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COMPREHENSIVE TECHNO-ECONOMIC ASSESSMENT AND PROCESS SIMULATION OF BIOETHANOL PRODUCTION FROM RICE STRAW VIA SUBCRITICAL WATER PRETREATMENT AND ENZYMATIC HYDROLYSIS

AHMAD MUSTAFA[1,2*], HUSSEIN N. NASSAR[2-4], M. SHAABAN SADEK[5], ABDALLAH R. ISMAIL[3], OZBEN KUTLU[6], KONSTANTZA TONOVA[7], CASSAMO U. MUSSAGY[8], BASMA A. ALI[9] AND YASMIN ELSHAMAKH[1]

1 Faculty of Engineering, October University for Modern Sciences and Arts (MSA), 26 July Mehwar Road intersection with Wahat Road, 6th October City, Giza, 12451, EGYPT
2 Center of Excellence, October University for Modern Sciences and Arts (MSA), 26 July Mehwar Road intersection with Wahat Road, 6th October City, Giza, 12451, EGYPT
3 Egyptian Petroleum Research Institute (EPRI), 1 Ahmed El-Zomor Street, El Zohour Region, Nasr City, Cairo, 11727, EGYPT
4 Biochemistry Department, Faculty of Physical Therapy, October University for Modern Sciences and Arts (MSA), 26 July Mehwar Road intersection with Wahat Road, 6th October City, Giza, 12451, EGYPT
5 Department of Chemical Engineering, Faculty of Engineering, Minia University, Minya Governorate, Minia, 61516, EGYPT
6 Ege University Solar Energy Institute, Evka-3, 119/1. Sk. No:2, Bornova/Izmir, 35100, TURKEY
7 Institute of Chemical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bldg. 103, Sofia, 1113, BULGARIA
8 Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Av. Brasil No. 2950, Valparaíso, 2340025, CHILE
9 General Organization for Export and Import Control (GOEIC), Cairo, 11522, EGYPT

Rice straw is a plentiful source of lignocellulosic biomass that has significant promise as a raw material for sustainable energy systems. The management of rice straw in Egypt presents considerable difficulties due to its indispensability and abundant availability. Efficient pretreatment methods are therefore necessary to overcome the resistive composition of lignocellulosic biomass in order to convert it into biofuels. This study suggests combining experimental and computational methodologies to enhance the combined impact of the green subcritical water (SCW) pretreatment and enzymatic hydrolysis of rice straw. This strategy aims to reduce the need for chemicals and energy consumption. The findings suggested that the most favorable parameters for the pretreatment process were a temperature of 160.3°C, an extraction time of 63.4 minutes and a water-to-rice straw mass ratio of 5:2. The application of enzymes led to a substantial 370% rise in the concentration of glucose after extracting it using subcritical water. Furthermore, the fructose concentration rose almost threefold, while the cellobiose levels increased twofold. After simulating the entire process with an annual plant capacity of 72,600 tons, economic indicators were determined. An investment opportunity that is financially feasible is evidenced by the following: a favorable internal rate of return (IRR) of 27%, a favorable net present value (NPV) of $152.6 million and a brief repayment period of 5 years. By employing a comprehensive approach, this endeavor aims to promote the development of biofuel manufacturing routes that are more environmentally friendly, sustainable and efficient, thereby mitigating the ecological consequences associated with meeting global energy demands. To the best of the authors' knowledge, this is the first paper that combines both the technical and economic feasibility of bioethanol production from Egyptian rice straw using a combination of subcritical water technology followed by enzymatic hydrolysis.

Keywords: subcritical water, enzymatic hydrolysis, rice straw, simulation

1. Introduction

The exponential advancement of human societies in the twenty-first century has resulted in a significant surge in energy requirements. This has occurred during a period when economically viable and readily accessible fossil fuels as well as nonrenewable resources, including coal, natural gas and oil, are becoming considerably scarcer [1]-[3]. As a result, a growing level of attention has been

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*Correspondence: ammhamed@msa.edu.eg

directed towards renewable energy sources. Biofuels such as bioethanol and methane are noteworthy alternatives among these [4]. In addition to being renewable and environmentally sustainable, they presently provide between 10 and 14 percent of energy needs worldwide [5]-[6]. Lignocellulosic biomass is predominantly composed of cellulose (40–50%), hemicellulose (25–30%) and lignin (15–20%) as its constituent parts. Cellulose consists of a linear polymer composed of glucose units joined by β-1,4 glycosidic bonds. On the other hand, hemicellulose is an intricate heteropolymer that is branched and comprised of a multitude of sugars, including D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid. Three phenolic substances are comprised of lignin: syringyl, p-hydroxyphenyl and guaiacyl [7]-[8]. Lignin covalently binds to hemicellulose to form the outermost layer of plant cell walls; cellulose, hemicellulose and lignin are interwoven within the structure of lignocellulosic biomass [9]. Nonetheless, in order to facilitate the conversion process, particularly into ethanol, cellulose must be broken down into glucose, a basic carbon source that is commonly utilized by microorganisms [10]. In order to deconstruct the intricate structure of lignocellulosic biomass prior to enzymatic hydrolysis and fermentation, a pretreatment phase is required. This procedure increases the accessibility of the enzymes and the exposure of cellulose. A multitude of pretreatment methodologies have been investigated such as steam explosion as well as alkaline and acidic approaches [11]. Rice straw, a prominent agricultural byproduct, has garnered considerable interest as a source of biomass owing to its plentiful accessibility and potential as a feedstock for renewable energy. For rice straw to be utilized in a sustainable manner, it is critical to implement pretreatment methods that are effective in breaking down its intricate structure. This process should liberate fermentable carbohydrates in the form of glucose, which can subsequently be converted into biofuels and other products with added value. Within this particular framework, the investigation of environmentally-friendly and sustainable pretreatment approaches has become a pivotal field of study with the objective of alleviating the adverse ecological consequences linked to traditional biomass processing methods.

Subcritical water (SCW) technology presents a multitude of benefits in comparison to conventional chemical approaches when it comes to the pretreatment of lignocellulosic biomass [12]. Operating at elevated pressures and temperatures ranging from 100 to 374 °C, subcritical water is a multipurpose solvent that can break down complex biomass into fermentable carbohydrates [13]. By reducing the production of noxious byproducts and eliminating the need for harsh chemical solvents, this method is environmentally friendly. Furthermore, by expediting hydrolytic reactions, the utilization of water under these conditions increases the efficiency of the biomass conversion process and, consequently, the overall yield of valuable biochemicals [14]. In addition, when compared to alternative thermal processes, SCW technology is comparatively energy-efficient since it utilizes the distinctive characteristics of water in the vicinity of its critical point, where it can efficiently solvate substances that are both hydrophobic and hydrophilic. This characteristic renders it highly compatible with the processing of various forms of biomass, providing a method for biofuel production that is scalable, environmentally friendly and sustainable [5].

The enzymatic hydrolysis of hydrolysates produced by SCW pretreatment technology offers numerous advantages, particularly with regard to the conversion of biomass into biofuels and bioproducts. An important benefit is the increased efficacy of sugar release. The SCW pretreatment process efficiently breaks down the intricate structures present in lignocellulosic biomass, resulting in the partial removal of lignin and hemicellulose as well as a reduction in cellulose crystallinity. The altered configuration is more receptive to enzymatic activity, thereby facilitating the access of enzymes to and conversion of cellulose into glucose more efficiently [11]. Furthermore, enzymatic hydrolysis is characterized by its exceptional specificity and operation at low temperatures, both of which contribute to preserving the integrity of liberated sugars and preventing the formation of degradation products that may impede fermentation processes [15]. This particularity additionally diminishes the necessity for severe chemicals and elevated temperatures, thereby conforming to sustainability objectives through the reduction of energy usage and the mitigation of ecological repercussions. Moreover, the integration of enzymatic hydrolysis and SCW pretreatment enables a more comprehensive as well as regulated conversion process, enabling the customization of both processes in order to optimize efficiency and productivity in accordance with the type of biomass employed. In conclusion, this integrated methodology improves the cost efficiency and practicability of biofuel and biochemical production as a whole, thereby establishing itself as a highly auspicious technology for industrial implementations [16].

The majority of published research on biomass pretreatment is primarily concerned with optimizing the technical aspects of the process with the economic viability of these methods when scaled up for industrial applications frequently being neglected [17]-[18]. In light of this knowledge deficit, our research endeavors to implement a thorough techno-economic analysis that encompasses not only the meticulous simulation of the pretreatment procedure utilizing SCW technology but also an elaborate economic evaluation. By adopting this methodology, it is possible to assess the practicability of the suggested approach on a significant magnitude. By incorporating economic sustainability and technological performance into our analysis, a more realistic and balanced perspective on the applicability of this technology is gained. By providing stakeholders with information regarding the potential costs, benefits and scalability challenges, our study aims to enable them to make more informed decisions regarding the implementation of this technology in industrial settings.

Figure 1: Experimental set-up of rice straw pretreatment using subcritical water technology

To the best of the authors' knowledge, this is the first paper that combines both the technical and economic feasibility of bioethanol production from rice straw using a combination of SCW technology followed by enzymatic hydrolysis.

2. Materials and methods

2.1. Rice straw

The rice straw originated from the Egyptian city of Fayoum. The gathered biomass was dried for 24 hours at 60 °C in an oven. After that, a Knife Mill fitted with a 20-mesh screen was used to grind it. Prior to being employed in experimental trials, the processed biomass was stored for up to two months.

2.2. Analysis of the moisture content and chemical composition of rice straw

To determine the moisture content, 5 grams of rice straw was placed in Petri dishes and dried in an oven at a temperature of 105 °C for 4 hours. After weighing the samples, they were placed back in the oven for an extra hour and then weighed once more. This procedure was iterated until a consistent mass was attained. In order to conduct a chemical composition analysis, 2 grams of rice straw was subjected to Soxhlet extraction by boiling it in a solvent consisting of a mixture of ethanol and toluene in a ratio of 2:1 for 4 hours. The extracted material was then carefully washed and subsequently dried overnight at a temperature of 105 °C in an oven with air circulation. The desiccated biomass was measured and split into two equal parts. Fraction A was simply weighed and did not undergo any further treatment. Fraction B underwent treatment with 24% potassium hydroxide to remove hemicellulose, after which its dry mass was recorded. The difference between Fractions A and B represented the hemicellulose content (A - B). Fraction B then underwent further treatment with 72% sulfuric acid to remove cellulose, leaving only the lignin behind. The remaining mass, referred to as Fraction C, was considered to be the lignin content. The difference between Fraction B (after the removal of hemicellulose) and Fraction C represented the cellulose content (B - C).

2.3. Rice straw pretreatment using SCW technology

The studies were carried out over two stages: i) hydrolysis reaction and ii) separation of hydrolysis products. The rice straw was hydrolyzed using water in a subcritical state under different conditions. The experimental configuration employed in this investigation is depicted in *Figure 1*. The optimization tests involved conducting SCW hydrolysis in a stainless-steel reactor made of AISI 316L. The reactor had an exterior diameter of 0.5 inches, was 6 inches in length and sealed using Swagelok caps. In order to begin an experimental run, the reactor tube was filled with water and rice straw with liquid/solid (L/S) mass ratios (g/g) of 5.0, 7.5 and 10.0. The tube was then completely sealed and placed in a prepared thermal oil bath (Mobiltherm 605). The hydrolysis process was carried out between 160 and 200 °C. The pressure inside the reactor was determined using steam tables specifically designed for subcritical conditions. Following the designated duration of the reaction, the reactor was promptly cooled by submerging it in a bath of cold water.

The hydrolysis products were isolated during the second step. The solid and liquid phases were isolated using the process of vacuum filtration followed by centrifugation. The resultant liquid underwent four washes and was further filtered using filter papers to eliminate any remaining particles. The ultimate blend was preserved in a cryogenic freezer at a temperature of -20 °C until examined. The experimental process is illustrated in *Figure 1*. The studies were conducted three

Table 1: The levels of three factors in the design matrix

Variables	Factor	Levels		
		-1	0	1
L/S mass ratio (g/g)	A	5	7.5	10
Temperature (°C)	B	160	180	200
Time (min)	C	60	105	150

times and the concentrations of reducing sugars reported as the average values.

2.4. Analysis of hydrolysate

2.4.1. Determination of the total reducing sugar DNS assay

1 mL of DNS reagent was added to two tubes: one containing 1 mL of a sugar solution and the other containing 1 mL of water as a reference sample. The tubes were immersed in a heated water bath and boiled for 10 minutes, after which they were allowed to cool down to the ambient temperature. Subsequently, 5 mL of distilled water was introduced into each tube. A spectrophotometer (Model Jasco V-570) was used to measure the change in color intensity from yellow to orange at a wavelength of 540 nm. Glucose was utilized as the standard. A calibration curve was generated using doses spanning from 25 to 2200 mg/L. The saccharification% was determined using the methodology outlined by Uma and Muthulakshmi [19], as per the following equation:

$$saccharification\ \% = \frac{RS \text{ x } 0.9 \text{ x } DF \text{ x } 100}{biomass\ weight} \quad (1),$$

where *RS* denotes the total reducing sugars and *DF* the dilution factor.

2.4.2. Sugars and bioproducts

The analytes were measured on a HPLC instrument which consisted of a pump (Knauer Smartline Pump 1000), a RI detector (Knauer AZURA RID 2.1L), a LC Control module for HPLC/CE control and a software program (Clarity Extensible CFR21/GLP). All the acids, sugars and furans were separated on a Sepax Carbomix H-NP10 column that was thermostated at 35 °C with a mobile phase of 2.5 mM H_2SO_4 at a flow rate of 0.6 mL/min. To achieve a greater degree of precision, the monosaccharides in the samples were secondarily analyzed in a Sepax Carbomix Pb-NP10 column at 75 °C with a mobile phase of deionized water at a flow rate of 0.6 mL/min. Using both columns, the volume of sample injected was 20 µL. For all measurements, the temperature of the RI detector was kept constant at 35 °C.

Each sample was analyzed at least twice. In each series, the deviation from the mean concentration was within 4% for all substances, except for furans for which it fell within 6%.

The concentration of reducing sugars (RS) in mg/L was obtained for each hydrolysis experiment. In addition, the yield of reducing sugars (Y_{RS}), measured in grams of glucose equivalents per 100 grams of raw material, was determined using:

$$Y_{RS} = \frac{M_{RS}}{M_{IN}} \text{ x } 100 \quad (2).$$

The efficiency (E), measured in grams of glucose equivalents per 100 grams of carbohydrates (hemicellulose and cellulose) was computed using the following equation:

$$E = \frac{M_{RS}}{M_{CA}} \text{ x } 100 \quad (3),$$

where M_{RS} represents the mass (g) of reducing sugars in the hydrolyzed solution, M_{IN} designates the initial mass (g) of rice straw placed in the reactor at the start of the operation and M_{CA} stands for the initial mass (g) of carbohydrates (hemicellulose + cellulose).

2.5. Time-dependent behavior of the pretreatment process

Before optimizing the pretreatment conditions, the appropriate extraction time was first determined. In this set of experiments, the concentration of reducing sugars was measured periodically every 30 mins. The experimental setup was the same as in Section 2.3. The kinetics experiments were carried out at a temperature of 200 °C and a L/S mass ratio (g/g) of 7.5. By the end of the kinetics experiments, the lower and upper extraction limits could be defined for the subsequent optimization study as detailed below.

2.6. Experimental design

In this project, the Box-Behnken experimental design was employed. The influence of three selected independent variables (L/S mass ratio, temperature and time) on the response (the concentration of reducing sugars) was investigated. A three-factor, three-level Box-Behnken design matrix with five replicate experiments at the central point was added, resulting in a total of seventeen experiments. The factor levels in the design matrix are provided in *Table 1*.

The influence of the variables on the response variable was assessed by applying Analysis of Variance (ANOVA). A quadratic polynomial equation was employed to establish a mathematical relationship between the independent factors and the response:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} . \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \quad (4),$$

where Y represents the reduced sugar concentration, while x denotes the independent variables, namely the L/S mass ratio, temperature and time. The subscripts i and j are used to distinguish between different factors, while the constants 0, i, ii and ij correspond to the regression coefficients for the intercept, linear, quadratic and interaction model terms, respectively. The regression model was constructed with a confidence level of 95%. The experimental design, ANOVA and optimization

were carried out using the Stat-Ease 360 Design of Experiments (DOE) software tool (Stat-Ease, USA).

2.7. Effect of enzymatic hydrolysis

In order to assess the effect of enzymatic hydrolysis on the amount of sugar produced, the biomass solution that had been treated with subcritical water at 200 °C was mixed with a solution containing 10% enzymes. The enzymes, Viscozyme L and a cellulase enzyme blend, were obtained from Sigma-Aldrich (Saint Louis, MO, United States). The enzyme blend was combined with the hydrolysates in a flask following the procedure outlined by Kim and Lee [11]. The ratio of enzyme blend to hydrolysates was 1:1 based on the weight of the biomass with the volumes measured in a volume-to-volume (v/v) ratio. The mixture was then placed in a shaking incubator and incubated at a speed of 170 rpm and a temperature of 45 °C for 48 hours. Once the enzymatic hydrolysis process had finished, samples of the liquid portion were taken for examination using High Performance Liquid Chromatography (HPLC).

2.8. Process simulation

The software Aspen Plus was utilized to simulate the rice straw-based production of the proposed second-generation bioethanol. Inserting chemical components, selecting the thermodynamic property model, determining the necessary operating units as well as configuring the input conditions for the streams and units (e.g. flow rate, temperature, pressure, etc.) comprised the majority of the process simulation steps. The process flowchart, as illustrated in *Figure 2*, is comprised of four stages:

i) SCW hydrolysis,
ii) cellulase-enzymatic hydrolysis,
iii) fermentation of the saccharification's product
iv) separation as well as purification.

In order to accurately represent the numerous processes involved in this procedure, the components used in the simulation were defined in accordance with the NREL report [20]. The Non-Random Two-Liquid (NRTL) method was selected as the property estimation method for analyzing the thermodynamic interactions between the components of the system. This method is renowned for its precision in describing non-ideal liquid mixtures, which allowed the behavior of the system to be accurately modelled.

In order to faithfully represent the vapor phase association of acetic acid with dimers, it was crucial to employ a local property method called NRTL-Hard Sphere Overlap Contribution (NRTL-HOC) to consider the intricate behavior of acetic acid under the given conditions.

The feed composition of rice straw was entered into Aspen Plus according to the analysis performed as per Section 2.2. The other missing elemental components were assumed to be identical to those composing the Egyptian rice straw [21]-[22] as shown in *Tables 2A and 2B*. The core of our simulation resided within the RStoic reactor model, strategically employed due to the availability of conversion data for the pretreatment reactions. These reactions, pivotal in the transformation of rice straw, were represented within this model. A summary of the components involved in the hydrolysis, enzymatic hydrolysis and fermentation reactions is shown in *Table 2C*.

The specifics of these reactions and their fractional conversions are meticulously shown in *Table 3A*, illustrating how key components such as cellulose, hemicellulose and lignin are progressively converted into various products. The fractional conversion values indicate the proportion of each component transformed in the simulation, reflecting the evolving nature of this hydrolytic process [23]. Furthermore, it is imperative to underline that our simulation was conducted under conditions mirroring the experimental setup. This entailed maintaining a constant temperature of 200 °C and a pressure of 15 atm for the hydrolysis with subcritical water stage. This entailed maintaining a constant temperature of 200 °C and a pressure of 15 atm for the hydrolytic stage with subcritical water.

Enzymatic hydrolysis and fermentation processes were also simulated using the RStoic model since the conversion rates of the reactions are available. The reactions and conversion rates are shown in *Tables 3B and 3C*. Cellulose was hydrolyzed by the enzyme cellulase into glucose and then fermented by *Saccharomyces cerevisiae* yeast to obtain ethanol. Finally, the fermentation product was separated and purified using DSTW and RadFrac distillation units established in Aspen Plus. The ethanol solution from the fermentation was separated in a purifying distillation column to obtain approximately 92 wt% ethanol as the final product.

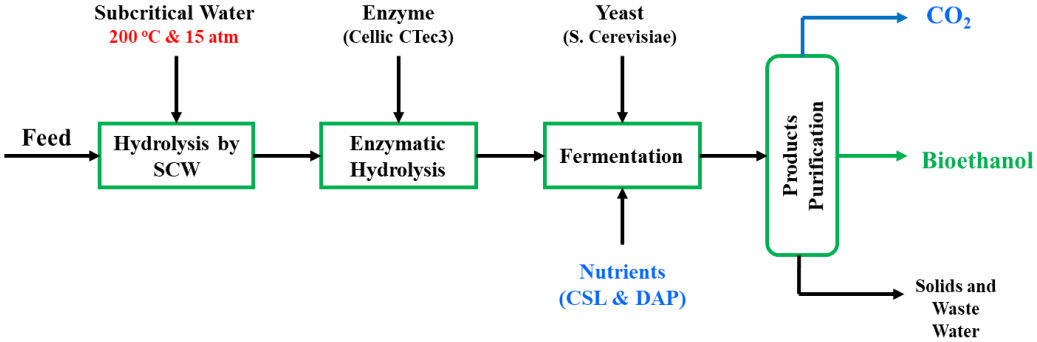

Figure 2: Process steps of rice straw conversion into bioethanol

Table 2A: Rice straw composition (this study)

Component	wt%
Cellulose	35
Hemicellulose	30
Lignin	12
Ash	13

Table 2B: Elemental analysis of rice straw available in Egypt [22]

Ultanal Element	wt%, dry basis	Proxanal Element	wt%	Sulfanal Element	wt%, dry basis
C	39.2	Volatile matter	60	Organic	100
H	3.8	Fixed carbon	20	Sulfate	0
Cl	0.9	Ash	20	Pyritic	0
N	0.3	Moisture	10		
Ash	20.0				

Table 2C: Summary of components involved in hydrolysis, enzymatic hydrolysis and fermentation reactions

Component	Description	Component	Description	Component	Description
CELLULOS	Cellulose	ARABOLIG	Arabino-oligosaccharides	ETHANOL	Ethanol
GLUCOLIG	Glucooligosaccharides	ARABINOS	Arabinose	CO2	Carbon dioxide
H2O	Water	ARABINAN	Arabinan	ZYMO	Zymomonas mobilis (biomass)
CELLOB	Cellobiose	LIGNIN	Lignin	GLYCEROL	Glycerol
GLUCOSE	Glucose	AACID	Acetic acid	SUCCACID	Succinic acid
HMF	Hydroxymethylfurfural	LGNSOL	Lignin soluble fraction	LACID	Lactic acid
HEMICELL	Hemicellulose	LEVUL-01	Levulinic acid	CSL	Corn steep liquor (nutrient)
FURFURAL	Furfural	FORMI-01	Formic acid	DAP	Diammonium phosphate (nutrient)
TAR	Tar	PROPI-01	Propionic acid		
D-FRU-01	D-fructose derivative	GLUCONIC	Gluconic acid		

Table 3A: List of subcritical water hydrolysis reactions with fractional conversion

Stoichiometry	Fractional conversion	Fractional conversion of component
CELLULOS(CISOLID) --> GLUCOLIG(MIXED)	0.0007	CELLULOS
H2O + 2 CELLULOS(CISOLID) --> CELLOB(MIXED)	0.012798519	CELLULOS
H2O + CELLULOS(CISOLID) --> GLUCOSE(MIXED)	0.012830795	CELLULOS
CELLULOS(CISOLID) --> HMF(MIXED) + 2 H2O(MIXED)	0.000264835	CELLULOS
HEMICELL(CISOLID) --> FURFURAL(MIXED) + 2 H2O(MIXED)	0.000977414	HEMICELL
H2O + HEMICELL(CISOLID) --> TAR(CISOLID)	0	HEMICELL
H2O + CELLULOS(CISOLID) --> D-FRU-01(MIXED)	0.019839841	CELLULOS
HEMICELL(CISOLID) --> ARABOLIG(MIXED)	0.00025	HEMICELL
H2O + HEMICELL(CISOLID) --> ARABINOS(MIXED)	0.01361932	HEMICELL
H2O + ARABINAN(CISOLID) --> TAR(CISOLID)	0	ARABINAN
LIGNIN(CISOLID) --> 2.57833 AACID(MIXED)	0.004242377	LIGNIN
3 H2O + FURFURAL --> TAR(CISOLID)	1	FURFURAL
3 H2O + HMF --> 1.2 TAR(CISOLID)	1	HMF
LIGNIN(CISOLID) --> LGNSOL(MIXED)	0.0021	LIGNIN
CELLULOS(CISOLID) + 2 H2O --> 1.7 LEVUL-01(MIXED)	0.000950471	CELLULOS
CELLULOS(CISOLID) + H2O --> 2 PROPI-01(MIXED) + O2(MIXED)	0.00102319	CELLULOS
LIGNIN(CISOLID) --> 3.36304 FORMI-01(MIXED)	0.003459427	LIGNIN
CELLULOS(CISOLID) + H2O --> 0.918367 GLUCONIC(MIXED)	0.001274245	CELLULOS

Table 3B: List of enzymatic hydrolysis reactions with fractional conversion

Stoichiometry	Fractional conversion	Fractional conversion of component
CELLULOS(CISOLID) --> GLUCOLIG(MIXED)	0.0007	CELLULOS
H2O + 2 CELLULOS(CISOLID) --> CELLOB(MIXED)	0.080323131	CELLULOS
H2O + CELLULOS(CISOLID) --> GLUCOSE(MIXED)	0.33318555	CELLULOS
CELLULOS(CISOLID) --> HMF(MIXED) + 2 H2O(MIXED)	0.0070934	CELLULOS
HEMICELL(CISOLID) --> FURFURAL(MIXED) + 2 H2O(MIXED)	0.007373716	HEMICELL
H2O + HEMICELL(CISOLID) --> TAR(CISOLID)	0	HEMICELL
H2O + CELLULOS(CISOLID) --> D-FRU-01(MIXED)	0.212763003	CELLULOS
HEMICELL(CISOLID) --> ARABOLIG(MIXED)	0	HEMICELL
H2O + HEMICELL(CISOLID) --> ARABINOS(MIXED)	0	HEMICELL
H2O + ARABINAN(CISOLID) --> TAR(CISOLID)	0	ARABINAN
LIGNIN(CISOLID) --> 2.57833 AACID(MIXED)	0.004860943	LIGNIN
3 H2O + FURFURAL --> TAR(CISOLID)	1	FURFURAL
3 H2O + HMF --> 1.2 TAR(CISOLID)	1	HMF
LIGNIN(CISOLID) --> LGNSOL(MIXED)	0	LIGNIN
CELLULOS(CISOLID) + 2 H2O --> 1.7 LEVUL-01(MIXED)	0.00103824	CELLULOS
CELLULOS(CISOLID) + H2O --> 2 PROPI-01(MIXED) + O2(MIXED)	0.000934299	CELLULOS
LIGNIN(CISOLID) --> 3.36304 FORMI-01(MIXED)	0.003418227	LIGNIN
CELLULOS(CISOLID) + H2O --> 0.918367 GLUCONIC(MIXED)	0.00129263	CELLULOS
CELLOB + H2O --> 2 GLUCOSE(MIXED)	1	CELLOB

Table 3C: List of fermentation reactions with fractional conversion

Stoichiometry	Fractional conversion	Fractional conversion of component
GLUCOSE --> 2 ETHANOL(MIXED) + 2 CO2(MIXED)	0.9	GLUCOSE
GLUCOSE + 0.04696 CSL + 0.018 DAP --> 6 ZYMO(CISOLID) + 2.4 H2O(MIXED)	0.04	GLUCOSE
GLUCOSE + 2 H2O --> 2 GLYCEROL(MIXED) + O2(MIXED)	0.004	GLUCOSE
GLUCOSE + 2 CO2 --> 2 SUCCACID(MIXED) + O2(MIXED)	0.006	GLUCOSE
GLUCOSE --> 3 AACID(MIXED)	0.015	GLUCOSE
GLUCOSE --> 2 LACID(MIXED)	0.002	GLUCOSE
D-FRU-01 --> 2 ETHANOL(MIXED) + 2 CO2(MIXED)	0.9	D-FRU-01
D-FRU-01 + 0.04696 CSL + 0.018 DAP --> 6 ZYMO(CISOLID) + 2.4 H2O(MIXED)	0.04	D-FRU-01
D-FRU-01 + 2 H2O --> 2 GLYCEROL(MIXED) + O2(MIXED)	0.004	D-FRU-01
D-FRU-01 + 2 CO2 --> 2 SUCCACID(MIXED) + O2(MIXED)	0.006	D-FRU-01
D-FRU-01 --> 3 AACID(MIXED)	0.015	D-FRU-01
D-FRU-01 --> 2 LACID(MIXED)	0.002	D-FRU-01
3 ARABINOS --> 5 ETHANOL(MIXED) + 5 CO2(MIXED)	0.8	ARABINOS
ARABINOS + 0.03913 CSL + 0.015 DAP --> 5 ZYMO(CISOLID) + 2 H2O(MIXED)	0.04	ARABINOS
3 ARABINOS + 5 H2O --> 5 GLYCEROL(MIXED) + 2.5 O2(MIXED)	0.003	ARABINOS
3 ARABINOS + 5 CO2 --> 5 SUCCACID(MIXED) + 2.5 O2(MIXED)	0.015	ARABINOS
2 ARABINOS --> 5 AACID(MIXED)	0.014	ARABINOS
3 ARABINOS --> 5 LACID(MIXED)	0.002	ARABINOS

3. Economic assessment

The assessment of the financial feasibility of industrial processes is a crucial function performed by techno-economic studies. These studies focus on three fundamental goals: preliminary identification of potential sources of revenue, a thorough evaluation of project expenses related to administration as well as execution, and a final prediction of the overall viability of the project [24]-[26]. By conducting an exhaustive analysis, they offer invaluable insights into the anticipated financial flows, thereby exerting a substantial impact on the processes by which decisions are made.

The present study, which is presented as a techno-economic analysis, intends to provide a thorough and intricate examination of the suggested 2G ethanol manufacturing process. Its primary objective is to function as an indispensable asset for the ethanol manufacturing community. Through providing insights into the economic environment pertaining to this technology, relevant parties can acquire the necessary knowledge to formulate well-informed judgments concerning the viability and strategic trajectory of forthcoming 2G ethanol manufacturing undertakings.

3.1. Total capital investment (TCI)

To launch a project, a crucial factor is understanding the total upfront financial commitment, known as the total capital investment, which represents the combined cost of building the physical plant (fixed capital investment), covering initial operations (working capital) and any one-time startup costs [27].

3.1.1. Fixed capital investment (FCI)

The FCI serves as a cornerstone for initiating projects, encompassing all expenses related to establishing the physical plant (e.g. construction, planning, installation of equipment and modifications to the facility) [24]. Accurate FCI estimates are crucial for effective project planning and preventing operational setbacks. The FCI can be further broken down into four main cost categories: inside battery limits, outside battery limits, engineering costs and contingency costs [28].

Inside battery limits (ISBL)

The accurate estimation of the ISBL cost is crucial for evaluating the overall economic viability of a project. Bridgewater's method [29] offers a suitable approach for estimating the ISBL costs in processes involving liquid and solid streams, particularly when detailed design information is lacking. This method leverages a correlation between the plant costs and the number of processing steps.

Bridgewater's method requires the following information: plant capacity (metric tons per year), reactor conversion (mass of desired product per mass of feed) and the number of functional units within the process. Functional units encompass major unit operations that significantly contribute to the overall plant costs, typically excluding pumps and heat exchangers. Based on the proposed flowsheet analysis, this project necessitates 6 functional units. The designed capacity of the plant is 72,600 tons per year with a projected reactor conversion of 46%.

For plant capacities in excess of 60,000 tons per year, the following equation from the Bridgewater's method is applicable [24]:

$$C = 4{,}320\, N \left(\frac{Q}{S}\right)^{0.675} \quad (5),$$

where C represents the ISBL capital cost (US$), N refers to the number of functional units, Q denotes the capacity of the plant (metric tons per year) and S is the reactor conversion rate (desired mass of product in relation to the mass fed into the reactor).

The selection and application of this specific equation will be presented in the subsequent section.

Outside battery limits (OSBL)

The OSBL cost encompasses expenses associated with facilities and utilities located outside the plant boundary but are essential for its operation. This includes storage tanks for raw materials and products, waste treatment facilities, utility lines for water, electricity and steam as well as any off-site modifications to infrastructure [27]. Due to the limited information regarding the condition of the existing site and its infrastructure, an estimated proportion of the ISBL cost is often used to approximate the OSBL cost. This value typically ranges from 10 to 100% of the ISBL, depending on the specific requirements of the project [24],[26]. In this study, considering the lack of detailed site information, a conservative estimate of 30% of the ISBL cost was assumed for the OSBL. This value will be refined as the project progresses and more site-specific data becomes available.

Estimation of engineering costs (EC)

EC encompass the fees associated with the professional services required to bring a project to life. This includes chemical engineers, mechanical engineers and other specialists involved in designing the layout of the plant and process control systems, selecting equipment as well as other technical aspects.

Estimating the EC can be challenging due to factors like the prevailing economic climate and the complexity of the project. However, for large-scale industrial processes, a rule-of-thumb approach can be employed. In this project, the EC is estimated to be 10% of the combined direct capital cost (DCC = ISBL + OSBL). This estimation will be further refined as the project progresses and more detailed engineering design information becomes available.

Estimation of contingency costs (CC)

The CC are included in budgets of the project to account for unforeseen circumstances that may arise when executing the project, including unexpected fluctuations in material prices, delays in the delivery of equipment or

unforeseen technical challenges encountered during its construction.

Although the minimum recommended CC for projects is typically 10% of the DCC, this value can be adjusted based on the level of technological uncertainty associated with the project. In this study, considering the established nature of the technologies employed (many processes are similar to at existing bioethanol production facilities), a CC of 10% of the DCC was assumed. This value reflects the anticipated level of project risk associated when utilizing proven technologies. As the project progresses and further engineering details are finalized, the estimate of contingency costs may be refined to account for any project-specific risks that are identified [30].

3.1.2. Estimation of the working capital (WC)

In order to simplify the estimation of costs in this study, the WC will be calculated as 15% of the total direct capital cost. This method is in line with established practices in the field, as referenced in [24]. As the project progresses, a more refined estimate of the working capital may be developed based on a detailed analysis of anticipated operational costs.

3.1.3. Estimation of start-up costs

Start-up costs encompass one-time costs incurred when initially commissioning the plant and during the ramp-up phase, including training personnel with regard to operational procedures and safety protocols, initial product quality testing and analysis to ensure adherence to specifications, permitting costs associated with waste disposal or environmental regulations as well as consumables and utilities required when starting up the plant and during the initial production period.

To predict the initial costs, start-up costs are typically estimated to be a percentage of the direct capital cost. In this study, a conservative estimate of 10% of the DCC was assumed for the start-up costs. This value will be further refined as the project progresses and a more detailed commissioning plan is developed.

3.2. Operating expenditure (OPEX)

The OPEX represents the ongoing costs associated with running a production facility. These expenses are crucial for sustaining day-to-day operations and directly impact the profitability of the project.

3.2.1. Fixed cost of production (FCOP)

The FCOP encompasses expenses incurred regardless of the production rate within the operational range of the plant. These costs are independent of the production volume and remain constant during its operation. The FCOP significantly impacts the economic viability of the project and necessitates careful consideration when estimating costs. Optimizing plant operations to reduce the FCOP is generally limited.

Labor costs represent a major component of the FCOP and encompass the salaries as well as wages of personnel, including operators and supervisors, required to maintain a continuous operation. The number of personnel needed per shift position significantly influences labor costs.

The variables considered to estimate the FCOP are detailed in *Table 4*, along with the assumptions made for each variable during the calculations. A thorough understanding of the FCOP is crucial for ensuring the project is economically feasible and developing strategies to manage operational expenses effectively [24].

3.2.2. Variable cost of production (VCOP)

The VCOP directly correlates with the output of the bioethanol plant, encompassing expenses like raw materials, utilities, consumables, waste disposal as well as packaging and shipping the product. As the production process design and operational plan are further refined, estimates of the VCOP become more accurate. Importantly, the VCOP can lead to optimization by improving the design or operation, emphasizing the value of continuous cost reduction efforts.

3.2.3. Revenues

Project revenues will be generated from the sale of products manufactured by the bioethanol production facility, including income from the primary product, namely bioethanol, and any potential revenue streams from by-products generated during the process. While carbon dioxide (CO_2) is a common by-product of bioethanol production, its economic viability as a source of revenue needs further investigation. The feasibility of capturing and selling CO_2 will depend on factors like market demand, transportation costs as well as potential applications for the captured CO_2.

3.2.4. Gross margin

A key metric in this analysis is the gross margin, which represents the difference between the revenue generated by the project and the cost of raw materials. This can be expressed mathematically as follows:

$$gross\ margin = revenues - raw\text{-}material\ cost \quad (6).$$

The gross margin indicates the proportion of sales revenue that remains after accounting for the direct cost of the primary feedstock. A higher gross margin signifies a more profitable project as it reflects a greater contribution towards covering other operational expenses and generating profit [31].

3.2.5. Profitability analysis

A profitability analysis is a critical aspect of project evaluation, assessing its potential to generate financial returns. A key metric in this analysis is net profit, which represents the profit remaining after accounting for all expenses.

Table 4: Breakdown of fixed cost of production (FCOP) components

Parameter	Assumption	Explanation
Operating labor	$60,000 USD/year (based on average salary per shift position)	This value represents the estimated average annual salary for a single shift position within the plant. The plant will produce liquid products (biodiesel and glycerol) and solid coffee pellets.
Number of shift positions	4	The minimum estimated number of shift positions required for a continuous operation is 3 with an additional position assigned to the dedicated handling of solids.
Supervision costs	25% of operating labor	This value represents the estimated cost of plant supervision as a percentage of the total operating labor costs.
Overhead costs	50% of (operating labor + supervision costs)	This is a conservative estimate of overhead costs, encompassing indirect labor and other administrative expenses associated with the plant operation. It accounts for 50% of the combined operating labor and supervision costs.
Maintenance costs	5% of ISBL investment	This value reflects the anticipated higher maintenance costs due to the presence of significant moving equipment within the plant such as conveyor belts and machinery handling solids.
Property taxes and insurance	2% of ISBL investment	This assumption incorporates potential property tax and insurance costs associated with the plant facilities.
Rent of land	2% of (ISBL + OSBL)	This cost is included as the land is assumed to be rented not purchased. It represents 2% of the combined direct capital costs (ISBL + OSBL).
General plant overheads	65% of (operating labor + supervision costs + direct overhead costs + maintenance costs)	This value represents the estimated cost of various miscellaneous expenses associated with the overall plant operation calculated as a percentage of the sum of operating labor, supervision, direct overhead (e.g. office supplies) and maintenance costs.
Environmental charges	1% of (ISBL + OSBL)	This cost provision accounts for potential charges associated with environmental regulations and Superfund payments.

3.2.6. Cash cost of production (CCOP)

The CCOP is a crucial parameter for estimating the profitability of the project, reflecting the total cash outflow associated with producing the bioethanol and excluding any return on the invested equity capital. Mathematically, the CCOP can be expressed as follows:

$$CCOP = VCOP + FCOP - \text{by-product revenue} \quad (7),$$

where by-product revenue = revenue generated from the sale of any by-products.

3.2.7. Profit calculation

The gross profit of the project can be calculated by subtracting the CCOP from the total revenue generated from bioethanol sales and represents the profit before deducting taxes.

The net profit is the ultimate measure of project profitability, reflecting the profit remaining after accounting for all expenses, including taxes. It can be calculated as per the following equation:

$$\text{net profit} = \text{gross profit} - \text{tax expenses} \quad (8),$$

$$\text{tax expenses} = \text{taxable income (gross profit} - \text{depreciation)} \times \text{tax rate} \quad (9).$$

The specific tax rate applicable to the project will need to be determined based on the relevant tax regulations and accounting practices. Depreciation, a common tax allowance, can be deducted from the gross profit to determine the taxable income used for tax calculations.

3.2.8. Corporate tax

Corporate tax is a levy imposed on the profits generated by corporations. The specific tax rate applicable to a project can vary depending on the geographical location, industrial sector and prevailing tax regulations. In the United States, corporations paid a federal tax rate of 21% on their profits in the year 2018 [32].

3.2.9. Depreciation

Depreciation is an accounting expense that does not involve actual cash. It refers to the progressive decrease in the value of physical assets over time due to normal wear and tear. This technique reduces the recorded value of the asset on financial statements and provides a tax benefit by reducing taxable income. The Modified Accelerated Cost Recovery System (MACRS) is a commonly used depreciation technique in the United States for tax purposes. The MACRS classifies biorefineries as chemical manufacturing facilities and

assigns them a standard class life of 9.5 years. Typically, when estimating project costs, a 5-year recovery period is commonly assumed, factoring in the depreciation expenses and analyzing the cash flow. It is important to acknowledge that the most effective technique of reducing the value of an asset over time and the length of time it takes to recover the cost of the asset may vary depending on the specific accounting rules and tax laws in place. Hence, it is recommended to seek guidance from a tax expert in order to ascertain the most accurate method for a specific project.

3.2.10. Discount rate

A discount rate, or interest rate, of 11% is assumed to account for the time value of money in the financial analysis of the project, reflecting the minimum expected return on investment for undertaking this project.

3.3. Financial performance metrics

This section examines essential financial indicators employed to assess the economic feasibility and future profitability of the project. These measurements take into account the concept of the time value of money, which recognizes that a dollar today is worth more than a dollar in the future.

3.3.1. Net present value (NPV)

The NPV is an essential measure that takes into account the concept of the time value of money in order to evaluate the profitability of an investment. The calculation involves determining the current value of all anticipated future cash inflows related to the project before subtracting the current value of all cash outflows [24]. A positive NPV signifies a project that is anticipated to have a favorable return on investment. The next equation is utilized to compute the NPV in the following manner:

$$NPV = \sum_{n=1}^{n=t} \frac{CF_n}{(1+i)^n} \quad (10),$$

where CF_n = cash flow in year n, i = discount rate (interest rate) and t = project lifespan in years.

3.3.2. Return on investment (ROI)

The ROI is a financial measure that indicates the profitability of an investment as a percentage. It quantifies the increase in or profit generated from the initial capital invested in a project. A higher ROI percentage is indicative of a more appealing investment prospect, suggesting that its profits are likely to surpass its costs [24]. The ROI is determined by applying the following equation:

$$ROI = \frac{average\ annual\ profit}{initial\ investment} \text{ x } 100 \quad (11).$$

3.3.3. Internal rate of return (IRR)

The IRR is the rate at which the NPV regarding the cash inflows of a project is equal to the NPV of its cash outflows. Put simply, it denotes the lowest satisfactory rate of return for the project to reach a break-even point, taking into account the concept of the time value of money [24]. A greater IRR typically signifies a more favorable investment prospect. The equation for IRR can be solved by an iterative process according to the steps outlined as follows:

$$IRR = \sum_{n=1}^{n=t} \frac{CF_n}{(1+i')^n} = 0 \quad (12).$$

3.3.4. Payback period

The payback period is a metric that quantifies the period of time needed for an investment to recoup its initial expenditure. It offers a clear evaluation of its liquidity and risk characteristics. A reduced payback period is indicative of a diminished investment risk, since the initiative swiftly recoups its initial investment. The payback period can be determined by applying the formula below:

$$payback\ period = \frac{total\ investment}{average\ annual\ cash\ flow} \quad (13).$$

Although each statistic provides useful insights, it is advisable to evaluate them collectively when making investment decisions. NPV and IRR take into account the concept of the time value of money, whereas ROI offers a perspective on the return expressed as a percentage. The payback period provides a rapid evaluation of the project's schedule for recovering its initial investment. By combining these measurements, a thorough comprehension of the project's prospective profitability and risk profile can be obtained, which in turn facilitates well-informed financial decisions to be made.

4. Results and analysis

4.1. Time-dependent approach of rice-straw pretreatment with SCW technology

Time plays a pivotal role in determining how the RS concentration changes. In this proposed study, as shown in *Figure 3*, it was observed that increasing the

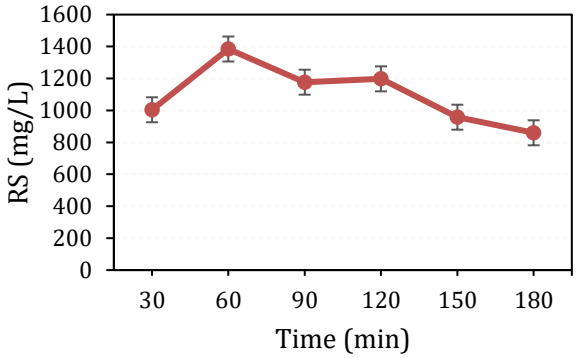

Figure 3: Reducing sugars concentration against time using subcritical water technology. Extraction temperature = 200 °C, L/S mass ratio = 7.5

Table 5: Experimental results of the pretreatment of rice straw

Run	Factor levels	A: L/S mass ratio [g/g]	B: temperature [°C]	C: time [min]	Response: reducing sugar [g/L]
1	0 – +	7.5	160	150	2.91
2	+ + 0	10.0	200	105	1.84
3	0 0 0	7.5	180	105	2.85
4	0 + +	7.5	200	150	2.32
5	0 + –	7.5	200	60	2.76
6	+ 0 –	10.0	180	60	2.35
7	– 0 +	5.0	180	150	3.34
8	– + 0	5.0	200	105	3.30
9	+ – 0	10.0	160	105	2.30
10	0 0 0	7.5	180	105	2.85
11	– – 0	5.0	160	105	4.09
12	– 0 –	5.0	180	60	4.00
13	0 0 0	7.5	180	105	2.85
14	0 – –	7.5	160	60	3.30
15	0 0 0	7.5	180	105	2.90
16	0 0 0	7.5	180	105	3.30
17	+ 0 +	10.0	180	150	1.99

pretreatment time from 30 to 60 mins increased the concentration of reducing sugars. However, beyond 60 mins, the concentration of the RS unfavorably declined, possibly due to the degradation of some reducing sugars into other bioproducts and inhibitors as a result of the long pretreatment time.

4.2. Statistical analysis results

4.2.1. Verification of model equations

The results obtained in accordance with the designed experimental matrix are presented in *Table 5*. Although the higher order polynomial equations model is often suitable, the linear equation model is more suitable for predicting the concentration of reduced sugar based on the factor levels. The linear model equation is formulated as follows, relying on the actual factor values:

$$reduced\ sugar = +8.46 - 0.31 \cdot A - 0.015 \cdot B - 0.005 \cdot C \quad (14),$$

where A denotes the L/S mass ratio (g/g), B stands for the temperature (°C) and C represents the time (min).

This model equation can be employed to estimate the amount of reduced sugar corresponding to any factor level. It is important to note that these levels should be defined in their original units.

In the model designed with a second-order polynomial equation, the p-values for some interaction terms did not yield significant results, indicating that the interaction terms of the selected variables hardly influence the model equation. Accordingly, the insignificant terms were removed from the quadratic equation to obtain the linear model equation in which the sign of the regression coefficients also indicates the direction effect on the response [33]. At this point, it is understood that all three variables negatively affect the reduced sugar concentration. According to the ANOVA results provided in *Table 6*, the fit of the model is evident from all four different outcomes (R-squared, F-value, p-value and lack of fit). The p-value for the linear model was calculated to be less than 0.0001 and remained lower than 0.05 [33], indicating that the model and its equation can be considered significant. The F-value is approximately 100, demonstrating that it is not affected by any statistical noise, thereby strengthening its case as a significant model.

On the other hand, the lack of fit p-value is greater than 0.05 and the F-value of 0.30 indicates that the lack of fit is not significant relative to the pure error. This result further supports the adequacy of the model, which was previously deemed suitable for the experimental data. Moreover, R-squared of the preferred linear model equation was calculated as 95.9%. Before removing the terms of the insignificant model, R-squared of the quadratic model was calculated to be 97.3%. However, considering the other results, its reduction by 1.5% is deemed acceptable and R-squared of the linear equation also supports a strong fit. The difference between the predicted and adjusted R-squared is reasonable (<0.2) [34]-[35] and consistent. Additionally, the high Adequate Precision result serves as evidence that this model equation can be used for predictions within its design boundary. In addition to determining that the linear model equation was appropriate, the effects of the variables on the response were also examined based on their F-values. The parameter with the highest F-value was observed to be the L/S mass ratio, indicating that the highest impact on reduced sugar could be achieved by varying the mass ratio. The temperature correlated with

Table 6: ANOVA output of the fitted model (Adequate precision: 31.43)

	SS	df	MS	F-value	p-value	R-squared (R^2)	Adjusted R^2	Predicted R^2
Model	6.01	3	2.00	99.98	<0.0001	0.9585	0.9489	0.9391
Residual	0.2604	13	0.02					
Lack of fit	0.1054	9	0.0117	0.3022	0.9372			
Pure error	0.1550	4	0.0387					
Cor total	6.27	16						

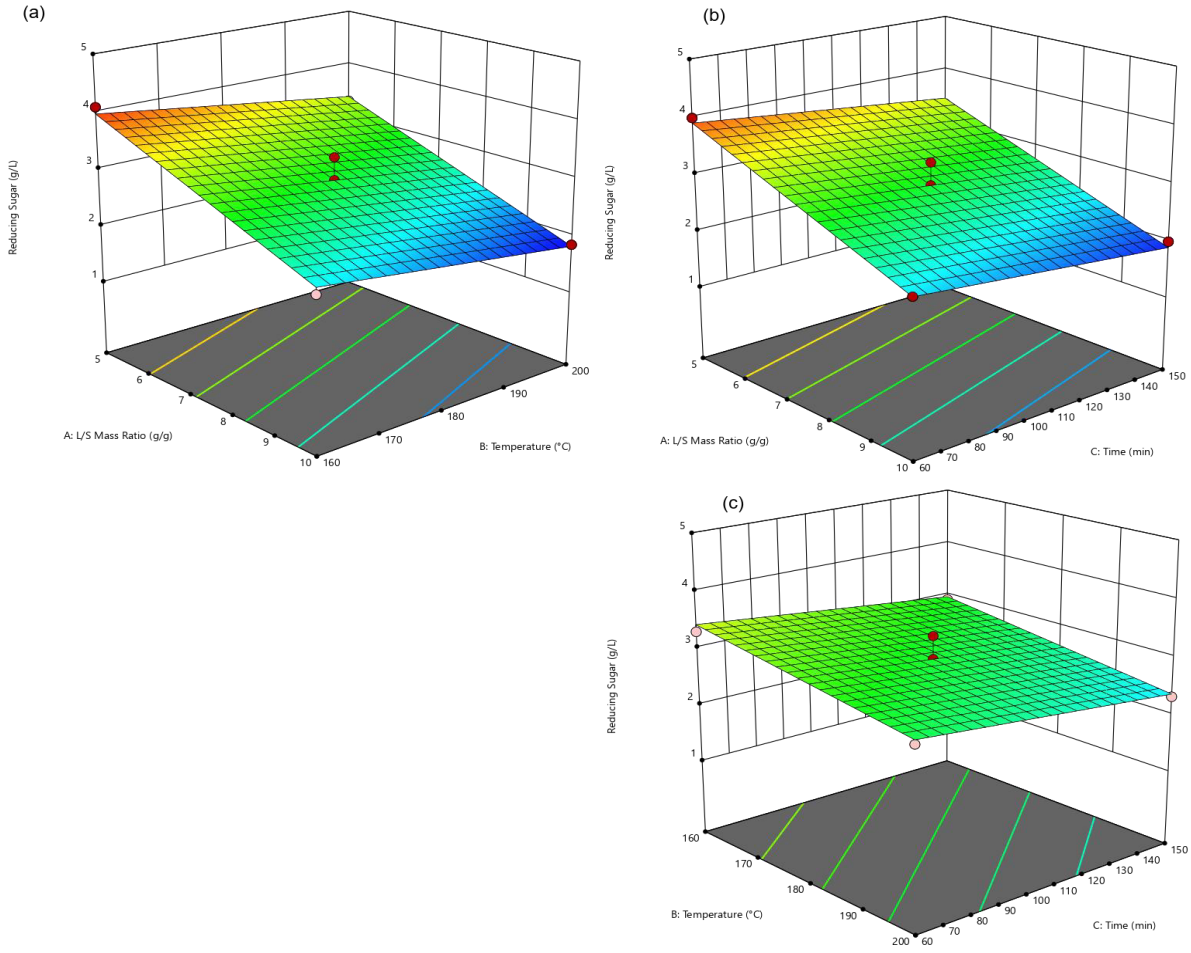

Figure 4: Pairwise interaction plots (3D surface plots) while one factor was kept constant at its zero-factor level; the interaction between the mass ratio & temperature (a), mass ratio & time (b) and temperature & time (c)

the mass ratio, moreover, the extraction time was identified as the parameter that had the lowest effect among these factors. In similar research, carried out by Muharja et al. [36], using coconut husk, it was reported that the mass ratio had the highest impact, even at higher factor levels than the present study. However, it was observed that the ranking of other factor effects varied in similar research due to the use of different raw materials and the selection of other parameters at lower factor levels.

4.2.2. Assessment and optimization of parametric interactions

The potential effects of factors on the response were evaluated using three-dimensional surface plots. As observed in *Figure 4*, each pairwise interaction generated linear contour plots and it was observed that the maximum reduced sugar concentration was obtained when the factor levels for all parameters were at their minima. This result was observed when evaluating the signs of the model in the optimization study conducted with the adequate model equation. The results observed in the pairwise interaction plots were confirmed. During optimization, the aim was to maximize the reduced sugar concentration without imposing any constraints on the variables within the experimental range. Consequently, a total of 100 solutions were obtained, where the reduced sugar concentration ranged from 3.88 to 4.15 g/g. Regarding the optimal solution that provided the highest performance, it was calculated that a reduced sugar concentration as high as 4.12 g/g could be produced. The optimal conditions for this amount were determined to be a mass ratio of 5.2, an extraction time of 63.4 minutes and a temperature of 160.3 °C. If a constraint is introduced to minimize the most critical parameter in the model, namely the mass ratio, in this optimization scenario, only the extraction time would increase to 78.5 minutes.

In terms of the response, factor *A*, which exhibits the highest individual effect, also exerts a significant impact as a result of its combined interactions (*AB* and *AC*) with other factors. In the *AB* and *AC* plots, a decrease in time and temperature, respectfully, both result in a consistent effect yielding the same gradient and an increase in the reduced sugar concentration. When examining the *BC* plot, it is evident that these factors do not significantly influence the reduced sugar concentration at the selected factor levels. Although both the temperature and extraction time influence the model, at the chosen factor levels, neither parameter nor their interactions seem to have a significant positive effect on increasing the reduced sugar concentration.

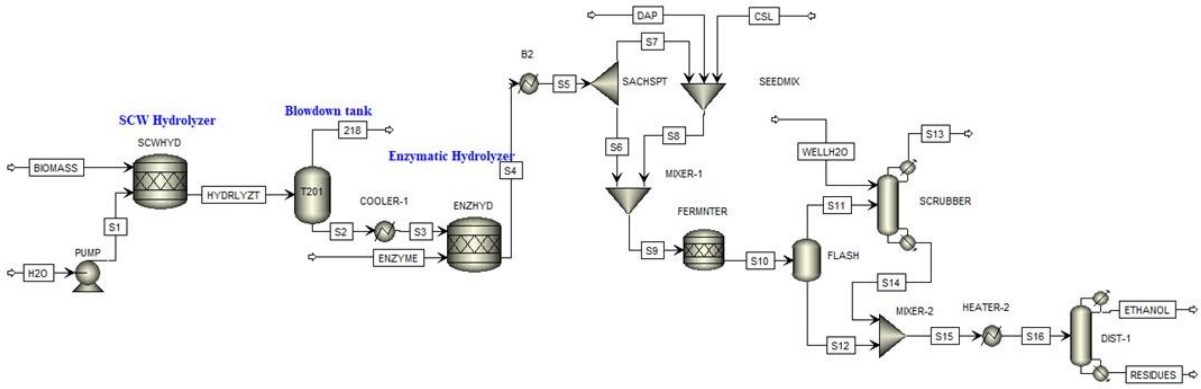

Figure 5: Simulation of rice straw pretreatment using subcritical water and Aspen Plus software

At this juncture, it is apparent that the mass ratio is the most influential factor in the pretreatment of rice straw. However, caution should be exercised in managing this variable to increase the reduced sugar concentration.

4.3. Effect of enzymatic hydrolysis

Enzymatic hydrolysis of the hydrolysate obtained from the SCW extraction of rice straw notably enhanced the yield of fermentable sugars. Notably, the concentration of glucose, a pivotal sugar when producing biofuels, exhibited a dramatic increase from 3.827 g/L in the initial extract to 17.988 g/L following enzymatic treatment, representing an approximate 370% increase and underscoring enzymatic efficiency in the saccharification of polysaccharides. Likewise, fructose levels nearly tripled, ascending from 3.112 to 10.084 g/L. The concentration of cellobiose, which is a dimer of glucose, also nearly doubled from 2.162 to 4.243 g/L, indicating the effective enzymatic breakdown of β-1,4 glycosidic bonds. Xylose, a five-carbon sugar, showed a modest increase from 0.630 to 0.696 g/L, suggesting that the enzymatic treatment was less effective at hydrolyzing hemicellulose into xylose or that the majority of xylose had already been released during the subcritical water extraction process. These findings are in line with those in the literature, for instance, Kim & Lee [11] observed a similar pattern where enzymatic hydrolysis significantly improved the yield of monosaccharides from lignocellulosic biomass.

4.4. Process simulation results

4.4.1. Rice-straw pretreatment using SCW technology: process simulation

The pretreatment of rice straw using subcritical water was simulated using Aspen Plus software and the corresponding process flow diagram is presented in *Figure 5*. The reactor (SCW Hydrolyzer) received a main feed stream (BIOMASS) at a flow rate of 80 tons/h at 20 °C and a pressure of 1 atm. To increase the pressure of the water stream (H_2O) from 1 to 15 atm, a 27.58 kW pump (PUMP) was employed before feeding the water into the reactor (SCW Hydrolyzer). Additionally, the heat duty of the reactor was approximately 18.229 MW. In addition, the software supplied all the data regarding mass and energy balances. The material exiting the pretreatment reactor undergoes flash cooling to 1 atm in a blowdown tank (T201). Subsequently, the bottom stream is used in subsequent processes such as saccharification, fermentation and ethanol production.

4.4.2. Enzymatic hydrolysis and fermentation

As shown in the flowsheet simulation, the same reactor was used during both stages. The pretreated stream (HYDRLYZT) was cooled to 47 °C (COOLER-1), that is, the operating temperature for the enzymatic process.

The enzyme cellulase was introduced at a rate of 15 mg cellulase/g dry biomass [20],[37].

Following the hydrolysis simulation step, the resulting slurry was cooled in a dedicated cooling unit (B2) to ensure optimal fermentation conditions typically at 35 °C. After cooling, one tenth of the hydrolyzed slurry was diverted via a stream splitter unit (SACHSPT) into a dedicated seed train tank in which yeast (*Saccharomyces cerevisiae*) is cultivated – the industry standard for fermenting glucose into ethanol. To promote the robust growth of dry yeast, the process utilizes corn steep liquor (CSL), a source of nutrients containing water, protein and lactic acid alongside diammonium phosphate (DAP) as an additional nutritional supplement. The DAP concentration within the broth was assumed to be 0.67 g/L while CSL was introduced at a loading rate of 0.5 wt% [37]. The fermentation process itself achieved a conversion rate of 90%, transforming glucose into the desired biofuel – ethanol. Notably, a small portion (4%) of the glucose was utilized, along with lactic acid and DAP, to cultivate the *Saccharomyces cerevisiae* yeast culture itself.

The fermentation stage utilizes a reactor (FERMNTER) for the purpose of process modeling. The temperature in the reactor was meticulously maintained at 35 °C, ensuring the optimal conditions for yeast activity. Within this controlled environment, glucose underwent fermentation and was efficiently converted into ethanol at a conversion rate of 90% [20].

4.4.3. Ethanol recovery and purification

The subsequent stages focus on recovering and purifying the ethanol produced during fermentation. A two-pronged approach was employed to achieve a final ethanol purity of 99.5%. Distillation served as the primary separation technique, while adsorption onto a molecular sieve provided final purification.

The raw fermentation broth, also known as "beer," was distilled within a designated column (DIST-1) to separate the ethanol from the by-products of fermentation. To maximize efficiency and reduce energy requirements, a preheating step utilizing a dedicated heater (HEATER-2) elevated the temperature of the mixed stream (S15) to near its boiling point before entering the column. The distillation column operated with a reflux ratio of 6 and a distillate to feed ratio of 0.208 utilizing 22 theoretical stages. This configuration achieved a high recovery rate, extracting essentially all the ethanol fed into the system and concentrating it to approximately 92.5 wt%.

Following distillation, adsorption onto a molecular sieve unit achieved final purification by removing any remaining water from the nearly azeotropic ethanol-water mixture through a vapor phase adsorption process. Regeneration of the adsorption columns necessitates the ethanol-water mixture to be recycled back into the distillation process for ethanol recovery.

4.4.4. Ethanol vent recovery

To minimize ethanol loss, fermentation vent streams, primarily containing CO_2 but also trace amounts of ethanol, were directed through a water scrubber (SCRUBBER) which efficiently captured nearly all the ethanol with the resulting effluent being reintroduced into the distillation column (DIST-1) alongside the fermented beer. Overall, this multistage approach ensured the efficient recovery and final purification of 99.5% bioethanol from the fermentation process, suitable for various applications.

4.5. Economic analysis

4.5.1. Capital expenditure (CAPEX)

The initial investment of the project is a critical factor for manufacturers. As expected, the ISBL plant cost, representing the expense of constructing the core facility, forms a significant proportion of the total capital expenditure. Utilizing Bridgewater's method, the process proposed in this study resulted in an ISBL cost of $83.6 million.

4.5.2. Production costs

Beyond the initial investment, ongoing production expenses significantly impact project feasibility, which reached $1321 per ton, playing a crucial role in the overall financial assessment.

4.5.3. Profitability analysis

The NPV considers the time value of money and assesses the disparity between the present values of future cash inflows and outflows over a specified period (in this case, 15 years). A positive NPV signifies a project that is strongly economically viable.

The project timeline commences with the design stage in year 1, marking the initial cash outflow. The construction and installation phases, anticipated in year 2, are factored into the evaluation. By year 3, the facility reaches its maximum production capacity. However, depreciation costs lead to a subsequent decline in gross profit over time.

4.5.4. Favorable investment metrics

In this work, the proposed enzymatic process exhibited a positive NPV of $121.2 million alongside a promising ROI of 422, strongly suggesting a successful and highly profitable investment opportunity.

4.5.5. Rapid capital recovery

The project's IRR stands at 25%, further emphasizing its attractiveness. Additionally, the projected payback period for this investment is just 7 years, indicating a relatively rapid timeline of capital recovery. These combined outcomes convincingly support the financial feasibility and allure of commencing production of second-generation bioethanol using the proposed enzymatic process. Overall, the economic analysis paints a promising picture for this project. The positive NPV, high ROI, attractive IRR and rapid payback period all point towards a financially sound investment opportunity.

5. Conclusions

In conclusion, this study underscores the significant potential of using subcritical water pretreatment combined with enzymatic hydrolysis for converting rice straw into valuable biofuels, addressing the increasing global demand for sustainable energy. Through the optimized application of the Response Surface Methodology (RSM) and simulation techniques, it was successfully demonstrated that green subcritical water pretreatment can efficiently convert rice straw into reducing sugars with high yields, thereby minimizing energy consumption and chemical use. The enzymatic treatment following this pretreatment significantly enhanced glucose concentrations by approximately 370%, proving the effectiveness of the enzymes used and further increasing the yield of crucial biofuel precursors such as fructose and cellobiose. The economic analysis supports the viability of scaling up this process to a plant capacity of 72,600 tons per year, highlighting its financial attractiveness with a positive net present value of $152.6 million, a high return on investment of 665%, an attractive internal rate of return of 27% and a rapid payback period of 5 years. These results not only affirm the economic benefits of the proposed green route but

also its potential to contribute significantly towards the development of more sustainable and efficient biofuel production pathways. Ultimately, this approach marks a promising step forward in meeting the 21st century's pressing energy requirements while reducing the environmental impact.

Acknowledgements

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