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RESEARCH ARTICLE

PRODUCTION OF BIO-ETHANOL BY ANAEROBIC FERMENTATION OF GREEN TOBACCO LEAVES (*NICOTINA TOBACCUM*) BY USING *SACCHAROMYCES CEREVISIAE*

[1]Vaishali A. Gargade, [1]Nilesh. R. Yemul, [1*]Nitin. N. Bolabatin, [1]Prathusha. S. Chegu and [2]Dilip G. Kadam

[1]Walchand College of Arts and Science, Solapur, Maharashtra- 413006
[2]DBF Dayanand College of Arts and Science, Solapur, Maharashtra- 413002

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ABSTRACT

Tobacco (*Nicotina tobaccum*) can generate a large amount of inexpensive biomass more efficiently. The current research is focused on using green tobacco as a feedstock for ethanol production. Acid pretreatment of green tobacco leaves increases efficiency of saccharification by releasing high amount of sugar monomers from complex polymer. It is easiest way to produce bio-ethanol from green tobacco leaves by using *Saccharomyces cerevisiae* as there is no need of any additional supporting media.

INTRODUCTION

Bioethanol is a renewable energy source made by fermenting the sugar and starch obtained from plant such as sugarcane and crops like grain by using yeast. Today, it is blended with petrol to make a truly sustainable transport fuel, it's used in cosmetic and other manufacturing processes, and it creates the clean burning, beautiful dancing flame with heat, steam and carbon Dioxide. Carbon dioxide is absorbed by plants. It is then processed via. photosynthesis to help the plant growth. This infinite cycle of creation and combustion of energy makes bio-ethanol a carbon neutral fuel source. Ethanol has been made since ancient times by the fermentation of sugars. The fermentation reaction, represented by the simple equation,

$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$

The global production of ethanol in 2011 was 84.5 billion liters representing about 4% of the global gasoline consumption (Lichts, 2012). The United States is the largest producer of bio-ethanol, accounting for nearly 47% of global bio-ethanol production, followed by Brazil (37%). About 60% of global bioethanol production comes from sugarcane and sugar beet.

****Corresponding author: Nitin. N. Bolabatin,***
Walchand College of Arts and Science, Solapur, Maharashtra-413006

Brazil exclusively uses sugarcane for bio-ethanol production while the United States and Europe mainly use starch from corn (USA), and from wheat and barley (Europe). The concept and advantages of tobacco biomass for ethanol production have been proven by a Virginia start-up company, Floyd Agricultural Energy Cooperative Ltd., in the early 1980's during the surge of oil prices caused by an Organization of Petroleum-Exporting Countries embargo (Andrianov et al, 2010). Tobacco (*Nicotina tobaccum*) can generate a large amount of inexpensive biomass more efficiently similar to other agricultural wastes.

MATERIALS AND METHODS

Collection and pretreatment of fresh, healthy Tobacco leaves

Fresh green tobacco leaves were collected from a farm near Solapur city. These leaves (100 gm) were first washed with distilled water to remove all adhering substances. Then shade dried and smashed by grinder into the powder (particle size <1mm) & stored into the air tight container at 4°C. The 10g of tobacco leaves powder was pre-treated with the 4% 300 ml sulphuric acid (H_2SO_4) & autoclaved at 121°C, 15 lb for 60 min. After pretreatment, sample was neutralized with 1N

Sodium hydroxide (NaOH) followed by washing with tap water and dried at 70°C for six hours & stored at 4°C.

Enzymatic saccharification of tobacco leaves powder

Then the 5 gm of pretreated tobacco leaves powder was taken in 250 ml of distilled water and 2.5 mg of Unienzyme tablet containing alpha-amylase was added in it. The mixture was heated at 90ºC in a water bath for one hour saccharfication and cooled to room temperature. After cooling, pH of the mixture was adjusted to 5.4 with phosphoric acid and 5 mg of Unienzyme containing glucoamylase was added in it. Then it was heated at 60 ºC for 90 min for further saccharification (Liimatainen *et al*., 2004). Then the final mixture was centrifuged at 6000 rpm for 10 min and 100 ml supernatant was used as hydrolysate for ethanol production.

Estimation of reducing sugar concentration from pre-treated tobacco leaves powder

The estimation of reducing sugar in pretreated leaves powder hydrolysate was carried out by 3, 5 – dinitrosalicylic acid (DNSA) method (Miller, 1959). Standard glucose solution (500 µg/ml), distilled water and DNSA were added in a test tube and kept in boiling water bath for 5 min. After cooling, optical density of each sample was measured at 530 nm on colorimeter.

Preparation of inoculums

Two gram of Baker's yeast (*Saccharomyces cerevisiae*) was added into the 50 ml of nutrient broth and kept in an incubator at 28ºC for 48 hours. After incubation the broth was centrifuged and pellet was used as inoculum for fermentation.

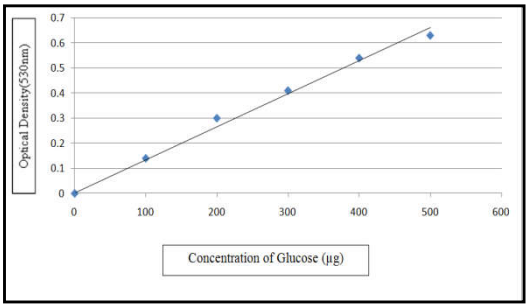
Fermentation of pretreated tobacco leaves powder

The inoculum was added aseptically into 250 ml Erlenmeyer flask containing 100 ml of saccharified hydrolysate and plugged it with cotton followed by pouring paraffin wax on it. It was covered with aluminum foil and kept for anaerobic respiration of *Saccharomyces cerevisiae*. The fermentation was continued up to 10 days.

After completion of fermentation, the fermentation medium was filtered through muslin cloth. The obtained filtrate was subjected for estimation of alcohol concentration by potassium dichromate method (William's *et al*, 1950).

RESULTS AND DISCUSSION

Bioethanol is an ideal fuel to substitute for petrol. The production of ethanol by fermentation received special attention to search alternative biomass sources apart from traditional sources like molasses, starchy crops. In this study the tobacco leaves are used for production of bioethanol by using *Saccharomyces cerevisiae*. The 10 gm of tobacco leaves powder was pretreated with 4% sulphuric acid (H_2SO_4) which enhances saccharification process. Fermentation is carried out at temperature 28ºC, pH 5.5 and 10 days of incubation in anaerobic conditions. The alcohol concentration in the filtrate of fermented broth was estimated by potassium dichromate method (William's et.al 1950). According to this method the concentration of alcohol was 2.5 %. Tobacco leaves contains 20 mg of reducing sugar per 1 gm which was utilized for production of bioethanol.



Graph 1. Concentration of Glucose vs. O.D (530nm)

Table 1. Estimation of Reducing Sugar Concentration (Miller, L.G. 1959)

Std. Glucose Solution: 500µg/ml

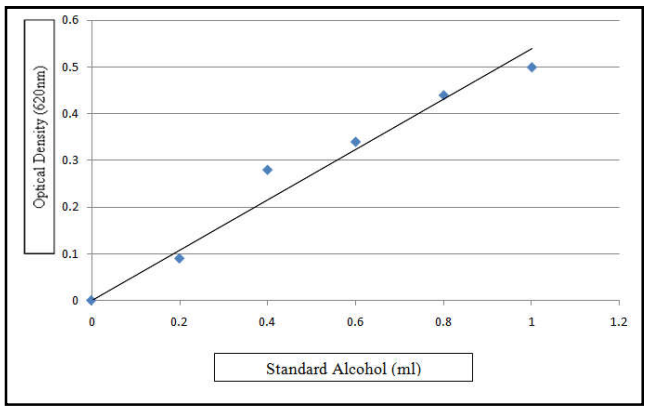
Sr. No.	Std. Glucose Solution (ml)	D/W (ml)	Conc. Of Glucose(µg)	DNSA (ml)		O.D (530 nm)
1	0.0	1.0	000	2.5	Keep in boiling water bath for 5 mins. & cool it.	0.00
2	0.2	0.8	100	2.5		0.14
3	0.4	0.6	200	2.5		0.30
4	0.6	0.4	300	2.5		0.41
5	0.8	0.2	400	2.5		0.54
6	1.0	0.0	500	2.5		0.63
7	1.0	0.0	-	2.5		0.55

Table 2. Alcohol Estimation (William's et.al 1950)

Sr. No	Std. Alcohol (ml)	Distilled water (ml)	Potassium dichromate (ml)	Conc. Sulphuric acid (ml)	O.D (620nm)
1	0.0	1.0	4	1	0.00
2	0.2	0.8	4	1	0.09
3	0.4	0.6	4	1	0.28
4	0.6	0.4	4	1	0.34
5	0.8	0.2	4	1	0.44
6	1.0	0.0	4	1	0.50
7	(A)0.5	0.5	4	1	0.52

(A)- Fermented Filtrate

From graph: The concentration of reducing sugar is 400 µg per 1 ml of sample. Thus 5 gm of tobacco leaves 100 mg of reducing sugar, therefore 1 gm leaf contain 20 mg.



Graph 2. Std Alcohol estimation vs. O.D (620 nm)

From graph: Alcohol concentration in sample A is 2.5% in the filtrate of fermentation broth.

Conclusion

From present investigation it was concluded that the green tobacco leaves can be used for the production of bioethanol by using Saccharomyces cerevisiae. As per this study, there is no need of any supporting media for production of bioethanol. Thus, it is possible to get high yield of bioethanol having good quality in affordable conditions. It might serves as an alternative to all petroleum based fuels. By optimization of process parameters may yield maximum production of bioethanol by this method which could be promising technology in future.

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