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To study the optimization of bio-ethanol production from agronomic wastes by using trichoderma isolates

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ABSTRACT
This research was aimed at bio-ethanol production by fungi capable of producing cellulases and to convert pre-treated lingo-cellulosic material to fermentable sugars. The lingo-cellulosic material such as sugarcane bagasses, sugarcane leaves, rice husk or wheat bran were used as substrates. Fungi were isolated from soil samples collected from various regions. The pure cultures were screened for the ability to degrade cellulose. The fungi capable of cellulose production were identified as *Trichoderma sp* based on colony characters, microscopic observation and identification. The substrates were powdered and pretreated with fungal isolates using Mandels' and Reese media. The substrates were used as a carbon source. Then optimization studies were carried out by using five bio-mass substrates at different pH, temperature and incubation period. Analysis was done by using Gas Chromatography. Sugarcane bagasses, Juice waste, Rice husk, Wheat bran, and Dry leaves were treated with *Trichoderma* isolates. Sugarcane bagasse and juice waste have shown highest concentrat ion of reducing sugars of 45.95 mg/g and 40.56 mg/g respectively and ethanol yield of 51.15 % and 46.5 % respectively. Dry leaves, Wheat bran and Rice husk have shown less reducing sugars of 33.32 mg/g, 30.32 mg/g, and 29.45 mg/g and ethanol yield 11.1 %, 7.15 %, and 6 % respectively as compared with sugarcane bagasse and juice waste.

Introduction

Long-term economic and environmental concerns have resulted in a great amount of research in the past couple of decades on renewable sources of liquid fuels to replace fossil fuels. Burning fossil fuels such as coal and oil releases CO_2, which is a major cause of global warming (Yat*et al.*, 2008). Conversion of abundant lingo-cellulosic biomass to bio-fuel as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions (Wyman, 1999). Several reviews have been published on the theme of fuel ethanol production especially from lingo-cellulosic biomass (Lin and Tanaka,2006) Ligno-cellulosic material from different crop residues have been used for conversion to ethanol (Cardona and Sánchez,2007*;* 2008). The major lingo-cellulosic material found in great quantities to be considered, especially in tropical countries, is sugarcane bagasse, the fibrous residue obtained after extracting the juice from sugar cane (*Saccharumofficinarum*) in the sugar production process (Martín *et al.*, 2007) and sugarcane trash, the left-over residue of leaves and tops. The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to bio-fuel. For the conversion of biomass to bio-fuel, the celluloses and hemicelluloses must be broken down into their corresponding monomers (sugars), so that microorganisms can utilize them (Kumar*et al.*, 2009). But these require pre- treatment for obtaining reducing sugars and conversion of the same to ethanol. The various types of pretreatments and efficient microorganisms have been reviewed here.

Material & method:

Isolation & Screeningof cellulolytic fungi *Trichoderma sp* for conversion of agronomic biomass into fermentable sugars, from soil samples: by dilution plate methods & screen on Mandels' and Reese agar medium(Selective media).

Substrate Treatment

Five substrates namely Sugarcane bagasses, Juice wastes, Dry leaves, Rice husk and Wheat bran were collected. Each substrate was powdered and sieved into a 1mm sieve. All wastes were taken and dried in a hot air oven at 100^{o}C for two days. The powder of each substrate was used as carbon source.

Optimization

Optimization of the substrate, inoculation time, pH, and temperature for the production of Bio-Ethanol was carried forward.

Analytical methods:

After spore inoculation, the samples were collected to check ethanol production at regular alternative days .The supernatants were collected and the Bio-ethanol assay was carried out using Gas Chromatography method.

Assay Method:

The sample showing the highest production value, was considered as the best solid substrate. The best solid substrate was selected and used in subsequent experiments for optimization.

Distillation & Ethanol estimation:

The ethanol, produced from the fermentation process was purified by fractional distillation &was estimated by Gas chromatography analysis. Estimation of total carbohydrate, Reducing and Non reducing sugar.

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Table 1: Sugar estimation results

sr. no.	substrate name	before fungal inoculation			after fungal inoculation		
		reducing sugar (mg/ml)	non-reducing sugar (mg/ml)	total sugar (mg/ml)	reducing sugar (mg/ml)	non-reducing sugar (mg/ml)	total sugar (mg/ml)
1	dry leave	0.62	1.07	1.69	33.32	21.02	54.34
2	juice waste	0.88	1.15	2.03	40.56	31.34	71.90
3	rice husk	0.56	0.92	1.48	29.45	18.86	48.31
4	sugarcane bagasse	0.98	1.27	2.25	45.95	30.05	76.0
5	wheat bran	0.51	0.90	1.41	30.32	19.09	49.41

Calculation

$$\text{Ethanol concentration (µL/ 0.2 µL)} = \frac{\text{Area of Sample} \times \text{Vol. of Std Ethanol}}{\text{Area of Std Ethanol}}$$

$$\% \text{ of Ethanol} = 100 - \left\{\frac{\text{Vol. of Control} - \text{Vol. of Sample}}{\text{Vol. of control}} \times 100\right\}$$

Determination of Total Carbohydrate

The carbohydrate content of untreated and pretreated raw material in the culture broth was measured by phenol sulphuric acid method (Krishnaveni*et al.*, 1984) using glucose as standard. The amount of total sugars present in the sample is calculated using the standard curve.

Determination of Reducing Sugars:

Reducing sugars in untreated and pretreated raw material in the culture broth were determined by DNS method (Miller, 1972) with glucose as standard. The amount of reducing sugars present in the sample is calculated using the standard curve.

Determination of Non-reducing Sugars

The concentration of non reducing sugars was determined by taking the difference in concentrations of Total sugars and reducing sugars.

Non-reducing sugar = (Total sugar – Reducing sugar)

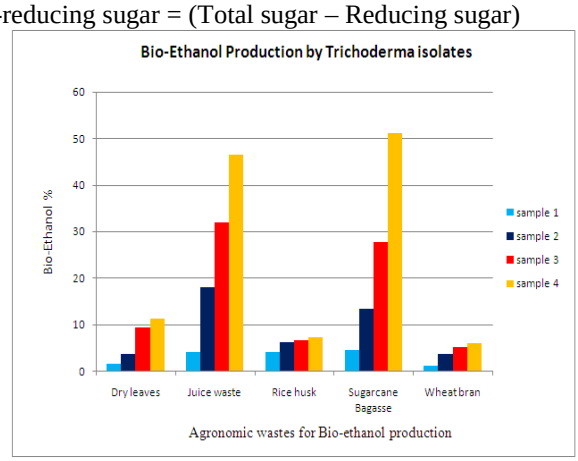

Figure 1: Substrate optimization result analysis

Result & discussion:

Among the five substrates, sugarcane bagasses pre-treated with *Trichodermasp*isolate gave maximum ethanol yield (51.15 %) followed by juice waste pretreated with the same culture 46.5 %. The other substrates (Wheat bran, Rice husk, Dry leaves) pretreated with *Trichodermasp*isolate moderately increased the ethanol content as shown in figure 1. At optimum condition, the bio-ethanol concentration of sugarcane bagasse distilled sample was 87 % at pH 6 and temperature 30 ^{0} C after 13 days incubation. Similar results, (Frain*et al., 1982)*have also obtained by solid state fermentation using*Trichodermareesei*for cellulase production on agro residues around ~13 days incubation. Earlier studies have revealed that fungi required slightly acidic pH for optimum growth. pH is known to affect the synthesis and secretion of cellulase for degradation of cellulose (Ting *et al. 2005*).

Sugar estimation:

Total sugar, reducing sugar, non-reducing sugar content of each substrate was determined using Phenol sulphuric acid method and DNS method respectively. Estimation of sugars was done for untreated and pretreated samples and the concentrations of sugars were compared. Concentration of reducing sugar, non reducing sugar and total sugar of treated samples as compared with the untreated (control) samples is shown in Table 1

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