BIO-ETHANOL PRODUCTION FROM FINGER MILLET (RAGI) HUSK BY DIFFERENT PRETREATMENT METHODS, SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

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Introduction: Energy consumption has surged over the past century as the world population has rapidly increased. The depletion of fossil energy resources and concern over CO_2 emission have further shifted the global trend towards developing a new and consistent bio-based resources. Biofuel is a high priority alternative energy source because of rapid depletion of fossil fuel and other environmental issues. (Scheidel & Sorman, 2012). This instigated an attention to produce bio fuels from renewable resources, Ethanol productions from cellulosic materials offer a solution to some of the recent environmental, economic, and energy problems facing worldwide. Nationally, energy costs are on the rise and forecasts of petroleum supply disruptions are once again making news (Srivastava *et al*., 2014)

Finger millet husk is a by-product derived at the time of harvesting of finger millet crop. Finger millet husk is the major agro waste in south India especially in Karnataka, which is cultivated around 7.0-8.0 lakh hectares per year. Which produce large amount of husk which is not used for any purpose at present except for using as a manure, as in many regions burning is the main practice. According to Umakantha (2007), Tumkur district covers an area of 175115 ha., followed by Bangalore Rural 146944 ha., Hassan 115582 ha., Kolar 100903 ha., and Mysore 79533 ha.The bioethanol production involves saccharification for breakdown of cellulose to monosaccharides and conversion to ethanol by the process of fermentation.

Objectives:

- Collection of samples from different district of Karnataka.
- Estimation of total sugar, reducing sugar,
- Applying different pretreatment methods.
- Production of bioethanol.
- Qualitative and quantitative analysis of bioethanol.

Materials and methods:

Sample collection: Finger millet husk collected from 4 different districts of Karnataka namely, Kolar, Bengaluru rural, Tumkur, Hassan. Samples were chopped, sieved, washed and dried, stored for the further use**.**

Analysis of samples: All the 4 samples were estimated for, Reducing sugar by DNS method (miller, 1972), Total sugar by phenol H_2SO_4 method, Cellulose contents by Updegroff (1969) method.

Organisms used: The *Cellulomonas fumi* which is reported for the production of cellulase enzyme is procured from NCIM pune (NCIM 5015) cultured on LB media for the saccharification of husk. The yeast required for the fermentation is isolated from musk melon using YEPDA media.

Saccharification and fermentation: To evaluate the best pre-treatment methods different pretreatments was carried out (physical, chemical and enzymatic) according to Rawinder *et al.,* (2017). Followed by

simultaneous saccharification and fermentation was done according to Cassells *et al.,* (2017). The raw and pretreated lignocellulosic feedstocks were analyzed using Fourier transform infrared spectroscopy (Kim *et al.,* 2013)

Qualitative and quantitative analysis of bioethanol: Determination of ethanol content was done by spectrophotometric method (Caputi *et al* 1968). Quantitative analysis of bioethanol was done by GC (Mushimiyimana *et al.,* 2016).

Results:

Collected samples

Raw finger millet husk collected from Kolar, Bengaluru rural, Tumakur and Hassan districts.

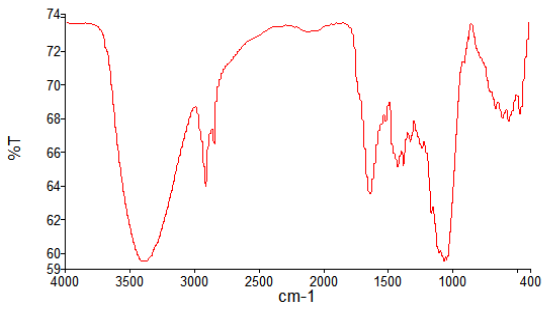
Chopped, sieved, washed and dried finger millet husk.

Analysis of samples

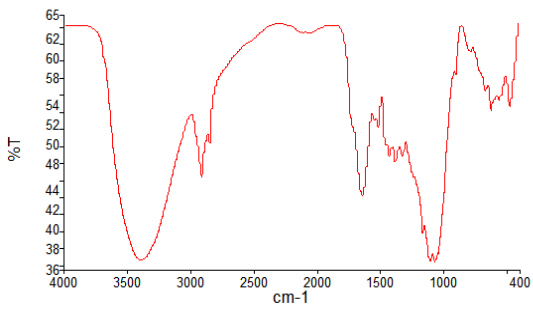
The reducing sugar was found to be highest in Tumakur sample (63.80 µg/ml), lowest in kolar sample (45.20 µg/ml). Total sugar content is maximum in Tumakur sample (19.60 µg/ml), minimum in Hassan sample (9.10µg/ml) and the cellulose content is more in Bengaluru rural sample (150.00 µg/ml), less in Tumakur sample (95.00 µg/ml).

Sl.no.	**sample**	**Reducing sugar** (µg/ml)	**Total sugar** (µg/ml)	**Cellulose** (µg/ml)
1	Kolar	45.20	12.30	135.00
2	Bengaluru rural	47.30	17.20	150.00
3	Tumkur	63.80	19.60	95.00
4	Hassan	59.00	9.10	110.00

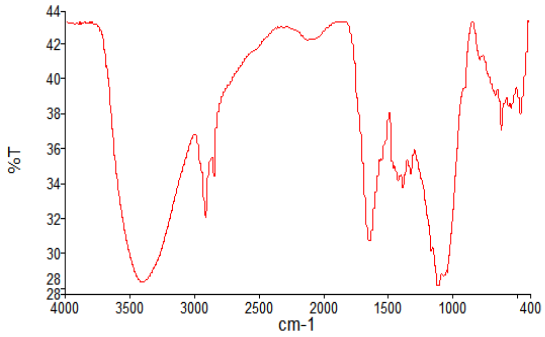
FTIR analysis



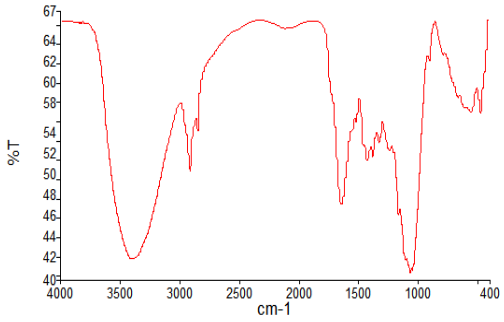
Physical treated



Acid treated



Alkali treated



Enzymatic treated

The FTIR analysis of the Finger millet husk (Tumakur sample) after Saccharification and filteration shows the Region of 1000 – 1200 cm^{-1}, which is commonly used to study the fine structural characteristics of cellulose. The spectra of regenerated cellulose from the ionic liquids and untreated ragi husk are presented.

GC analysis

Saccharification method	% of ethanol
physical	3.0
acid	3.9
alkali	3.6
enzymatic	5.1

The GC analysis confirms that the amount of ethanol was highest in enzymatic treated sample (5.1%), followed by Acid treated (3.9 %), alkali treated (3.6 %) and physical treated (3.0%).

Outcome of the project: One of the largest ago waste in Karnataka is finger millet husk it can be utilized for the production of bioethanol. Pre-treatment of finger millet husk will be a potential option for bioethanol production in the future. Pretreatment strategies help to increase the accessibility of enzymes to the cellulose to convert it into sugars, enzymatic pre-treated (*Cellulomonas fumi)* was the best suited method. The GC analysis confirms that the amount of ethanol was highest in enzymatic treated sample (5.1%), followed by Acid treated (3.9 %), alkali treated (3.6 %) and physical treated (3.0%). The result indicates that enzymatic treated is best suited for ethanol production. As in many regions burning is the main practice. If this huge amount of husk is used by industry as a raw material for bioethanol production it will be helpful.

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