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Extraction of bioethanol from plant leaves

S. Sharmila*, L. Jeyanthi Rebecca, Ankush, Satadru Dutta Ray and E. Kowsalya

Department of Industrial Biotechnology, Bharath University, Chennai, Tamil Nadu

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ABSTRACT

Ethanol is a clear liquid alcohol that is made by the fermentation of different biological materials. This alcohol is known to have many uses, but one in particular is becoming more popular. Ethanol, the most widely used biofuel. There are many sources used for the production of ethanol. The main of this study was to find out new source for ethanol production and to optimize the various factors to increase its concentration. The production of bioethanol involves two processes which are enzymatic breakdown of starch into glucose and fermentation of glucose by yeast. Leaves of Pongamia pinnata were collected from Bharath University campus, Selaiyur Chennai, Tamil Nadu. Qualitative confirmation of ethanol was checked by litmus test, ester test and iodoform test. Maximum ethanol concentration was found to be 1.52M at 26^{o}C and for 24hrs fermentation at 5.7 pH.

Keywords: Bioethanol, *Pongamia pinnata*, pH, temperature and time.

INTRODUCTION

Ethanol or ethyl alcohol has existed since the beginning of recorded history. The ancient Egyptians produced alcohol by naturally fermenting vegetative materials. Also in ancient times, the Chinese discovered the art of distillation, which increases the concentration of alcohol in fermented solutions. Ethanol was first prepared synthetically in 1826, through the independent effort of Henry Hennel in Britain and S.G in France. Michael Faraday prepared ethanol by the acid-catalyzed hydration of ethylene in 1828, in a process similar to that used for industrial synthesis of ethanol today. The most common substrate used for nearly 99% of ethanol production in the United States today is starch from agricultural crops, primarily corn [1]. The evolution of new biofuel production technologies could help alleviate some of the concerns regarding the use of food for fuel by facilitating the use of non-food feedstock's, and could alleviate some of the environmental concerns associated with grain ethanol production. In particular, cellulosic ethanol is believed to hold great promise in this regard, even though there are currently no commercial scale plants in the United State [2]. According to Atchison and Hettenhaus [3], over 240 million dry tons of corn Stover is produced each year in the United States. Brechbill and Tyner [4] found through research that corn Stover collection risk soil loss from wind erosion and runoff from water erosion depending on the amount of corn Stover collected [2]. However, as cellulosic ethanol technologies advance the use of organic content of the municipal solid waste as a transportation fuel feedstock and simultaneously reduce externalities associated with waste disposal.

MATERIALS AND METHODS

Collection of samples

The samples *Pongamia pinnata* were collected from the Bharath University campus, Selaiyur Chennai, Tamil Nadu and where identified by Dr. Narasimhan, Head, Department of Botany, MCC, Chennai, Tamil Nadu.

Preparation of samples
The leaves of *Pongamia pinnata* were collected and were wiped with cotton dipped in ethanol to clean the surface and were ground into a fine powder.

Production and extraction of bioethanol
Ethanol was produced by hydrolyzing plant leaves by α-amylase then fermented using yeast. After fermentation, ethanol was extracted using phosphate buffer.

Confirmation test
Litmus test, iodoform test and ester test were performed to confirm the presence of ethanol [5].

Optimization of Time
Fermentation was carried out at different time intervals such as 24 hours, 48 hours, 72 hours, 96 hours and 120 hours with each of the three samples keeping the sample weights constant.

Optimization of Temperature
Fermentation was carried out at different temperature such as room temperature of 26^0C and controlled temperatures in incubator (32^0C), and refrigerator (2^0C).

Optimization of pH
The pH was optimized by taking ten grams of sample for each of the three different plants and was maintained at acidic pH of 4, neutral pH of 7 and alkaline pH of 9 by 0.1N Sulphuric acid and 0.1N Sodium hydroxide.

RESULT AND DISCUSSION

In this study, ethanol was extracted from the yeast fermentation of *P.pinnata* and the presence of ethanol was confirmed by litmus, ester and idoform test (Table.1). Results revealed that the concentration of extracted ethanol was maximum at 24hrs of fermentation.

Table.1 Confirmation test for ethanol

Time	Ester	Iodoform	Litmus Test
24	+	+	+
48	+	+	+
72	+	+	+
96	+	+	+
120	+	+	+

Table.2 Effect of time

Sl.No	Time (hrs)	Concentration of Ethanol (M)
1	24	1.52
2	48	1.44
3	72	1
4	96	0.7
5	120	1.26

The optimum temperature for maximum concentration of ethanol was found to be room temperature (26^oC). which was coincide with the results of Hossain and Fazliny [6] who extracted bioethanol from pine apple waste (30^oC) (Table.3)

Table.3 Effect of temperature

Sl.No	Temperature (^{o}C)	Concentration of Ethanol (M)
1	RT (26^0C)	1.52
2	2^0C	1
3	32^0C	1.2

The pH is one of the most important factors for any fermentation processes and depends upon microorganisms because each microorganism possesses pH range for its growth and activity. The ethanol concentrations of the peak valued samples were optimized for pH and were kept at acdic pH of 4, neutral pH and alkaline pH of 9.

Table.4 Effect of pH

Sl.No	pH	Concentration of Ethanol (M)
1	4	0.72
2	7	0.7
3	9	0.8
4	5.7	1.5

It was observed that the highest ethanol concentration was found at acidic pH 5.7 (1.5M) (Table.4) which satisfies the results obtained by various researchers. Fadel [7] reported that high ethanol production was obtained by using initial pH of 5.0-6.0. Graves et al., [8] observed that no ethanol production exist lower than pH 4.0.

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