, positioning KA as a promising resource in the transition to renewable energy sources.

4 CONCLUSION

This study highlights the potential of using KA for bioethanol production, demonstrating that optimizing hydrolysis conditions can maximize sugar yields and pave the way for more sustainable and efficient biofuel technologies. Integrating statistical tools and machine learning models enhances process precision and scalability, making bioethanol production from KA more feasible and economically viable. Future studies should focus on identifying industrial yeast strains capable of efficiently fermenting galactose to produce ethanol from the macroalgal biomass while also integrating machine learning algorithms to optimize the research and development process for scaling efforts. Although this study presents promising results, there remain opportunities for improvement in biomass pretreatment optimization to further enhance efficiency and yield. These findings contribute to sustainable and economically viable biofuel solutions, highlighting the significant potential of *Kappaphycus alvarezii* in reducing environmental impacts and supporting energy security.

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