RESEARCH PAPER

BIOTECH STUDIES

Optimizing of acidic and alkaline pretreatments for enhanced sugar release of coffee silverskin: a taguchi method approach

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Abstract

Coffee by-products are promising reservoirs of antioxidants and fermentable sugars. Coffee silverskin (CSS) is a significant by-product of coffee bean roasting. In this study, glucose release from CSS was optimized to investigate the pretreatment parameters using the Taguchi method. For this purpose, four different acids (HCl, H_2SO_4, HNO_3, and CH_3COOH) and two alkalis (NaOH and KOH) were tested. The factors were acid/alkali type and amount (1, 2, 3, and 4%), CSS amount (2.5, 7.5, 12.5, and 17.5%), and treatment time (15, 30, 45, and 60 min). Sixteen experimental runs were performed using the L16 orthogonal array. The conditions under which maximum glucose release was obtained and the effects of these conditions on the process were examined. The maximum glucose yield (Y_g= 0.037 $g_{glucose}/g_{biomass}$) was obtained by autoclaving a 4% (v/v) HCl solution containing 17.5% CSS (w/v) for 60 min. This study evaluated the effectiveness of pre-enzymatic CSS treatments to produce fermentable sugars that are valuable for various industrial applications.

Introduction

According to recent data from the International Coffee Organization, global coffee consumption was expected to reach 177.0 million bags (ICO, 2023). Coffee is the second-largest traded commodity after petroleum. Approximately 2 million tons of roasted coffee are produced annually in Europe, generating substantial by-products in the coffee industry (Procentese et al., 2018a). These by-products are potential sources of antioxidants and fermentable sugars (Procentese et al., 2019). Among them, coffee silverskin (CSS) and spent coffee ground (SCG) are the primary residues of bean roasting and instant coffee preparation, respectively, with CSS comprising

approximately 4.2% of coffee beans. CSS contains significant quantities of cellulosic and hemicellulosic fibers, caffeine, and polyphenolic compounds. Therefore, CSS is a good source of cellulose, dietary fiber, and antioxidants. It can also be used as fuel, compost, and fertilizer (Hijosa-Valsero et al., 2018). CSS is a lignocellulosic material composed of 29% lignin, 24% cellulose, and 17% hemicellulose (Andrade Mota et al., 2021). Hemicellulose comprises a diverse array of sugars including arabinose, galactose, glucose, mannose, and xylose. Moreover, the sugar profile and composition of hemicellulose exhibit substantial variation among the different biomass residues. Notably, glucose and xylose

are the predominant sugars in CSS (Mirzoyan et al., 2022).

The majority of CSS are employed as firelighters or are deposited in landfills. On the other hand, the high levels of caffeine, polyphenols, and tannins pose significant environmental challenges. For these reasons, environmentally friendly waste management options should be explored to convert this residue into a valuable resource while adhering to green practices (del Pozo et al., 2021). In this context, the production of biofuels and value-added products from lignocellulose provides a sustainable solution for agricultural waste management, reduces toxic substances in the environment, and supports circular economic goals and growth (Ojo, 2023). In previous studies, CSS has been used to produce value-added products such as biobutanol (Hijosa-Valsero et al., 2018), bioethanol (Mussatto et al., 2012), amylase (Murthy et al., 2009), xylanase (Murthy and Naidu, 2012), fructooligosaccharides and β-fructofuranosidase (Mussatto et al., 2013), biohydrogen (Renaudie et al., 2022), acetone-butanol-ethanol (Procentese et al., 2019), butanol and succinic acid (Niglio et al., 2019), butanol and isopropanol (Procentese et al., 2018b), and fillers for composite production (Garcia and Kim, 2021).

The biomass conversion process includes three steps: pretreatment to reduce inhibitory compounds for enzymatic and microbial action, enzymatic saccharification of biomass to yield monomeric sugars, and conversion of sugars into fuels or value-added chemicals through chemical or biological pathways (Pulidindi and Kim, 2018). Agro-food waste are characterized by high sugar and low lignin contents, which makes them well suited for pretreatment (Procentese et al., 2018a). For the extraction of fermentable glucose, effective exposure and accessibility of cellulose within lignocellulosic biomass to cellulase enzymes are imperative. Pretreatment is a pivotal strategy for facilitating this process (Chen et al., 2015). Saccharification is the conversion of lignocellulose into sugars, and typically involves a combination of chemical pretreatment and enzymatic hydrolysis. Thermochemical pretreatment is critical for making lignocellulosic substrates amenable to enzymatic digestion (Zhang et al., 2019). Lignocellulose, a plant-derived material composed of hemicellulose, cellulose, and lignin, is a promising alternative to fossil fuels for the production of fuels and various valuable chemical by-products. The efficacy and underlying mechanisms of pretreatment techniques vary, with hot water/auto-hydrolysis pretreatment being optimal for biomass with low lignin content, whereas acid/alkaline pretreatment is more suitable for biomass with high lignin content (Gundupalli et al., 2022).

The complex lignocellulosic composition of CSS makes it resistant to enzymatic and microbial digestion. Therefore, their use can be limited in biorefinery processes (Niglio et al., 2020). Various pretreatment methods, such as deep eutectic solvents (Procentese and Rehmann, 2018; Procentese et al., 2018a), mild hydrothermal (Conde and Mussatto, 2016), organosolv (Smyrnakis et al., 2023), steam explosion (Gondim et al., 2024), acidic (Niglio et al., 2017; Dadi et al., 2018; Morales-Martínez et al., 2021), and alkaline (Procentese et al., 2018b; Niglio et al., 2019; Procentese et al., 2019) methods, have been suggested to disrupt the lignocellulosic structure of CSS. Dilute acid hydrolysis is a widely adopted and highly efficient chemical pretreatment method. It serves two purposes: enhancing lignocellulose accessibility to cellulolytic enzymes, and directly yielding fermentable sugars. The cost-effectiveness and suitability of industrial-scale applications are also important for this process (Dadi et al., 2018). Alkali pretreatment removes lignin and acetyl groups without carbohydrates loss or sugar reduction. This process induces the swelling of fibrous cellulose and cleaves the crosslinks between hemicellulose and other constituents, thereby augmenting the specific surface area and porosity, and ultimately facilitating enzymatic hydrolysis (Ebrahimian et al., 2023).

Enzymatic hydrolysis involves the cooperative action of cellulolytic and hemicellulolytic enzymes to break down the carbohydrate polymers in biomass into sugars. The composition, degree of polymerization, and crystallinity of biomass influence the enzymatic hydrolysis process. Residual lignin following pretreatment may form nonspecific bonds with enzymes, thereby impeding the efficiency of hydrolysis of the pretreated biomass. Additionally, the sugars (cellobiose and glucose) released during hydrolysis can inhibit cellulolytic enzymes (Niglio et al., 2019). The effectiveness of pretreatments such as acid/alkali is important for many processes, the next step of which may be enzymatic hydrolysis. Therefore, this study focused on optimizing the acid and alkali pretreatment methodologies for CSS pretreatment using the Taguchi method to enhance the yield of fermentable sugars for prospective biotechnological applications. Literature on CSS pretreatment is limited, and existing studies have typically been conducted prior to enzymatic hydrolysis without optimization. This study is important for future pretreatment and enzymatic hydrolysis studies, because it simultaneously evaluates multiple factors.

Material and Methods

Sample collection

CSS supplied from the coffee roasting process was acquired from Miko Gıda, Antalya, Turkey, stored in sealed bags at room temperature (approximately 22 °C), and shielded from light exposure. The samples were sieved to obtain subsamples, as shown in Figure 1.

Biomass pretreatments

For acidic pretreatment, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), nitric acid (HNO_3), and acetic acid (CH_3COOH) were used. Sodium hydroxide (NaOH) and potassium hydroxide (KOH) were preferred for alkaline

pretreatments. CSS pretreatment was carried out in 200 mL Duran bottles, and sieved CSS sample was mixed in acid and alkaline solutions and then autoclaved (HiClave HV-25 autoclave, HMC, Japan) at 121 °C. After removal from the autoclave, the liquid portions of the samples were filtered through a coarse filter paper, separated for glucose determination, and filtered through a syringe filter with a porosity of 0.45 μm before analysis.

Figure 1. Non-sieved and sieved CSS samples.

Determination of glucose concentration and glucose yield

Glucose concentrations were determined using an Agilent 1260 Infinity series HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a quaternary pump (Agilent, G1311C), autosampler (Agilent, G1329B), column thermostat (Agilent, G1316A), and Refractive Index Detector (RID) (Agilent, G1362A). MetaCarb H Plus column (300 × 7.8 mm) and 0.0085 N H_2SO_4 as the mobile phase at a flow rate of 0.4 mL/min were used. The wavelength of the RID detector was set at 210 nm. The glucose yield (Y_G ($g_{glucose}/g_{biomass}$)) was determined as the ratio of the glucose obtained after pretreatment to the pretreated biomass amount.

Optimization of pretreatment conditions

Combinations of experimental factors were chosen from the L16 orthogonal test table to determine the optimal pretreatment conditions. As only two alkalis were used in the alkaline pretreatment, the [L16(2^1 × 4^3)] trial pattern was created using the mix-level design option in the Taguchi model. The factors and levels for each process are listed in Table 1 and Table 2.

Table 1. Factors and levels for the optimization of acid pretreatment of CSS

Factors	Level 1	Level 2	Level 3	Level 4
Acid type	HCl	H_2SO_4	HNO_3	CH_3COOH
Acid (%, v/v)	1	2	3	4
CSS (%, w/v)	2.5	7.5	12.5	17.5
Treatment time (min)	15	30	45	60

In experiments designed using the Taguchi method, the optimal conditions were determined based on the signal-to-noise (S/N) ratio values. This ratio reflects the deviation of the experimental results from the desired performance outcomes and offers insights into how closely the results align with the ideal. The purpose of this analysis was to maximize the S/N ratio (Alavi-Borazjani et al., 2021). Minitab statistical software

(Version 19) was employed to design the experiments and analyze the experimental data statistically. Analysis of variance (ANOVA) was used to ascertain the statistical significance of the experimental parameters.

Table 2. Factors and levels for the optimization of alkaline pretreatment of CSS

Factors	Level 1	Level 2	Level 3	Level 4
Alkaline type	NaOH	KOH		
Alkaline (%, w/v)	1	2	3	4
CSS (%, w/v)	2.5	7.5	12.5	17.5
Treatment time (min)	15	30	45	60

The experiments were conducted using two parallel bottles, with each bottle containing three replicates. Subsequently, the results were analyzed by calculating the mean of the two bottles.

Results and Discussion

Optimization of the acid pretreatment of CSS

The glucose concentration, glucose yield (Y_g), and S/N ratio for acidic pretreatment are listed in Table 3. The lowest glucose concentration was 49.56 mg/L after autoclaving with a 4% (v/v) CH_3COOH solution containing 2.5% CSS (w/v) for 45 min. There was no difference in glucose yield when CH_3COOH was used in the experiments, and these values remained at the lowest level. The conditions under which maximum glucose concentration was achieved were 4% (v/v) HCl, 17.5% (w/v) biomass loading, and 60 min. Under these conditions, the highest glucose concentration and glucose yield were 6467.59 mg/L and 0.037 g/g, respectively. The standard deviations of the glucose yields were 0.000.

Acidic pretreatment enhances the solubility of hemicelluloses and overall biomass permeability. This increased permeability increases the accessibility of cellulose, rendering it more susceptible to enzyme penetration. Temperature, acid concentration, and solid loading are critical parameters that influence the effectiveness of acid-based biomass pretreatments. A higher solid loading is advantageous for bio-economy, as it yields elevated concentrations of sugars. On the other hand, it is imperative to ascertain the maximum solid loading capacity for acid-based pretreatment to mitigate the challenges associated with mass transfer limitations (Hoang et al., 2021). During acidic pretreatment, elevated temperatures, prolonged reaction times, and increased concentrations tend to diminish the yield, crystallinity, and thermal stability of cellulose (Batista et al., 2022). A successful pretreatment strategy should improve enzyme efficiency, decrease carbohydrate loss, and inhibit by-product formation (Zhang et al., 2016).

The plots of the main effects of the S/N ratio are shown in Figure 2. The objective of optimizing the process parameters is to enhance the S/N ratio to

Table 3. Designed experiment and results for acidic pretreatment optimization

Exp. No	Acid	Acid (%, v/v)	CSS (%, w/v)	Time (min)	Glucose concentration (mg/L)	Y_S ($g_{glucose}/g_{biomass}$)	S/N ratio
1	HCl	1	2.5	15	800.56±24.81	0.032	58.0679
2	HCl	2	7.5	30	1565.28±55.21	0.021	63.8918
3	HCl	3	12.5	45	3472.99±298.67	0.028	70.8141
4	HCl	4	17.5	60	6467.59±98.30	0.037	76.2148
5	H_2SO_4	1	7.5	45	655.81±65.52	0.009	56.3356
6	H_2SO_4	2	2.5	60	323.26±27.28	0.013	50.1910
7	H_2SO_4	3	17.5	15	1638.15±59.07	0.009	64.2871
8	H_2SO_4	4	12.5	30	1510.87±86.97	0.012	63.5845
9	HNO_3	1	12.5	60	1025.88±38.37	0.008	60.2219
10	HNO_3	2	17.5	45	928.20±67.95	0.005	59.3528
11	HNO_3	3	2.5	30	323.99±23.04	0.013	50.2106
12	HNO_3	4	7.5	15	858.26±53.87	0.011	58.6724
13	CH_3COOH	1	17.5	30	331.01±21.93	0.002	50.3968
14	CH_3COOH	2	12.5	15	238.90±23.14	0.002	47.5643
15	CH_3COOH	3	7.5	60	161.43±6.04	0.002	44.1597
16	CH_3COOH	4	2.5	45	49.56±2.23	0.002	33.9026

achieve better results. The optimal level for each control factor was established to minimize noise variability and maximize the glucose concentration. The peak S/N ratio for each control factor was observed for HCl, 4% (v/v) acidic solution, 17.5% (w/v) biomass loading, and 60 min.
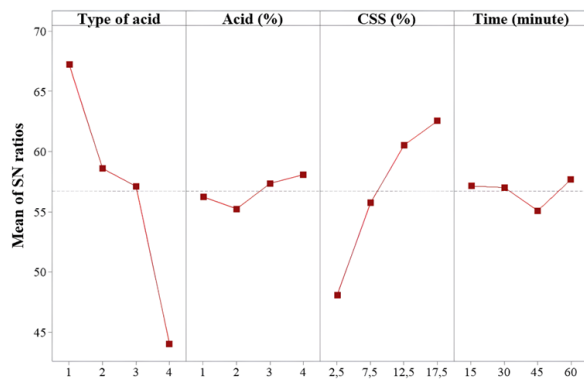
Signal-to-noise: Larger is better

Figure 2. The plot of the S/N ratio for release of glucose after acidic pretreatment (Acid no 1: HCl, 2: H_2SO_4, 3: HNO_3, CH_3COOH).

Under elevated temperature conditions, dilute acids with concentrations below 4% hydrolyze hemicellulose into monomers. However, a drawback associated with the use of diluted acids at high temperatures is their potential to inhibit fermentation (Zborowska et al., 2021). The use of concentrated acids can entail exposure to elevated temperatures of approximately 100 °C for 2-10 hours. This situation may cause various drawbacks, including corrosion risks, high toxicity levels, and necessity for chemical product disposal and/or recycling. In contrast, diluted acids serve as catalysts for cellulose and hemicellulose degradation at elevated temperatures ranging from 100 to 240 °C for shorter times (Nava-Valente et al., 2023). In this study, a maximum time of 60 min and maximum biomass loading of 17.5% (w/v) were used. When this amount was used, an almost solid appearance was obtained before autoclaving with acidic or alkaline solutions adjusted to various percentages. Therefore, a higher CSS rate has not been reported. Nevertheless, higher solid loadings are favored in industry, as they result in reduced expenses associated with reactors and enhanced product recovery due to economies of scale. It has also been indicated that the hydrolysis of cellulose causes glucose release (Dutra et al., 2018). Similarly, the disruption of lignocellulose facilitates the hydrolysis of hemicellulose and cellulose into monosaccharides (Corrêa et al., 2021). Based on the results obtained in our study, it can be stated that HCl induces greater cellulose hydrolysis than H_2SO_4, HNO_3, and CH_3COOH, respectively. Ravindran et al. (2017) arrived at the conclusion that spent coffee waste subjected to acid pretreatment for 20 min at high temperatures exhibited a notable increase in reducing sugar release, irrespective of the acid concentration. This observation was attributed to the detrimental effects of prolonged treatment on the polysaccharide fraction of the lignocellulosic biomass.

Glucose concentration is determined using the liquid phase during biomass pretreatment and the solid phase during enzymatic hydrolysis. In our study, increasing the solid ratio further enhanced sugar release. At this point, obtaining more glucose with HCl pretreatment could result in a decrease in glucose release after enzymatic hydrolysis. As indicated by Li et al. (2022), an optimal pretreatment process should prevent carbohydrate degradation or loss. Toquero and Bolado (2014) compared thermal autoclaving, dilute acid autoclaving, dilute alkali autoclaving, and alkaline peroxide pretreatment for enzymatic hydrolysis of wheat straw. The experimental results revealed significantly lower glucose and xylose release in the hydrolysates with these pretreatments, except for dilute acid autoclaving. In contrast, the concentrations of cellulose and hemicellulose retained in the solid fraction were higher. It can be assumed that the maximum sugar

Table 4. Analysis of variance for acidic pretreatment of CSS

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F	P
Acid type	3	1104.63	67.08%	1104.63	368.209	96.20	0.002
Acid (%, v/v)	3	18.72	1.14%	18.72	6.242	1.63	0.349
CSS (%, w/v)	3	496.46	30.15%	496.46	165.485	43.24	0.006
Time (min)	3	15.39	0.93%	15.39	5.128	1.34	0.408
Error	3	11.48	0.70%	11.48	3.827		
Total	15	1646.68	100%				

DF: Degrees of freedom, Seq SS: Sequential sums of squares, Adj SS: Adjusted sum of square, Adj MS: Adjusted mean square (R^2:99.30%, R^2adj:96.51%)

release observed in our study was due to the lower retention of cellulose and hemicellulose in the solid fraction. Therefore, if acidic pretreatment is applied before glucose is obtained from CSS by enzymatic hydrolysis, treatment with CH3COOH is recommended.

The ANOVA results (Table 4) showed that among the selected factors, the type of acid had a stronger influence (67.08%) on glucose concentration, whereas autoclave time had the least influence (0.93%). The effects of acid percentage and autoclaving time were not statistically significant ($P > 0.05$).

Optimization of the alkaline pretreatment of CSS

Table 5 shows the experimental design and results of the alkali pretreatment. The highest glucose concentration obtained with 1% (w/v) KOH and 17.5% (w/v) CSS after 60 min was determined to be 364.76 mg/L. The second-highest value of 327.38 mg/L was obtained after 30 min of autoclaving with a solution containing 3% (w/v) KOH and 17.5% (w/v) CSS. Notably, the lowest glucose concentration (2.56 mg/L), was obtained by autoclaving 2.5% (w/v) CSS in a solution containing 3% (w/v) NaOH for 45 min. Overall, KOH was more effective than NaOH for the production of glucose from CSS. Finally, the glucose yield varied between 0 and 0.002 g/g. The standard deviations of the glucose yields were 0.000.

The primary impact of alkali pretreatment is lignin removal, which leads to an increased reactivity of the remaining polysaccharides. This process involves altering the lignin structure by breaking the ester and glycosidic side chains within the biomass using an alkaline solvent, which results in cellulose swelling and decrystallization. Although higher alkaline concentrations generally promote biomass delignification, excessively high concentrations ranging from 6-20% (w/w), may lead to cellulose dissolution and hinder lignin removal (Loow et al., 2016). In contrast to alternative chemical pretreatment methods, alkaline hydrolysis offers the advantage of operating at lower temperatures and pressures, resulting in minimal sugar degradation compared with acid pretreatment. This process is effective when hexoses and pentoses are utilized as substrates in the subsequent fermentation following enzymatic hydrolysis (Rabemanolontsoa and Saka, 2016). Other advantages include lower operating costs, lower energy consumption, lower yield of sugar degradation, less corrosion compared to acid treatment, lower lignin content, and fewer inhibitors (Tan et al., 2021).

The plots of the main effects of the S/N ratios are shown in Figure 3. The peak S/N ratio for each control factor was 17.5% (w/v) CSS, 2% (w/v) alkaline solution, KOH, and an autoclaving time of 30 min. Niglio et al. (2020) aimed to optimize ultra sound-assisted alkaline pretreatment of CSS for maximizing the reducing sugar yield. Under optimal conditions (11% (w/v) biomass loading, 5 min sonication time, 5% (w/v) alkali concentration, and 75 min), the maximum glucose yield was 0.61 g/g. Moreover, the NaOH concentration and incubation time were the most significant process parameters.

Table 5. Designed experiment and results for alkaline pretreatment optimization.

Exp. No	Alkaline	Alkaline (%, w/v)	CSS (%, w/v)	Time (min)	Glucose concentration (mg/L)	Y_S ($g_{glucose}/g_{biomass}$)	S/N ratio
1	NaOH	1	2.5	15	20.75±1.84	0.001	26.3404
2	NaOH	1	7.5	30	105.09±7.06	0.001	40.4312
3	KOH	1	12.5	45	229.62±16.19	0.002	47.2202
4	KOH	1	17.5	60	364.76±28.77	0.002	51.2401
5	KOH	2	2.5	30	57.25±4.09	0.002	35.1555
6	KOH	2	7.5	15	100.32±8.22	0.001	40.0278
7	NaOH	2	12.5	60	111.58±5.29	0.001	40.9517
8	NaOH	2	17.5	45	299.91±17.32	0.002	49.5398
9	NaOH	3	2.5	45	2.56±0.13	0.000	8.1648
10	NaOH	3	7.5	60	16.55±0.85	0.000	24.3760
11	KOH	3	12.5	15	144.37±8.44	0.001	43.1895
12	KOH	3	17.5	30	327.38±29.42	0.002	50.3010
13	KOH	4	2.5	60	21.25±1.48	0.001	26.5472
14	KOH	4	7.5	45	119.68±10.21	0.002	41.5604
15	NaOH	4	12.5	30	233.22±10.08	0.002	47.3553
16	NaOH	4	17.5	15	175.45±8.12	0.001	44.8831
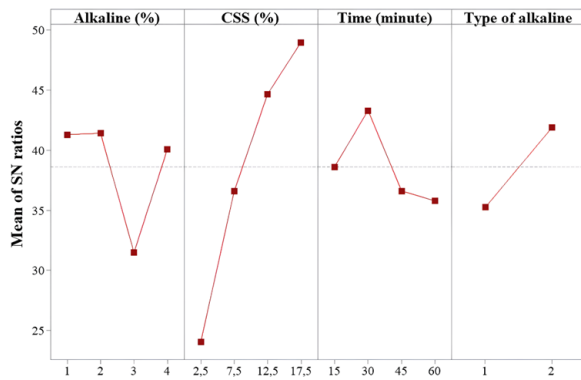
Signal-to-noise: Larger is better

Figure 3. The plot of the S/N ratio for release of glucose after alkaline pretreatment (Alkaline no 1: NaOH, 2: KOH).

Murthy et al. (2009) assessed coffee pulp, coffee cherry husk, CSS, spent coffee, and mixed waste (all the substrates in equal ratios) as sole carbon sources for α-amylase production with solid-state fermentation using *Neurospora crassa* CFR 308. They found that steaming was the most effective pretreatment method for enhancing the enzymatic activity of coffee pulp, and mixed waste; however, NaOH pretreatment and enzyme hydrolysis did not increase the enzyme activity. In another study, response surface methodology (RSM) was applied to optimize CSS pretreatment with alkaline hydrogen peroxide to enhance the quality, shelf life, sensory attributes, and appearance of Barbari flat bread. The optimal conditions determined using the desirability function method were mixing time of 1 h, solution ratio of 4.77, and particle size of 116.41 μm. The results also indicated that alkaline hydrogen peroxide treatment of CSS could be effective in reducing the caloric density and increasing the dietary fiber content of bread (Pourfarzad et al., 2013). Soontornchaiboon et al. (2016) stated that cellulose and hemicellulose can be destroyed at a concentration of 2% NaOH (w/v) but it causes carbohydrate loss and a decrease in sugar concentration. Lee et al. (2021) used the RSM for the optimization of alkali pretreatment of spent coffee grounds, and optimal conditions were found as follows: 75 °C temperature, 3% (w/v) KOH concentration, and a duration of 2.8 h. Jin et al. (2020) identified the optimum conditions for fermentable sugar production using RSM as 0.5% (w/v) NaOH, 100 °C, and 5% (w/v) spent coffee grounds. In a study conducted by Hijosa-Valsero et al. (2018) on biobutanol production, CSS was initially

subjected to autohydrolysis pretreatment. Autohydrolysis, combined with enzymatic hydrolysis, was subsequently applied, resulting in an increase in glucose concentration from 0.51 g/L to 21.83 g/L.

Table 6 presents the ANOVA results for alkaline pretreatment. CSS amount had a stronger influence on glucose concentration, with a maximum contribution of 69.22%, whereas autoclaving time had the least influence, with a contribution of 6.54%. In addition, the effect of autoclaving time was not statistically significant ($P > 0.05$).

Conclusions

Pretreatment is a pivotal stage in biomass conversion, with the purpose of efficiently generating fermentable sugars, thereby facilitating enzymatic saccharification, while ensuring cost-effectiveness. On the other hand, the Taguchi method is useful in biotechnology, in which experiments are costly, time-consuming, and sensitive to many interacting factors. Additionally, the Taguchi method focuses on improving the process robustness by identifying the optimal conditions that yield consistent results despite external variability, which is essential for scaling up biotechnological processes. While this study focused on optimizing the pretreatment stages of CSS, future research can aim to optimize the enzymatic hydrolysis stage and identify the conditions for maximizing the fermentable sugar content. Through these efforts, it is anticipated that waste resources such as CSS can be utilized with maximum efficiency in diverse processes, including bioethanol production. In this study, considering the advantages of alkaline pretreatment over acid pretreatment and glucose release concentrations, it can be concluded that CSS should be treated with alkaline pretreatment before enzymatic hydrolysis.

Author contributions

İzzet Özhamamcı: Formal analysis, review&editing; Bilge Sayın: Conceptualization, formal analysis, writing–original draft.

Conflict of interest

The authors declare no conflicts of interest.

Table 6. Analysis of variance for alkaline pretreatment of CSS

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F	P
Alkaline type	1	176.89	8.49%	176.89	176.89	15.51	0.011
Alkaline (%, w/v)	3	271.14	13.01%	271.14	90.38	7.93	0.024
CSS (%, w/w)	3	1442.31	69.22%	1442.31	480.77	42.16	0.001
Time (min)	3	136.26	6.54%	136.26	45.42	3.98	0.085
Error	5	57.01	2.74%	57.01	11.40		
Total	15	2083.61	100%				

DF: Degrees of freedom, Seq SS: Sequential sums of squares, Adj SS: Adjusted sum of square, Adj MS: Adjusted mean square (R^2:97.26%, R^2adj:91.79%)

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