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Production and Quantification of Bioethanol from *Saccharum officinarum* and biowaste materials using *Saccharomyces cerevisiae* in solid and submersed fermentation.

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ABSTRACT :

The raw materials, rotten waste materials and agricultural wastes could be utilized for commercial production of ethanol, as an alternative source of green energy. In this study *Ethanol was extracted from* sugarcane, wood chips, potato, rice, rotten apple to determine their respective and comparative yield and also to determine its fuel composite qualities. The rate of ethanol production in different substrate was found more efficient in submerged fermentation by *Saccharomyces* in comparison to solid fermentation. The aim of the present study is to highlight on major agricultural, industrial and urban waste, which could be used for ethanol production in an ecofriendly and profitable manner. Primarily, the utilization of these wastes for ethanol production will reduce dependency on foreign oil and secondly, this will remove disposal problem of wastes and make environment safe from pollution.

KEYWORDS: Biofuel, Ethanol, Alternate energy resource, Agriculture, Industry, Fermentation.

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INTRODUCTION:

Renewable energy is energy which comes from natural resources such as sunlight, wind, rain, tides, geothermal heat *etc*. It is an alternative to fossil fuels and is commonly called as *alternative energy*. About 16% of global energy consumption comes from renewable with 10% coming from traditional biomass and 3.4% from hydroelectricity [1-3]. New renewable like wind, solar, geothermal, and biofuels accounted for another 3% and are growing very rapidly.

A non-renewable resource is a natural resource which cannot be produced, grown or generated if once depleted and there is no more available for future needs because of their limited amount. These resources have been consumed much faster than nature can create them[4-5]. Fossil fuels (such as coal, petroleum, and natural gas), nuclear power (uranium) and certain aquifers are examples. Eventually natural resources will become too costly to harvest and humanity will need to find other sources of energy.

Bioethanol is made by fermentation of carbohydrates (sugar or starch) produced in crops such as corn or sugarcane [6,7]. However, cellulosic biomass derived from non-food sources such as trees and grasses and biomass wastes are also being used to produce bioethanol. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin from which ethanol can be produced by the hydrolysis and sugar fermentation process [8,9]. The principal fuel used as a petrol substitute or to blend petrol is bioethanol. It is mainly produced by the fermentation of sugar by *Saccharomyces cerevisiae* a species of yeast having been used for baking and brewing since ancient times[10-13].

Sugarcane ethanol is used as fuel for vehicles and is made from sugarcane. Brazil is the World's leading producer of sugarcane ethanol. One of the reasons that sugarcane is a great stock for ethanol is that the sugarcane does not need to be converted into sugar for the fermentation process because it is already naturally rich in sugars. While corn and other bio wastes not rich in sugars, and needs to be treated to get the carbohydrates in the crop to convert into sugars which can be used for ethanol production[14-17]. Even after conversion, corn contains less sugar than sugarcane, making it an inferior feedstock for ethanol production.

Since, sugarcane ethanol is made with one less step than many other types of ethanol, it is much more efficient and it is estimated that this form of ethanol produces eight times more energy than is used to make it, in contrast with other bioethanol. The advantage of bioethanol in general is a renewable source of energy because it can be produced from crops[18]. In addition to being renewable, it has high calorific value, good flammability and produces less emission[19-22]. Its production from bio waste is helpful in treating bio waste and it has commercial value too.

One of the goals of using bio fuels is to contribute with net reduction of greenhouse gases (GHG) emissions and thus not affecting carbon stock negatively in different sub-systems of production, below and above ground biomass (roots, branches and leaves) and in the soil (carbon fixed in clay, silt, sand and organic matter). The ethanol from sugarcane reduces 86% of the GHG emissions when compared to gasoline[23-25]. Another application of ethanol is as a feedstock to make ethers, most commonly ethyl tertiary-butyl ether (ETBE), an oxygenate with high blending octane used in gasoline. ETBE contains 44 percent ethanol [26]. A last application, that we mention here, is the use of ethanol in diesel engines.

MATERIAL AND METHODS:

Sample Collection:

The samples taken were sugarcane, wood chips, potato, rice, rotten apple. All the samples were collected from the local market of Lucknow, Uttar Pradesh, India. The samples are taken as the waste, dumped material, and rotten material. The samples washed thoroughly, air dried, grinded to powder and stored at room temperature in sterile vessels.

Production of Bioethanol:

For submerged fermentation, samples mixed with water (10% w/v), autoclaved and inoculated with *Saccharomyces cerevisiae,* incubated at 37 ^{0}C for 5-10 days before analysis. For solid state fermentation, sterile, moist substrate was supplemented with suspension culture of producer organism before incubation.

Quantification of Ethanol (Ethanol Assay): To quantify ethanol, different concentrations of ethanol solutions (1ml each), 2.0 ml of (0.5 %) potassium dichromate was added followed by addition of 1.0 M sulphuric acid. Tubes were boiled at 80-85° C for 1 hr in water bath. 5.0 ml of DW was added to the tubes after cooling at room temperature and OD was recorded at 600 nm.

Effect of cellulose on production of ethanol: To observe the effect of cellulose on production of ethanol, fermentation of cellulosic wastes was done by co-cultivation of *S. cerevisiae* and *Bacillus megaterium,* a potent cellulose producer.

Effect of various elicitors on ethanol production: Fermentation was carried out under influence of various growth regulators of the producer organism to optimize better fermentation ratio. Different, pH, temperature, organic compounds, metal ions was used to optimized it [27].

RESULT AND DISCUSSION:

Ethanol Production from Different Sources:

Different sources were selected in present study and fermented for ethanol production. After quantification of ethanol by ethanol assay, separate production pattern was observed for all sources.

Table 1. Effect of different carbohydrate source on ethanol production

Sr. No	Samples	Solid (OD)	Submerged (OD)
1.	Sugarcane	0.097 nm	0.123 nm
2.	Woodchips	0.011 nm	0.033 nm
3.	Potato	0.081 nm	0.082 nm
4.	Rice	0.012 nm	0.222 nm
5.	Apple	0.303 nm	0.093 nm

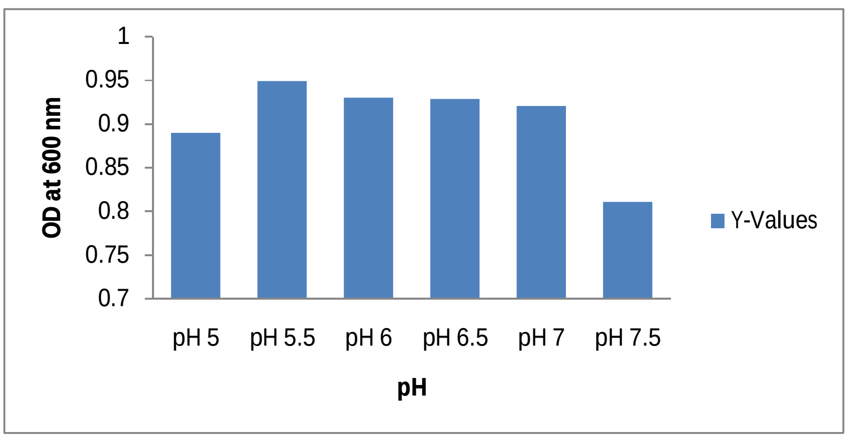
Effect of pH:

To see the effect of pH on ethanol production, different pH such as 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5were maintained before autoclaving the substrate, and after fermentation (5 days) produced ethanol was quantified by ethanol assay. Results are shown in table 4.

Table 2. Effect of pH

pH	O.D at 600 nm
5.0	0.890
5.5	0.949
6.0	0.930
6.5	0.929
7.0	0.921
7.5	0.811

Figure 1. Effect of pH.



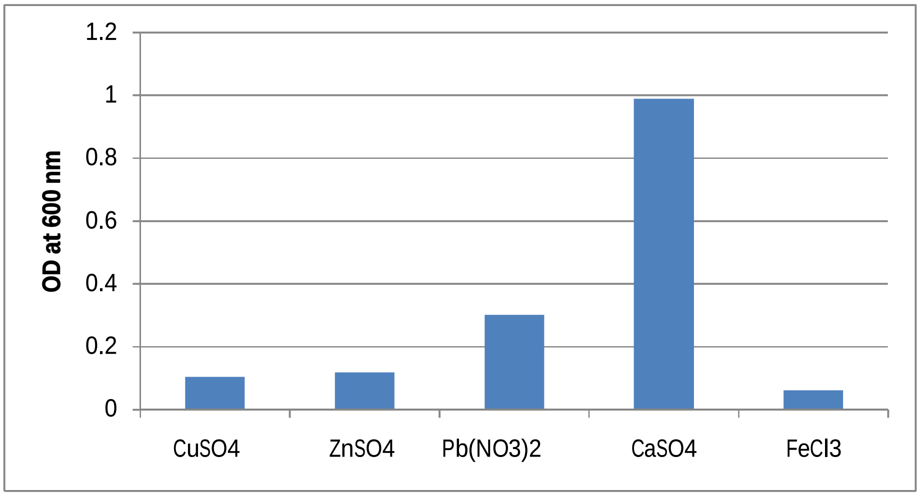
Effect of metal ions:

To see the effect of metal ions on ethanol production, different metal ions such as copper sulphate ($CuSO_4$), zinc sulphate ($ZnSO_4$), lead nitrate $Pb(NO_3)_2$, calcium sulphate ($CaSO_4$), ferric chloride ($FeCl_2$) (0.1% each) were added along with substrate and after fermentation (5 days) produced ethanol was quantified by ethanol assay. Results are shown in table 3.

Table 3. Effect of metal ions

Metal ions (0.1%)	O.D at 600 nm
Copper sulphate	0.105
Zinc sulphate	0.119
Lead nitrate	0.301
Calcium sulphate	0.991
Ferric chloride	0.061

Figure 2. Effect of metal ions.



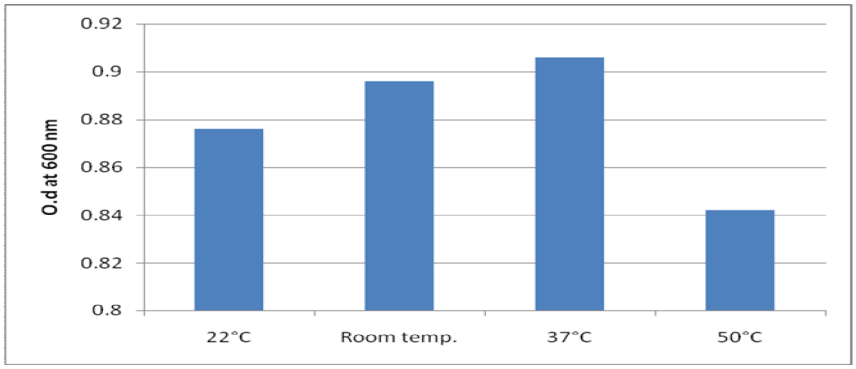
Effect of temperature:

To see the effect of temperature on ethanol production, different temperature 22°C, Room temp., 37°C, 50°C were maintained along with substrate and after fermentation (5 days) produced ethanol was quantified by ethanol assay. Results are shown in table 4.

Table 4. Effect of temperature

Temperature	O.D at 600 nm
22°C	0.876
Room temp.	0.896
37°C	0.906
50°C	0.842

Figure 3. Effect of temperature.



Effect of cellulase on ethanol production:

Cellulase was found to be potent in regard rate of fermentation as there was an increased percentage of ethanol in presence of cellulose producing organism[28-30].

Table 5. Effect of cellulose

Sample	Yield
without cellulase	0.07
with cellulase	0.09

CONCLUSION:

Our data suggest that raw materials, rotten waste materials and agricultural wastes could be utilized for commercial production of ethanol, as an alternative source of green energy. The rate of ethanol production in different substrate was found more efficient in submerged fermentation by *Saccharomyces* in comparison to solid fermentation. Also, the rate of ethanol production was found more positive when samples were inoculated with two microorganisms simultaneously. The cellulase produced by first organism was found potent in providing extra amount of utilizable substrate for fermentation.. Fermentation was slight variable at different pH, different temperature, although calcium ion was found to have great positive impact on fermentation process[31-33]. This data suggest that rate of fermentation could be easily managed and controlled during industrial and commercial production of ethanol as it will require less maintenance and could provide a durable and long term setup for generating green energy.

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