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BIOETHANOL PRODUCTION FROM CORN COBS AND TO STUDY THE POTENTIAL INCREASE IN THE PRODUCTION USING MAGNETIC NANOPARTICLES

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Abstract: The conventional method for the production of ethanol is the usage of fossil fuels. A constant use of fossil fuels can lead to global warming and many other environmental problems. An alternative method for the production of ethanol is the use of biological sources. In the current study, Corn cobs, a potential waste product is used for the production of bioethanol. Iron oxide nanoparticles which are synthesized from the medicinal plant, *Andrographis paniculata* is incorporated to the fermentation medium in order to increase the production of ethanol. Both nanotechnology and ethanol production are important for today's society and the combination of both can be an answer for many of the environmental issues faced today, such as increased fuel demand etc.

Index Terms **- Bioethanol, Iron Oxide Nanoparticles, *Andrographis paniculata*, Fermentation**

I. INTRODUCTION

Energy is one of the most significant variables to worldwide prosperity, in perspective on consistently rising oil expenses and reliance upon fossil fuel resources, considerable focus has been centered around alternative energy resources, henceforth the production of biofuels which has been supported as an economical alternative to handle the issues related with rising unrefined petroleum costs, global warming and diminishing petroleum reserves. Bioethanol produced from renewable biomass has received significant consideration in current years; it is one intends to decrease non-renewable energy source use and outflows of ozone harming substances. Utilizing ethanol as a gas fuel added substance just as transportation fuel assists with mitigating a worldwide temperature alteration and ecological contamination [1-3].

Bioethanol is a high octane number biofuel which is produced through fermentation process. Fermentation of sugar-based crude/raw materials is known as **First Generation Bioethanol**, while the utilization of lignocelluloses crude materials is called **Second Generation Bioethanol**. Production of bioethanol from algal biomass is known as **Third Generation Bioethanol**.

In the current study, corn cobs are utilized as a substrate for the production of bioethanol and nanoparticles were added to increase the production of ethanol. The usage of corn cobs as a biomass feedstock offers promising possible results for sustainable energy production. A feedstock utilized in bio-energy conversion must have sufficient energy content. While corn cobs are not as energy dense as the petroleum products that society knows about, they have a comparable energy density to different biomass feedstock and less energy dense coals, the two of which are effectively used as energy feedstocks around the globe. The substance properties and physical qualities of corn cobs make it appropriate as a feedstock for energy generation [4]. Different physical, chemical and physical methods are employed in the synthesis of nanoparticles. Fungi, Bacteria, Algae, Yeast, Actinomycetes, Sea weeds and plants fill in as a reasonable source in the green synthesis of nanoparticles. Green synthesis with plant extract is increasingly favored as it is profitable, less difficult and safe to deal with**. In the current study, iron oxide nanoparticles were synthesized from the medicinal plant, *Andrographis paniculata***.

Nanoparticles are described as nanofibers, nanorods, nanowires, nanoclusters of metal and metal oxides, and so forth. These nano catalysts are unique in relation to conventional bulk catalysts as far as their size that gives exceptionally large area to-volume proportion and the resultant enormous active surface for substance response to occur [5]. Nanomaterials can quicken the response by giving the active sites for reactants in strong, liquid, or gaseous phase. Use of nanoparticles during the alcohol production helps in improving the general adequacy of the procedure by increasing the efficiency of pretreatment, enzymatic hydrolysis, and increasing the reaction rate during the fermentation stage. The fundamental disadvantages of conventional methods for bioethanol production are low reaction rate, significant expense of biomass processing, and low product yield. To overcome these issues, nanoparticles have been effectively utilized for bioethanol production and are advancing towards improving the productivity [6].

II. EXPERIMENTAL

2.1 Milling and Ammonia Steeping

40 grams of milled corn cobs were mixed in 200mL of 2.9M NH_4OH solution in a 500mL conical flask and the mixture was incubated in a shaker for 24 hours at 30°C. After the incubation period, the contents were filtered into a 500mL conical flask using a filter paper and the contents were further rinsed twice with distilled water. The ammonia steeped corn cobs were dried at room temperature [7].

2.2 Dilute Acid Hydrolysis

The corn cobs were delignified by treating with 0.3M HCl solution at 121°C for 1 hour and after 1 hour and then neutralized using 0.5M NaOH. The pre-treated cellulosic residue was then washed with distilled water to remove the residual acid [7].

2.3 Estimation of Reducing Sugar by DNS Method Before Fermentation

Different aliquots of standard glucose solution was taken in different test tubes and in one test tube, the acid hydrolyzed corn cobs were taken. The volume in each test tube was made upto 1ml using distilled water. 0.5ml of DNS solution was added to all the test tubes and the tubes were incubated in boiling water bath for 15 minutes. After the incubation period, the tubes were cooled and 10ml of distilled water was added to all the tubes. The absorbance of the solution was read at 540nm against a blank.

2.4 Preparation of Yeast Culture

Contents	Quantity
Agar	2 g
Malt Extract	0.3 g
Peptone	0.5 g
Glucose	1 g
Distilled Water	100 ml

Table 1 : Composition of Yeast Maintenance Agar

The yeast maintenance culture media was prepared according to the above composition and autoclaved at 121°C for 15 minutes. The autoclaved media was poured into sterile petri plates and allowed to solidify. Yeast Granules were dissolved in luke warm water and loopful of it was used to streak the culture medium. After inoculation, the cultures were inoculated at 30°C for 3 days.

2.5 Preparation of *A. paniculata* leaf extract

About 100 grams of *A. paniculata* leaves were collected, shade dried and powdered using a kitchen blender. The powdered mixture was soaked in 300ml of double distilled water overnight at 4°C. After 24 hours, the mixture was boiled for 10 minutes and the extract was cooled to room temperature and then filtered using Whatman filter paper (No. 42) [8].

2.6 Synthesis of Iron Oxide Nanoparticles Using *A. paniculata* leaf extract

$FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ were measured in 1:2 molar ratios and dissolved in 200ml of double distilled water and heated at 80°C with mild stirring using a magnetic stirrer under atmospheric pressure. After 10 minutes, 40ml of the leaf extract was added (color change to dark brownish color). After 10 minutes, 20ml of aqueous NaOH solution was added to the mixture at a rate of 3ml per minute which allowed iron oxide to precipitate consistently. The mixture was then cooled down to room temperature. The iron oxide nanoparticles obtained by decantation was subjected to centrifugation at 10,000 rpm for 10 minutes. The magnetites formed were washed with double distilled water and ethanol for 3 minutes each and further air dried at room temperature [8].

2.7 Bioethanol Fermentation

100ml of hydrolysate were added to three different conical flasks, taking one as **Control** and the other two marked as **0.2g** and **0.4g of nanoparticles**. 0.2g and 0.4g of iron oxide nanoparticles along with *Saccharomyces cerevisiae* were added to two different conical flask with the hydrolysate. The **Control** flask had only the hydrolysate and *Saccharomyces cerevisiae*. The samples were then subjected to incubation in a shaker incubator for 72 hours at 180 rpm [7].

2.8 Estimation of Reducing Sugar by DNS Method After Fermentation

0.6 ml aliquots of the three fermented medium were taken in three different test tubes and marked as **Control** (without nanoparticles), the second medium with **0.2g of nanoparticles** and the last with **0.4g of nanoparticles**. The volume in each tube was made upto 1 ml using distilled water. 0.5ml of DNS solution was added to all the test tubes and the tubes were incubated in boiling water bath for 15 minutes. After the incubation period, the tubes were cooled and 10ml of distilled water was added to all the tubes. The absorbance of the solution was read at 540nm against a blank.

2.9 Distillation Using Soxhlet Apparatus

After fermentation and DNS estimation, the fermented medium was subjected to distillation using the Soxhlet Apparatus. The procedure is as follows : The fermented medium individually were taken into three different round bottom flasks. The flasks were then adjusted in the Soxhlet Apparatus and the temperature was adjusted to the boiling point of ethanol in order to extract ethanol. After adjusting the temperature, the beaker slowly heated up and after hours of distillation, the ethanol was collected in the distillation tube [7].

3.0 Ethanol Estimation by Specific Gravity Method

The specific gravity bottle was washed, dried and the weight of the empty bottle with the stopper was taken. The bottle was then completely filled with water and the bottle was covered with the stopper. The water on the outer surface of the bottle was thoroughly wiped and the weight of the bottle was taken. The bottle was emptied, dried and then completely filled with ethanol and the bottle was covered with the stopper. The ethanol on the outer surface of the bottle was thoroughly wiped and the weight of the bottle was taken. The specific gravity of ethanol was calculated using the formula:

$$\{W_3 - W_1 / W_2 - W_1\} * \text{Density of water at room temperature}$$

W_1 : Weight of the empty specific gravity bottle

W_2 : Weight of the bottle with distilled water

W_3 : Weight of the bottle with ethanol

The percentage of ethanol was read against the corresponding value of specific gravity in the standard AOAC chart.

III. RESULTS AND DISCUSSIONS

3.1 Yeast Culture

The yeast culture (*Saccharomyces cerevisiae*) was prepared and kept for incubation. After incubation growth was seen on the plate and viewed under microscope for identification.

Figure 1: Control Plate

Figure 2: Test Plate

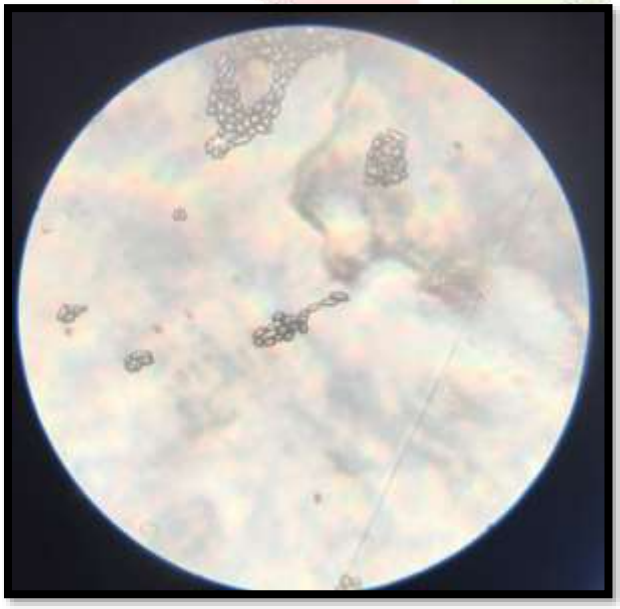


Figure 3: Microscopic view of budding yeast

3.2 Estimation of Reducing Sugar by DNS Method Before Fermentation

The reducing sugar estimation was done and the standard Graph was plotted to check the glucose concentration in the medium before fermentation.

S. NO	Std. Glucose solution (ml)	Distilled water (ml)	DNS solution (ml)	Incubate in boiling water bath for 15 minutes	Distilled water (ml)	OD at 540 nm	Conc. Of Glucose (µg)
1.	0.0	1.0	0.5ml		5ml	0.0	00
2.	0.2	0.8				0.07	200
3.	0.4	0.6				0.17	400
4.	0.6	0.4				0.23	600
5.	0.8	0.2				0.26	800
6.	1.0	0.0				0.37	1000
Sample	0.6	0.4				0.88	2640

Observation Table no. 1

CALCULATION:

0.15 OD has glucose concentration as 450 µg (from graph)
So 0.88 OD has glucose concentration = (0.88* 450)/ 0.15 = **2640 µg**

From the calculation of the standard graph, the glucose concentration of the medium is **2640 µg** before fermentation.

3.3 Green Synthesis of Iron Oxide Nanoparticles Using Leaf Extract of *Andrographis paniculata*

In the present study, the iron oxide nanoparticles were prepared by green synthesis method using *A. paniculata* leaf extract. When *A. paniculata* leaf extract and aqueous $FeCl_3$ and $FeCl_2$ solution was mixed, the colour of the reaction mixture changed instantaneously from light green to dark brown colour. The blackish brown precipitates of iron oxide nanoparticles were obtained after centrifugation of the above solution, which was further purified and characterized to obtain the surface morphology and other functional groups. The characterization of the green synthesized iron oxide nanoparticles was done by UV-vis spectrophotometry, XRD, FTIR,SEM and HR TEM.

Figure 4: Powdered Green Synthesized Iron Oxide Nanoparticle

3.4 Characterization Result

UV-Vis Spectroscopy Analysis

The formation of iron oxide nanoparticle was further studied by measuring the absorbance with UV-Vis spectrophotometer over the range from 200 nm to 700 nm. The synthesized iron oxide nanoparticle has the absorption peak within the range of 260 nm to 280 nm, which is a characteristic of it. These results are harmony with of findings of Saranya *et al.*,2017 [9] who reported green synthesis of iron nanoparticles using aqueous extract of Musa ornata flower sheath.

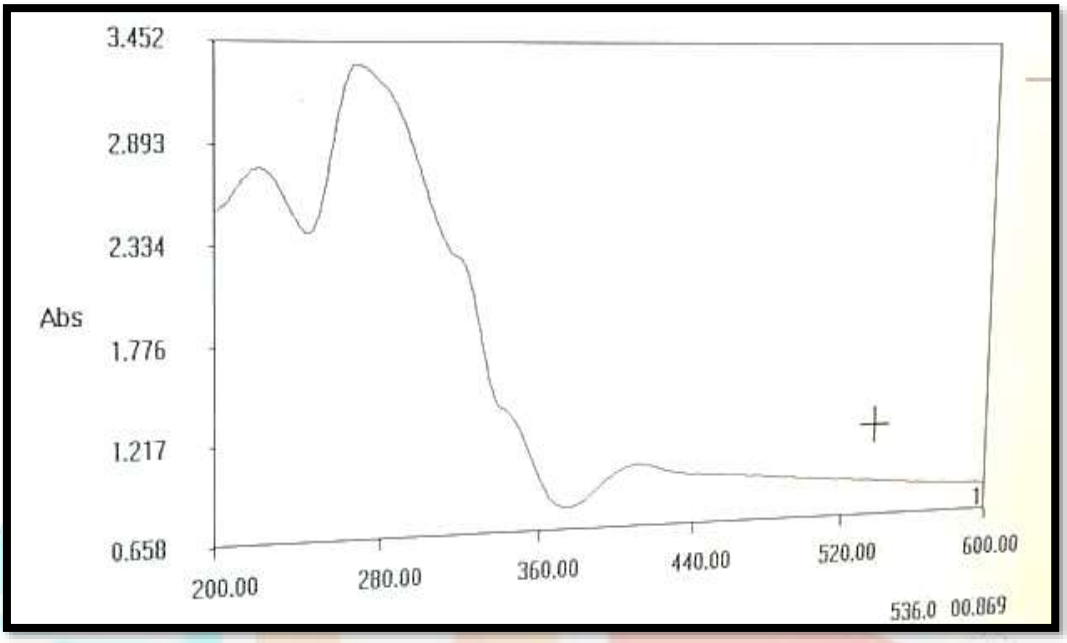


Figure 5 : UV-vis Spectrum of Synthesized Iron Oxide Nanoparticles

XRD Analysis

The powdered XRD pattern of the prepared iron oxide nanoparticles using *A. paniculata* leaf extract is shown in below figure 8. The powdered XRD pattern of the prepared iron oxide nanoparticles using A. paniculata leaf extract is shown in adjacent figure The major strong characteristic peaks of iron oxide particles are obtained at 2θ =17.0° , 26.4° , 28.26°, 28.62°, 34.90°, 39.219°, 45.371° . The major characteristic peaks of iron oxide nanoparticles corresponds to the crystalline structure of iron oxide nanoparticles. These findings are analogous with the crystalline nature of iron oxide nanoparticles Saranya *et al.*,2017 [9].

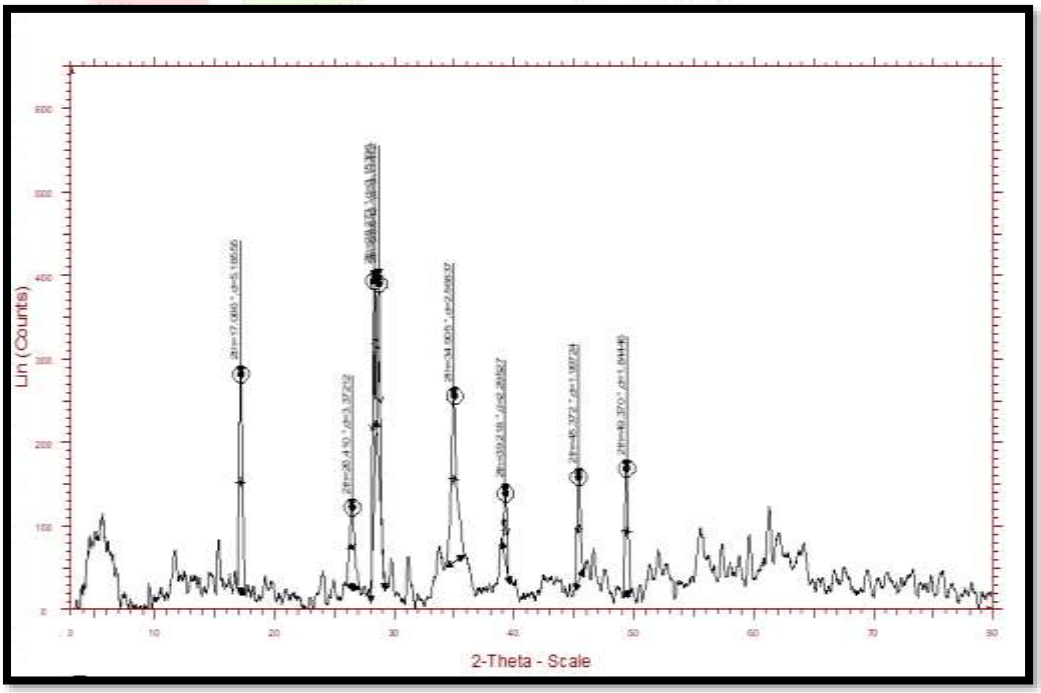


Figure 6 : XRD Analysis of Synthesized Iron Oxide Nanoparticles

FTIR Analysis

The FTIR analysis of synthesized iron oxide nanoparticles gave different stretching vibrations at different peaks which are shown in the below figure 9 . The FTIR analysis of synthesized iron oxide nanoparticles gave different stretching vibrations at different peaks , The presence of peak at 3384.62 cm−1 indicate the possible O-H stretching vibration of alcohol groups, 1630.84 cm−1 corresponds to C=C (alkene) stretching vibrations, 1415.15 cm−1 corresponds to C-H bending vibrations, 1384.6 cm−1 corresponds to N-O (nitro) stretching vibrations, 1205.04 cm−1 corresponds to C-N (amine) stretching vibrations, 1090.68 cm−1 corresponds to C-O (ether) stretching vibrations, 1016.36 cm−1 corresponds to C-F (alkyl halide) stretching vibrations, 846.89 cm−1, 630.98 cm−1 and 510.89 cm−1 corresponds to alkyl halide stretching vibrations of C-H,C-Cl, C-Br groups respectively. The identified functional groups are found in previous FT–IR analysis of iron oxide nanoparticles synthesized by green tea extract by Gottimukkala *et al*,.2017 [10].

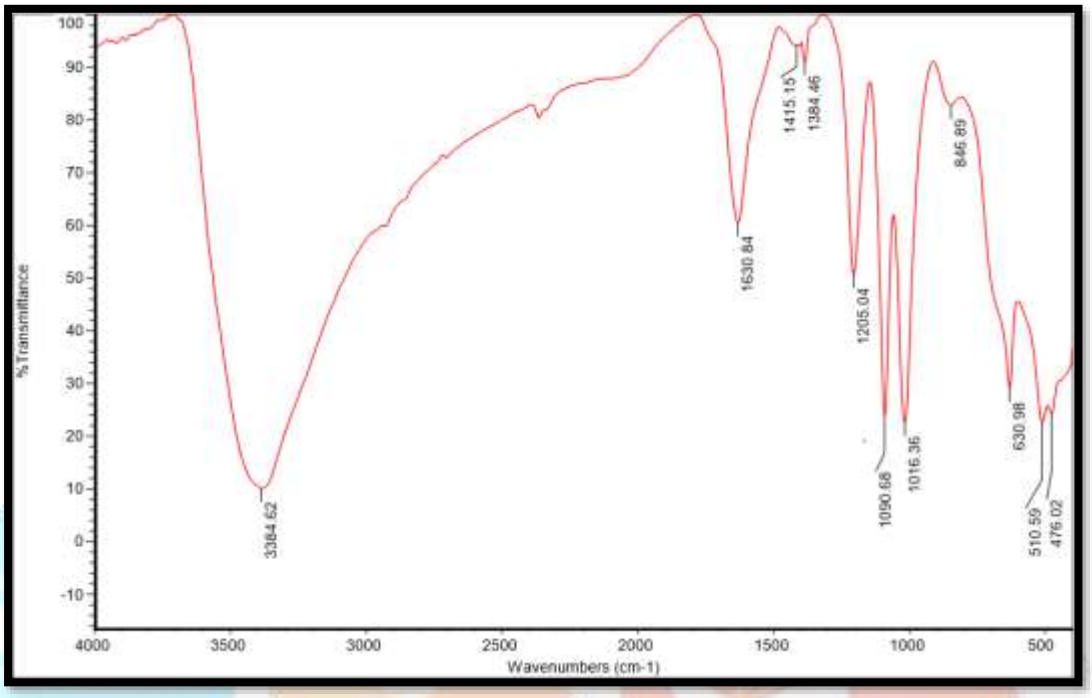


Figure 7 : FT-IR Analysis of Synthesized Iron Oxide Nanoparticles

SEM Analysis

The morphological dimensions of synthesized iron oxide nanoparticles were studied using the SEM (scanning electron microscopy). The study demonstrated that the shape of the nanoparticle is irregular spherical, which are agglomerated together. Detailed structural information was further confirmed by HR- TEM. The result is in harmony with Kuang *et al.*,2013 [11] used three different tea extracts, namely, green tea , oolong tea and black tea to synthesis iron nanoparticles and the SEM image revealed the irregular spherical iron nanoparticles.

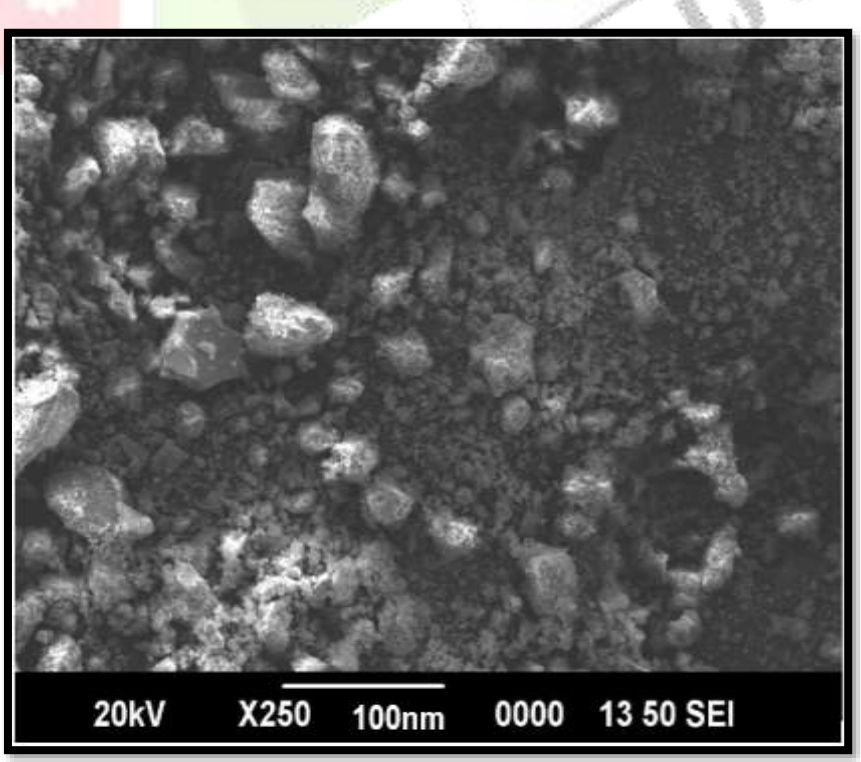


Figure 8: SEM Images of Synthesized Iron Oxide Nanoparticles by *Andrographis paniculata* Leaf Extract

HR-TEM Analysis

Morphologies of the synthesized nanoparticles during the bio-reduction were confirmed by employing HR-TEM analysis. Iron oxide nanoparticles exhibited spherical nanostructures with the average core diameter of 20nm. It is analogues to S. Sridhar *et al.*, 2018 [8] who reported spherical iron oxide particles with a diameter of up to 20 nm were formed by *Glycosmis mauritiana*.

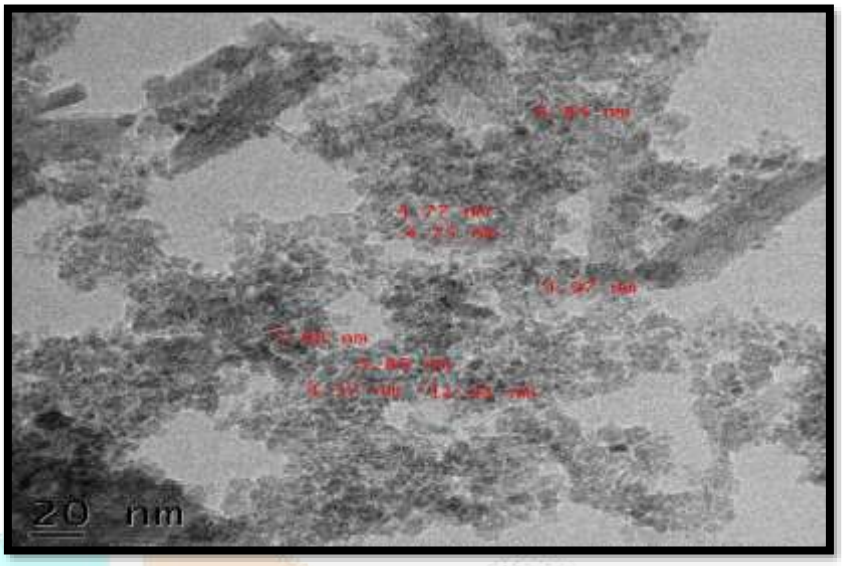


Figure 9: HR-TEM Images of Synthesized Iron Oxide Nanoparticles by *Andrographis paniculata* Leaf Extract

3.5 Bioethanol Fermentation

The control , 0.2g of nanoparticles added flasks and 0.4 g of nanoparticles added flasks were kept for fermentation in shaker at room temperature and after fermentation the sample was analyzed for reducing sugar concentration for checking the fermentation process.

FIGURE 10: Flasks Kept For Fermentation

3.6 After Fermentation

After the fermentation process for 72 hours , the three fermented flasks were taken out of the incubator shaker.

FIGURE 11: Flasks After fermentation

After fermentation, the flasks were subjected to DNS estimation to check the concentration of glucose.

3.7 Estimation of Reducing Sugar by DNS Method After Fermentation

Glucose estimation was done after fermentation to confirm the fermentation process has occurred.

S. NO	Std. Glucose solution (ml)	Distilled water (ml)	DNS solution (ml)	Incubate in boiling water bath for 15 minutes	Distilled water (ml)	OD at 540 nm	Conc. Of Glucose (µg)
BLANK	0.0	1.0	0.5ml		5ml	0.0	00
CONTROL	0.6	0.4				0.42	1260
0.2g NP	0.6	0.4				0.40	1200
0.4g NP	0.6	0.4				0.35	1050

Observation Table No. 2

FIGURE 12 : Estimation of Reducing Sugar by DNS Method After Fermentation

By plotting the OD values in the standard graph of DNS we got the following concentration of glucose in the three fermented flasks.

CALCULATION:

0.15 OD has glucose concentration as 450 µg (from graph)

- So, for the control flasks the OD value is 0.42 nm
  The glucose concentration for the control flasks : (0.42* 450) / 0.15 = **1260 µg**
- For the flasks with 0.2g of magnetic nanoparticle, the OD value is 0.40 nm
  The glucose concentration for the 0.2g NP flasks = (0.40* 450) / 0.15 = **1200 µg**
- For the flasks with 0.4g of magnetic nanoparticle, the OD value is 0.35 nm
  The glucose concentration for the 0.2g NP flasks = (0.35* 450) / 0.15 = **1050 µg**

The glucose concentration before fermentation was **2640 µg,** which after fermentation decreased tremendously. This shows that the fermentation has taken place with the help of *Saccharomyces cerevisiae*, which used glucose as a substrate to form the end product that is ETHANOL.

The glucose concentration in the control flask has decreased , but when compared to the flasks with the nanoparticles added, the glucose concentration in the control flasks is more. From this we can infer that the nanoparticles has aided the process of fermentation in the same time period as that of normal fermentation.

3.8 Distillation Using Soxhlet Apparatus

After the distillation with Soxhlet apparatus by adjusting the temperature to the boiling point of Ethanol to 79° C, the final product in the distillation tube was collected into small flasks and subjected to estimation of ethanol by specific gravity method.

3.9 Ethanol Estimation by Specific Gravity Method

The Specific Gravity Method helps to estimate the ethanol and tells the percentage of ethanol in the sample. In the current study the ethanol purity was estimated giving the following results:

- Weight of the empty bottle(W_1) : 24.48 g
- Weight of the bottle with distilled water (W_2) : 49.72g
- Weight of the bottle with ethanol (CONTROL) (W_{3a}) : 50.09g
- Weight of the bottle with ethanol (0.2g of NPs) (W_{3b}) : 50.15g
- Weight of the bottle with ethanol (0.4g of NPs) (W_{3c}) : 50.20g

By applying the Specific Gravity formula the percentage of alcohol in each flasks are as follows:

CONTROL:

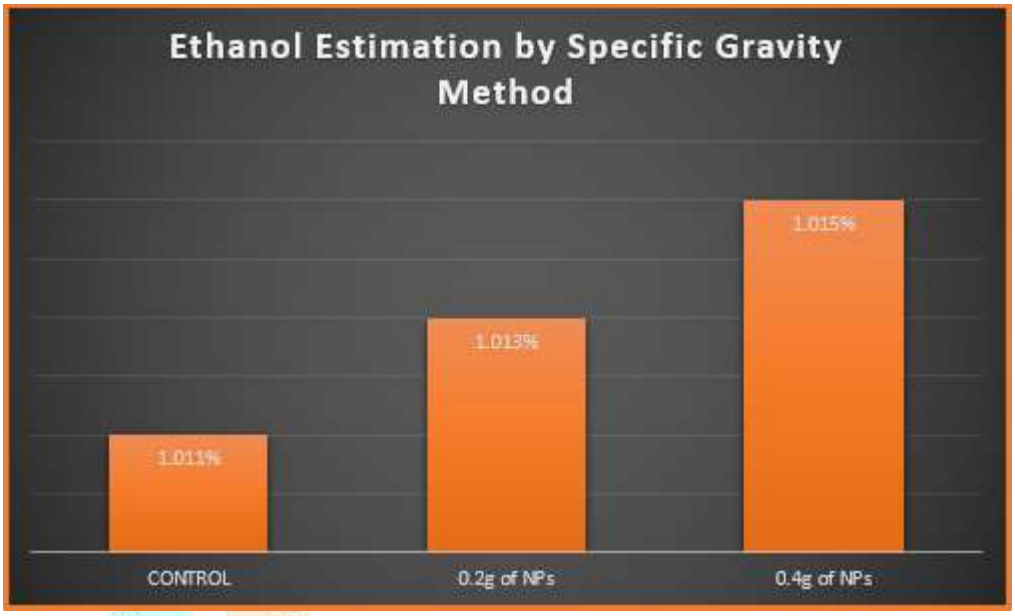
(W_{3a}-W_1/ W_2 – W_1) * DENSITY OF WATER AT RT
(50.09 -24.48/ 49.72 – 24.48) * 0.9965 = 1.011
Ethanol % = 1.011%

0.2g OF NPs:

(W_{3b}-W_1/ W_2 – W_1) * DENSITY OF WATER AT RT
(50.15 -24.48/ 49.72 – 24.48) * 0.9965 = 1.013
Ethanol % = 1.013%

0.4g OF NPs:

(W_{3b}-W_1/ W_2 – W_1) * DENSITY OF WATER AT RT
(50.20 -24.48/ 49.72 – 24.48) * 0.9965 = 1.015
Ethanol % = 1.015%



Graph 2: The Above Graph Shows The Ethanol Percentage In The Three Flasks By Specific Gravity Method

From the specific gravity method results, we can conclude that with increase in the nanoparticle concentration, the process rate as well as the ethanol percentage also increases.

IV. CONCLUSION

Energy consumption has increased during the last century due to the world population development and growth. One of the potential options to solve the environmental and energetic problems is the use of bio-ethanol. In an attempt to maximize waste product into useful material, this project focusses on the use of waste corn cobs in the production of bioethanol and also tries to incorporate the nanotechnological approach to increase its production in folds. The current project deals with usage of corn cobs as potential raw materials for the production of bioethanol by fermentation of the raw material with *Saccharomyces cerevisiae* and also simultaneously addition of different aliquots of iron oxide nanoparticles to study the potential increase in the production. The corn cobs have been kept for fermentation in three different flasks labelled as control (without nanoparticle), one with 0.2g of magnetic nanoparticles and the other with 0.4g of nanoparticles. After fermentation, the product (ethanol) formed was recovered by distillation with Soxhlet apparatus. By estimating the final product, ethanol, by Specific Gravity Method showed that with increase in the nanoparticle concentration in the fermentation medium the ethanol percentage also increases. Thus, the study focuses on the increasing the ethanol production by incorporating the nanoparticles. The nanoparticles are also formed by the green synthesis, so it is equally bio-friendly and cost – efficient. Both nanotechnology and ethanol production are important for today's society and the combination of both can be an answer for many of the environmental issues faced today, such as increased fuel demand etc.

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