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SYNTHESIS OF BIOETHANOL FROM PADDY STRAW USING *Pseudomonas sp.* AND ITS CHARACTERIZATION

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ABSTRACT

Bioethanol is presently found as common renewable fuel which can be produced using microbes from variety of feed stocks and wastes in all over the world. Hence, the aim of the study is to evaluating the bioethanol production using *Pseudomonas sp.* from paddy straw and the production could be utilized as energy resources as fuel for increasing the energy needs. The bulk amount of sustainable lignocelluloses in paddy straw is hydrolyzed with various enzymatic and saccharification processes to yield hemicelluloses, lignin and converted to biofuel. The quality of the product is determined by physiochemical methods. The results of the current study provide the information about substrate properties and the steps to produce a proper ethanol for commercialization.

KEY WORDS

Bioethanol , *Pseudomonas sp.* Paddy straw, Pre-treatment, Biofuel and Ethanol

INTRODUCTION

Fossil fuels and natural gases play a vital role as energy resources. These resources will deplete within the next few years. Ethanol is one of the important alternative second generation biofuels and produced from biodegradable fractions of products from agricultural crops like corn, wheat, sugarcane; sorghum plants, forestry as well as biodegradable fractions of industrial and municipal wastes. Hussain *et al.*, (2014) reported that the paddy straw is one of the most abundant lignocellulosic, non-food based, sustainable biomasses. Lignocellulose is composed of cellulose (40–50%),

hemicellulose (25-35%) and lignin (15-20%). The current status of bioethanol production from lignocellulosic biomass get much more attention in recent days. Lignocellulosic biomass has an advantage over other agriculturally important biofuel feedstock because of its fast and easy recovery of the product. Heinrich (2005) investigated that the neem tree contained 23% carbohydrate that yield high amount of ethanol than the edible sources. *Pseudomonas* sp. is a Gram negative, rod shaped, gamma proteobacteria, belonging to the family Pseudomonadaceae and containing 191 described species. These are ubiquitous, able to adapt worst conditions to survive and capable to degrade complex sugars into simple sugars for the production of biofuel. Sindhu *et al.*, (2009) reported that, pretreatment is a low cost and eco-friendly technique to treat lignocelluloses biomass prior to enzymatic and saccharification. The aim of the present study is to synthesis of bioethanol from paddy straw using *Pseudomonas* sp.

MATERIALS AND METHODS

Collection of raw materials

The paddy straw wastes were collected from nearby villages and washed with distilled water and made into a fine powder.

INOCULUM PREPARATION

Pseudomonas sp. was isolated from the soil by spread plate method using *Pseudomonas* selection agar media. The isolated culture was sub cultured in 50ml of Nutrient broth for characterizing the morphological and biochemical nature of an organism by various tests. Bacterial morphology was characterized by Gram staining technique and biochemical characters were confirmed by Indole production, MR-VP, Nitrate, Urease, Catalase and Oxidase tests.

CULTURE PREPARATION

Pseudomonas sp. (1ml) was inoculated in the conical flask containing sterilized 100ml Nutrient broth and incubated at 37^0C for 24 hours in an aerobic condition.

ENZYMATIC HYDROLYSIS

A known quantity, 100g of powdered paddy straw was washed with distilled water and sterilized in an autoclave at 180^0C. Following, 2ml of *Pseudomonas sp.* was inoculated into the sterilized paddy straw medium and incubated at 37^oC for 5 days.

DETERMINATION OF REDUCING SUGAR CONCENTRATION

After incubation, a series of test tubes like (0.1, 0.2, 0.3, upto 1.0ml) was prepared for determining the reducing sugar level of paddy straw. All these tubes were treated with 2ml of Anthrone reagent and kept in a boiling water bath for 5 minutes to develop blue colour. The absorbance of each test tube was measured at 620nm.

ANAEROBIC FERMENTATION OF REDUCING SUGAR

The culture inoculated with *Pseudomonas* sp. was autoclaved to determine the reducing sugar level. Then 1g of yeast pellets were added to the sterilized medium and incubated at 37^0C for 5 days under anaerobic condition.

DISTILLATION AND CONFIRMATION OF BIOETHANOL

The fermented samples were filtered using muslin cloth and distillated at 60^0C using soxhlet apparatus [4]. After distillation, the bioethanol was confirmed by various tests such as pH paper, Lucas test, Iodoform test, Sodium hydrogen carbonate test, Potassium dichromate method.

CHARACTERIZATION BY FTIR

Fourier Transform Infra Red Spectroscopy is one of the most instrumental methods used to elucidate the compositions and molecular structures of organic polymeric compounds. The components were analyzed by FTIR (BRUKER- K720, FTIR- ALPHA, ECO-ATR, German model).

RESULT AND DISCUSSION

Morphological and Biochemical characteristics of *Pseudomonas sp.*

Pseudomonas sp. showed pink colour colonies after Gram staining, so called Gram negative bacteria. The results of biochemical tests showed positive for Catalase, Nitrate reduction, Oxidase, Citrate tests and negative for Methyl red, Voges Proskauer and Urease tests and represented in (Table 1).

Table 1: The morphological and biochemical characteristics of *Pseudomonas sp.*

Tests for identification of bacteria	***Pseudomonas sp.***
Morphology	**Rod shape**
Gram staining	**Gram negative**
Citrate utilization	**Positive**
Urea test	**Negative**
Methyl red test	**Negative**
Voges Proskauer Test	**Negative**
Catalase Test	**Positive**
Oxidase Test	**Positive**
Indole production Test	**Negative**
Nitrate reduction test	**Positive**

REDUCING SUGAR ANALYSIS

In the present study, the amount of bioethanol in reducing sugar value is found that ≈500ug/ml as the OD value of the sample (1.7) corresponded with the standard value (0.7) of ethanol.

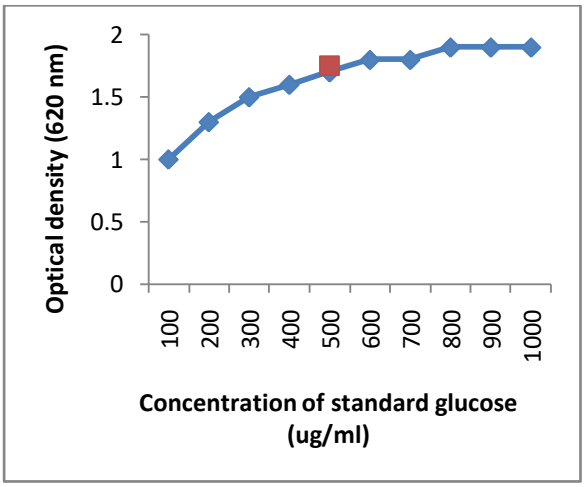

Fig 1: Plot for concentration of glucose sample Vs. Absorbance value

Confirmatory tests for ethanol

The pH paper, Lucas test, Sodium hydrogen carbonate, Iodoform test and Potassium dichromate test were confirmed the presence of ethanol. In Lucas test, the visible formation of turbidity indicated as a positive result for primary alcohol. The disappearance of brown colour indicated that a positive result for Iodoform test. In sodium hydrogen carbonate test, the effervescence formation showed as a positive. The appearance of green colour indicated the presence of ethanol in Potassium dichromate test.

FTIR ANALYSIS

The highest yield of bioethanol was subjected to FTIR spectroscopy. From the result, it has been inferred that the bonding nature and the peak formation of bioethanol almost resemble the standard as the strong and broad peak appeared at 3000 – 3500 cm, which indicates $–CH_2$ and $– CH_3$ stretching vibrations. The peak value depicted the presence of bioethanol and given in figure 2.

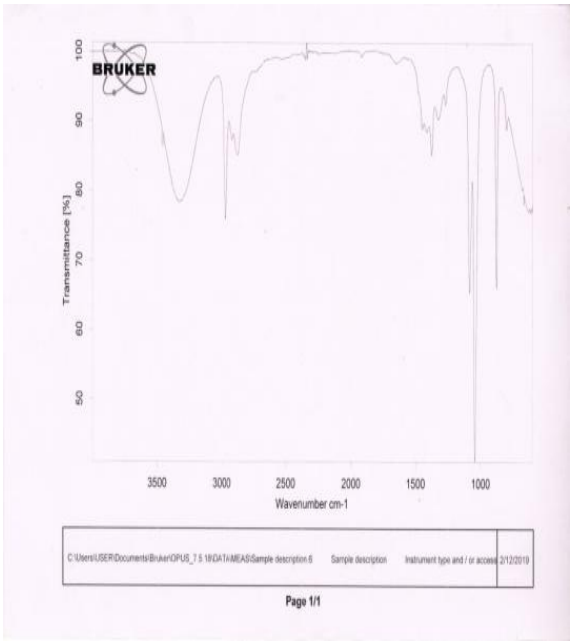

Fig 2: FTIR – ATR Result of Bioethanol obtained from rice straw

CONCLUSION

The population of human being is increased every year, hence the demand for energy source also increased. Vijayaraghava *et al.*, (2014) reported that, the level of reducing sugar concentration

in the sample of waste paper was 140mg/L. In the current research found that the reducing sugar value is high as (500mg/L) compared to the previous work. But in recent days, the usages of genetically modified microorganisms act as a good manipulator for this work and also able to get more and pure form of ethanol within few days without any further treatment. Currently, the predominant bioethanol is generally used blended with gasoline to reduce the usage of conventional fuel. This study suggested that the production of bioethanol from the sustainable raw material handling could be further improved by various technologies to satisfy the demands.

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REFERENCES

1. Abdi Hanra sebayang, Masjuki Haji Hassan, Hwai Chyuan Ong,Surya Dharma, Kusumo, Optimization of Reducing Sugar Production from Manihot glaziovii Starch Using Response Surface Methodology, *Energies -MDPI*, 2017.
2. Binod, P., Sindhu, R., Singhaniar, R., Vikram, S., Devi, L., Nagalakshmi, S., Kurein, N., Sukumaran, K.., Pandey, A., Bioethanol production from rice straw, *Bioresource technology*, 101, 4767-4774, 2009.
3. Heinrich Michael, Bremner Paul, Plants as medicines, *Research Gate*, 205 -238, 2005.
4. Hossain Zabed, Golam Faruq, Jaya Narayan Sahu, Mohd Sofian Azirun, Rosli Hashim, Amru Nasrulhaq Boyce, *The Scientific World Journal*, 1- 11, 2014.
5. Kundu, K.S., Kumari, S., Paddy as a substrate for Ethanol production, *Indian Journal of Microbiology*. 47, 26-29, 2007.

6. Muhammed Shuaib, Malikbadshah, Syed waqa shassan, Muhammed Faruq Hussain, Chaudery, Recent progress in Bioethanol production from lignocellulosic material, *International Journal of Green Energy*, 13(14), 1413-1441, 2016.

7. Noura EI- Ahmady EI- Naggar, Sahar Deraz and Ashraf Khalii, Ethanol production from lignocellulosic feedstocks based on enzymatic hydrolysis: Current status and developments, *Asian Network for Scientific Information*, 13(1): 1-21, 2014.

8. Rabah, A.B., Utilization of millet and Guinae corn husk for bioethanol production, *African Journal of Microbiology*, 5(31), 5721-5724, 2011.

9. Rosane Freitas, Ivanimaria, Karina, Teixeira. Ethanol production from deproteinized cheese whey fermentations by co-cultures of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. *African Journal of Microbiology Research*, 7(13), 1121 – 1127, 2013.

10. Sunil kumar, T., Anwar, Ashok kumar, Jogindersingh Duban. Bioethanol production from starchy part of tuberous plant (potato) using *Saccharomyces cerevisiae* MTCC -170. *African Journal of Microbiology*, 7(46), 5253-5260, 2013.

11. Vijayaraghava, Marimuthu, Rajashekaran, Synthesis of Bioethanol by *Saccharomyces cerevisiae* using lignocellulosic hydrolysate from pretreated waste paper fermentation, *International Journal of chemtech research*, 6(11), 4904-4912, 2014.