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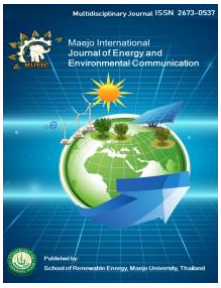

ARTICLE

Exploring golden shower foliage fermentable sugars untapped potential and promise for sustainable bioethanol development

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ABSTRACT

This study investigates the potential of golden shower foliage (GSF) fermentable sugars for sustainable bioethanol production. Utilizing chemical and biological pretreatments involving agents such as sodium hydroxide (NaOH), calcium oxide (CaO), and *Trichoderma reesei*. The research analyzed various aspects including reducing sugar coefficient, total sugar, degree of polymerization (DP), and derived energy. Results underscored the efficacy of a 3% NaOH solution over 72 hours in maximizing sugar and energy concentration, facilitating efficient lignocellulosic biomass conversion into sugar. However, the economic viability for large-scale deployment poses a challenge, directing attention to the cost-benefit of incorporating CaO due to its affordability compared to NaOH without notably diminishing output efficiency. The role of *T. reesei*, a notable entity in biomass decomposition and a staple in biofuel production, was also highlighted. The research further delved into the complexity of carbohydrate structures and the significant role of the degree of polymerization, influencing the classification of carbohydrates based on their monomer count. Therefore, this approach is the cost and competitiveness of the pretreatment on the hydrolysis phase of large-scale fermentable sugar is a hurdle.

1. Introduction

Biomass derived from lignocellulose encompasses various sources including agricultural remnants, byproducts of forestry and industry, and energy plants. A prominent example of this is straw biomass, a type of agricultural waste that is plentiful, affordable, environmentally friendly, safe, and renewable (Ramaraj et al., 2023a,b). Utilizing straw biomass as an alternative to traditional fossil fuels can effectively address the tension between energy production and food supply, positioning it as an optimal choice for sustainable energy development (Junluthin et al., 2021). This biomass can be a precursor to a range of high-value chemical products, contributing to environmental conservation and promoting economic sustainability (Bhuyar et al., 2022). Primarily, lignocellulosic biomass contains cellulose, hemicellulose, and lignin, which can yield fermentable sugars through sugar component hydrolysis, presenting a viable pathway to leverage this resource (Khammee et al., 2021; Kongchan et al., 2022).

Sugars serve as essential intermediates in the transformation processes of lignocellulosic biomass, but the stubborn nature of plant cell walls impedes their extraction (Bhuyar et al., 2021). Within lignocellulose, a significant portion of glucose is confined within rigid cellulose polymers, characterized by their highly crystalline structures. This cellulose is encased by hemicellulose, a polymer composed of various sugars including glucose and xylose, and lignin, a compound formed from intricate aromatic polymers

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that reinforce and shield the plant structure. A series of physical and chemical breakdown processes are indispensable to extract sugars from this diversified and complex material. Saccharification via enzymatic techniques is predominantly employed, entailing initial physical and chemical pretreatments succeeded by cellulases-facilitated hydrolysis, ultimately leading to sugar liberation (Nguyen et al., 2020). When optimized with the right blend of pretreatments and enzymes for the specific biomass, this method can facilitate high sugar yields from both the hemicellulose and cellulose segments.

However, this process is not devoid of challenges, including the substantial costs associated with pretreatments and enzyme procurement, which account for a considerable share of ethanol production expenses from cellulose (Manmai et al., 2019a). Furthermore, the generally low hydrolysis rates present another bottleneck in the efficiency of enzymatic hydrolysis, necessitating further innovations and optimizations in this field. Nevertheless, realizing the full potential of lignocellulosic biomass is challenging due to its intricate composition and the strong interconnections between different types of biomass, creating barriers to efficient utilization (Manmai et al., 2020; Nguyen et al., 2020). Consequently, overcoming these complexities remains pivotal to unlocking the vast potential embedded in this renewable source of energy and material. In the burgeoning bioenergy field, the exploitation of lignocellulosic biomass from unconventional sources is escalating, bringing forward the urgent need to scrutinize and optimize the methodologies employed in extracting fermentable sugars for the precursor to biofuel production.

Standing firm in this frontier, the golden shower tree (*Cassia fistula* Linn.) emerges as a focal point of interest, potentially transforming from an ornamental entity to a critical contributor in the green energy sector. Deepening our understanding of the biochemical dynamics involved in the pretreatment processes is instrumental in overcoming the hurdles to large-scale fermentable sugar production. The focal point of our research situates itself around the meticulous analysis of the GSF, which, post-shedding from the trees, undergoes a series of preliminary treatments before serving as the biomass substrate in our experiments (You et al., 2018). The leaves' journey from the tree to the laboratory epitomizes a dedicated approach to resource preparation, involving solar drying, size reduction, and hot air oven drying to facilitate moisture and water evaporation, creating a refined substrate ready for the subsequent pretreatment stages.

The investigative journey seeks to delve into the intricacies of chemical and biological pretreatments, wherein a nuanced manipulation of the process variables such as NaOH and CaO concentrations, *T. reesei* utilization, and treatment time plays a pivotal role. By operating under distinct concentrations and timeframes, we aspire to arrive at an optimized strategy that ensures the maximum release of fermentable sugars while balancing the economic implications of the process. Moreover, the research extends to understanding the degree of polymerization (DP) of a determinant in ascertaining the complexity of carbohydrate structures and a vital parameter influencing hydrolysis efficacy.

Utilizing a range of analytical methods including the 3,5-dinitrosalicylic acid (DNS) technique and the phenol–sulfuric acid method and the venture into a deep exploration of the sugar landscape that evolves post-pretreatment, deciphering the sugar yields and understanding the relationship between reducing sugar and total sugar concentrations (Miller, 1959). Each experiment is designed to unearth the underlying patterns that govern the fermentable sugar extraction process, dissecting the interplay between chemical structures and pretreatment conditions.

The competitive landscape of fermentable sugar production is fraught with challenges, chief among them being the pretreatment processes' economic viability and efficiency (Lee and Yu, 2021). Herein, we gauge the energetics associated with reducing sugar production, critically assessing the energy derived from various pretreatment conditions, thereby bridging the gap between laboratory research and potential industrial application. In navigating this complex terrain, we leverage advanced analytical tools, including a UV-Spectrophotometer, for meticulous sugar analysis to characterize yield coefficients and energy derivations (Dubois et al., 1956; Manmai et al., 2021).

This voyage of scientific inquiry is underpinned by a rich array of experimental designs that pivot around well-defined mathematical calculations, anchoring our findings on a solid quantitative foundation and providing avenues for informed decisions in process optimization. As we unfold the layers of this study, we thread a narrative that integrates scientific rigor with a vision for sustainable development, forging a path that may well see the GSF transition from a mere botanical entity to a powerhouse in the renewable energy sector. Therefore, this study aims to explore GSF, fermentable sugars' untapped potential, and fermentable sugar extraction from dried GSF using chemical and biological pretreatment methods for future sustainability, low-cost, and environment-friendly bioethanol development.

2. Material and methods

2.1 Collection and preliminary processing of C. fistula foliages

The foliages of the golden shower (*Cassia fistula* Linn.) was collected after falling off the tree depicted in Figure 1 near Maejo University in Chiang Mai, Thailand (18° 8' 98" N 99°0' 13" E). For three days, the foliages was dried in a solar drying building at Chiang Mai's ambient temperature (30-32 ºC).

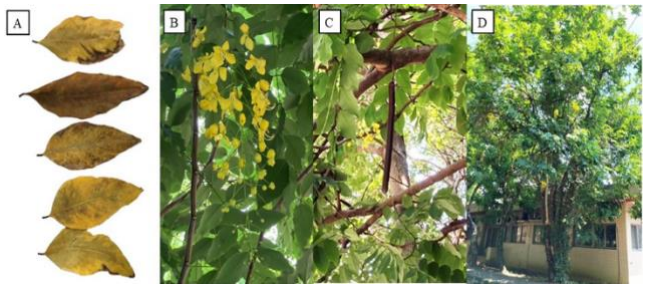

Figure 1. (A) The dried golden shower foliages, (B) The inflorescence of the golden shower, (C) The pod of the golden shower, (D) The golden shower tree

Cutting and blending equipment lowered their size in two steps. Dried and powdered golden shower tree foliage was dried for moisture and water evaporation in materials in a hot air oven at 60 ºC for 48 hours before being stored in plastic bags at room temperature for additional utilization (Manmai et al., 2019; 2020) were used to prepare all resources.

2.2 Sugar extraction

A beaker was filled with dried sunflower stalk powder (5 g), and 15 mL of NaOH, CaO, and *T. reesei* were added, with a solid-to-liquid ratio of 1:3, at various concentrations (1.0, 1.5, and 2.0% (v/v)). At room temperature, these beakers were covered with aluminum foil and extraction times with set points (24, 48, and 72 hrs.). After the extraction operations were complete, 20 mL of distilled water was added to the beakers. After being squeezed, the sugar solution was filtered from solids to calculate the reducing sugar yields using glucose as the standard by the DNS method. Determining total sugar yields by the phenol–sulfuric acid method.

2.3 Sugar analysis

The 3,5-dinitrosalicylic acid (DNS) technique was used to determine the reduction in sugar content. A UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) was used to measure the absorbance at 540 nm with a blank as the control (Miller, 1959). According to Dubois et al. (1956), the total sugar content was examined using phenol sulfuric techniques using a Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm. Sugars and related chemicals can be determined using a colorimetric technique. Calculating the reducing sugar concentration, reducing sugar yield coefficient, total sugar concentration, total sugar yield coefficient, and the degree of polymerization.

2.5 Analytical methods

The following calculations were performed to determine the total sugar and reducing sugar during pretreatment, and Thangavelu et al. (2014).

$$\text{Reducing sugar yield coefficient } \left(\frac{\text{g}}{\text{g}}\right)=\frac{\text{Reducing sugar } \left(\frac{\text{g}}{\text{L}}\right)}{\text{Dry biomass } \left(\frac{\text{g}}{\text{L}}\right)} \quad (1)$$

$$\text{Total sugar yield coefficient } \left(\frac{\text{g}}{\text{g}}\right)=\frac{\text{Total sugar } \left(\frac{\text{g}}{\text{L}}\right)}{\text{Dry biomass } \left(\frac{\text{g}}{\text{L}}\right)} \quad (2)$$

$$\text{Energy}_{\text{Reducing sugar}} \left(\frac{\text{kJ}}{\text{L}}\right)=\text{Reducing sugar } \left(\frac{\text{g}}{\text{L}}\right) \times 16 \quad (3)$$

$$\text{Degree of polymerization}=\frac{\text{Total suagr } \left(\frac{\text{g}}{\text{L}}\right)}{\text{Reducing sugar } \left(\frac{\text{g}}{\text{L}}\right)} \quad (4)$$

3. Results and discussion

3.1 Enhanced reducing sugar yields from GSF through strategic chemical and biological pretreatments

These cellulosic components, presented in the dry leaves in varying amounts, are desirable as possible sources of ethanol, gasoline, and other compounds. However, to convert lignocellulose into sugar, two additional processes—pretreatment and saccharification—must be carried out before biological fermentation because of the cell wall's remarkable recalcitrance (Lee and Yu, 2021). This research uses chemical and biological solutions to extract the reducing sugar presented in Figure 2. Since CaO is less expensive than NaOH and NaOH offers higher pretreatment alkalinity, biomass was cost-effectively pretreated using a combination of CaO and NaOH.

In previous research, biomass pretreated with a combination of CaO and NaOH could be converted to sugar more readily than biomass that had just been prepared with CaO (You et al., 2018). One of the most researched cellulolytic microbes is *T. reesei*. The biotechnology sector uses this fungus extensively to produce enzymes for biomass decomposition, primarily for biofuel generation (de Paula et al., 2018). The results from Figure 2 presented the highest total sugar concentration of pretreatment with NaOH 3% for 72 hrs. Estimating the total sugar concentration in different substances of pretreatment cannot be equivalent to the amount of reducing sugar and reducing sugar coefficient obtained from NaOH pretreatment illustrated in Figure 2 and Table 1.

Table 1 details the reducing sugar coefficient from dried GSF across a series of treatments — NaOH, CaO, and *T. reesei*, at concentrations of 1%, 2%, and 3% — measured at different time intervals (0, 24, 48, and 72 hours). The initial readings at 0 hours were quite low for all treatments with values ranging narrowly from 0.000714±0.000033 (1% CaO) to 0.001253±0.000065 (3% NaOH). However, as time progressed to the 72-hour mark, there was a prominent increase in the reducing sugar coefficient. The highest value was observed in the 3% NaOH treatment, registering a coefficient of 0.0598997±0.001612, followed closely by the same concentration of CaO and *T. reesei* treatments with coefficients of 0.044877±0.001168 and 0.036246±0.000487, respectively. The standard error margins indicate that the data is fairly precise, allowing for confident interpretation of the results.

This increasing trend over time across all concentrations and treatment types suggests a consistent enhancement in sugar release as the treatment duration prolongs. Moreover, higher concentrations of the treatment solutions generally resulted in higher sugar coefficients, highlighting a concentration-dependent

efficacy in sugar extraction. This data, presenting a substantial increase, especially in higher concentrations and longer durations, could be pivotal in understanding the pre-treatment strategies for biofuel production utilizing GSF foliage biomass.

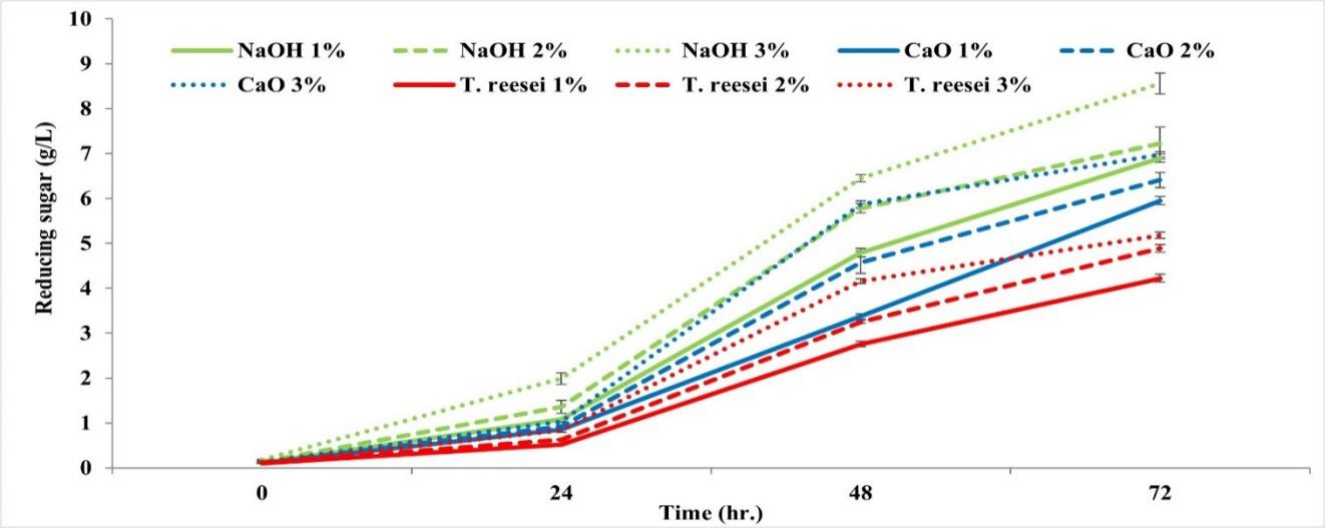

Figure 2. Reducing sugar from dried GSF

Table 1. Reducing sugar coefficient from dried GSF

Biomass	Time (hr.)	Reducing Sugar Coefficient (g/g)								
		NaOH (%)			CaO (%)			*T. reesei* (%)		
		1	2	3	1	2	3	1	2	3
C. fistula Linn.	0	0.000784 ±0.000075	0.000945 ±0.000068	0.001253 ±0.000065	0.000714 ±0.000033	0.000805 ±0.000033	0.000931 ±0.000019	0.000735 ±0.000041	0.000791 ±0.000011	0.000833 ±0.000007
	24	0.007600 ±0.000092	0.009505 ±0.000977	0.013922 ±0.000893	0.005978 ±0.000158	0.006447 ±0.000289	0.007189 ±0.000089	0.003619 ±0.000029	0.004368 ±0.000095	0.005845 ±0.000297
	48	0.033524 ±0.000641	0.040446 ±0.000707	0.045131 ±0.000565	0.023625 ±0.001056	0.032032 ±0.001712	0.041048 ±0.000562	0.019299 ±0.000415	0.022729 ±0.000219	0.029099 ±0.000369
	72	0.048292 ±0.000641	0.050590 ±0.002509	0.0598997 ±0.001612	0.041664 ±0.000576	0.044877 ±0.001168	0.048867 ±0.000428	0.029533 ±0.000632	0.034237 ±0.000613	0.036246 ±0.000487

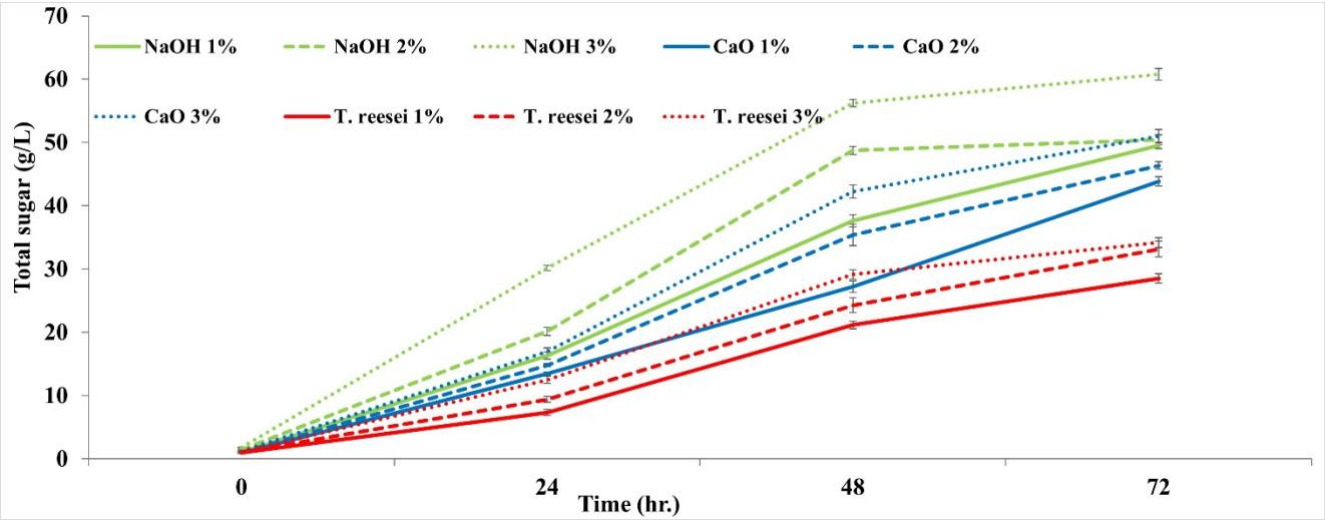

Figure 3. Total sugar from dried GSF

Table 2. Total sugar coefficient from dried GSF

Bio-mass	Time (hr.)	Total sugar coefficient (g/g)								
		NaOH (%)			CaO (%)			*T. reesei* (%)		
		1	2	3	1	2	3	1	2	3
C. fistula Linn.	0	0.00723 ±0.000869	0.010203 ±0.000443	0.012589 ±0.000423	0.006699 ±0.000537	0.007192 ±0.000359	0.008666 ±0.000227	0.006858 ±0.00072	0.007376 ±0.000058	0.007691 ±0.000138
	24	0.114294 ±0.003761	0.141118 ±0.004618	0.211147 ±0.002955	0.094531 ±0.001601	0.103647 ±0.00152	0.118906 ±0.004018	0.051233 ±0.003512	0.065962 ±0.003176	0.087426 ±0.004097
	48	0.263378 ±0.006997	0.341115 ±0.004667	0.393642 ±0.003927	0.190395 ±0.006484	0.247704 ±0.011767	0.295764 ±0.007418	0.148198 ±0.004103	0.169862 ±0.008267	0.203977 ±0.005259
	72	0.346726 ±0.003392	0.352735 ±0.005611	0.425316 ±0.006373	0.307067 ±0.005194	0.324437 ±0.004092	0.356908 ±0.007574	0.199646 ±0.005246	0.232059 ±0.008597	0.239240 ±0.005442

Table 3 Degree of polymerization from dried GSF

Biomass	Time (hr.)	Degree of polymerization								
		NaOH (%)			CaO (%)			*T. reesei* (%)		
		1	2	3	1	2	3	1	2	3
***C. fistula* Linn.**	**0**	9.217	10.79661	10.047095	9.38248	8.93474	9.3079	9.33129	9.32496	9.23297
	24	15.03896	14.84733	15.166065	15.8131	16.0768	16.5399	14.1566	15.1011	14.9574
	48	7.856485	8.433848	8.7221877	8.05904	7.733	7.20531	7.67906	7.47336	7.00975
	72	7.179733	6.972378	7.1004734	7.37007	7.22946	7.30367	6.76009	6.778	6.60047

Table 4 Energy from reducing sugar from dried GSF

Biomass	Time (hr.)	Energy from reducing sugar (kJ/L)								
		NaOH (%)			CaO (%)			T. reesei (%)		
		1	2	3	1	2	3	1	2	3
C. fistula Linn.	0	1.792 ±0.171	2.160 ±0.155	2.864 ±0.148	1.632 ±0.076	1.840 ±0.076	2.128 ±0.042	1.680 ±0.094	1.808 ±0.024	1.904 ±0.016
	24	17.371 ±0.211	21.725 ±2.234	31.822 ±2.040	13.664 ±0.360	14.736 ±0.660	16.432 ±0.203	8.272 ±0.067	9.984 ±0.216	13.360 ±0.678
	48	76.626 ±1.465	92.448 ±1.617	103.157 ±1.290	54.000 ±2.414	73.216 ±3.914	93.824 ±1.285	44.112 ±0.950	51.952 ±0.500	7.01 ±0.844
	72	110.382 ±1.465	115.635 ±5.734	136.914 ±3.684	95.232 ±1.316	102.576 ±2.669	111.696 ±0.977	67.504 ±1.445	78.256 ±1.401	6.601 ±1.113

It points towards a preference for NaOH treatment at a 3% concentration for 72 hours to attain the optimal reducing sugar coefficient. The findings set a substantial ground for future studies aiming at the commercial exploitation of biomass for biofuel and other allied sectors.

3.2 Enhanced total sugar yields from GSF through strategic chemical and biological pretreatments

Pretreatment minimizes the physical barrier to biomass movement by reducing the biomass's macroscopic stiffness (Taechawatchananont et al., 2022). Figure 3 and Table 2 compared The effect of three different pretreatments on the total sugar and the total sugar coefficient in the dried GSF powder to NaOH, CaO, and *T.reesei*. The quantity of the total sugar and the total sugar coefficient were measured with tree pretreatments, whereas the amount of the total sugar and the total sugar coefficient increased with increasing concentrations of three types of substrates for pretreatment and pretreatment times from the pretreatment with NaOH 3% for 72 hrs. is the best pretreatment condition.

This indicates that sodium hydroxide sufficiently altered the material's structure and increased the amount of the total sugar released. Table 2 outlines an experiment on dried GSF, testing sugar coefficients derived from treatments with NaOH, CaO, and *T. reesei* at concentrations of 1%, 2%, and 3% over 0, 24, 48, and 72-hour intervals. The results, paired with standard error measurements, indicate a notable increase in sugar coefficient over time and with higher concentration treatments, mainly with NaOH. The 72-hour, 3% NaOH data point stands out, showcasing the highest coefficient and a small error margin (±0.006373), suggesting the treatment's effectiveness and reliability. Further statistical tests like ANOVA using this error data can help more precisely determine the best strategy for sugar extraction from this biomass, which holds significant implications for fields.

3.3 Degree of polymerization in carbohydrates

Carbon, hydrogen, and oxygen are the components of carbohydrates. Monosaccharides, formed by various polymerization forms to create complex sugars, starches, and fibers, are minor carbohydrate structures (Saïed et al., 2023; Trejo et al., 2023). In addition to categorizing carbohydrates according to physiological characteristics, carbohydrates' chemical-structural qualities and the degree of polymerization are also considered (Panahi et al., 2022). A molecule's number of monomers is related to the degree of polymerization. The four categories used to organize carbohydrates are sugars, oligosaccharides, polysaccharides, and polyols (Manmai et al., 2019b). Table 3 presents the DP values that belong to the classification of oligosaccharides and polysaccharides which is a large molecule suitable for hydrolysis.

3.4 Energy from reducing sugar

The energy of the reducing sugar production results were presented in Table 4 from different chemical and biological pretreatment solutions and the time of pretreatments. The results showed that the energy from pretreating the dried GSF with NaOH 3% for 72 hrs. is the best condition for pretreatment when compared with the other two types of substrate. The energy derived from reducing sugar extracted from the dried golden shower (*C. fistula* Linn.) foliage using different pre-treatments: NaOH, CaO, and *T. reesei*, at concentrations of 1%, 2%, and 3% observed over a series of time intervals: 0, 24, 48, and 72 hours. From the outset at 0 hours, the energy values were relatively low across all treatments, ranging from 1.632±0.076 kJ/L (1% CaO) to 2.864±0.148 kJ/L (3% NaOH).

As time progressed to the 72-hour mark, a remarkable escalation in energy values was noted, with the highest energy being extracted under the treatment of 3% NaOH, registering an energy value of 136.914±3.684 kJ/L. This was followed by 3% *T. reesei* and 2% NaOH treatments, demonstrating energy values of 111.696±0.977 kJ/L and 115.635±5.734 kJ/L respectively. Interestingly, while the trend demonstrates a general increase in energy values over time for most treatments, the values for 3% *T. reesei* decreased at the 72-hour mark compared to 48 hours. It's pertinent to note the standard error reflected alongside each data point, indicating precision in the measurements (Elshobary et al., 2021). Despite higher error margins, the data portrays considerable reliability (e.g., 115.635±5.734 kJ/L in the 72-hour, 2% NaOH treatment). This data elucidates a considerable potential in harnessing energy from GSF, with prolonged treatment times and higher concentrations generally fostering higher energy yields, especially under NaOH treatment.

The outcomes here exhibit a promising avenue for renewable energy sourcing and set a rich ground for further research to optimize the energy extraction process from such biomasses, potentially playing a pivotal role in green energy solutions (Ganguly et al., 2021; Mejica et al., 2022). This study shows that NaOH concentration and reaction time affect the process of lignocellulose plants producing sugar because, in the case of low NaOH concentrations (1%), lignocellulose has not yet been converted to oligosaccharides despite the sample being pretreated with 3% of NaOH for 72 hrs, which oligosaccharides are suitable for enzyme hydrolysis to produce fermentable sugar. The dried GSF is therefore a waste product that may be used to produce cheap, sustainable biomass for biofuel and possibly new types of materials in the future.

3.5 Bioethanol production from cellulosic feedstocks: A detailed overview

Bioethanol production from cellulosic feedstocks, a renewable resource obtained from plant biomass, involves sophisticated procedures to convert the cellulose and hemicellulose present in the biomass into fermentable sugars, which are then converted to ethanol (Hassan et al., 2021; Solarte-Toro et al., 2019; Trejo et al., 2023). Below, we delve deeper into the individual processes and the science behind each step:

Biological pathway, Pretreatment:

- Physical pretreatment: This involves mechanical actions such as milling and chipping to break down the complex structure of biomass, increasing the surface area for the enzymatic action in the subsequent steps.
- Chemical pretreatment: Chemical agents like acids and alkalis break down the lignin and hemicellulose structures, thereby exposing the cellulose fibers. Solvents can also be employed in dissolving lignin and partial hemicellulose, preparing the feedstock for enzymatic hydrolysis.
- Biological pretreatment: Certain fungi and bacteria are known to secrete enzymes that can naturally and selectively degrade lignin and hemicellulose, making cellulose more accessible.

Hydrolysis:

- Enzymatic hydrolysis: Complex enzymatic cocktails containing cellulases break down cellulose into simpler sugars, primarily glucose. This process is optimized for high temperature, pH, and enzyme loading yields.
- Acid hydrolysis: Strong acids can hydrolyze cellulose into glucose; however, this method is less favored due to the production of inhibitory compounds that can affect subsequent fermentation.

Fermentation:

- Simultaneous saccharification and fermentation (SSF): This method synergizes the enzymatic hydrolysis and fermentation processes, reducing the production time and increasing the yield by avoiding end-product inhibition.
- Separate hydrolysis and fermentation (SHF): In this method, hydrolysis is carried out first, followed by fermentation, allowing for optimized conditions for each process but potentially reducing yield due to end-product inhibition.

Distillation:

- Ethanol recovery: Once fermentation is complete, the ethanol is recovered from the mixture through distillation, often followed by dehydration processes to obtain high-purity ethanol.

Thermochemical pathway, Gasification:

- Partial oxidation: Biomass undergoes partial oxidation to

produce synthesis gas or syngas, a mixture of carbon monoxide (CO) and hydrogen (H_2), which can be used as a substrate for microbial fermentation.

Syngas Fermentation:

- Microbial fermentation: Specialized microbes ferment syngas components to produce ethanol and other valuable products.

Pyrolysis:

- Fast pyrolysis: The biomass is rapidly heated in an oxygen-free environment, decomposing it into gases, liquids (bio-oil), and char. The bio-oil can be further processed to produce fermentable sugars.
- Bio-oil upgrading: The bio-oil undergoes a series of upgrades to remove impurities and break down complex molecules into simpler fermentable sugars.

Hydrogenation:

- Hydroprocessing: Hydrogen is introduced to reduce the oxygen content in the liquids obtained from pyrolysis, yielding hydrocarbons that can be fermented to produce ethanol.

Hybrid Pathways:

- Innovative combinations: Researchers are constantly developing hybrid pathways integrating biochemical and thermochemical methods to enhance ethanol production's overall productivity and yield.

Each pathway has challenges, including high costs, energy demands, and complex setup requirements (Hassan et al., 2021; Solarte-Toro et al., 2019). The ongoing research in this field aims to optimize these processes and foster the development of more efficient and sustainable solutions for bioethanol production from cellulosic feedstocks.

4. Conclusion

The research studied the potential of GSF as a source for sustainable bioethanol production through chemical and biological pretreatments. The optimum results were achieved using a 3% NaOH solution over 72 hours to maximize sugar and energy concentrations derived from processed golden shower foliage. This methodology surpassed other agents including CaO and *T. reesei*, albeit the latter presented a cost-effective alternative. The study highlights the critical role of pretreatment in enhancing the yield of essential precursors for bioethanol, namely total sugars and reducing sugars. Despite pointing towards promising avenues in biofuel and potential new materials, it acknowledges the economic challenges and the necessity for control over the variables in scaling the process. Encouraging further research, the study identifies GSF as a promising yet underutilized resource for bioethanol production, with economic viability and environmental sustainability ramifications.

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Conflict of Interest Declaration

The authors assert that no conflicts or personal affiliations might be construed as impacting the outcomes shared in this research.

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