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CONVERSION OF NATURAL WASTES INTO SUGAR BY *TRICHODERMA HARZIANUM*

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ABSTRACT

The objective of this study was to determine the influence of natural wastes substrates such as rice straw and grasses to produce the high sugar content by *Trichoderma harzianum*. The organism was isolated from soil and identified based on the cultural morphological characteristics and was used for the sugar production. The natural wastes such as rice straw and grasses were subjected to the cellulolytic action of the intact cells of *Trichoderma harzianum* grown in 5% wheat bran medium in presence of glucose as major carbon source. Rice straw and grasses have pH 3, 4 and temperature 28°C, 30°C respectively. In the present study, highest moisture content (90.9%), ash content (21.6%), cellulose (46.6%), carbohydrate (66.6%), protein content (13.6%), total sugar (40.6%) and reducing sugar (40%) were noted in rice straw. Maximum sugar was produced due to the degradation of cellulose present in natural wastes by cellulase producing *Trichoderma harzianum*.

Key words: Rice straw, Grasses, *Trichoderma harzianum,* Sugar, Cellulose, Glucose, Protein and Ash.

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INTRODUCTION

Living organisms consume materials and eventually return them to the environment, usually in a different form, for reuse. Municipal solid waste consists of materials from plastics to food scraps. Generally, the most common waste product is paper (about 40% of the total).Other common components are green waste (yard waste) such as pineapple peels, rice straw, wheat bran, rice bran, maize bran *etc.* (Angenent *et al.*, 2004; Das and Singh, 2004; Haight, 2005; Hamer, 2003; Humpheryet al., 1977; Vanwyk, 2001) plastics, metals, wood, glass and food waste. The agrowaste such as sugarcane bagasse, pineapple peels, rice straw, wheat bran, rice bran, maize bran etc, can be used as the best substrate for sugar production (Pandey et al., 1992).

Trichoderma spp., are free – living fungi that are common in soil and root ecosystems. The chemical composition of rice straw ash is similar to that of many common organic fibers, containing, Cellulose (C5H10O5), a polymer of glucose, Lignin (C7H10O3), polymer of phenol, Hemicellulose, a polymer of xylems, SiO_2, the primary component of ash. Grasses are monocots, and the grass family *Gramineae* The chemical composition of grasses as follows, Cellulose (C5H10O5), a polymer of glucose, Lignin (C7H10O3), polymer, Hemi cellulose, a polymer of xylems, Carbohydrate (CHO), and Starch. These natural waste is converted various useful products such as fermentable sugars, paper making, biofuels, chemicals, cheap energy sources for fermentation improved animal feeds and human nutrients (Chatterton et al., 1986).

From the above said point of view rice straw and grasses is used to best substrate for sugar production. The present study was aimed the high amount of sugar yields from agricultural residue such as rice straw and grasses by *Trichoderma harzianum.*

MATERIALS AND METHODS

Sample collection

Soil samples were collected from an agricultural waste compost area at Thenparai, Thiruvarur district, Tamilnadu.

Isolation and identification of fungi

Fungi were isolated and identified by serial dilution and wet mount technique (Aneja, 2005).

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Collection of natural wastes

The rice straw and grasses were collected from agricultural wastes compost area at Thenparai, Thiruvarur, Tamilnadu.

Determination of the initial moisture content of natural wastes

25gms of rice straw and grasses were placed in an oven at 105°C for 24hrs. The moisture was present and evaporated to a constant weight of the sample. The dried samples were cooled and weighted. By comparison of the two weights the percentage of moisture present in the samples was calculated by using following formula,

$$\% \text{ of age loss} = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100$$

Culture and inoculums

Trichoderma harzianum was grown on solidified potato dextrose agar slants at 8°C on the culture medium. Approximately 10 ml of medium was poured in each tube. All the test tubes were cotton plugged. The medium was sterilized in a pressure cooker at 15psi for 15 minutes. The test tubes were allowed to set for 24hrs to prepare the slants. The slants were inoculated with a sterilized needle loop and incubated at 30°C. The slants were preserved in a refrigerator. The inoculums were prepared and, the slants were washed carefully with sterilized distilled water and thus a spore suspension was obtained. The spores were centrifuged at 2500 rpm for 20 minutes in a sterilized centrifuge tube. The supernatant was discarded and the pellet was suspended in an adequate volume of the sterilized distilled water. The optical density of the suspension was read in a spectrophotometer. The suspension of the same optical density was transferred each time to keep the total population of spores constant 10 to 20 ml of spore suspension was transferred to each of the flasks containing 250 ml wheat bran medium and 30 ml glucose solution (Herr, 1980).

Fermentation medium

Wheat bran was chosen for the growth of *Trichoderma harzianum* as it was considered to be a suitable medium for the production of extracellular cellulose. 250ml of wheat bran medium was taken in five different 500ml of conical flasks. One of these flasks was used as a blank. To each flask then was added 30ml of sterilized glucose solution to make final concentration of glucose 1%.The flasks were cotton plugged and were ready for inoculation.

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Fermentation

Trichoderma harzianum was grown by surface culture technique. The flasks were inoculated using 10ml of inoculum and subsequently incubated in an incubator. The growth temperature was 30°C. After three or four days, when the growth of organisms had started, about 5ml of suspension was taken out with a sterilized pipette. The suspension was filtered, to determine the physico – chemical characteristics such as pH, temperature, cellulose content, ash content, carbohydrate content, protein content, total sugar content, and reducing sugar content.

Determination of pH

The pH was determined by using Ph paper (Farinas, 2010).

Determination of temperature

Temperature was measured by mercury filled Celsius thermometer gratitude from 0°C to 100°C. The surface temperature was measured by dipping the thermometer directly into the sample for about a minute and the temperature was recorded (Farinas, 2010).

Determination of ash content

1ml of fermentation samples (W1) were taken in each flask. Crucible with 5ml of nitric acid and heated continuously at low flame until the material begins to char. After charring the sample, kept in muffle furnace at 650°C for 4hrs and weighed (W2). The formula was used to determine the percentage of ash contents (Ishtiaq Ahmed et al., 2010).

Determination of cellulose content

1ml of fermentation samples (W1) were refluxed with 10ml of 80% acidic acid and 1.5ml of nitric acid for 20 minutes. The mixture was dried at 105°C by using hot air oven. The residue was then transferred to a pre-weighed (W2) dry porcelain crucible and heated at 650°C for 6hrs. After cooling down, it was weighed (W3). The following formula was used to determine the cellulose content (Rodriguez et al., 2005).

Determination of carbohydrate

Various concentration of the working standard solution in a series of the test tube from 0.2 ml to 1ml was prepared (10µg to 100µg). Make up the volume 1ml with distilled water. All the tubes are kept in an ice bath and slowly 5ml of anthrone reagent was added and mixed properly. Green colour was developed and measured the optical density at 620nm. Blank and test also measured optical density at 620nm and plotted the standard graph to determined the carbohydrate content (Hedge and Hofreiter, 1962).

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Determination of protein by Lowry's method

Pipette out various concentration of working standard solution into a series of test tubes and make up the volume to 0.2ml with distilled water (10µl to 100µl). 1ml of the mixed reagent was added to each test tubes and mixed thoroughly allowed to stand at room temperature for 10minutes or longer. 0.3ml of diluted folin – ciocalteau reagent was added rapidly and mixed properly. Then the tubes were incubated for 60mintues at room temperature. Blue colour was developed. Measured the optical density of the standard and test solution at 660nm and plotted the standard graph. Test protein the sample is performed as like the standard and calculated the amount of protein present in the sample (Lowry et al., 1951).

Estimation of total sugar

Phenol sulfuric acid method was used to measure the total sugar in the sample. Various concentration of the sample was taken in the test tubes from 0.2ml to 1ml. Made up t10he volume to 1ml with distilled water. Then 1ml of 5% phenol and 5ml of concentrated H2SO4 was added. All the test tubes were stayed for 10 – 15 min. Then optical density was checked at 550nm to measured colour density (Dubosis et al., 1956).

Estimation of reducing sugar

100ml of the sample were taken and extract the sugars with the hot 80% ethanol twice (5ml each time). Supernatant was collected and evaporated it keeping it on a water bath at 80°C. 10ml of water was added and dissolved the sugars. Pipette out 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard solution into a series of the test tubes. Make up the volume in both standard and sample tubes to 2ml with distilled water. Pipette out 2ml of distilled water in a separated tube to set a blank. 1ml of alkaline copper tartarate reagent was added in each tube. All the tubes are placed in a boiling water bath for 10 min. Then the tubes are cooled and 1ml of arsenomolybdic acid reagent was added in the each tubes. Measured the optical density of the standard and test sample at 660nm and plotted the graph. Test sugar sample is performed as like the standard and calculated the amount of reducing sugar present in the sample (Nelson, 1994).

RESULTS AND DISCUSSION

Fungal species were isolated from soil sample. The strains were identified based on the cultural and morphological characteristics;

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finally the isolated fungal species were confirmed as *Trichoderma harzianum*. This strain is used for enhanced the sugar production from natural wastes. The initial moisture content of natural wastes such as rice straw and grasses were determined. Rice straw contains high moisture content (91.75%) than grasses (84.71%) (Table -1**)**

Table-1: Initial moisture content of the natural wastes

Natural wastes	**Moisture content (%)**
Rice straw	90.9± 0.90
Grasses	86.9 ± 0.005

Values are represented as Mean ± Standard deviation

Before and after inoculation of natural wastes (Rice straw and grasses) pH was noted. Before and after inoculation of natural wastes have pH 4 and pH 3 respectively. Before and after inoculation of natural wastes (Rice straw and grasses) temperature was determined as the temperature 30°C and 28 °C respectively.

In our study correlated with the findings of the variation of sugar concentration during the growth of *Trichoderma harzianum* with the incubation time at 30°C wheat bran medium containing different agricultural wastes due to sugar consumed by the cells and produced as a result of degradation of cellulosic materials (Beguin and Anbert, 1994).

Determination of ash content

Before and after inoculation of natural wastes such as rice straw and grasses ash content was determined. Before inoculation of natural wastes have similar amount of ash content (7.30). After inoculation of natural wastes such as rice straw and grasses determined as 20% and 6.5 %.

Rice straw have high amount of ash content compared than other (Table 2).

Determination of cellulose content

Cellulose content was determined from before and after inoculation of natural wastes .Maximum cellulose content was noted at after inoculation of rice straw (50%) and grasses (48%), compared than before inoculation of natural wastes. Before inoculation of natural wastes (rice straw and grasses) have similar amount of cellulose (39.4%) (Table 2).

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Table – 2: Determination of ash and cellulose Content

Substrates	Before inoculation		After inoculation	
	Ash %	Cellulose %	Ash %	Cellulose %
Rice straw	7.4±0.07	41.3±0.07	21.6±0.1	46.6±0.14
Grasses	7.4±0.07	41.3±0.07	5.3±0.07	44.3±0.07

Values are represented as Mean ± Standard deviation

In our study similar to findings of the sugar concentration subsequently rises incase of all natural wastes. This rise may be due to the degradation of cellulose present in the natural wastes by the cellulose produced by *Trichoderma harzianum* (Rafiq khan and Bursha Perveen, 2010).

Determination of carbohydrate content

Carbohydrate content was determined from the rice straw, grasses and before inoculation of natural wastes fermentation. Carbohydrate content from rice straw (60%) and grasses (40%) have high amount. Carbohydrate content from before inoculation of natural wastes fermentation have lowest amount (30%) compared than after inoculation of natural wastes. The results were presented in the Table – 3.

Determination of protein content

Before inoculation of natural wastes such as rice straw and grasses have similar amount of protein content (5.07). After inoculation of rice straw and grasses it was determined as 10.5% and 7.5% respectively. Maximum amount of protein content was noted as rice straw (Table 3).

Table – 3: Determination of carbohydrate and protein content

Substrates	**Before inoculation**		**After inoculation**	
	Carbohydrate %	Protein %	Carbohydrate %	Protein %
Rice straw	30.6 ± 0.42	6.3± 0.07	66.6 ± 0.14	46.6±0.14
Grasses	30.6 ± 0.42	6.3± 0.07	38.6 ± 0.14	6.6±0.14

Values are represented as Mean ± Standard deviation.

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Determination of total sugar content

Sugar was estimated from the before inoculation and after inoculation of natural wastes. Maximum sugar production was noted at after inoculation of rice straw (30%) compared than other Results were presented in the Table 4.

Determination of reducing sugar content

In this study, reducing sugar was estimated from the before inoculation of natural wastes and after inoculation of natural wastes. Maximum reducing sugar production was noted at after inoculation of rice straw (38%) compared than other. Results were presented in the Table 4.

Table – 4: Determination of total sugar and reducing sugar content

Substrates	Before inoculation		After inoculation	
	Total sugar %	Reducing sugar %	Total sugar%	Reducing sugar %
Rice straw	7.3±0.07	6.3±0.07	40.3±0.14	40±0.07
Grasses	7.3±0.07	6.3±0.07	23.3±17.7	30.9±0.14

Values are represented as Mean ± Standard deviation

In our study similar to the findings of leaf waste consists of three basic polymers; cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses should be provided as precursor for fermentable sugars. Different pretreatments were applied to increase the degradation activity of the from *cellulomonas spp*. The highest reducing sugar concentration was obtained from the leaf waste (Sanchez and Cardona, 2008).

Biological digestion of rice straw and grasses during the growth of organisms

At the end of fermentation the natural wastes such as rice straw and grasses were removed, and weighed. The initial and final weights were compared to determine the percentage of age loss. Among this study, highest percentage of age loss was noted at rice straw (94.59%) and the lowest percentage of age loss was noted at grasses (90.33%). Among this study, the results were represented as the maximum amount of ash, cellulose, protein, carbohydrate, total sugar, and reducing sugar content also noticed at after inoculation of rice straw.

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CONCLUSION

Finally it was concluded that *Trichoderma harzianum* strain produced high levels of cellulose enzyme. This enzyme degrades the cellulose to form fermentable sugar. In this study, indicated that all the wastes showed some tendency to degradation, but rice straw was the substrate as it exhibited the highest loss in weight, during the fermentation. It indicates remarkable cellulose degradation into sugar by *Trichoderme harzianum* from rice straw and grasses an agricultural by - product which has sufficient amount of sugar (38%), used as an alternative energy source for microorganisms, which is renewable, efficient, safe, ideally an inexpensive and abundantly available carbon source. Cellulose is degraded to fermentable sugar through cellulase enzyme. In conclusion attempt was made, to find the optimum fermentation conditions for successful cultivation of *Trichoderma harzianum*, and also towards an enhanced production of cellulase. However, the suitability of the enzymes for biotechnological applications can be investigated through kinetic characterization of the purified enzymes as thermo – stability is a desired characteristic of an enzyme for its possible use in industry.

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