Ethanol from Cellulose

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ABSTRACT- Due to limited landfill capacity, paper makes up a significant portion of municipal solid waste and constitutes a substantial disposal challenge in countries that are industrialized as well as developing. An alternative approach for utilizing newspaper, composed mainly of cellulose, involves its conversion into bio-ethanol. This offers potential advantages over ethanol derived from corn, including the prospect of replacing fossil fuels, minimizing greenhouse gas emissions, and reducing costs. The objective of this initiative is to mitigate newspaper waste within municipal solid waste by efficiently employing it in bio-ethanol production. In order to accomplish this, pre-treatment procedures have been improved through experimental research, with the goal of achieving effective microbial hydrolysis and the transformation of cellulose into sugars by organisms capable of breaking down cellulose. The multi-step process involves pretreatment, hydrolysis, and fermentation processes. To improve the hydrolysis process, hemicellulose, lignin, and cellulose are separate. Pre-treatment conditions with a 1.5% H2SO4 concentration for 50 minutes at 100°C were found to be ideal. Cytophaga Huchnosonni bacteria were used in the hydrolysis step to help convert cellulose to sugars. Dinitrosalicilic acid was used for the analysis of the resultant sugars. Then, Saccharomyces cerevisiae yeast is used in fermentation to reduce sugars in order to make bio-ethanol. HPLC techniques and specific gravity were used to evaluate the yield. The goal of this thorough procedure is to aid in the reduction of waste while generating a sustainable energy source.

Keywords *— **Paper waste, Bioethanol production, Greenhouse gases, Enzymatic hydrolysis, Fossil fuels, Fermentation***

I. INTRODUCTION

Over the past century, the global population has experienced significant growth, leading to a corresponding increase in energy consumption. Predominantly fueled by crude oil, meeting the escalating energy demand has become a formidable challenge. However, current forecasts suggest a decline in global oil production from 5 billion barrels globally instead of 25 billion, underscoring the necessity to explore alternative, non-petroleum-based energy sources to address the imminent energy crisis. Biomass emerges as one of the most promising substitutes for petroleum-based energy, originating from the biological fixation of carbon and containing sugar, starch, or cellulose. Bioethanol, a biomass derivative, serves as a greener fuel with lower carbon monoxide emissions than traditional gasoline, offering a viable option for those seeking to minimize their carbon footprint.

While first-generation biofuels, like sugarcane and corn, have been extensively utilized, the substantial increase in the manufacturing of ethanol from these flora has strained the worldwide supply of food. In contrast, second-generation fuels can be derived from agricultural residue, industrial waste, and other sources. Municipal garbage has grown to be a major problem in both developed and developing countries., with waste paper serving as a cost-effective resource for bioethanol production. Stringent environmental regulations have prompted the untimely completeness of existing dumping and increased costs for establishing new ones. Municipal solid waste comprises various components, including food waste, rubber, textile, leather, wood, glass, and paper. Approximately 40% to 45% of the metropolis. solid waste is comprised of paper, offering the potential for conversion into bioethanol, thereby mitigating waste and creating a sustainable energy source.

II. LITERATURE REVIEW

Around the 20th and 21st centuries, transportation fuels primarily relied on crude oil derivatives, although earlier Holocaust-powered vehicles were initially fueled by ethanol. In 2006, A total of 85 million barrels of Mb/d was expected to be needed worldwide for petroleum & liquid fuels. Projections indicate an increase to 105.4 Mb/d in 2030, for the transportation sector accounting for 80% of the augmented crude oil consumption, as indicated by research conducted by Suhail J. C. et al. in 2013.

Store enhancements in energy efficacy standards and subdued requirements resulting from the 2008-09 global economic slowdown, worldwide petroleum consumption continues to rise by oversight 1.1% per year. This trend is basically attributed to growing demand in China and India, as noted by Bishnu J. et al. in 2011. But, the escalating requirement for crude oil is anticipated to deplete world fuel reserves, leading to fuel scarcity and considerable price increases.

Vehicular and industrial carbon dioxide (CO2) emissions are substantial contributors to global warming, underscoring the importance of developing alternative energy sources like biofuels. Currently, bioethanol and biodiesel stand out as the only globally significant non-fossil liquid fuels.

Clean-burning and high in octane, ethanol from biomass presents itself as a perfect replacement for gasoline and is thus a potential fuel option for both transport and gasoline enhancement. When bioethanol is burned, polluting substances

such as benzene, formaldehyde, and 1-3 butadiene are significantly reduced. According to research done in 2006 by Sadashivam S. M., blending petrol with ethanol may increase the mixture's octane and lower the amount of carbon monoxide (CO) emissions by 10 to 30%.

Greenhouse gases (GHGs) such as carbon dioxide (CO2) can be generated from biomass, a resource that can be produced again, and bioethanol can help reduce pollutants in the air in towns and cities. Ethanol produced from renewable organic raw materials that absorb through CO2 growth is predicted to replace petroleum and cut CO2 emissions by 90–100%. according to the American Society for Testing and Materials (ASTMD) in 1993. This positions bioethanol as a promising alternative to traditional fossil fuels, offering environmental benefits and a sustainable solution to the rising energy demand.

The development of biofuels has gained importance due to the imperative to reduce pollution, establish renewable energy sources, and address economic and social challenges. Biofuels hold the potential to enhance the economic value of forests, alleviate poverty and unemployment, and provide a sustainable alternative to traditional fossil fuels. While current bioethanol production focuses on sugar and starch crops, limitations in meeting the increasing demand and potential competition with food production make it imperative to shift towards the bioconversion of lignocellulose biomass. This resource provides a more cost-effective and readily available substitute. It is sourced from solid waste from municipalities, different underutilized industry wastes, and farming and forest residues.

Lignocellulose materials, composed of cellulose, lignin, and hemicellulose, are widely available and relatively inexpensive compared to other feeds tocks for bioethanol production. However, the encapsulation of cellulose and hemicellulose molecules by lignin makes extraction challenging. Cellulose, consisting of long chains of glucose molecules with a distinct structural configuration, presents greater hydrolysis challenges compared to starchy materials.

Hemicellulose, comprising long chains of sugar molecules with the addition of pentose, exhibits varying compositions depending on the plant type, complicating the extraction process. Given that pentoses constitute a significant portion of available sugars, their recovery, and fermentation into ethanol are critical for process efficiency. Recent advancements involve genetically engineered microorganisms capable of fermenting pentose's into ethanol with relatively high efficiency.

Therefore, the development of bioconversion of lignocellulose biomass and the use of genetically engineered microorganisms for pentose fermentation are crucial in bioethanol production. These endeavors not only mitigate pollution and provide a sustainable alternative to traditional fossil fuels but also enhance the economic value of underutilized biomass resources, reduce poverty and unemployment, and support indigenous biomass production

PROBLEM STATEMENT:

Excessive use of paper products is a major contributor to environmental waste and pollution. Therefore, it's crucial to find sustainable methods to recycle and repurpose paper waste. One promising solution is to extract ethanol from paper, which not only reclaims valuable resources but also offers a sustainable substitute for traditional ethanol production techniques... However, efficient and cost-effective techniques for extracting ethanol from paper need to be explored and optimized. This project aims to address this challenge by investigating innovative methods for the extraction of ethanol from paper waste, promoting both environmental sustainability and the utilization of renewable resources.

OBJECTIVES:

The primary objectives of this project are as follows:-

- The primary goal of the current project is to reduce the amount of waste newspapers that end up in municipal solid trash by effectively using discarded newspapers for pretreatment, hydrolysis, and fermentation to produce bioethanol. Reducing Dependence on Fossil Fuels.
- To improve and optimize the technology for converting cellulose into ethanol.
- To investigate the scalability of the process to meet the demands of the energy market and contribute to reducing greenhouse gas emissions.
- To raise awareness about the benefits of cellulosic ethanol as a renewable energy source and to encourage its adoption.
- To ensure that the project complies with relevant regulations and standards for biofuel production and environmental safety.

III. MATERIALS AND METHODS

Equipment

The necessary equipment for conducting experiments involving fermentation, distillation, and hydrolysis includes:

1. Plastic bags: employed for the collection of samples.
2. Digital pH meter: utilized to measure the pH of hydrolytes before the fermentation process.
3. Thermometer: employed to control the temperature of the sample during both fermentation and distillation, maintaining it at the designated set point.
4. Containers: used for the storage of materials and supplements required for testing.
5. Gradient cylinders of varying capacities: utilized to quantify volume.
6. Cylinders of different volumes: employed for accurate volume measurement.
7. Autoclave: utilized for sterilization and hydrolysis processes.
8. Beaker: employed for density measurement.
9. Fermentation and distillation setups: dedicated equipment for conducting fermentation and distillation processes, respectively.

Collection of substrate

In the pursuit of sustainable alternatives to conventional fossil fuels, a research endeavor was undertaken to assess the viability of using household newspapers as a substrate for bioethanol production. To maintain the purity of the substrate, the newspapers were gathered under dust-free and fungus-free conditions. Subsequently, they underwent meticulous sun-drying and were finely cut into little pieces to enhance the bioconversion process. Finally, to safeguard the quality of the substrate, the small pieces were kept in sealed polythene bags, prepared for utilization as a renewable energy source

Fig. 1. Waste Raw Paper

Chemical Analysis of the substrate

Before initiating the pretreatment process, an extensive analysis was performed on the composition and properties of the substrate. The cellulose contains and complete carbohydrates were assessed using the enthrone method, a widely accepted and accurate technique in the industry [13]. Additionally, the humidity and content of ash in the under layer which is determined with the help of recognized standard procedures in the field of substrate analysis [20]. This detailed analysis contributed to a thorough understanding of the substrate's characteristics, playing a crucial role in designing an optimal pretreatment process.

Fig. 2. Filtered solution of cellulose.

Pretreatment process optimization:

In the pre-treatment phase of our experiment, we implemented various measures to optimize the substrate for subsequent processing. This involved employing different concentrations of diluted sulfuric acid 0% to 5%, and varying the heating durations at 15, 30, 45, 60 minutes, all conducted under a pressure of 15lb and a temperature of 121°C.

The procedure commenced by combining 1gm of substrate with dilute 10ml sulfuric acid with 1gm of substrate 10:1 ratio. The mixture was then subjected to the specified temperature and time settings. Throughout the experiment, we vigilantly monitored the reactions, recording our observations.

To assess the efficiency of the pre-treatment process, we analyzed the cellulose released during the procedure using the enthrone method, following the protocol outlined by Zahid Anwar et al. in 2011. The process continued until we achieved the maximum release of cellulose possible.

Upon obtaining the optimized substrate, we proceeded to the hydrolysis stage using the solution derived from the pre-treatment process.

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Fig. 3. Shredding & Pulping if waste

The pretreated substrate undergoes hydrolysis:

Throughout the pretreatment process, it was noted that the microorganisms that break down cellulose isolated from the substrate demonstrated a superior ability to hydrolyze cellulose compared to other microorganisms. To remove any remaining acidic residues, the pre-treated substrate underwent multiple rinses with distilled water before being dried in an oven until a consistent weight was achieved. Following this, the substrate's pH was adjusted to 7.0 to create optimal conditions for the proliferation of cellulose-degrading bacteria.

For a comparative analysis, a pure culture of Cytophaga hutchisonni (CH) (NCIM) was employed, sourced from the National Collection of Industrial Microorganisms (NCIM) in Pune. This was done to evaluate the efficacy of the isolated cellulose-degrading bacteria, with the isolated organism obtained from the Department of Biotechnology at BEC Bagalkot.

The pure CH culture and the separated cellulose-degrading bacteria were added to the substrate that had been prepared, and they were both left to incubate for a full day. Every 24 hours, the Dinitrosalicylic Acid (DNS) technique was used to assess the reducing sugars that were generated during substrate hydrolysis [19]. To create bioethanol, the maximum amount of sugars released during this time were fermented further...

This study underscores the superior cellulose-hydrolyzing ability of the isolated bacteria, positioning it as an ideal candidate for bioethanol production. The findings from this study could catalyze further research in this domain and advocate for the utilization of cellulose-degrading bacteria in biofuel production.

Fig. 4. .waste cellulose container

Fermentation of hydrolyzed broth:

In the process of bioethanol production, the fermentation stage employed a commercially available yeast strain called Saccharomyces cerevisiae. To initiate fermentation, the pH of the hydrolyzed broth was adjusted to 4.6. Following this adjustment, an inoculum of active yeast in the logarithmic growth phase was introduced into the broth. Fermentation proceeded at a temperature of 36°C until the maximum conversion of sugar to bioethanol was achieved. Quantification of reduced sugar consumption during fermentation was conducted using the DNS method [19], while the production of bioethanol was analyzed through the
specific gravity method.

Fig. 5.waste cellulose fermentation container

Calculations

The specific gravity (SG) is determined using the formula

(W2 – W1) / (W3 – W1),

Where,
- W1 is the empty weight of the specific gravity bottle,
- W2 is the combined weight of the sample and the specific gravity bottle, and
- W3 is the combined weight of the distilled water and the specific gravity bottle. bottle

The assessment of ethanol yield in a given sample utilized HPLC with the aid of the advanced "SHIMADZU-C-10AVP" instrument. HPLC analysis was executed employing a action exchanger SugarPak column (C18) for ethanol detection. To ensure precision, a mobile phase of acetone nitrate and water (80:20) flowed at a rate of 1mL/min, maintaining the column temperature at 90°C. Prior to HPLC analysis, all samples underwent filtration through a 0.45µm filter to eliminate impurities. The injection volume stood at 5µl, with a refractive index detector employed for ethanol yield detection and assessment at 50°C. This analytical approach was selected for its heightened sensitivity, accuracy, and precision, establishing it as a dependable tool for gauging ethanol yields across diverse samples.

Block diagram

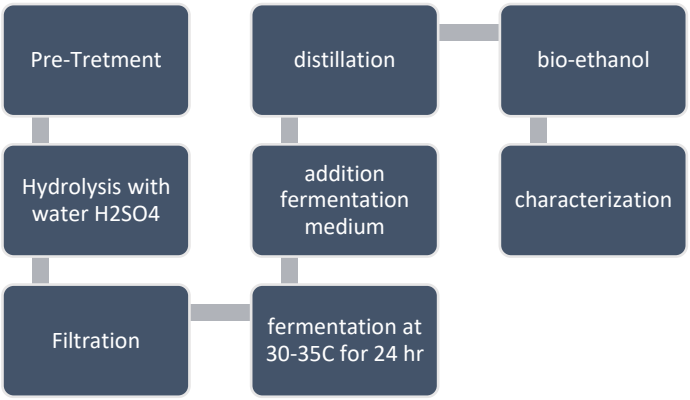

Fig. 6.Block diagram

IV. RESULTS AND DISCUSSIONS

Fig. 7.Distilltion column

4.1. Chemical analysis:

After thorough analysis, it was ascertained that the substrate comprises 45% cellulose, a complex carbohydrate crucial for the structural integrity of plant cell walls. Moreover, the substrate was identified to have 5.17% ash content, representing the inorganic residue post-incineration, and 6% moisture content, signifying the amount of water within the substrate.

Fig. 8. Ethanol

4.2 Optimizing the substrate's pretreatment*:*

To efficiently convert carbohydrate polymers into fermentable sugars, we initiate the process by subjecting cellulosic biomass to dilute sulphuric acid treatment. This treatment alters the biomass structure, facilitating enzyme access to cellulose. To refine this pre-treatment process, a series of experiments with varying dilute H2SO4 conc. (ranging from 0% to 5%) and different heating periods (15, 30, 45, 60 minutes) were conducted. The outcomes of these experiments, depicted in Figure 1, offer detailed insights into the efficacy of diverse pre-treatment conditions.

Following pre-treatment, the sample contains inhibitors like acetic acid and furfural that can hinder yeast metabolism. To ensure efficient biomass conversion into fermentable sugars, the pre-treated substrate underwent multiple washes with distilled water. Our experiments reveal that maximum cellulose recovery (45%) is achievable with a 1.5% Dilute H2SO4 concentration and a 50-minute heating period at 120 °C.

Previous studies have indicated that Dilute H2SO4 concentrations below 4% can enhance the affordability and effectiveness of the pre-treatment process (Ajeetkumar S P et al., 2014). This information, combined with our experimental data, holds the potential to drive the development of more efficient and cost-effective processes for converting cellulosic biomass into fermentable sugars.

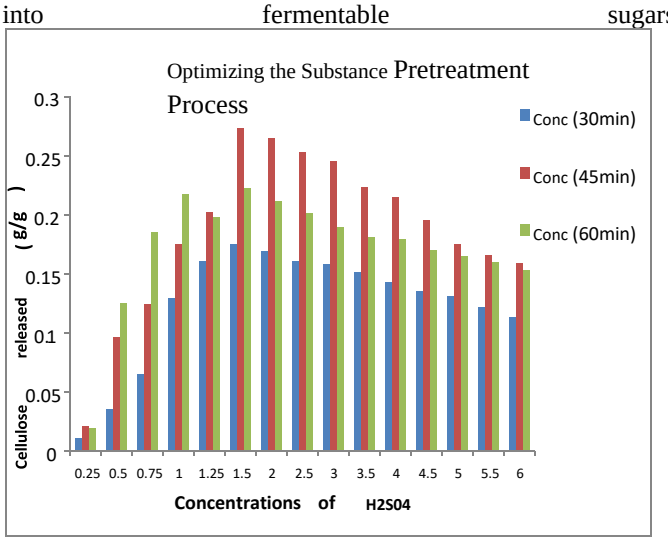

Fig. 9 : Optimization of substrate pretreatment

4.3 hydrolysis process

During the hydrolysis process, cellulose within substrate undergoes a chemical transformation, yielding ethanol, facilitated by cellulose-degrading organisms. In this investigation, bacteria were chosen over enzymes for their cost-effectiveness. Hydrolysis transpired under neutral pH conditions to ensure optimal reaction parameters. To gauge the efficiency of the pure and isolated bacterial cultures, the study focused on two bacterial strains—Cytophaga hutchisonni (CH) (NCIM 2338) and WG3. The isolated bacterial culture, WG3, came from the Faculty of The field of biotechnology BEC Bagalkot, which, whilst the pure culture of Cytophaga hutchisonni (CH) (NCIM 2338) had been obtained from the National Collection of Industrial Microorganisms (NCIM) in Pune. For a more understandable comprehension of the data, the study includes graphic illustrations that represent the collected results.

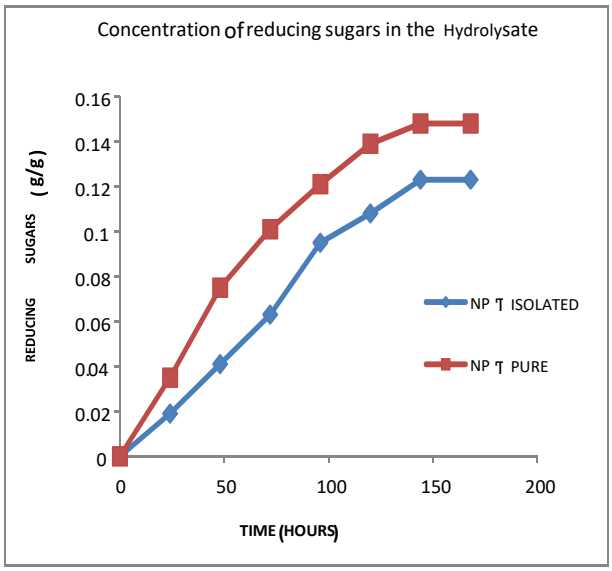

Fig. 10. Hydrolysis of pretreated substrate

The investigation into cellulose degradation revealed that the pure culture of Cytophaga hutchisonni exhibited greater effectiveness in the maximum degradation of Comparing cellulose to the organism that was isolated. The study showed that whereas the isolated culture of Cytophaga hutchisonni only released 0.110g of reducing sugars per gram of substrate, pure cultural expression of the same organism released 0.140g. Within 24 hours of the inoculation, this conversion was seen, with the highest conversion being noted on the fifth day. After that, there was no discernible rise in the decreasing sugar content, as Figure 4.2 shows.

In a study published by Ajeet Kumar SP et al. in 2014, investigations into cellulose hydrolysis using the fungus Trichoderma reesei revealed a cellulose yield of 0.184g per gram of rice husk as the substrate. In a separate study, substrates including rice straw, bagasse, and wheat straw were subjected to breakdown by fungi such as Phanerochaete chrysosporium and Aspergillus wamori. The cellulose released from these substrates amounted to 62.7 mg, 73.7 mg, and 52.4 mg per gram of substrate, respectively (Sahail J. et al., 2011).Further, in a different investigation, the isolated fungus Aspergillus Niger that was isolated from a soil sample produced 200 mg of reducing carbohydrates per gram of ground walnut shell substrate and 185 mg of rice husk intermediate upon hydrolysis of the aforementioned materials (Srivastava et al.). Significant research efforts have been dedicated to exploring the biological hydrolysis of pre-treated substrates utilizing various enzymes. According to Mir Naiman et al. (2011), findings from a specific study indicated that an inverse bacterium discharged 30.46 mg of reducing sugars per gram of sugarcane bagasse feedstock.

With these results in mind, it appears that the cellulose-degrading microorganism Cytophaga Hutchinson utilized in this research is more effective in turning cellulose into dextran. Therefore, it can be concluded that this research has provided valuable insights into the potential use of Cytophaga Hutchinson for cellulose degradation, and further exploration in this field could lead to significant advancements in biotechnology.

4.4 Fermentation of the hydrolyzed broth:

In the bioethanol production process, the yeast Saccharomyces cerevisiae played a crucial role in fermenting the sugars released during substrate hydrolysis. The fermentation process occurred at an optimal temperature of 35°C and a pH of 4.5, providing the ideal conditions for yeast activity. To initiate fermentation, a fresh yeast inoculum was introduced to the hydrolyzed broth at a ratio of 5% v/v. This fermentation process extended over six days, during which samples were collected at twenty-four-hour intervals for monitoring and analyzing substrate utilization via the DNS method for reducing sugar. Both the specific gravity method and the HPLC method were employed to assess bioethanol production. Figure 4 illustrates the bioethanol proportion determined through the specific gravity method, while Figure 3 depicts the results obtained through the HPLC method. shows a graphical depiction of the analysis results, showing sugar use throughout the fermentation process.

In the bioethanol production process, the Specific Gravity method was employed to calculate the maximum percentage of bioethanol achievableThe findings suggested that a bioethanol production of 5.49% v/v was achievable with pure culture organisms, whereas isolated culture organisms exhibited a higher yield of 6.10% v/v. Furthermore, HPLC analysis conducted at 24-hour intervals revealed ethanol yields of 5.02% for pure culture organisms and 5.11% for isolated culture organisms. Notably, the capacity to convert both the Pentose and Hexose sugars in the Hydrolysate into Bioethanol would improve the fermentation process's economic feasibility. Nevertheless, the fermentation yeast Saccharomyces cerevisiae is limited to fermenting hexose carbohydrates into ethanol. Earlier studies utilizing Saccharomyces cerevisiae for the fermentation of these materials demonstrated a production of 0.122g per gram of groundnut oil and 0.108g per gram of rice husk. Another research endeavor documented a yield of 0.18g per gram from a cellulosic waste blend consisting of office paper, newspaper, and cardboard in a 1:1:1 ratio.. Similarly, 0.110 mg/L of ethanol (alkaline pre-treated) and 0.265 mg/L (acid pre-treated) of ethanol were produced by using sugarcane leaf litter as feedstock. When the cellulase enzyme hydrolyzes the substrate bagasse pith, the bioethanol produced was 6.1% (v/v).

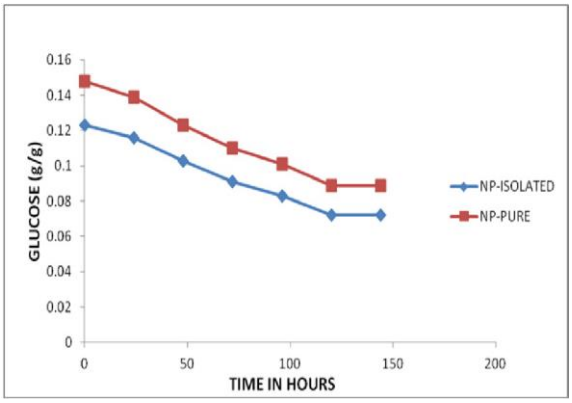

Fig 11. Use Of Sugars That Reduce During Fermentation

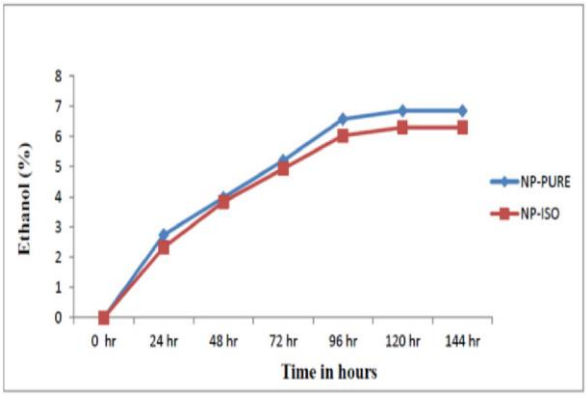

Fig. 12. Percentage Of Bioethanol Generated Using The Specific Gravity Technique

CURRENT STATUS AND CHALLENGES

Over the years, many studies have been conducted to enhance the biodegradability of biomass materials by pretreating them with biomass. Efficient pretreatment methods have been developed for a wide range of lignin-based biomass feedstocks. Despite these advancements, none of the techniques for producing ethanol from cellulosic materials have been successfully commercialized due to limitations on economic sustainability.

A few pretreatment techniques have been tested on a demonstration scale, including steam explosion and diluted acid. One of the most well-known demonstration-scale cellulose ethanol facilities is run by Iogen Industries in Canada. It produces 340 litres of ethanol per tonne of fibre by utilising a modified steam-explosion pretreatment developed by Iogen Corporation.

The United States Department of Energy supports various Biofuel Programs through Bioenergy Laboratories and centers like the National Renewable Energy Laboratory, Great Lakes Bioenergy Research Centre , and Oak Ridge National Laboratory. These programs focus on developing various pretreatment techniques, obtaining process performance data, and assessing financial viability.

The environment, delignification, co-fermentation of hexose and pentose into ethanol, detoxification by hydrolysis, separation of hemicellulose and cellulose, digestibility of pretreatment products, energy requirements, and processing costs are some of the techno-economic challenges that pretreatment research is currently facing. It is imperative that future studies tackle these obstacles in order to progress towards the commercialization of cellulosic ethanol.

FUTURE WORK

Developing effective pretreatment methods for producing highly palatable biomass for conversion into cellulosic ethanol requires a significant amount of work. A cost-effective and ideal pretreatment technique should possess several essential qualities, such as ensuring the recovery of a sufficient quantity of fermentable carbohydrates, reducing the number of inhibitors that are generated during carbohydrate degradation that occurs during pretreatment, having no negative impact on the environment, requiring minimal pretreatment like washing, detoxification, neutralization, using minimal amounts of water and chemical substances, having low reactor capital costs, requiring a relatively low energy input, exhibiting a relatively high treatment rate, and generating high-value byproducts.

As pretreatment research progresses in the coming years, it will focus on specific areas. Initially, the emphasis will be on using less water and fewer chemicals. Secondly, efforts will be made to recover carbohydrates and produce byproducts with added value to improve economic sustainability. Thirdly, clean delignification methods that enable co-fermentation of hexose and sugars containing pentose will increase the pretreatment's economic effectiveness. Fourthly, it is essential to have a basic understanding of pretreatment mechanisms and how they relate to the enzymatic hydrolysis process and biomass structure. Lastly, since inhibitors like furfural, 5-HMF, and acetic acid can seriously obstruct cellulose fermentation and enzymatic hydrolysis, research should focus on lowering their production.

V. CONCLUSION

The main objective of the project was to generate bioethanol from waste newspaper, a substantial component of municipal solid waste. The experiment encompassed three primary phases: substrate fermentation, hydrolysis, and pretreatment from the hydrolysate. The optimal conditions for substrate prior to treatment were identified as a 1.2% H2SO4 concentration at 120ºC for 50 minutes, leading to a cellulose recovery of 45%. Two bacterial strains, Cytophaga Hutchnisonni acquired via NCRM. Pune, and To compare hydrolysis efficiency, an organism obtained from Bagalkot's Department of Biotechnology BEC was usedThe amount of reducing sugar produced by Cytophaga Hutchnisonni was 0.140g per gram of substrate, which was more than the 0.110g yield of the isolated organism... The resulting reducing sugar was subsequently fermented into bioethanol using yeast Saccharomyces Cerevisiae. The estimated ethanol yield was 5.49% (v/v) with Cytophaga Hutchnisonni and 5.011% (v/v) with the isolated organism. HPLC analysis confirmed ethanol yields of 5.10% and 5.02%, respectively, for Cytophaga Hutchnisonni and the isolated organism. These findings suggest that the biological cellulose hydrolysis by Cytophaga Hutchnisonni bacteria exhibited superior efficiency compared to the isolated bacteria.

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