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Energy

BIOCONVERSION OF LIGNO CELLULOSIC BIOMASS IN TO ETHANOL

K. Nalini Associate Professor, Microbiology Department, Ayya Nadar Janaki Ammal College, Sivakasi.

D. Jeyachitra Microbiology Department, Ayya Nadar Janaki Ammal College, Sivakasi

ABSTRACT The present study is aimed with an objective to produce bio ethanol from agrowaste. Paddy straw bio mass is collected from agricultural land and utilized as feed stock for ethanol production after subjecting them to acid, alkali and enzymatic pre treatments. Two bacterial strains (R2 and R3) and one yeast strain (S6) were isolated from soil samples collected from paddy field dumped with straw and from sugarcane bagasse dumped soil. In addition to this, cellulose degrading bacteria strain (C1) is isolated to carry out the enzymatic pre treatment. Based on the fermentation efficiency tested, S6, R2 and R3 strains were inoculated in eight different pretreated biomass filtrate medium for fermentation process. Ethanol concentration were quantified at a period of five days interval up to twenty days. Ethanol concentration increased from 5th day to 20th day and 5th day to 15th day in yeast and bacteria inoculated medium. After recovery through distillation, yeast strain S6 inoculated medium is reported with more concentration of ethanol in alkali pre treated bio mass filtrate medium. There is no significant difference between alkali pre treated biomass and combined alkali & enzyme pre treated bio mass inoculated with yeast strain at 0.001 level of significance. The overall product extracted is in the range of 5-12%. Ethanol extracted from yeast S6 is reported to have disinfectant effect against bacteria. FTIR spectrum analysis reported the presence of similar peaks to that of standard ethanol

KEYWORDS : Bio-ethanol, Bio Mass, Lignocellulose, Pre Treatment, Fermentation

INTRODUCTION

Energy is an important source of 21th century and the world depends upon the fossil fuel which is decreasing proportionately with increased demand. The fossil fuel contributes a dramatic effect on pollution, production of agriculture and global warming. The world is moving towards an alternate to fossil fuels. Methyl tertiary butyl ether (MTBE) is also used as an additive to petrol which is reported as a poisonous chemical compound in causing pollution to the groundwater system and now it is eradicated by US Environmental Protection Agency.[1] Pandemic onset in 2020 resulted in a decrease in the world's energy consumption, but since 2021, the need has been dramatically increased on the rise as the world's fossil fuel resources are exhausting.[2] Ethanol and biodiesel are the two most commonly available commercial renewable liquid transportation fuels alternate to the fossil fuel.[3] Bioenergy such as bioethanol, biodiesel, biohydrogen can be produced from lignocellulosic biomass. In agricultural sector, bioethanol can reduce the environmental pollution and imparts a positive energy worldwide. 92 million barrels oils are used for per day in 2022. In 2030, the oil consumption will be raised to 116 million barrels. Bio fuel can be obtained from agricultural residues like sugar crop, rice straw, corn stalk, sugarcane bagasse, and wheat straw.[4] Lignocellulosic biomasses act a good alternative renewable bioenergy source and are abundantly available in nature. Rice straw is one of the most abundant lignocellulosic biomass sources in India to be used as a feedstock for the manufacture of bioethanol.[5] Lignocellulosic biomass consists of polysaccharides which should be broken down to monosaccharides for efficient utilization by microorganisms for fermentation. Acid / Alkali diluted hydrolysis and or enzymatic hydrolysis is most commonly used for pre treatment. High yield of ethanol is derived from pre treated samples rather than untreated biomass.[6] The bacterial and fungal species are generally expected to degrade the lignocellulosic biomass.[7] After the fermentation process of the Lignocellulosic biomasses (agricultural residues), can be used as fertilizers.[8]

MATERIALS & METHODS

Sample Collection: Soil sample were collected in agricultural land in Sivakasi for bacteria isolation and for yeast isolation, soil sample were collected from sugarcane bagasess waste dumped soil in Sivakasi. Compost sample were collected from raw vegetable waste dumped site inside the college campus for isolating cellulose degrading bacteria.

Pre treatment of biomass

Acid Pre-treatment- The 90% dried rice straw was chopped with 1-2 cm length. 10 gram of rice straw was treated with acid (1% Hydrochloric acid). Ten gram of rice straw sample was soaked in 100 ml of acid solution for 1 hour. Then the suspension was autoclaved at 121°C, 15 lbs for 15 minutes. [9]

Alkali Pre-treatment- Ten gram of rice straw sample was soaked with 5% alkali (NaOH) solution for 1 hour. Then the suspension was autoclaved at 121°C, 15 lbs for 15 minutes.

Combined Pre treatment (Acid and Enzyme / Alkali and Enzyme) - 5% of cellulose degrading bacteria culture was inoculated in nutrient broth containing 1% of cellulose powder. The contents in the flask were incubated at 37°C in the shaker for a period of 7 days. Then, the mixture was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was used as the source of cellulase enzyme. The acid / alkali treated biomass sample adjusted to pH 7 was mixed with enzyme source for a period of 24 hours at room temperature. Then the biomass was filtered and the filtrate was used for fermentation.[9]

Fermentation- Pre treated bio mass filtrate (acid, alkali, Combined acid+ enzyme and combined alkali + enzyme was inoculated with 1% of the inoculum (R2, R3 and S3) in separate brown bottles and the contents were incubated for a period of 20 days in anaerobic condition. The contents in the flask were periodically assessed at a period of five days for analysing the concentration of sugar by Dinitro Salicylic method[10] and ethanol by Dichromate method.[11]

Concentration of ethanol is calculated by comparing the absorbance of 95% standard ethanol. Gram of ethanol = 95g X Absorbance of bioethanol / Absorbance of standard Ethanol.

Fractional Distillation- The fermented broth was dispensed into round-bottom flasks fixed to a distillation column enclosed in running tap water.. A heating mantle with the temperature adjusted to 78°C was used to heat the round-bottomed flask containing the fermented broth.

FT-IR- FT-IR spectroscopy is used for the detection of the different functional groups present in the given sample.

RESULTS & DISCUSSION

Two bacterial cultures R2 and R3 are gram negative rod shaped and one yeast culture S6 used in the present study were isolated from soil samples collected from paddy field dumped with straw and from sugarcane bagasse dumped soil. Cellulose degrading gram positive cocci shaped bacteria C1 was isolated from compost soil for hydrolyzing the biomass.

Bio fuels are considered as the most promising alternative source of fossil oil energy sources, as they can be produced from abundant natural renewable sources and it is also an eco friendly alternative.[12]. India has decided with a goal of blending 20% of ethanol by the year 2025. In January 2021, the government of India has declared to increase the ethanol blend 25% by 2030. From June 2023, Indian government has asked the oil marketing companies (OMCs) to sell 20% ethanol. Many polices of Indian Government has supported to improve the production of ethanol and reduce the import levels. In the year 2020, US is the major supplier of ethanol to India.[13] The most prominent method for producing economically viable bioethanol is to use agro waste and to optimise the industrial medium and fermentation conditions in a better manner.[14]

Pre treated biomass filtrate is incubated with 5% inoculum of the bacterial strain R2, R3 and Yeast strain S6 for fermentation of hydrolysed cellulose to Ethanol.

Second-generation biofuels from lignocelluloses rich bio mass has reduced the utilization of food sources and in addition has economic and environmental benefits. Ethanol production is mainly by yeasts Saccharomyces cerevisiae and bacteria E.coli, Zymomonas mobilis and Klebsiella oxytoca. The microbes are either used separately or in combined form.[15] Microbial conversion of various agricultural wastes as substrates for second-generation ethanol production emerged to reduce the first-generation feed stocks for food, productive agricultural land, and fresh water.[16]

In the present study, paddy straw bio mass is collected from agricultural land, dried and pre treated through chemical (acid and Alkali) and biological (crude cellulose enzyme) methods in separate and combined manner. The pre treated bio mass filtrate is then fermented with two bacteria and one yeast strain isolated from soil obtained from agricultural land and from bagasse deposited site. Ethanol production is assessed at an interval of five days up to a period of twenty days.

With effective from January 2021, India has permitted to use surplus rice (available through the Food Corporation of India) and maize as source of ethanol and blend with gasoline under the Ethanol Blending Project program.

Gram negative bacteria like Z. mobilis, K. oxytoca and E. coli have reported to produce ethanol more effectively form many carbohydrate sources.[17] These bacteria are more effective in fermenting sucrose, glucose and fructose molecule at a rapid rate than that of S.cerevisiae. Similar to the above report, gram negative bacteria and yeast culture is employed for ethanol production.

Many eukaryotic microbes like Yeast, Candida sp., Mucor sp., Pichia, sp. can ferment sugars more efficiently than Prokaryotic microbes. Wild type yeast Saccharomyces cerevisiae is more commonly used for ethanol production due to its ease cultivation and maintenance.[15]

The efficiency of fermentation was assessed by estimating the concentration of ethanol by comparing with the standard 95% ethanol in a period of five days interval up to twenty days. Based on the observations from pilot study, bacterial Strain R3 is used for fermenting acid treated bio mass and combined alkali & enzyme pre treated bio mass. Similarly bacterial Strain R2 is used for fermenting alkali treated bio mass and combined acid & enzyme pre treated bio mass.

The concentration of crude ethanol in the acid and combined enzyme pre treated biomass is reported in figure 1. Ethanol concentration in acid pre treated biomass with yeast S6 increased from 5th day to 20th day. The concentration increased more during the period of 15th day to 20th day of fermentation.Ethanol concentration in acid pre treated biomass with bacteria R3 increased from 5th day (97g) to 15th (195g) day and decreased slightly on 20th day (164g). Ethanol concentration in acid and enzyme pre treated biomass with the bacterial strain R2 increased slowly from 5th (227g) day to 15th day (257g) and decreased slightly on 20th day (251g). Ethanol production is more in the period of 5th day to 10th day.

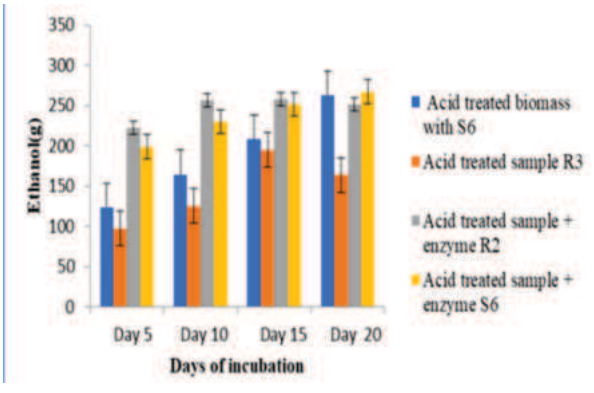

Figure 1 Ethanol Concentration in acid And Enzyme Treated Biomass

Ethanol concentration in acid and enzyme pre treated biomass with yeast S6 increased moderately from 5th day (198g) to 20th day (267g). Ethanol production is more in the period of 5th day to 10th day.

Based on the results obtained, more concentration of ethanol is reported in the yeast strain S6 inoculated strains (around 260g) than that of the bacterial strains and combined pre treatment is reported with more ethanol than acid pre treatment alone.

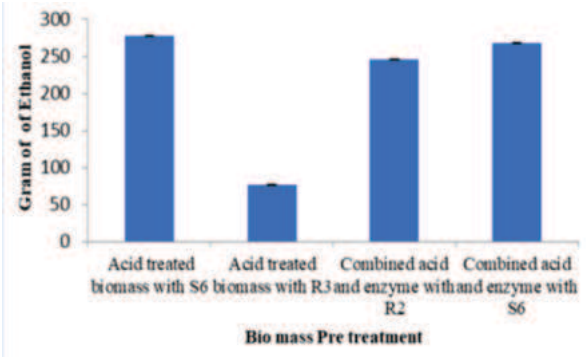

Figure 2 Ethanol Concentrations After Distillation of Acid Pre Treated Fermented Medium

The ethanol from fermented medium was separated through distillation and the concentration is reported in figure 2. Yeast strain S6 inoculated medium is reported with more concentration of ethanol. There is no significant difference between acid pre treated biomass and acid and enzyme pre treated bio mass inoculated with yeast strain. Ethanol collected after first distillation will have high proportion of water and the specific gravity of the distillate helps to understand the presence of water and the distillation can be repeated for complete removal of water and improve the quality of bio ethanol.[8]

The concentration of ethanol in alkali pre treated biomass medium is reported in Figure 3. Ethanol concentration in alkali pre treated biomass with yeast S6 strain increased from 5th day (106g) to 20th day (248g). Ethanol concentration in alkali pre treated biomass with bacterial strain R2 increased from 5th day (127g) to 15th day (210g) and decreased to 101g during the period between 15th day and 20th day of fermentation. Ethanol concentration in alkali and enzyme pre treated biomass with yeast strain S6 increased from 5th day (149g) to 15th day (250g) and decreased to 243g on 20th day of fermentation. Ethanol concentration in alkali pre treated biomass with bacterial strain R3 increased from 5th day (167g) to 15th day (211g) and decreased to 161g on 20th day of fermentation.

Based on the results observed, all alkali pretreated biomass filtrate ethanol production decreased after 15 days of fermentation.

The ethanol from fermented medium containing alkali pre treated bio mass was separated through distillation and the concentration is reported in figure 4. Yeast strain S6 inoculated medium is reported with more concentration of ethanol. There is significant difference between alkali pre treated biomass and alkali & enzyme pre treated bio mass inoculated with yeast strain

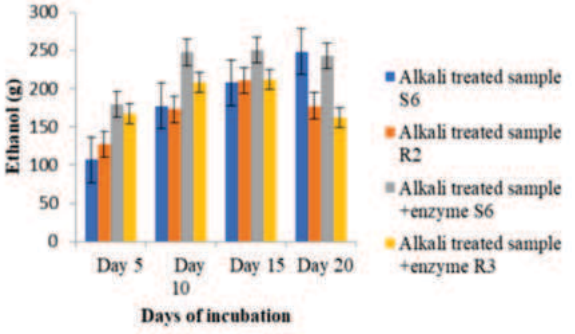

Figure 3 Ethanol Concentration in Alkali and Enzyme Treated Biomass.

Lignocellulose is the major composed of the bio mass followed by cellulose and lignin. It is one of the most non hydrolysable forms of

biological materials in nature. Structural polysaccharides like cellulose and lignin requires a efficient enzyme and heavy treatment for saccharification in to simple sugars.

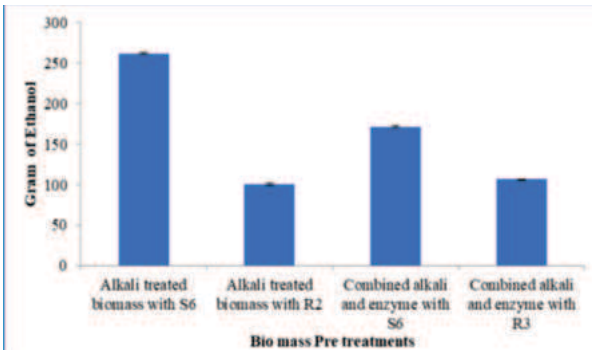

Figure 4 Ethanol Concentration After Distillation of Alkali Pre Treated Fermented Medium

Chemical pretreatments use alkali, acids, ozone, organic solvent and ionic liquids. Biological pretreatments utilizes microbial enzymes like cellulases, hemicellulases etc., Combination of physicochemical with enzymatic (biological) pretreatment is reported with higher conversion of sugar to ethanol. [18]

Cellulose $(C_6H_{10}O_5)x$ is the a main constituent of lignocellulosic biomass and it contains linear chain of D-Glucose linked by beta(1,4)-glycosidic linkage. Intramolecular and intermolecular hydrogen bonds in cellulose are linked together.[19] The dried and chopped paddy straw biomass was treated with 1% of Hydrochloric acid It increases the accessibility of cellulose to enzymatic hydrolysis by hydrolyzing polysaccharides, particularly hemicelluloses, to simple sugars. Acid pre treatment is generally carried at high temperature for low acid concentration or low temperature for high acid concentration. [20]

5% of sodium hydroxide is used for alkali pre treatment. Alkali pre treatment breaks the ester bond in the cellulose, hemicelluloses and lignin.[21]. Among different pre treatment employed alkali pretreatment is effective for fermentation studies followed by combined enzyme and alkali treatment.

Acid pre treatment is supportive in the hydrolysis of lignocellulose, but the main negative aspect in this is the release of by-products, such as furfural and 5-hydroxyfurfural.[22] .Enzymatic hydrolysis is becoming more accepted than acid hydrolysis due to the lack of corrosion and consumes less energy with pleasant and convenient environment with very less inhibitors of fermentation.[23] Several microorganisms utilise the hydrolysed plant based biomass as a source of fermentable sugars. Suitable microorganisms individually or in combined manner ferment both pentose and hexose sugars by improving the industrial use of lignocelluloses for the generation of bioethanol.[24]

Bioethanol Quantity is directly proportional with fermentation time. Increase in fermentation time improves the activity of enzymes, more time for to interact with the reactants and increased cell density and carbon content used for ethanol production.[25]

Complete recovery of 95% pure ethanol requires at least three distillation process since the water in the fermentation medium has higher boiling point (100°C) than that of the ethanol (78°C) from the water in just one distillation process.[26].

Fermentation of ethanol is easier than the purification in bioethanol production. Ethanol and total water are the major components of the fermentation medium after alcoholic fermentation. Thus, Separation is needed to remove water from the bioethanol present in the fermentation broth. [27]

After 20 days of fermentation, eight fermented medium under study was distilled and the ethanol was extracted through distillation. Ethanol recovery from the fermented medium is more in yeast treated medium and alkali pre treated bio mass. The overall product extracted is in the range of 5-12% which is very low. Bacterial strain R2 and R3 has lower rate of fermentation in the range of 5-7%.

FTIR spectrum analysis study is done for standard ethanol (Figure 5) and ethanol produced from Yeast S6 (figure 6) fermented alkali pre treated biomass filtrate. More peaks were observed in bio ethanol than that of the standard ethanol.

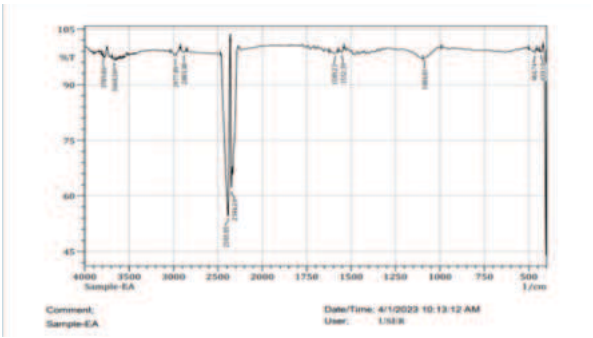

Figure 5 FTIR Spectrum of Standard Ethanol

Eleven peaks are reported in standard ethanol and twenty four peaks are observed in the ethanol extracted from pre treated bio mass.

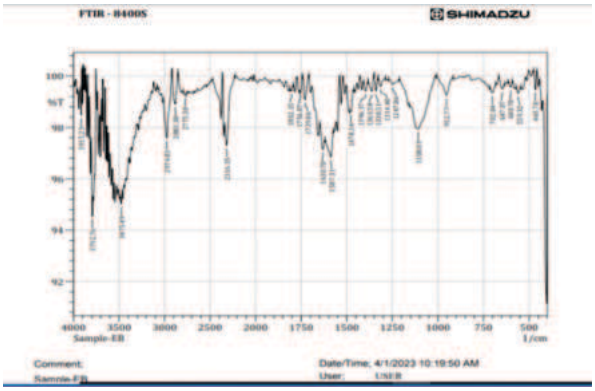

Figure 6 FTIR Spectrum of Ethanol Recovered From Pre Treated Bio Mass

According to the reports available, Brazil is one of the largest producers of ethanol from the sugarcane in the world. Sugarcane is reported to be an efficient source of ethanol production than that of the corn grain.[28] Fivefold more ethanol is produced by the bacteria Z. mobilis compared to that of the Yeast S. cerevisiae. Z. mobilis is bacterium is having the capacity to utilize fructose and glucose and convert them to ethanol but it cannot utilize pentose.[29]. But in the present study yeast cells fermented more efficiently than the bacterial strains

CONCLUSION

The present study produced bio ethanol from paddy straw waste collected from agricultural land using two bacteria and one yeast strain. High concentration of ethanol is produced in alkali pre treated bio mass fermented by yeast strain. Ethanol production has to be optimised with different physical and chemical parameters. Yeast strain can be improved and the production levels can be increased.

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