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## PRODUCTION OF BIOETHANOL FROM *TECTONA GRANDIS*, *PTEROCARPUS MARSUPIUM* AND PAPER

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### ABSTRACT

Currently one of the world's primary energy sources, fossil fuels are also causing an increase in environmental pollution. Besides pollution, these fossil fuels are also diminishing in quantities which increase the need for alternative fuels. This rising need lead to the development of alternative renewable energy resources. A diverse range of sources are available that can be efficiently utilized to produce biofuels, in this research we utilize *Tectona grandis* (teak wood), *Pterocarpus marsupium* (malabar kino) and paper as biomass sources to produce bioethanol using *Saccharomyces cerevisiae* fermentation. These biomasses were said to be lignocellulosic substrates rich in cellulose, hemicellulose and lignin. Simultaneous saccharification and fermentation (SSF) are used to in the conversion of substrate to ethanol in which the polysaccharides in the biomass are converted into simple sugars by hydrolysis. After which *Saccharomyces cerevisiae* used these basic sugars for the development of ethanol by fermentation. A bioethanol yield of 14.1%, 10.81% and 7.5% was obtained from *Tectona grandis*, *Pterocarpus marsupium* and paper respectively, which was analyzed using dichromate titrimetric method. The bioethanol yield from *Tectona grandis* is predominantly when compared with other biomasses.

**Keywords :** Bioethanol, wood, fermentation, *Saccharomyces cerevisiae*

### Introduction

Fossil fuels such as coal and petroleum have been conventional energy sources for decades and it also causes a variety of environmental pollution. As fossil fuels are declining, there has been substantial work over the years in creating renewable energy sources, which needs to decrease reliance on oil and greenhouse gas. (Xingang and Pingkuo, 2013). Currently, biofuel production from different sources is being studied worldwide. Biofuels are being scrutinized as potential substitutes for currently available high pollutant fuels (Akia *et al.*, 2014). In recent times, one of the most commonly used biofuels is bioethanol. Bioethanol has been utilized since ancient times as alcohol, solvent, germicide, antifreeze agent and fuel. Since bioethanol production is expensive, its popularity reduced through the years. However, the usage of these fuels will reduce the energy dependence on fossil fuels, allow mitigation of greenhouse gases and also offer new employment opportunities in the biofuel producing industries (Torres-Jimenez *et al.*, 2011).

The global market for bioethanol has entered a phase of rapid and transitional growth due to the depleting crude oil reserves and shifting their investments and focus towards the renewable source of power production. The trend is extending to transport fuel as well. Bioethanol can potentially act as a valuable replacement of gasoline in the transport fuel market. Each country prefers and uses raw materials with its availability, technology and economic viability (Corro and Ayala, 2008; Kumar *et al.*, 2018).

Various compounds such as sugar, starch, cellulose or other plant fibres are used as biomass for the production of bioethanol to replace fossil fuel. Raw materials used in the first-generation bioethanol production in different regions includes sugar beet, corn, sugarcane, cassava and wheat (Alonso-Gómez *et al.*, 2020). However, these raw materials are not preferable due to their high requirements of land and water for their growth (Mendoza, 2009). Also, the disjunction between bioethanol production and food security has been an issue treated covering topics, such as the soaring of food products in different countries (Tenenbaum, 2008). Thus, alternative raw materials for the production of bioethanol without risking food security were searched at national, sub-national, household and individual levels (Gomes *et al.*, 2018).

To overcome these issues, second-generation sugars or lignocellulosic biomass came into practice. Lignocellulosic materials are renewable, low cost and are abundantly available. It includes crop residues, grasses, sawdust, wood chips, etc. (Quintero *et al.*, 2013; Farahani *et al.*, 2016). These biomasses required a pretreatment process to yield the product. Simultaneous saccharification and fermentation (SSF) is one of the most important advances in the bioethanol production process. In the present work, the biomasses are subject to degradation by hydrolysis of polysaccharides into simple sugars; these sugars are simultaneously utilized in the fermentation.

## Materials and Methods

### Sample Collection

Biomasses used in the experiments are waste papers, wooden pieces of *Tectona grandis* and *Pterocarpus marsupium*. Waste papers were collected locally and the wood wastes were bought from a carpentry shop at Chennai. All chemicals used in the experiments are analytical reagent grade.

### Pretreatment of Samples

Paper, *Tectona grandis* and *Pterocarpus marsupium* were taken as biomass samples and ground to powder. The powdered sample is dried in an oven at 100°C for an hour to remove the moisture content in it. The homogenized samples were stored in a separate sterile container and labelled for further process.

The ground samples are subjected to chemical treatment using a freshly prepared solution (Sarkar *et al.*, 2012). To prepare the solution, add 0.98 mL of 1N H<sub>2</sub>SO<sub>4</sub> and makeup to 100mL using 99.2 mL of H<sub>2</sub>O. Weigh 25 g of each sample and add 100mL of the prepared solution along with 1gm of Na<sub>2</sub>CO<sub>3</sub> into a clean conical flask. The conical flask is then plugged with cotton and placed in a water bath at 160-260°C. After 3 minutes, the samples were kept for cooling at room temperature (Solarte-Toro *et al.*, 2019).

### Preparation of Culture Conditions

Yeast-Peptone-Dextrose (YPD) medium was prepared by diluting 1 g of yeast, 2 g of peptone, 2 g of dextrose in 100 mL of distilled water and autoclave for 20 minutes at 121°C. All apparatus and glassware used were autoclaved (Bonatelli *et al.*, 2019).

### Preparation of Starter Culture

For the preparation of starter cultures, 100 mL of growth media (YPD) was taken in Erlenmeyer flasks (250 mL) and inoculated with the loop full of *Saccharomyces cerevisiae* culture.

### Fermentation of Samples

The pretreated samples 15 mL along with 50 mL of NaOH buffer are added and labelled in separate conical flasks. Starter culture of 5 mL is added to each conical flask. The optimum conditions for fermentation were maintained at pH 5 and 30°C. To prevent the bioethanol evaporation and to maintain an anaerobic condition these were placed in the flasks plugged with cotton plugs in a mechanical shaker covered with an aluminum foil sheet. After the fermentation, the pulp broth was filtered through whatman filter paper, and the filtrate of each flask was immediately subjected to distillation using the claisen condensation apparatus. At 78.5°C the fractions are collected and analyzed for bioethanol yield. Fermented broths were removed at 24-hour intervals and were analyzed for the bioethanol yield (Zhao *et al.*, 2020).

### Analytical Method

**Reagent 1 (Potassium Dichromate):** Potassium dichromate of 16.88 g is dissolved in 250mL of distilled water and then 162.5 mL of sulphuric acid is added to the solution. Later, the whole solution is made up to 500 mL by adding distilled water.

**Reagent 2 (Ferrous ammonium sulphate):** Ferrous ammonium sulphate of 67.5 g is dissolved in 375 mL of distilled water. Later, 12.5 mL of sulphuric acid is added to the solution and made up to 500mL by adding distilled water.

**Indicator (Phenolphthalein):** Prepare a 50% ethyl alcohol solution containing 10 mL of ethanol and water each. Weigh 0.5 g of phenolphthalein and dissolve it in 50% ethyl alcohol solution and store it in a dropper.

**Method of Analysis:** Each sample of 20 mL (fermented broth) was measured into separate conical flasks and 100 mL of distilled water was added to prepare a stock solution. Take 5 mL of each stock solution in a new conical flask separately and add 25 mL of reagent 1 (Potassium Dichromate) dropwise. Prepare a blank sample with 5mL of reagent 1 and 95-mL distilled water in a separate conical flask. Use the cotton plug to seal the flasks and place them in a water bath at 60 - 65° C for 30 minutes. Later, allow the samples and blank to cool at room temperature. Fill the burette with reagent 2 (Ferrous ammonium sulphate). Take the samples and blank for titrating it separately against reagent 2. Add 5 drops of phenolphthalein indicator and continue to titrate till colour change occurs and note down the burette reading (Biwer and Vanderwarker, 2015).

## Results and Discussions

### Pretreatment

Pretreatment is an important step in the production of bioethanol from lignocellulosic biomass. Lignocellulosic biomass is generally composed of cellulose, hemicellulose and lignin which are inaccessible to most microbes. Thus, pretreatment is needed to break down the cellulose and hemicellulose components to simple mono and disaccharides as *S. cerevisiae* is capable of utilizing only the simpler sugars. This process of breaking down complex sugars to simple sugars is known as saccharification. It is the most important process in the production of bioethanol from lignocellulosic biomasses as it solubilizes and separates the components in the biomass into more accessible. Thus, pretreatment helps the biomass to increase the susceptibility of complex sugar molecules available in biomass and helps in increasing the product yield (Özbay and Yaman, 2018).

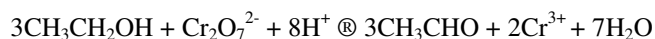
### Fermentation

*S. cerevisiae* is most commonly used in fermentation to convert glucose into bioethanol as it has an efficiency of 90-93%. *S. cerevisiae* in the starter culture utilizes the simple sugars that were obtained from pretreatment of cellulosic components in the samples. These simple sugars were converted into bioethanol and this complete process is termed as simultaneous saccharification and fermentation (SSF) (Farida *et al.*, 2015). The resulting bioethanol is subjected to purification to remove other substances and increase its purity. Bioethanol separation and purification involve distillation to obtain 95.6% pure ethanol from an ethanol-water binary azeotrope.

### Analytical Method

Dichromate titrimetric method is used for the estimation of bioethanol. Potassium dichromate oxidizes primary alcohols with an intermediate of an aldehyde to form the corresponding carboxylic acid. Initially, it requires the distillation of the sample into dichromate. This measures ethanol by titrating the excess dichromate with ferrous

ammonium sulphate after the conversion of ethanol to acetic acid. This reaction mainly depends on the hydrogen ion concentration for complete oxidation to occur. So, the redox reaction occurs as a two-step reaction (Michałowska-Kaczmarczyk and Michałowski, 2019).



Ethanol

Acetaldehyde



Acetaldehyde

Acetic acid

The reaction conditions of 60 - 65°C and a minimum of 30 minutes are favorable for reaction completion. Reduction of chromium from the [VI] oxidation state to the [III] oxidation state occurs due to the oxidation reaction, that can be observed with the help of the indicator has the colour changes from pink to purple.

Table 1 depicts the yield of the bioethanol production from *Tectona grandis*, *Pterocarpus marsupium* and paper as sources. The percentage of the produced bioethanol was estimated by the formula [% Yield = 25-25 (sample value / blank value)]. Both the sample value and the blank value are obtained from the dichromate titrimetric method.

**Table 1 :** Average Reading of Sample

	<i>Tectona grandis</i> (Teak Wood)	<i>Pterocarpus marsupium</i> (Malabar kino)	Paper
Sample Value	13	17	9
Blank Value	30	30	30
% Bioethanol = 25-25 (Sample Value/ Blank Value)	14.1%	10.83%	7.5%

### Conclusion

This work concludes that the wood waste and paper waste are potential biomasses for the production of bioethanol by simultaneous saccharification and fermentation. These lignocellulosic biomasses have been projected to be one of the main resources for economically attractive bioethanol production. These feedstocks extracted from crop waste are abundant and have more available energy than basic sugars and starch. In addition to their selection and use, they may provide farmers with an additional source of income from established land. These biomasses are processed to break down the lignocellulosic components into basic sugars that can be quickly converted to ethanol by *S. cerevisiae* during fermentation. As a result, we obtain bioethanol from *Tectona grandis*, *Pterocarpus marsupium* and paper of yield 14.1%, 10.83% and 7.5% respectively. Among these *Tectona grandis* has the highest bioethanol yield compared to other biomasses. Since bioethanol is a clean and green biofuel used in various applications such as transport fuel and fuel cells. The production methodologies discussed in the above work and been optimized to improve productivity. The bioethanol productivity remained approximately the same when the scale-up was 10 times greater.

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