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Bioethanol production from newspaper waste using microorganisms

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Abstract
In this present study, an attempt was made to reduce the accumulation of solid waste and the production of bioethanol from the wastes i.e., Newspaper. In the bioethanol production process, microorganisms were used instead of commercially available cellulose enzyme which is very expensive. In comparison with various composition, the fermentative medium containing 30g of Newspaper + 400 ml of distilled water + 100 ml of chemically defined medium, showed the better bioethanol production [(1.56 OD at 660 nm) and 1.12 mg/l of ethanol concentration]. The result indicates that the newspaper will be a one of the better source for bioethanol production.

Keywords: Bioethanol production, newspaper waste, microorganisms

1. Introduction
Ethanol is an alcohol-based fuel produced by the fermentation of plant sugars. It can be obtained from many agricultural products and food wastes if they contain sugar, starch or cellulose, which can then be fermented and distilled into ethanol. In Brazil, which is the largest ethanol producer, ethanol is produced from sugarcane (Pandeya. 2009) [5]. Ethanol represents closed carbon dioxide cycle because after burning of ethanol the released CO_2 is recycled back into plant material because plants use CO_2 to synthesize cellulose during photosynthesis cycle (Brooks 2006 and Wyman, 1999) [1, 8].
In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources (Wyman, 1990) [7]. Ethanol derived from biomass in the only liquid transportation fuel that does not contribute to the green house gas effect (Foody, 1988) [2]. Bioethanol production from non edible lignocellulosic biomass such as wheat straw, rice straw, bagasse, corn stover, wood, peels of fruits and vegetables is attracting keen interest. (Latika Bhatia, *et al*., 2012) [4]. In this present studies, waste papers like Newspapers were used as a substrate for the production of ethanol. The Newspaper contains 12% of cellulose, 61.3% of hemicelluloses and 9.8% of lignin (Kim & Moon, 2003) [3].

2. Materials and Methods
The fermentative production of bio ethanol was carried out in two types: (i) Saccharification (ii) fermentation. In our study an attempt was made to design an economical process by the use of intact fungal organism as a source of cellulase enzyme instead of commercially available enzyme. *Aspergillus niger* grows on the cellulosic substrates and hydrolyzes cellulose of the substrate and release simple sugars which can be fermented to produce bioethanol.

2.1 Pretreatment of Substrate
The size of the Newspaper was made into 2 x1cm and the substrates were treated chemically with 1% NAOH for a period of 2 hours.

2.2 Isolation and Identification of *Aspergillus niger* for Saccharification
The fungal culture *Aspergillus niger* was screened from 1g of soil sample of groundnut field in Nagarpuram. Soil sample was added with 99ml of distilled water. The sample was serially diluted and inoculated into Potato Dextrose Agar (PDA) by spread plate technique. The plates were incubated at 30 ºC for 72 hours until the mycelium sporulates black conidia.

The fungi were morphologically identified by using microscope. Inoculum was produced in 250 ml conical flask containing 100 ml Potato dextrose broth by transferring 2 discs from the PDA plates. The flask was incubated for another 72 hrs at 30ºC till the mycelial mat develops. This mycelial mat was used as inoculums in further saccharification experiments.

2.3 Preparation of *Saccharomyces cerevisiae* culture for fermentation

The yeast *Saccharomyces cerevisiae* pellets were sporulated by using hot water and inoculated into the PD broth and then culture was ready to use for fermentation.

2.4 Fermentation of substrate

In order to optimize bioethanol production the substrate were taken in three different variations the following manner (Table -1). Chemically defined medium(CDM) [L-glutamic acid-0.06 g, Sodium nitrate-0.28 g, Potassium phosphate-0.4 g, Calcium choloride-0.06 g, Magnesium sulphate-0.06 g, Proteose peptone-1.5 g, Ferrous sulphate-1.00g, Manganese sulphate-1.00g, Zinc sulphate-0.32 g, Tween 80-4ml] was used in this experiment.

Table 1: Experimental Setup for fermentation

S. No.	Newspaper			White Paper
1	10g of substrate + 200ml of distilled water (T1a)	20g of substrate + 300ml of distilled water (T2a)	30g of substrate + 400ml of distilled water (T3a)	10g of substrate + 100ml of distilled water (T4a)
2	10g of substrate + 200ml distilled water +0.5ml lactose (T1b)	20g of substrate +300ml of distilled water+0.5ml lactose (T2b)	30g of substrate+400ml distilled water+0.5ml lactose (T3b)	10g of substrate +400ml distilled water+0.5ml lactose (T4b)
3	10g of substrate + 200ml of distilled water +100ml of chemically defined media (T1c)	20g of substrate + 300ml of distilled water+100ml of chemically defined media (T2c)	30g of substrate + 400ml of distilled water+100ml of chemically defined media (T3c)	10g of substrate + 100ml of distilled water+100ml of chemically defined media (T4c)

* All the flasks were autoclaved at 15lbs for 15minutes.

Saccharification was carried out in stationary and shaking methods for a period of six days at 28ºC using *Aspergillus niger*. *Aspergillus niger* grows on the cellulosic substrates and hydrolyzes cellulose of the substrate and release simple sugars which can be fermented to produce bioethanol. After six days of saccharification mycelial mat of *Aspergillus niger* was removed under aseptic conditions and 10% of *Saccharomyces cerevisiae* was added to all the flasks. The process was carried out for a period of six days at 28ºC. After fermentation process samples were taken for the estimation of bioethanol by potassium di chromate method.

3. Results and Discussion

The ethanol content of the sample collected from all the setup was estimated for six days by optical density and the concentration of ethanol was calculated and tabulated (Table-2).

Table-2: Concentration of ethanol in the fermentative medium on 6th Day

Setup	Amount of substrate	Medium supplemented with	OD	Conc. of ethanol in sample (mg/l)
I set	10g of substrate (Newspaper) + 200ml of distilled water	H_2O (T1a)	0.292	0.21
		Lactose (T1b)	0.280	0.20
		CDM (T1c)	0.223	0.16
II set	20g of substrate (Newspaper) + 300ml of distilled water	H_2O (T2a)	0.446	0.32
		Lactose (T2b)	0.361	0.26
		CDM (T2c)	0.436	0.31
III set	30g of substrate (Newspaper)+ 400ml of distilled water	H_2O (T3a)	0.716	0.51
		Lactose (T3b)	1.68	0.21
		CDM (T3c)	1.56	**1.12**
IV set	10g of substrate (White paper) +100ml of distilled water	H_2O (T4a)	1.0	0.72
		Lactose (T4b)	0.730	0.52
		CDM (T4c)	0.324	0.23

In comparison with all the other treatments, T3c (30g of Newspaper + 400 ml of distilled water + 100 ml of chemically defined medium) showed the better result [(1.56 OD at 660 nm) and 1.12 mg/l of ethanol concentration]. This result indicates newspaper will serve as a better alternative substrate for bioethanol production.

4. Conclusion

In this present study, an attempt was made to reduce the accumulation of solid waste and the production of bioethanol from the wastes i.e., Newspaper. In this bioethanol production process, the cellulolytic and fermentative microorganisms were used instead of commercially available cellulose enzyme which is very expensive. The results indicate that the newspaper will be a one of the better source for bioethanol production.

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