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BIOETHANOL PRODUCTION FROM AGRICULTURAL WASTE MATERIALS

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ABSTRACT

Bioethanol is the alcohol made by Fermentation which is extremely environmental friendly, cheap, pollution free that can be obtained by the use of microorganism using Sweet sorghum, Sweet potato and Grasses as substrates. The fermentation was carried out maintaining the substrate inoculated with the *Saccharomyces cerevisiae* in the specific medium at various pH and with/without salt content such as pH 5, 5.5, 6 and with and without NH_4Cl and $(NH_4)_2SO_4$. Sweet potato as substrate was inoculated with *Trichoderma* and *Saccharomyces cerevisiae* as the sources for the production of Bioethanol. The fermentation was maintained at 37°C which was found to be optimum for the effective production of Bioethanol. Distillation process was done followed by the titration after the Fermentation. The Bioethanol produced was collected in a beaker in the form of vapors which was then titrated against the Sodium thiosulphate using starch as indicator. Finally, the Bioethanol produced using Sweet sorghum as substrate was found to be maximum at 22.5 ml as titre value at pH 5 and found to be minimum at pH 6. The bioethanol produced using Sweet potato as substrate was found to be maximum at 26.5 ml at pH 5 using *Trichoderm*a as microorganism and 23 ml at pH 5.5 using *Saccharomyces cerevisiae* as microorganism.

KEYWORDS: Bioethanol, Sweet sorghum, Sweet potato, Grasses, *Saccharomyces cerevisiae. Trichodermaviridae.*

1. INTRODUCTION

Biofuel produced from Lignocellulosic materials, and second generation bioethanol shows energetic, economic and environmental advantages in comparison to bioethanol from starch

or sugar. physical and chemical barriers caused by the close association of the main components of lignocellulosic biomass, hinder the hydrolysis of cellulose and hemicellulose to fermentable sugars. The main goal of pretreatment is to increase the enzyme accessibility improving digestibility of cellulose (P. Alvira et al., 2010).

Biofuels are solid, liquid or gaseous fuel derived from recently dead biological material and is distinguished from fossil fuels, which are derived from long dead biological material. Theoretically, biofuels can be produced from any carbon source although the most common sources are photosynthetic plants. Various plants and plant-derived materials are used biofuel manufacturing. Use of biofuels for energy generation is increasing nowadays because they allow migration of green house gases, provide independence and offer new Employment possibilities. Biofuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources (Archana Mishra and N.C. Mishra, 2013).

Bioethanol is a form of quasi renewable energy that can be produced from agricultural feedstocks. It can be made from very common crops such as sugar cane, potato, manioc and corn. There has been considerable debate about to the useful bioethanol will be in replacing gasoline. Concerns about its production and use relate to increased food prices due to the large amount of arable land required for crops as well as the energy and pollution balance of the whole cycle of ethanol production, especially from corn. Recent developments with cellulosic ethanol production and commercialization may allay some of these concerns (Matthew L. Wald, 2010).

2. Aim and Objectives

To study bioethanol production from agricultural raw materials by using *Saccharomyces cerevisiae* and *Trichodermaviridae*.

The present study was carried out

- To produce bioethanol by using sweet sorghum as substrate.
- To produce bioethanol by using sweet potato as substrate.
- To produce bioethanol by using grasses as substrate.

3. RESULTS AND DISCUSSION

Baker's yeast is the common name for the strains of yeast commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present

in the dough into carbon dioxide and ethanol. Baker's yeast is of the species *Saccharomyces cerevisiae*, which is the same species (but a different strain) commonly used in alcoholic fermentation, which is called brewer's yeast.

3.1. Sweet Sorghum As Substrate

Sweet sorghum is the type of grass in which sorghum plant which has a high sugar content. Sweet sorghum was used as a substrate for the high yield of Bioethanol. At the above particular point, the end point was achieved at various pH respectively indicating the production of Bioethanol.

Table 1: Bioethanol produced using with sweet sorghum.

PH	Bioethanol Produced/Titre Volume (ml)
Blank	24.5
5	22.5
5.5	21.3
6	20
5 + $(NH_4)_2SO_4$	21
5.5 + $(NH_4)_2SO_4$	20.1
6 + $(NH_4)_2SO_4$	22.1

The bioethanol produced by the sweet sorghum was estimated by the titration. The titre volume was found to be high at pH 5 and the amount of Bioethanol produced was 22.5 ml.

Figure: 1 Blank titration with sweet Sorghum.

Figure: 2 Titration without salt PH 5 with sweet sorghum.

Figure: 3 Titration of salt PH 5 with sweet sorghum.

From above the titration was estimated from sweet sorghum were blank, with salt and without salt had been titrated, various pH was indicated. Which the pH 5.0 is higher amount of ethanol to be produced (Figure:1-3).

Bio ethanol from sweet sorghum is the best choice to be implemented under hot and dry climatic condition regarding both economic and environmental considerations. The sweet sorghum has higher tolerance to drought, water logging and salt. In addition, sweet sorghum has high amount of sucrose and invert sugar (P Saranraj et al., 2012) which are easily converted to ethanol and maintained at 37°C.the report was observed (AyeleKefale et al., 2012).

3.2. Sweet Potato As Substrate

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family Convolvulaceae. Its large, starchy, sweet tasting, tuberous roots are aroot vegetable. It was used as a substrate for the bioethanol production. At the above particular points, the end point was acheived at various pH respectively indicating the production of Bioethanol. At the above particular points, the end point was acheived at various pH respectively indicating the production of Bioethanol.

Table2: Bioethanol produced using with sweet potato from *Saccharomyces cerevisiae.*

PH	Bioethanol Produced/Titre Volume (Ml)
Blank	26.5
5	21.2
5.5	23
6	21
5 + NH_4Cl	21.5
5.5 + (NH_4Cl	18.1
6 + NH_4Cl	23

Table 3: Bioethanol produced using with sweet potato from*Trichodermaviridae.*

PH	Bioethanol Produced/TitreVolume (ml)
Blank	23.2
5	26.5
5.5	24.3
6	23.3
5 + NH_4Cl	22
5.5 + NH_4Cl	23.5
6 + NH_4Cl	24.5

The Bioethanol Produced by sweet potato was at higher yield using *Trichoderma* of about 26.5 ml at pH 5 and using *Saccharomyces cerevisiae* of about 23ml at 5.5.

Figure: 4

Blank titration with s.cerevisiae

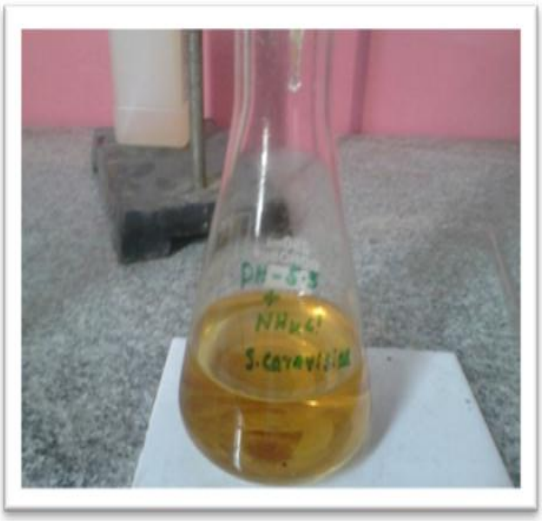

Figure: 5

Titration with salt PH5.5 from S.cerevisiae

Figure: 6

Blank Titration of trichoderma with sweet potato

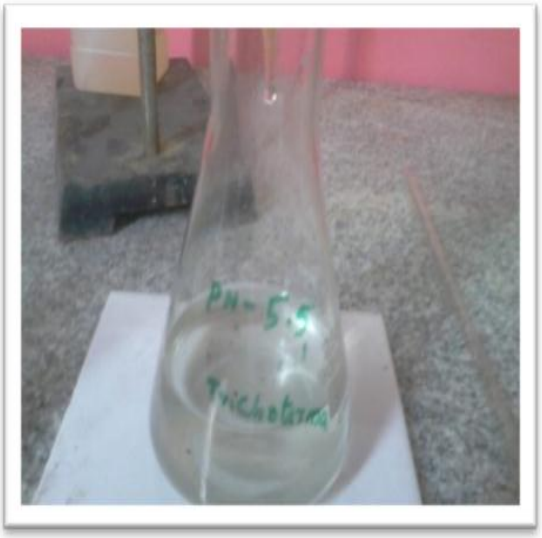

Figure: 7

Titrationof trichoderma without salt PH5.5 from sweet potato

From above the titration was estimated from *s.cerevisiae* and *trichodermaviridae* with sweet potato were blank, with salt and without salt had been titration and various pH was indicated which the pH 5.5 is higher amount of ethanol to be produced(Figure:4-7).

Fresh sweet potato and dried at 55°C were assayed. At ratios of 1:8, similar results for fresh sweet potato and flour in terms of ethanol concentration was observed(Zane R et al., 2008).

The yield of ethanol production was observed 0.31–0.37 at pH 4, 30°C and 150 rpm during 13 days fermentation period the report was found (Rizalinda L. De Leon, 2012).

3.3. Grasses As Substrate

Grasses, or more technically graminoids, are monocotyledonous, usually herbaceous plants with narrow leaves growing from the base. Grasses when used as a substrate with the microorganism, yielded Bioethanol. At the above particular points, the end point was acheived at various pH respectively indicating the production of Bioethanol.

Table 4: Bioethanol produced using with grasses.

PH	Bioethanol Produced/Titre Volume (ml)
Blank	12.5
5	8.5
5.5	10.1
6	13.1
5 + $(NH_4)_2SO_4$	10.1
5.5 + $(NH_4)_2SO_4$	9.5
6 + $(NH_4)_2SO_4$	10

The bioethanol produced by grasses was about 13.1 ml at pH 6. This substrate also yielded proteins of about 16mg/ppm. The cellulase produced was about 0.9 mg/ppm. The xylanase produced was found to be 45 mg/ppm. The reducing sugars was about 0.7mg/ppm (Table- 4).

Figure: 8. Titration of ethanol using grasses with PH 6.

From the above titration was estimated from grasses using ethanol were pH 6 had been higher amount ethanol to be produced(Figure:8). Grasses as substrate which was biologically-pretreated enzyme hydrolysate had better ethanol conversion efficiency, which was 18.5 g/g

(Thangprompan P et al., 2013). Yields from a grass that delivered an average of 13.1 megajoules of energy as bioethanol (Oyeleke, S.B et al., 2012).

3.4. Determination of Protein

Proteins were produced by the grases as substrate. A protein assay, were, measures the concentration with amount of a protein by using BSA as standard curve as (Table-5).

Tabel 5: Standard Curve of BSA.

Concentration (ppm)	Optical Density
0	0
2	0.124
3	0.175
4	0.218
5	0.282
6	0.331
7	0.428
8	0.512
9	0.574
10	0.603
11	0.723
12	0.856
13	1.235
14	1.524
15	1.741
16	1.847
17	1.953

Table 6: Estimation of Protein.

Optical Density At 600 Nm	Amount Of Protein Present In The Sample
1.8198	16 mg/ppm

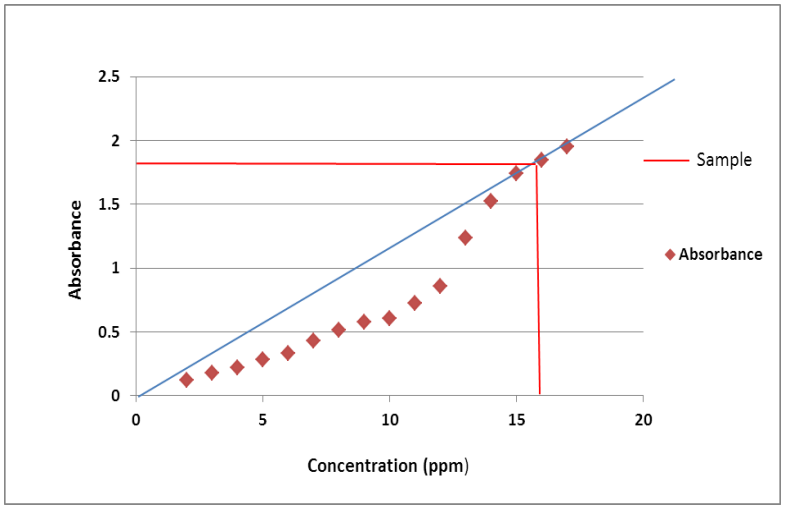

Figure: 9 Determination of Proteins.

The amount of protein was determined using Bovine Serum Albumin (BSA) as standard at 600 nm. The amount of protein present in the sample was found to be 16 mg/ppm.(Table:6).

Then the value was plotted to the graph were concentration against with absorbance (Figure-9).

3.5 Determination of Cellulase

Cellulase are enzymes that are used in the fermentation of biomass into biofuels. It was measured by using glucose as standard.(Tabel-7).

Table 7: Standard Curve of Glucose.

Concentration (Ppm)	Optical Density
0	0
0.5	0.0206
1.0	0.0511
1.5	0.0520
2.0	0.0948
2.5	1.0141

Tabel: 8 Estimation of Cellulase.

Optical Density At 560 Nm	Amount Of Cellulase Present In The Sample
0.3627	0.9 mg/ppm

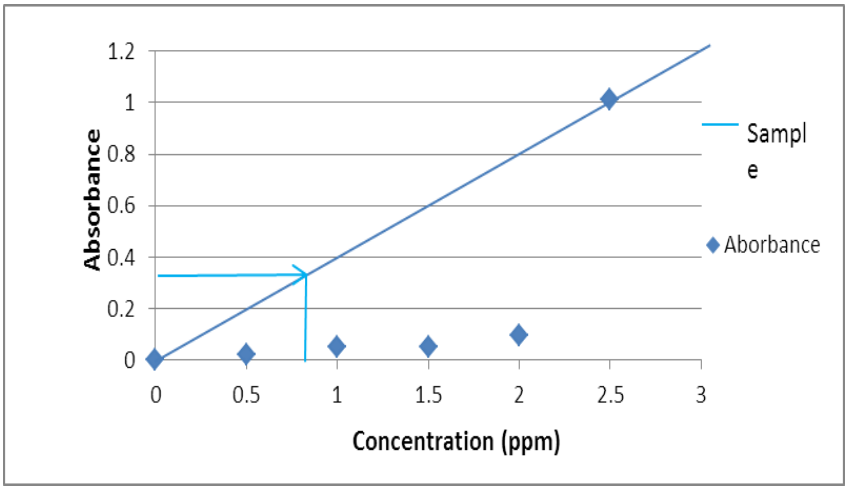

Figure 10: Determination of Cellulose.

The amount of cellulase was determined using Glucose as standard and CMC (Carboxy Methyl Cellulose) as substrate at 560 nm. The amount of cellulase present in the sample was found to be 0.9 mg/ppm.(Table-8).

Then the sample value was plotted to the graph with concentration against absorbance (Figure-10).

3.6. Determination of Reducing Sugar

A reducing sugar is any sugar that either has an aldehyde group. The aldehyde functional group allows the sugar to act as a reducing agent. It is measured by the DNS method.(Table-9).

Table 9: Standard Curve of Glucose.

Concentration (ppm)	Optical Density
0	0
0.5	0.0206
1.0	0.0511
1.5	0.0520
2.0	0.0948
2.5	1.0141

Table 10: Estimation of Reducing Sugar.

Optical Density At 540 Nm	Amount Of Reducing Sugarpresent In The Sample
0.2517	0.7 mg/ppm

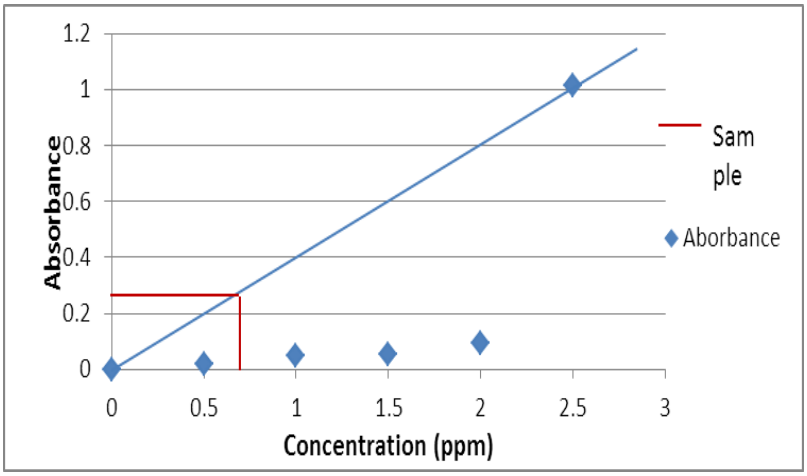

Figure 11: Determination of Reducing Sugars.

The amount of reducing sugar was determined using Glucose as standard using DNS method at 540 nm. The amount of reducing sugar present in the sample was found to be 0.7mg/ppm(Table:10).

Then the sample value was plotted to the graph with concentration against absorbance(Figure:11).

4. CONCLUSION

Grasses as substrate produced Bioethanol at 13.1 ml at pH 6 which was found to be lower compared to the other substrates. Grasses as substrate produced proteins, cellulase and xylanase and reducing sugars. The amount of protein determined was 16 mg/ppm. The amount of cellulase was determined as 0.9 mg/ppm and xylanase as 45 mg/ppm. The reducing sugars was found to be 0.7 mg/ppm. It was concluded that the Sweet sorghum proves to be the best substrate for the high production of Bioethanol compared to Sweet potato and Grasses.

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