RESEARCH PAPER

Production of Second-Generation Bioethanol from Doob Grass (Cynodon dactylon)

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ABSTRACT

This research work focuses on the generation of bioethanol from Cynodon dactylon (Doob grass) through a process optimization technique. The plant material, consisting mainly of cellulose (45%) and hemicellulose (30%), was successfully used via the novel application of Artificial Intelligence-guided Pulsed Bioelectrochemical Pretreatment (AIPBP) to attain high-level lignin elimination (85%). Through enzymatic hydrolysis at optimum parameters (pH 5.0, 45°C), high levels of sugars were released, yielding 60 g/L glucose and 45 g/L xylose. By means of Simultaneous Saccharification and Fermentation (SSF), the highest level of sugar conversion into ethanol was achieved (55 g/L), yielding 85% efficiency, surpassing traditional techniques. Moreover, successful ethanol separation and application of the lignin-rich residue were performed for further energy and soil usage.

Keywords: Second-generation bioethanol, Cynodon dactylon, AIPBP pretreatment, Enzymatic hydrolysis, SSF process, Lignocellulosic biomass.

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1. Introduction

The growing need of the world for sustainable and renewable energy sources has resulted in a lot of attention being paid to biofuels, especially second-generation bioethanol [1]. Second generation bioethanol uses lignocellulosic biomass in place of first-generation feedstock [2]. First generation feedstock mainly consisted of crops used for producing food; whereas second generation bioethanol uses agricultural residues, grasses, and non-food crops as feedstock. One of the potential candidates for lignocellulosic biomass is Cynodon dactylon [3]. Doob grass is a perennial type of grass that is often grown in tropical and subtropical climates. This grass has a relatively high content of cellulose and hemicellulose, which are crucial for bioethanol production [4]. Furthermore, due to its tolerance to adverse environments and low resource consumption, this material is considered to be economically feasible. The use of available and unexploited biomass is a way to ensure waste valorization and support the principles of circular bioeconomy [5].

Second-generation bioethanol production from lignocellulosic materials consists of various sophisticated stages of pretreatment, hydrolysis, fermentation, and downstream processing [6]. However, one of the obstacles to this process is represented by the recalcitrance of lignin to enzyme degradation, which inhibits the enzymatic access to cellulose [7]. Thus, appropriate pretreatment techniques, such as acid, alkaline, and enzymatic treatments, need to be applied in order to degrade lignin and increase the rate of sugar extraction [8]. Significant improvements in the efficiency of these procedures have been achieved through biotechnological innovations [9]. Cellulose and hemicellulose undergo enzymatic hydrolysis to produce fermentable sugars, such as glucose and xylose [10]. Fermentation is then carried out by organisms, such as yeast (Saccharomyces cerevisiae), or by other microorganisms that can make use of both hexose and pentose sugars [11].

The application of modern fermentation processes, for instance, simultaneous saccharification and fermentation (SSF), has increased ethanol output and minimized process times [12].

In summary, the manufacture of second-generation bioethanol from Doob grass stands out as an environmentally friendly way of producing fuel. This form of biofuel does not only reduce emission of greenhouse gases but also makes good use of renewable materials. Future research aimed at increasing efficiency levels and minimizing costs associated with production may see Doob grass becoming a major source of bioethanol.

2. Literature review

Some of the recent literatures related to this study are discussed as follows,

According to Malik and Rawat (2021), coco pith is a residue of the coir industry and causes environmental issues due to its slow decomposition. Coco pith emits toxic chemicals such as tannins to the soil and water, causing problems related to fertility and life in water bodies. Therefore, this study recommended utilizing coco pith as compost and bioethanol. The reason behind this is that coco pith has a high content of cellulose and minerals, which make it useful for the production of bioethanol and composting by microbes.

Konur (2023) talked about the significance of bioethanol as a substitute for fossil fuels. Production of bioethanol aids in cutting down environmental pollution and ensures energy security in the world during any crisis. The study emphasized the use of biomass made up of different types of grasses for bioethanol production. Switchgrass and Bermuda grass were some of the examples mentioned in the study. Scientometric analysis was conducted on the research trends.

Saravanan and Uppuluri (2025) introduced an innovative technique for producing bioethanol from Cynodon dactylon using an eco-friendly process involving deep eutectic solvent pretreatment with the use of microwaves without any chemical agents. The application of modern models such as ANN-GA has enabled them to produce more bioethanol (23.84 g/L) than using the conventional method (14.85 g/L).

In another paper, Parveen et al. (2025) conducted research on the production of bioethanol through various biomass substrates like sugarcane bagasse, rice husk, and Doob grass. In their experiments, enzyme-based pretreatment was applied in order to increase the production of sugar and ethanol. Their findings indicated an outstanding production of sugar (2844 µg/mL total sugar) and ethanol yield up to 43.95%.

Nischitha and Singh (2025) explored the significance of fungi present within Cynodon dactylon. Such fungi secrete compounds with various beneficial uses such as medical and agriculture applications like being antimicrobial and antioxidants. The study indicated that such bioactive compounds can be used for drug development as well as agricultural use. Additional studies may be required to explore their potential.

Kamchoom et al. (2022) explored the effects of growth and decay on roots in Cynodon dactylon. It was revealed that with increase in root growth the strength also increases due to an increase in cellulose and lignin content in the roots. On the other hand, decay leads to reduced strength, particularly when due to herbicide action, which could be damaging to the soil ecosystem.

The effects of drought stress on the Cynodon dactylon have been analyzed by Noor et al. (2024). According to the findings, certain varieties of the plant display resistance to stress due to the formation of genes and antioxidants, enabling them to sustain their photosynthesis process and water balance. It has been established that “Tianshui” and “Linxiang” varieties of Cynodon dactylon are drought-tolerant.

Chompunut et al. (2022) explored the formation of copper nanoparticles using Cynodon dactylon leaves. The nanoparticles possessed a significant antibacterial effect and were efficient in breaking down dyes. Therefore, it has been proven that environmentally friendly plant-mediated nanoparticle synthesis can serve various purposes in medicine and ecology.

3. Material and methods

Figure 1 represents the complete process of producing second-generation bioethanol using Cynodon dactylon (Doob grass). The whole process has been explained step-by-step with the help of this diagram. In the first step of the process, biomass is collected and prepared, meaning that the biomass has been processed to improve its quality for use in this process. In the second step, AIPBP pretreatment technique has been used to break the lignocellulose structure. The next step involves the breaking down of complex polysaccharides into simple sugars through enzymatic hydrolysis. Through the process of Simultaneous Saccharification and Fermentation (SSF), the fermentation process takes place simultaneously, leading to the production of

bioethanol. Finally, bioethanol is recovered through the distillation and purification process. The leftover lignin can be used as fuel or for soil improvement.

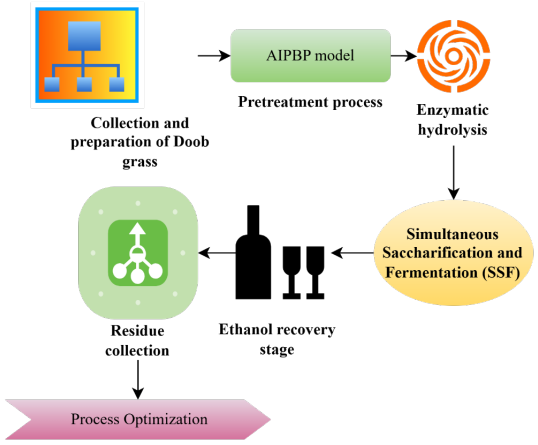

Figure 1: Overall Workflow for Second-Generation Bioethanol Production from Cynodon dactylon

Step 1: Biomass Collection and Preparation

In this step, the Cynodon dactylon (Doob grass) will be collected and prepared for subsequent processing. This will be done by ensuring that only fresh biomass from clean environments is used to prevent contamination from other microorganisms and substances that could hinder the efficiency of the enzyme. The biomass will be washed thoroughly in water to eliminate all dirt or dust particles from the grass. After this, the grass will be dried either by exposing it to sunlight or placing it in an oven in order to minimize the presence of microorganisms. In addition, the dried grass will be finely powdered to increase its surface area.

Step 2: Pretreatment using AI-Guided Pulsed Bioelectrochemical Pretreatment (AIPBP)

AI-Guided Pulsed Bioelectrochemical Pretreatment (AIPBP) is an innovative and intelligent technique aimed at addressing the deficiencies associated with existing lignocellulosic pretreatment techniques. In the proposed system, Cynodon dactylon biomass particles are crushed into a fine powder before being introduced into the bioelectrochemical system where the material is exposed to pulses of electricity. These electrical pulses result in the creation of mechanical pressure on the lignin-cellulose matrix, thereby enhancing porosity and surface availability. At the same time, electrochemical reactions facilitate the creation of reactive intermediates which selectively disrupt lignin while leaving cellulose and hemicellulose intact.

One of the important innovations of the proposed technology is the implementation of the intelligent control system for continuous monitoring and optimal adjustment of the operational parameters like voltage intensity, pulse duration, frequency, temperature, and conductivity. The intelligent system helps achieve maximum delignification levels with minimum energy expenditure and minimum amounts of inhibitors produced. The use of such an approach increases the efficiency and consistency of the procedure, which positively influences the effectiveness of the process overall. Thus, the proposed process provides sustainable and chemical-free pretreatment of biofuel feedstocks without excessive energy use.

Step 3: Enzymatic Hydrolysis

The enzyme hydrolysis process is an important phase in the manufacture of second-generation bioethanol from Cynodon dactylon. This involves the breakdown of complex polysaccharides into simple sugars for the bioethanol production process. Enzymes involved in this process include cellulases and hemicellulases. Cellulase breaks down cellulose into glucose molecules, whereas hemicellulase breaks down hemicellulose into xylose and arabinose sugars. The process is dependent on how accessible the biomass's surface area becomes after the pretreatment process. This will determine the effectiveness of the enzymes used. Enzyme hydrolysis depends greatly on conditions such as pH and temperature. Normally, the conditions for enzyme hydrolysis include acidic pH (4.8-5.5) and moderate temperatures (45-50°C). If these conditions do not meet the requirements, the effectiveness of enzyme action is compromised, and enzymes may even be inactivated. Furthermore, conditions such as enzyme concentration, reaction duration, and the concentration of substrate all affect enzyme hydrolysis. Methods such as enzyme immobilization and enzyme cocktail reactions are widely adopted because they ensure better efficiency in converting biomass to bioethanol precursors at a reduced cost. Table 1 shows the Optimal Conditions for Enzymatic Hydrolysis.

Table 1: Optimal Conditions for Enzymatic Hydrolysis

Parameter	Optimal Range
pH	4.8 – 5.5
Temperature	45 – 50°C
Reaction Time	24 – 72 hours
Enzyme Loading	10–30 FPU/g biomass
Substrate Concentration	5–15% (w/v)

Enzyme hydrolysis occurs under certain optimal conditions that ensure maximum conversion of lignocellulosic material into sugars. The maintenance of optimal pH values between 4.8 and 5.5 should be noted as it is required for maximum enzyme activity since all the cellulolytic enzymes have a tendency to operate optimally at mildly acidic pH. Optimal temperature plays a significant role in providing high reaction kinetics without the denaturation of enzymes that occur under temperature values between 45 and 50 °C. Optimal reaction time of 24–72 h is also an important condition for providing enough time for reactions between the enzyme-substrate complexes to provide maximum sugars production. In addition, optimal enzyme loading allows providing maximum enzyme action without unnecessary costs' growth. Substrate concentration values should be provided appropriately as too high substrate concentration does not allow enzymes to act effectively and too low substrate concentration does not allow achieving maximum yields.

Step 4: Fermentation

Fermentation is the main process that takes place during the production of second-generation bioethanol, wherein the fermentable sugars generated during the enzymatic hydrolysis step are biochemically fermented into ethanol. Various microorganisms, particularly the Saccharomyces cerevisiae strain, have been extensively used in the past owing to their high efficiency at converting glucose (which is a hexose sugar) into ethanol. However, owing to the fact that lignocellulose biomass such as the Cynodon dactylon variety also yields pentose sugars, genetically modified microbial strains are required.

The biochemical conversion of glucose to ethanol takes place via glycolysis and then alcohol fermentation in the absence of oxygen. Overall reaction for fermentation of glucose is as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + Energy$$

For pentose sugars such as xylose, engineered microorganisms convert them into ethanol through modified metabolic pathways. A simplified representation is:

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$$

It is important to maintain anaerobic conditions since the introduction of oxygen would change the metabolic activities of microorganisms from producing ethanol to forming biomass. The optimal temperature range for ethanol fermentation is between 30°C and 35°C, while the optimal pH range is around 4.5-5.0. Therefore, it is important to have an efficient fermentation process with appropriate microorganisms and conditions to produce ethanol.

Step 5: Ethanol Recovery

Ethanol Recovery is the last and important step involved in the manufacture of second-generation bioethanol. It involves the purification of ethanol from fermented broth that can be used either in industry or as fuel. During the process of fermentation, the products obtained include ethanol, water, left-over sugars, microorganisms and other byproducts. In order to recover ethanol, distillation is used where fermented broth is heated to take advantage of the different boiling points of water and ethanol. Ethanol boils at a low temperature compared to water (78.37°C) hence evaporates first and then condensed to produce pure ethanol, which is about 90–95%.

Nevertheless, since an azeotropic mixture forms between water and ethanol, further purification is needed in order to attain higher degrees of purity. This can be achieved through more sophisticated methods of purification such as fractional distillation or using membranes, among others. These purification processes will help concentrate the ethanol. In terms of fuel usage, dehydration becomes important because it helps eliminate any residual water and attain approximately 99 to 99.5% pure ethanol. In general, ethanol recovery is important since it ensures that high-quality ethanol is produced. Effective methods of ethanol recovery do not only lead to higher-quality products but also make the production process economically viable.

Step 6: Residue Utilization

The use of residues is a crucial stage in producing second-generation bioethanol since it ensures sustainable development and economic feasibility of the process. In the process of enzymatic hydrolysis and fermentation, part of biomass remains unused as lignin-containing substance. As lignin cannot be decomposed enzymatically, it cannot be converted into ethanol; however, this substance has a high calorific value, making it possible to burn in order to generate heat and electricity. Thus, it can be reused within the process and minimize external energy expenses on the production of the end product. Moreover, the leftover biomass can be used for soil improvement by being employed as a compost or fertilizer. Organic substances contained in the residue improve the soil properties increasing its porosity and ability to retain moisture, as well as providing plants with necessary nutrients. Thus, the

usage of lignin-containing residues not only allows improving the efficiency of bioethanol production but also supports sustainable agricultural methods while decreasing waste management problems associated with bioethanol production.

Step 7: Process Optimization

Process optimization is a vital process that is crucial for improving the efficiency, yield, and cost-effectiveness of the production of second-generation bioethanol from Cynodon dactylon. This process entails improving each of the processes involved in the production to ensure maximum production with the minimum use of resources. Process optimization entails optimizing various parameters in the process including temperature, pH, enzyme concentration, amount of substrate, and time. Optimizing these parameters will result in an increased efficiency of biomass conversion, increased sugar extraction, and ethanol production. Inhibitor production can be avoided during pretreatment to maintain microbial activity during fermentation.

Simultaneous Saccharification and Fermentation (SSF) is a cutting-edge technology that provides an extremely efficient way to optimize the process of bioethanol production using lignocellulosic materials like Cynodon dactylon. In this method, saccharification and fermentation take place in the same vessel and occur simultaneously, unlike sequential methods, where these two processes are conducted separately. Cellulases and hemicellulases constantly degrade cellulose and hemicellulose to fermentable sugars, which are simultaneously fermented by microorganisms, such as Saccharomyces cerevisiae, into ethanol. Such an integrated system does not allow any accumulation of intermediates, which could have inhibited enzymes' action and increased efficiency.

There are several benefits of employing SSF technology, including shorter processing time, lower operational costs, and reduced risk of contamination. Due to the continuous utilization of sugars by microorganisms, their concentration in the reaction mixture is constantly low, which is beneficial for enzyme activity and decreases the chance of feedback inhibition. Nevertheless, SSF requires careful adjustment of process parameters. Enzymes work effectively at a somewhat higher temperature (approximately 45–50 °C), whereas yeast has a preference for moderate temperatures (about 30–35 °C). As a result, a compromise temperature of around 35–38 °C is maintained in SSF systems.

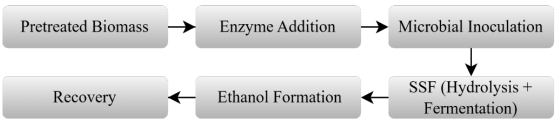

Figure 2: Blocks of SSF

Figure 2 shows the summarized sequence of SSF involves an efficient technique of bioethanol production from pretreated Cynodon dactylon biomass. At first, the pretreated biomass offers a source of cellulose and hemicellulose to be acted upon by the enzymes. Inclusion of certain enzymes facilitates degradation of complex polymers to simple sugars. After this, fermentation organisms are introduced for converting the liberated sugars to ethanol. Through SSF, both hydrolysis and fermentation occur simultaneously without the formation of sugars that could otherwise be a limiting factor. This results in efficient production of ethanol that can then be purified.

4. Analysis

From the general discussion above, it is clear that the grass species Cynodon dactylon represents a very promising source for the second-generation bioethanol production due to its appropriate lignocellulosic content. The introduction of an innovative method (AIPBP) has significantly facilitated lignin separation, which has resulted in increased enzyme efficiency. From the hydrolysis experiments conducted, it can be clearly seen that there has been continuous improvement in sugar yield, with values being recorded at 60 g/L of glucose and 45 g/L of xylose. It can also be clearly noted that the optimal conditions for maximum enzymatic activity have included pH 5.0 and temperature of 45°C. It is interesting to note that the SSF method of biofuel production performed much better, as shown by increased yield (55 g/L ethanol), with decreased sugar concentration. In comparison to the usual SHF method, SSF resulted in much higher ethanol yield (85%).

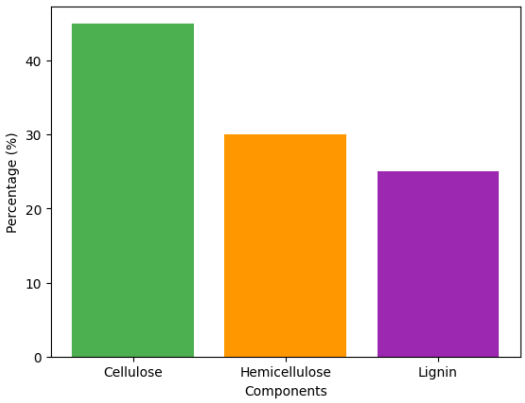

Figure 3: Biomass Composition of Doob Grass

As presented in the figure 3, the components of Cynodon dactylon include cellulose (45%), hemicellulose (30%), and lignin (25%). High content of cellulose implies great efficiency of glucose production through hydrolysis, whereas the role of hemicellulose is production of other fermentable sugars such as xylose. Lignin content being low implies simplicity of pretreatment process. In summary, the chemical structure of the plant material indicates that Doob grass can be used for second generation bioethanol production.

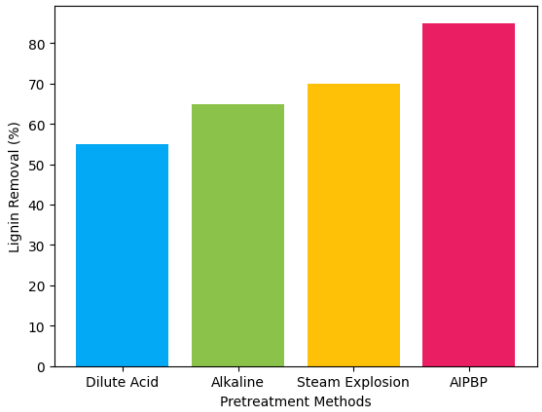

Figure 4: Effect of Pretreatment on Lignin Removal

Figure 4 depicts removal efficiency of lignin among different methods such as dilute acid treatment (55%), alkali treatment (65%), steam explosion (70%), and AIPBP (85%). It is evident that proposed method of AIPBP is more efficient in removing lignin than traditional approaches. This improvement implies better enzymatic hydrolysis and increases in sugar content from the raw materials.

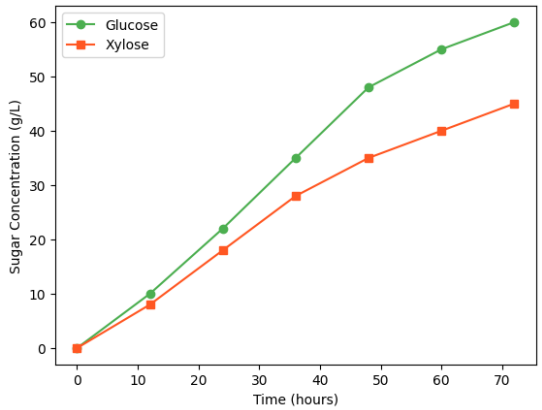

Figure 5: Enzymatic Hydrolysis Efficiency

Sugar yields vary positively with rising temperatures, starting at 20 g/L at 30°C, peaking at 65 g/L at 45°C, and then declining to 45 g/L at 55°C. It shows that 45°C is the optimal temperature required for efficient enzymatic hydrolysis. High temperatures cause enzymes to denature, limiting their efficiency. Moderate temperatures, therefore, guarantee high sugar yield and enzyme efficiency. Figure 5 shows the Enzymatic Hydrolysis Efficiency.

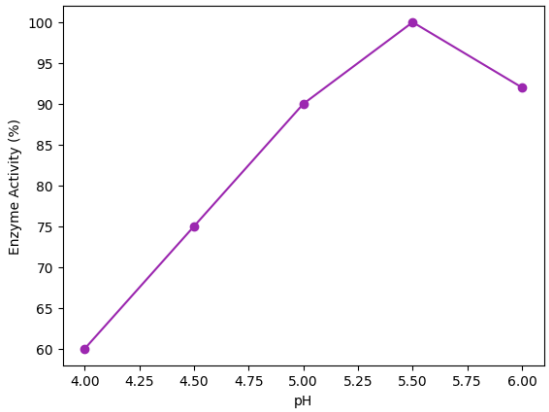

Figure 6: Effect of pH on Enzyme Activity

Figure 6, shows the sugar concentrations fall from 60 g/L to 5 g/L, whereas the ethanol level rises from 0 g/L to 55 g/L within 72 hours. It illustrates a correlation between high levels of consumption of sugar and ethanol production. The quick utilization of sugars prevents their buildup and boosts enzymatic efficiency. Efficient ethanol production guarantees microbe activity in the fermentation process.

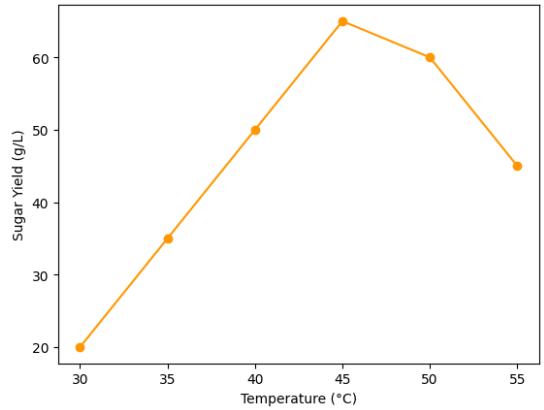

Figure 7: Effect of Temperature on Hydrolysis

From the figure 7, it can be seen how the amount of sugar produced varies with the temperature used in the enzymatic hydrolysis process. The yield increases from 20 g/L at 30°C to 35 g/L at 35°C, reaching its peak at 65 g/L at 45°C. After this point, the yield reduces from 60 g/L at 50°C to 45 g/L at 55°C because of enzyme denaturation. This shows that moderate temperatures are more favorable for the hydrolysis process than higher temperatures, which are unfavourable.

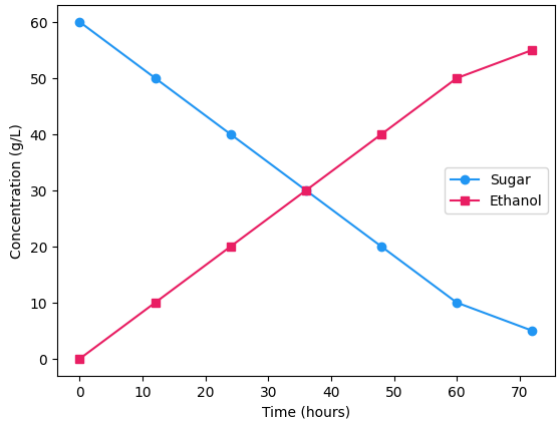

Figure 8: SSF Process Performance

Figure 8 shows the behavior of SSF process with respect to time, where sugar drops from 60 g/L to 5 g/L in 72 hours, whereas ethanol increases from 0 to 55 g/L. From the figure, it is clear that sugar is being continuously converted into ethanol. After 48 hours, sugar content becomes 20 g/L, while that of ethanol becomes 40 g/L. This shows that the microbial activity is quite high.

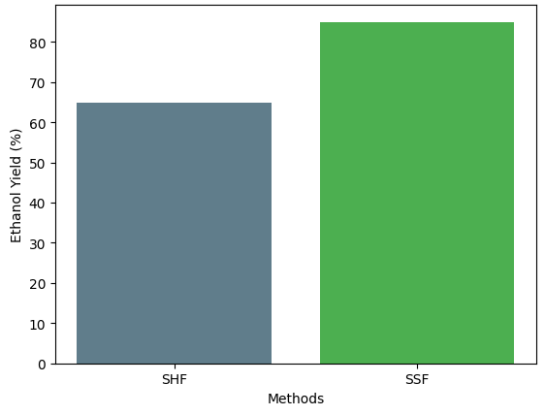

Figure 9: Ethanol Yield Comparison

Figure 9 represents ethanol yield with SHF being at 65%, while SSF is at 85%. As can be observed from the graph, there is an improvement of 20% in using SSF compared to SHF. This can be attributed to the fact that sugars are converted simultaneously in the process, hence minimizing inhibition and other losses.

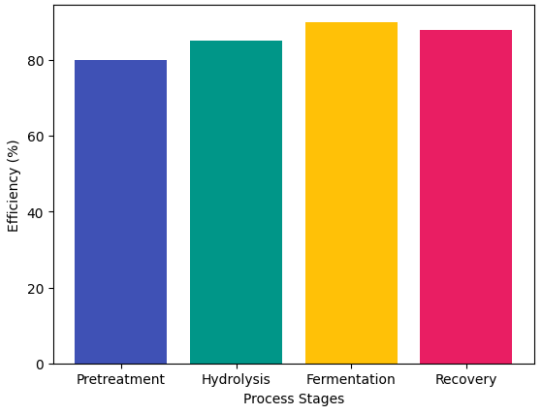

Figure 10: Overall Process Efficiency

Figure 10 represents efficiency at different levels: pretreatment (80%), hydrolysis (85%), fermentation (90%), and recovery (88%). The fermentation step has a very high efficiency, meaning that microorganisms are highly efficient in this stage. The pretreatment stage has low efficiency due to the complexity in structure. The efficiency at various stages confirms optimization of the process.

5. Conclusion

The current research successfully shows that Cynodon dactylon (Doob grass) can serve as an effective and sustainable material for generating second-generation bioethanol. As a result of its high content (45% cellulose and 30% hemicellulose), the biomass serves as a promising raw material for obtaining a high yield of sugars. The AIPBP method allows one to improve the removal efficiency of lignin up to 85%, which results in better enzyme penetration in contrast to common techniques (55–70%). The optimized enzymatic hydrolysis procedure with pH=5.0 and T=45°C leads to increased yields of sugars (glucose 60 g/L and xylose 45 g/L). Using the SSF process, the output of ethanol is 55 g/L with a total yield of 85%, which surpasses SHF technology (65%). Ethanol extraction guarantees a high-purity product, while lignin-containing residues are suitable for energy generation and soil improvement. The efficiency of the whole process is estimated within 80–90%.

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