https://doi.org/10.48047/AFJBS.6.12.2024.1477-1485

African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com
Research Paper Open Access

Production of Bioethanol using diverse lignocellulosic biomass for sustainable future

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Article History
Volume 6 Issue 12, July 2024
Received: 25 May 2024
Accepted : 25 June 2024
doi:
10.48047/AFJBS.6.12.2024.1477-1485

Abstract:

Ethanol produced by the use of fermentation of different biological materials is a clear liquid alcohol. As a fuel, bioethanol has significant characteristics, including a high octane number and rapid vaporization rate. In the present day, bioethanol is the most commonly produced biofuel, mainly derived from the first generation, resulting in a competition between food and biofuel. Many developing countries are currently facing this challenge while lignocellulosic waste is not being utilized. Since the second generation of bioethanol production uses non-edible feedstock derived from agricultural, municipal, domestic and forestry waste, it fills the gap created by the first generation of bioethanol production. The major aim of this study was to compare the yield of ethanol produced by *Saccharomyces cerevisiae* from three different types of lignocellulosic biomasses: sugarcane bagasse, paper waste and domestic waste. Sugarcane bagasse, domestic wastes were dried and ground to powder and pretreated with 4% H_2SO_4 at 60℃ for 2 hours followed by the hydrolysis using cellulase enzyme. The paper waste was cut into pieces, hydrolyzed using 5% H_2SO_4 at 121℃ for 120 mins. The composition of the substrates was analyzed before and after pretreatment. All the three substrates were fermented for 72 hours at 35℃. Among all these tested substrates, paper waste produced maximum ethanol followed by sugarcane bagasse and domestic waste.

Keywords: Bioethanol, Distillation, Lignocellulosic biomass and *Saccharomyces cerevisiae.*

1. INTRODUCTION

Energy demands for transportation, heating and industrial processes will pose the greatest challenge of the 21st century. The continuous increase in the population has directly upsurge the demand of energy from fossil fuels, which are exploiting at a great speed. Thus, there has been renewed interest in the expansion of alternative energy sources (Nisha J. et al., 2022). Ethanol is a widely used fuel in the automotive industry as well as for other potable purposes. Additionally,

it is also recognized as a suitable substitute for fossil fuels. Currently, majority of the ethanol is produced from sugar and starchy materials, but the usage of such feedstock for ethanol production is exceedingly discussed owing to their effect on environment and the food supply (Ibrahim, N.A. et al., 2013). As a result, attempts must be made to improve its production in the future. Ethanol is made mostly by yeast or bacteria fermenting sugar or starch from agricultural crops (Kaur, S.N. et al., 2021). There have been a number of initiatives to utilize lignocellulose residues for the production of enriched compounds and energy, furthermore attempts to lower their toxicity while adding value to them. Lignocellulosic materials mostly consist of cellulose (30–60%), hemicelluloses (20–40%) and lignin (15–25%). Along with these components ashes and extractives are also present in trace amounts (Balat,M. 2011). Many developing nations currently face fuel shortages because lignocellulose biomass waste is not being effectively used.

Solid sugarcane bagasse is left behind as a waste during the process of sugarcane juice extraction. In addition to its high content of sugar, the sugarcane bagasse is considered to be renewable, cost efficient, and a widely available feedstock, creating it to be an ideal feedstock for the production of ethanol (Sabiha H.S., and Halim, N.A.A. 2018). Since sugar cane bagasse (SCB) and sugar cane trash (SCT) contain high amount of hemicellulose, cellulose, and lignin but lack nutritional value, they are considered as potential sources of raw materials for the industrial production of bioethanol (Zhihong, F. and Holtzapple M.T., 2010). The amount of sugarcane bagasse produced every year around the world is estimated to be 540 million metric tons (Zhao, Y. et al., 2015). After Brail, India is thought to produce the second most sugarcane, with production of 101.3 million metric tons sugarcane bagasse every year (Kumari, S. and Das, D. 2015). Bioethanol made from SCB provides a number of advantages over fossil fuels, including being less carbon intensive, which reduces pollution (Canilha, L. et al., 2012).

The use of domestic wastes can serve as an alternative and valuable source of raw materials intended for the generation of biofuels (Matsakas, L., et al, 2014). This domestic waste can be discharged from kitchens, hotels and residues from food industries (Hafid, H.S. et al., 2011). About 35 million tonnes of food waste are produced in China which is considered as Asian economic giant. Whereas India also produces about 3500×10^4 tonnes per year which is a great concern to the country (Halimatun, S. F. et al., 2017). Domestic waste is considered as highly organic and biodegradable consisting of about 60% Carbohydrate, 20% Protein and 10% Lipid (Pleissner, D. et al., 2013), therefore it could be utilized as a potential feedstock for the synthesis of bioethanol. In addition to these substrates a large amount of waste papers are also generated and hence it could also be employed as a promising substrate for the bioethanol production.

Pulp and paper are among the largest global structure since they consume woody biomass on a global scale and also generate substantial amount of waste (Bajpai P. 2012 and Stoklosa R. J. et al., 2014). The waste paper contains comparatively high amount of carbohydrates that can be potentially converted into bioethanol, and their price is relatively low when compared to other biomass feedstocks. The use of waste papers to make bioethanol could provide a useful and valuable alternative to the conventional disposal of these materials since they are easily digestible without aggressive physical or chemical pretreatments (Lei, W. et al., 2012).

Pretreatment is required as a preliminary step to convert the complex structure of paper into fractions that can be easily hydrolyzed (Eliana Cardona et al., 2016). It is necessary to convert lignocellulosic resources into monomeric sugars and then into ethanol while keeping costs low and yields high. Therefore, the kind of pretreatment and how it affects the process as a whole are crucial (Mosier et al., 2005). Both the separate hydrolysis and fermentation (SHF)

method and the simultaneous saccharification and fermentation (SSF) approach can be used to make ethanol from pretreated lignocellulose material. Cellobiose and glucose are the two compounds created during the hydrolysis phase of a SHF process, block both the cellulase enzyme and the organisms that ferment the material. However, in SSF, microorganisms simultaneously metabolize the glucose produced during hydrolysis, effectively resolving issues brought on by product inhibition (Alfani et al., 2000). Additionally, different pretreatment techniques are known to result in varying yields and enzyme treatment requirements (Rosgaard L. et al., 2007 and Saha B. C. et al., 2009). The methods of hydrolysis also show substantial differences in terms of pH, temperature, holding duration and the types of chemicals utilized (Mosier N. et al., 2005 and Olsson L. et al., 2005).

The main objective of this study was to utilize alternative and economically feasible renewable substrates for bioethanol production using chemical and enzymatic means of degradation. The use of such feedstocks also bridges the gap which is caused by the ethanol production from first generation since the substrate used in the second generation are non-edible biomass.

2. MATERIALS AND MEHODS:

Collection of the Substrates

The materials used in the study were Sugarcane Bagasse, Paper Waste and Domestic waste. The sugarcane bagasse was collected from local juice shop, the waste papers were collected from the households and the domestic waste was collected from college canteen and restaurants.

Isolation and Identification of yeast

Different spoiled fruit juices were used for the isolation of yeast. The juices were plated on YEPD medium using spread plate technique. In the medium small amount of Chloramphenicol was added to avoid bacterial growth. After inoculation the YEPD plates were incubated at 30℃ for 3 days. By examining the morphological characteristics of the isolated yeast strain as Provided by Kurtzman and Fell (1999) and the assimilation and fermentation of carbohydrate. (Swe. Z. Y. et al., 2018).

Dilute acid Pretreatment

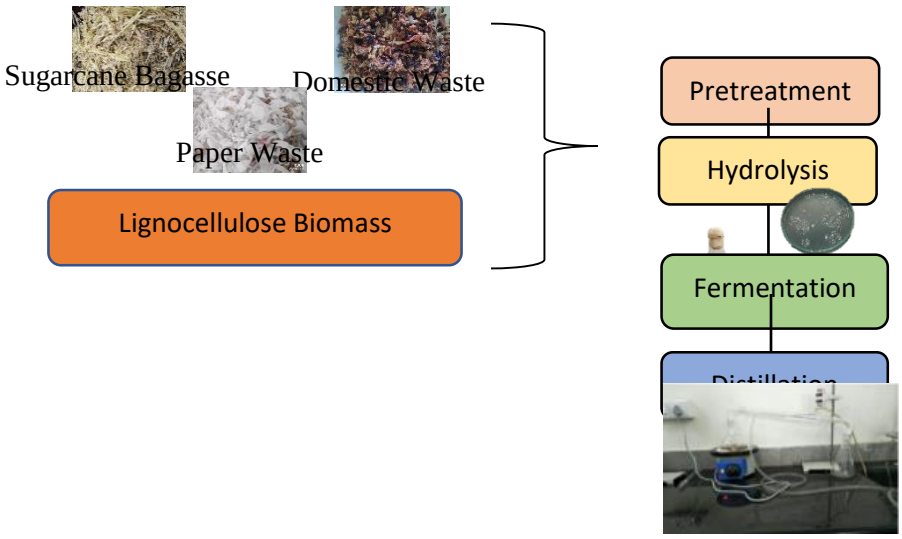
All the three substrates were first dried at 60℃ for 48h and the dried sugarcane bagasse and domestic waste were powdered for pretreatment whereas the papers were cut into small pieces and used. In a 250ml flask the substrates were taken and treated with 1:10 (w/v) ratio of 5% H_2SO_4 and kept at 121℃ for 60 minutes. After pretreatment the samples were passed through the cheesecloth. The Chesson method was used to analyze the chemical composition of the substrates (Cellulose, Hemi-cellulose, and Lignin) both before and after pretreatment. (Hernawan, R. et al., 2017).

Hydrolysis of Pretreated Substrates

Due to the acidic pretreatment of the substrates, the pH of the pretreated solution was very low and hence, the pH was neutralized by adding 8.5M NaOH solution. To the neutralized pretreated solutions of all the three different substrates C1184 Cellulase enzyme from *Aspergillus niger* (Sigma Aldrich) was added at a concentration of 7.5% and incubated at 35℃ for 2 hours.

Fermentation

The hydrolyzed samples of the three substrates were inoculated with 5% of 24 hours old *Saccharomyces cerevisiae* culture and allowed to ferment for 72 hours under anaerobic conditions. After fermentation, various substrates were subjected to simple distillation, a method that involves separating constituent chemicals from a liquid mixture through selective vaporization and condensation. The ethanol yield was determined using Chromic acid method.



3. RESULTS AND DISCUSSION:

Five different yeast strains were isolated from spoiled fruits juice in the first stage based on their morphological characteristics and were named as NCP01, NCP02, NCP03, NCP04 and NCP05. The morphological characteristics of the isolated strains are given in table 1 and Table 2 displays the assimilation characteristics of the five yeast isolates on different sugars.

Table 1. Morphological characteristics of Yeast Isolates

Yeast isolate	**Colony color**	**Colony nature**	**Appearance**	**Elevation**	**Margin**	**Colony size**	**Budding Cells**
NCP 01	Whitish cream colored	Smooth	Yeast like	Elevated	Wavy	6.0×13.0	++
NCP 02	Whitish cream colored	Smooth	Yeast like	Elevated	Wavy	6.0×13.0	++
NCP 03	Light cream colored	Smooth	Ovoid	Elevated	Entire	3.8×16.0	++
NCP 04	Cream colored	Smooth	Yeast like	Convex	Entire	5.1×12.0	++

NCP 05	Cream colored	Smooth	Glabrous and yeast like	Smooth	Entire	4.8×15.0	++

Table 2. Carbohydrate assimilation characteristics of isolates

Carbon sources	NCP 01	NCP 02	NCP 03	NCP 04	NCP 05
Glucose	+	+	+	+	+
Sucrose	+	+	+	+	+
Galactose	+	+	_	+	+
Lactose	_	_	_	_	_
Xylose	+	_	+	+	+
Arabinose	+	_	+	_	+

The yeast *Saccharomyces cerevisiae* have the potential to ferment 6C sugars but cannot ferment or assimilate 5C sugars like xylose and arabinose. In this study, from the selected strain NCP 02 was assumed as *Saccharomyces cerevisiae* using Carbohydrate assimilation tests and stored at 4℃ until further use.

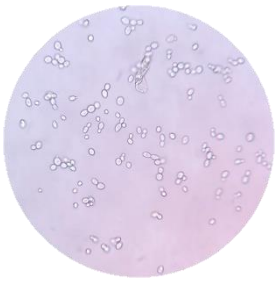


Figure 1: Isolate NCP 02 showing budding cells

Most of the hemicelluloses and celluloses in sugarcane bagasse, waste papers, and domestic waste were hydrolyzed during the pretreatment process, but only a small quantity of lignin was hydrolyzed. Respectively, all the three substrates showed solubilization of 60 to 85 % of hemicellulose and 20 to 35 % of total lignin. As a result, after dilute acid pretreatment maximum amount of cellulose and lignin was recovered which were hydrolyzed by the means of enzyme. The results of the study were found to be in relation with the work of Chen. S. et al., (2014). The chemical analysis of the three substrates, before is shown is figure 2 and after pretreatment is presented in figure 3.

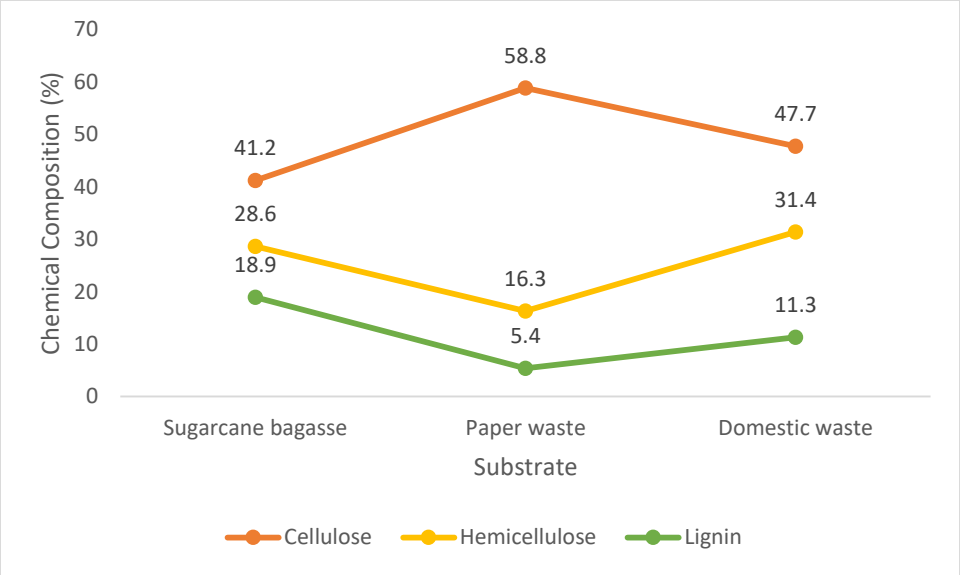


Figure 2. Chemical analysis of substrates before pre-treatment

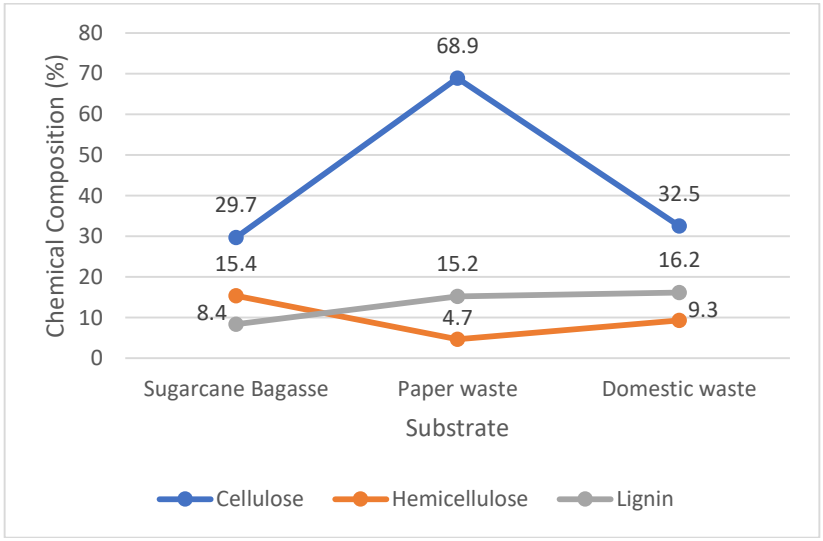


Figure 3. Chemical analysis of substrates after pre-treatment

According to Chen. S. et al (2014) research, increasing acid content and residence duration leads to increased hemicellulose hydrolysis. Acid pretreatment of lignocellulosic materials results in lower levels of hemicelluloses and lignin in the residues and higher levels of cellulose. Ultimately, such changes would improve the enzymatic hydrolysis and increase the yield of the reducing sugar (Sindhu et al. 2011). Sugar such as xylose and arabinose have been found in the substrate after dilute acid pretreatment. It was observed that in the dilute acid treatment, hemicellulose is converted into monomeric sugars through the dissolution of covalently bond between acetyl groups and dissociation of glycosidic chain links (Kamireddy et al. 2013).

The formed sugars and cellulose of the substrates which was left unhydrolyzed during pretreatment were further hydrolyzed using the cellulase enzyme. The findings of Alexandre De A. G. et al., (2018) reflected that hydrolysis of cellulose and reducing sugar highly depends on the concentration of cellulase enzyme. Increase in the concentration of enzyme will parallelly

increase the yield. After hydrolysis, all the three substrates were carried out using NCP 02 isolate for about 72 hours at 35℃ and distilled at 78℃.

While comparing the ethanol production of substrates, maximum yield of ethanol was found to be from waste papers followed by sugarcane bagasse and domestic waste. This might be due to the that papers consist of high amount of cellulose, hemicellulose and very low amount of lignin content. Work of Rocha, J. et al., (2014) revealed that nearly 0.16 g of ethanol can be made from 1g of waste office paper.

The yield of bioethanol obtained from the three different substrates is shown in figure 4. The time duration of hydrolysis should also be taken care of since, it effects the yield of ethanol. The results of Kumar A., et al., (2012) also shows that maximum ethanol production was seen when hydrolysis was done for 120 mins. Similar results were seen in the study of Maceiras, R. et al. (2016).

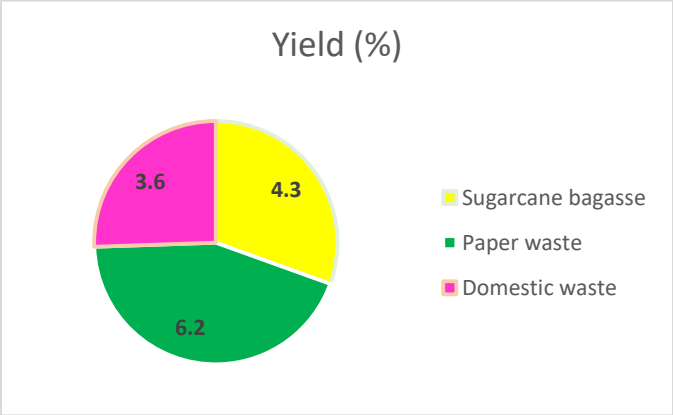


Figure 4. Percentage of ethanol produced using different substrates

Kaur Singh Nehra and her co-workers also made a comparative study for ethanol production from Sugarcane juice, Molasses and paddy straw in which sugarcane juice produced maximum ethanol followed by molasses and paddy straw (Kaur, S.N. et al., 2021). Studies of Matsakas, L., and Paul C., (2015) compared the efficiency of enzymatic hydrolysis on domestic waste which showed yield of 19.24 g/L (hydrolyzed) and 5.98 g/L (non- hydrolyzed).

4. CONCLUSION

This study concludes that waste lignocellulosic biomass has been identifies as a potential and cost-effective substrate for bioethanol production. The effectiveness of the ethanol production depends largely on the strain or enzyme utilized for hydrolysis and the type of lignocellulosic biomass used in the production. Although the percentage of bioethanol produced in this study was not high to meet the increasing demand of biofuel but by through appropriate usage of a pretreatment process and scaleup studies, the production of bioethanol can certainly be increased and a biodegradable waste for production of a biofuel (ethanol) it is likely to oblige as a sustainable substitute for energy production and diminishing the requisite for crude oil.

5. ACKNOWLEDGEMENT

I would like to acknowledge the support and facilities received from my college Dwaraka Doss Goverdhan Doss Vaishnav College, Chennai, Tamilnadu, India. It gives me an immense pleasure to express the deepest appreciation to my guide Dr. P. Vidya for her guidance and persistent help. I am extremely grateful to all the faculty members of the microbiology department. I am also very thankful to all my friends and non-faculty members of the department.

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