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## Full Length Research Paper

# Nanobiotechnology for the production of biofuels from spent tea

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**Bioenergy is the only alternative and cheap source of energy which can be made easily available to the world. The present experiment included three steps for the conversion of spent tea (*Camellia sinensis*) into biofuels. In the first step, spent tea was gasified using Co nano catalyst at 300°C and atmospheric pressure. Catalytic gasification of spent tea yielded 60% liquid extract, 28% fuel gases and 12% charcoal. Gaseous products contain 53.03% ethene, 37.18% methanol and 4.59% methane. In the second step of the experiment, liquid extract of spent tea obtained from gasification, on transesterification gave 40.79% ethyl ester (biodiesel). In the third step, *Aspergillus niger's* growth on spent tea produced 57.49% bioethanol. This study reports an interesting finding that spent tea (solid waste) could be used not only for the production of biodiesel and bioethanol but also hydrocarbon fuel gases. The world today is consuming several million tons of tea yearly. The present technology could be utilized to produce alternate energy.**

**Key words:** Black tea, biodiesel, bioethanol, Co nano particles, hydrocarbon gases, catalytic gasification.

## INTRODUCTION

Used black tea could become a cheap and environmentally friendly source of biofuels. The main growers of black tea are Turkey, China, Kenya, Malawi, Sri Lanka, Indonesia, India and Bangladesh. In 2008, total production of black tea was more than 3.8 million tons. Spent tea contains milk and sugar. In south Asia, tea full of creamy milk and sugar is preferred. Commonly, one cup of tea may contain 3 - 5 teaspoons of sugar. Fresh tea leaves contain 4 - 7% cellulose and hemicellulose, 5 - 6% pectin, 5 - 6% lignin, 14 - 17% proteins and 3 - 7% lipids. The black tea beverage contains cellulose, hemicellulose, pectin, lignin, proteins, organic acids, carotenoids, tea fibre and caffeine. Cow milk contains more than 2 - 6% fat, 2.9 - 5% protein, 3.6 - 5.5% lactose. Fat and protein content of cow milk increase after 80 days of lactation. More than 12 types of fatty acids are present in milk triglycerides. Large amount of Ca is present in milk. *Aspergillus niger* grows on milk. Beefalo's milk is richer in fats ([http://www.ilri.org/InfoServ/Webpub/Fulldocs/ILCA\\_](http://www.ilri.org/InfoServ/Webpub/Fulldocs/ILCA_)

[Manual4/Milkchemistry.htm#TopOfPage](http://www.ilri.org/InfoServ/Webpub/Fulldocs/ILCA_Manual4/Milkchemistry.htm#TopOfPage)). Gasification and pyrolysis is the thermal decomposition of solid biomass at a temperature of 650-800 K at 1-5 bars in the absence of air to yield liquid extract, solid charcoal and gaseous compounds (Navarro, et al., 2009). On gasification the quantity of liquid extract from the tea source was about 60% depending upon type of tea, quantity of milk and sugar. On gasification, million tons of tea may produce million tons of liquid extract. On transesterification of liquid extract, million tons of tea may give several million tons of biodiesel and charcoal. A computer based model predicted that a high gas production can be achieved during gasification when a high temperature (750°C) was applied (Aye and Yamaguchi, 2006). All methods for the production of fuels have their own limitations but for better fuel formation, the use of a pyrophoric catalyst is required. However, there is strong need to develop new green catalyst that should be efficient, involve an easy work-up and afford greater yields in shorter reaction times. In this regard, cobalt (Co) has extensively been used for catalytic gasification. He et al. (2008) studied the influence of catalyst and temperature on yield and product composition during gasification. They used temperature range of 750 - 950°C. However,

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reactions catalyzed by Co were very slow owing to the low surface area of the catalyst. It was therefore worthwhile to develop a new green catalyst that could enhance the reaction rate. Work in the field of Co metal nano particles as catalysts in synthetic organic chemistry has gained much attention. Recent literature has shown that the application of nano particles as catalysts in organic synthesis has been little explored (Kidwai et al., 2006). The role of nano particles in catalysis under well-controlled environment was studied by Valden et al. (1998). Gates (2000) and others have employed molecular nano particles in catalysis. Cobalt nano particle catalysts may be influenced by their size and structure and by other additional components such as Si, Ni and Mg. For example, Co or Co oxide nanostructures are known to be effective catalysts (Yang et al., 2001; Kesavan et al., 2001; Son et al., 2002). Cobalt nano particles, in particular being cheap, need mild reaction conditions for high yields of products in short reaction times as compared to the traditional catalysts. *A. niger* is the best cellulolytic fungi that can be isolated from soil. *A. niger* is a commonly found fungus. It is a network of brownish black mycelia and can easily grow on milk and sugar containing spent tea. It can easily produce bioethanol by its versatile enzymes. *A. niger* which can be isolated from different sources is responsible for bioethanol production (Ali and El-Dein, 2008). Presence of milk and sucrose in large quantities are responsible for its growth on spent tea.

Cellulase is an induced enzyme and its production increases with increase in fungal biomass over the incubation period and as simple sugar in the substrate diminished. There is an increase in saccharification from 0 to 24 h. Cellulase enzymes of *A. niger* are responsible for the hydrolysis of cellulose which results into ethanol after fermentation. There is a progressive increase in enzyme activity from 24 to 144 h after incubation (Nieves et al., 1998). Cellular hemi-cellulase enzymes can be used in fermentation. The sucrose is enzymatically converted into ethanol (Gray et al., 2006). Cellulase, hemicellulase and pectinase enzymes are responsible for conversion of sugars of plant origin into ethanol (Bhat, 2000). The ethanol producing enzymes are cellulase and hemicellulose-degrading. These may be isolated from *A. niger* (Thygesen et al., 2003). Cellulose changes into glucose by using the enzymes of fungi. It is then converted into ethanol. Hemicellulose hydrolyzed into xylose and mannose etc. These are converted into ethanol by enzymes. More than 90 - 95% of ethanol cannot be obtained by fermentation. Some other by-products like acetic acid and glycerol are also possible. The xylose is converted into ethanol by enzymes like xylose isomerase's (Nag, 2008). Cellular hemi-cellulases can be used in fermentation. Sucrose is enzymatically converted into ethanol (Gray et al., 2006)

For the analysis of biofuels already documented techniques like GC-MS (Gas Chromatography-Mass Spectrometry) (Li et al., 2009; Tsukatani et al., 2009), GC

(Gas Chromatography) (Warden et al., 2009) and FTIR (Fourier Transform Infra Red spectroscopy) was used. The properties of nano particles were studied by TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy) and XRD (X-Rays Diffraction) as documented in literature (Niasari, et al., 2007).

In the present study, spent tea was converted into hydrocarbon fuel gases, bioethanol biodiesel and charcoal. Previously, gasification was done using Co catalysts at high temperature (Pinto et al., 2009). Similar expensive reagents were used by Guru et al. (2008). Spent tea (*Camellia sinensis*) was converted into biofuels at room temperature. The byproduct tea charcoal can be used as biosorbent for waste heavy metal's removal from water as reported by Razmovski and Sciban (2008).

## MATERIALS AND METHODS

### Chemicals

The cobalt chloride and nickel chloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ) were purchased from Sigma Aldrich USA and 1-10 phenanthroline was purchased from Fluka. All solvents were of analytical reagent grade and were used without further purification.

### Synthesis of Co nano particles

For the synthesis of complex of cobalt, 0.5 molar solution of 1-10 phenanthroline and 0.5 molar solution of cobalt chloride were separately prepared in 1-propanol. The 1-10 phenanthroline solution was taken in dropping funnel and very slowly dropped to the cobalt chloride solution with constant stirring at a temperature of 40 - 50°C. The pink precipitate of cobalt/1-10 phenanthroline complex appeared after almost one third (1/3) of 1-10 phenanthroline solution was added to cobalt chloride solution. The addition of the 1-10 phenanthroline solution to the salt solution was continued till complete precipitate was formed in the reaction mixture. The precipitate was then filtered and washed two times with 1-propanol to remove the un-reacted 1-10 phenanthroline / cobalt chloride. The precipitate was dried under Infra Red lamp and then under vacuum. The complex was taken in a two-neck flask and was kept in tube furnace for decomposition. One side of the flask was connected to argon cylinder and the other was used as outlet for argon and decomposition gases. The temperature of the furnace was raised to 500°C at a heating rate of 0.5°C min<sup>-1</sup> and the contents were kept at 500°C for 24 h and then allowed to cool to room temperature under an inert atmosphere of argon gas to yield Co nano particles.

### Characterization of synthesized nano particles using SEM, XRD and particle size measurement

The particle morphology and size was studied by SEM (Scanning Electron Microscope- Zeiss Supra 50VP with EDS Oxford) and XRD. The X-ray diffraction pattern of the particles powders were collected with PANalytical, Netherlands, diffractometer (Model 3040/60 X<sup>1</sup> pert PRO) equipped with a Cu K $\alpha$  radiation source. Using Scherrer formula, based on line broadening, the mean crystal sizes of the powders were determined (Niasari et al., 2007).

### Gasification of spent tea

Spent black tea was collected from local residential area as a

kitchen waste. It was sun dried, weighted and placed in a round bottom flask. In 100 g spent tea, 0.15 g Co particles were mixed thoroughly. The flask was placed in Carbolite furnace (model-CWF-1100) and a "U" shaped tube was attached with flask and a liquid collector. For increase of temperature, the furnace was programmed on 5°C/min. When temperature reached 150°C, gaseous vapors evolution was started. At 300°C the condensed liquid was collected in a collector and its volume was measured.

#### Analysis of gaseous samples by GC-MS

The gaseous samples were collected and analyzed using gas chromatography mass spectrometer (Hewlett-Packard [Palo Alto, A] 5890 series II gas chromatograph with Hewlett-Packard 5972 mass selective detector) (Tsukatani et al., 2009). The pH of extract was measured using pH meter (Orion USA made model SA 720).

#### Transesterification of liquid extract obtained from gasification

The extract was filtered before analyzing on FTIR (Thermo-Nicolet Nexus 670 Spectrophotometer). It was transesterified with ethanol and NaOH catalyst by using Kondamudi et al. (2008) procedure.

#### Analysis of diesel samples by GC-MS

Diesel sample was collected, filtered, diluted with chloroform and was analyzed on gas chromatography mass spectrometer (GC-MS). Resulting peaks were analyzed and the results were recorded.

#### Analysis of solid residue samples by XRD and TEM

The catalyst used left in charcoal was recovered for reuse and was analyzed by XRD X-ray diffraction pattern. The particles powders was collected with PANalytical, Netherlands, diffractometer (Model 3040/60 X<sup>1</sup> pert PRO) equipped with a Cu K $\alpha$  radiation source.

#### Measurement of particle size of tea residue

Using Scherrer formula, based on line broadening, the mean crystal sizes of the powders were determined. The morphology and particle size of the Co nano particles and charcoal or residue of tea (powder) after gasification were determined by TEM (Transmission Electron Microscope- FEI Tecnai F20 S-TWIN 200 FEG). For preparation of the TEM sample, the powder was dispersed in high-purity ethanol via ultrasonic equipment for 2.4 ks (Figure 1).

#### Isolation and identification of *A. niger* from spent tea

Samples of spent tea containing milk and sugar were placed in a beaker undisturbed for 3 - 5 days. After 3 - 5 days, a net work of brownish black *A. niger* was observed. A sample of *A. niger* containing tea inoculums was mixed with AR Grade chloroform in a ratio of 1:4. This sample was mixed thoroughly on magnetic stirrer and filtered. The aqueous layer was separated from non-aqueous layer.

#### Preparation of sample for estimation and confirmation of bio-ethanol from spent tea by GC-MS and GC

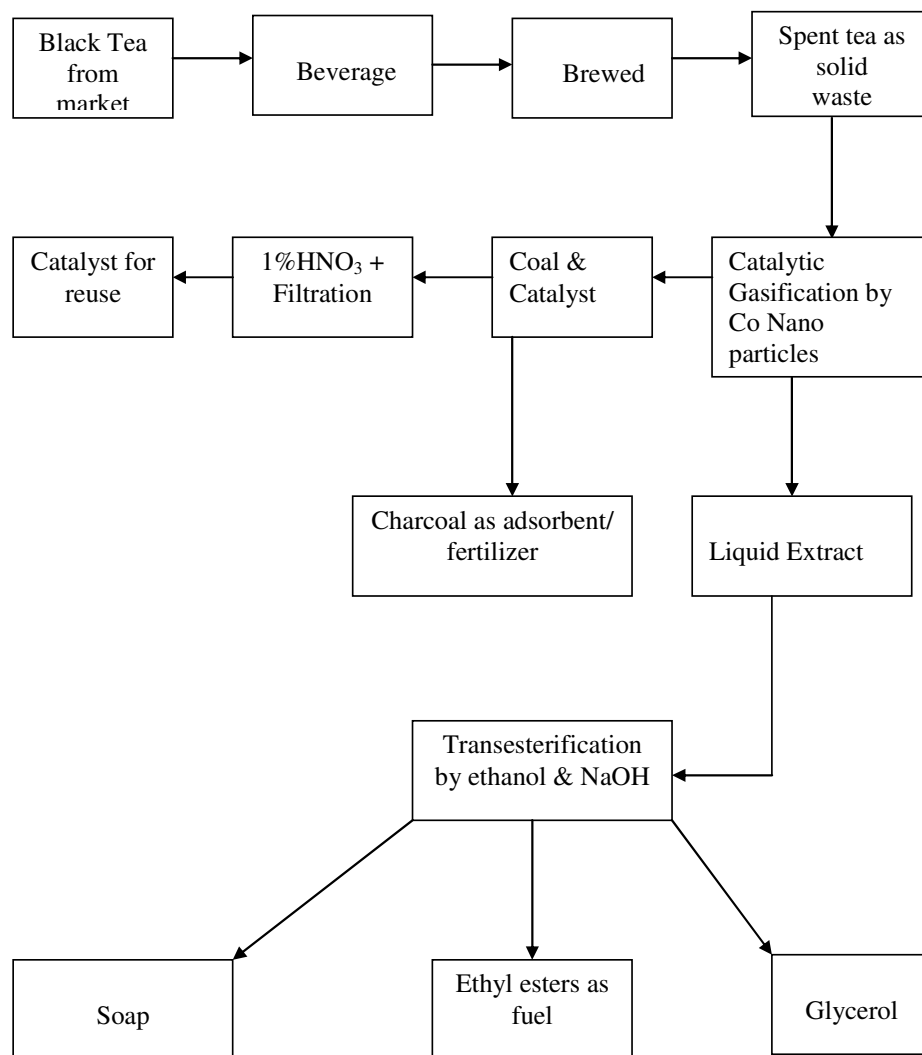
It was analyzed on GC-MS for ethanol. The crude sample was distilled and was also analyzed by GC. Another sample of *A. niger*

containing tea inoculum was mixed with Sabouraud dextrose media for growth, purification and isolation as described by Tabassum (2003). For identification, growth temperature, shape and colony morphology, mobility (Kausar, 2004), spore formation, H<sub>2</sub>S test (Tabassum, 2003; Kausar, 2004) and gram's staining, (Pandey, 1994) were performed (Figure 2).

## RESULTS AND DISCUSSION

The present technology was based on nano catalytic pyrolysis, gasification, transesterification, and fermentation. Pyrolysis is the thermal decomposition of solid biomass at high temperature and pressure (Navarro et al., 2009). In this study, low temperature due to high activity of Co nano particles was used. The thermal gasification produces hydrocarbons at 1000°C in the absence of air. The catalytic gasification decreased reaction temperature up to 650°C. The simplest type of gasification is the fixed bed countercurrent gasification. The major advantages of this type of gasification are its simplicity, high charcoal burnout, and internal heat exchange that led to low gas-exit temperatures and high gasification efficiencies (Quaak, et al., 1999). As Co nano particles due to high reactivity and higher surface area decreased the reaction temperature and activation energy. In the present experiment, gasification was carried out at 300°C which is the lowest possible temperature. Thermodynamic calculations in the degradation process showed that the cleavage of C-O bond took place at 288°C. Generally, thermal decomposition proceeds through either free radical or carbonium ion mechanism. Under these conditions of thermal decomposition, GC-MS analysis showed that approximately 73% of this product are made up of alkanes, alkenes, aromatics, carboxylic acids and alcohols with C numbers ranging from C=1 to more than 20 (Nag, 2008). On gasification, organic compounds were converted into CO<sub>2</sub> and H<sub>2</sub>O. Cobalt reacts to produce CoO after converting CO<sub>2</sub> to CO and H<sub>2</sub>O to H<sub>2</sub>. Now CO+H<sub>2</sub> are synthetic (syn) gases and Co is already known as Fischer-Tropsch catalyst for conversion of syn gas to hydrocarbons. The gaseous products of tea gasification thus obtained were analyzed by GC-MS and the results were given in Tables 1 and 2.

Reddy and Tucker (1983) gasified water hyacinth plant at 800°C. In the present investigation, low temperature due to high activity of Co nano particles was used. For their characterization SEM, TEM and XRD were used. SEM image demonstrates the morphology as well as crystallite size of metallic nano particles which were synthesized through hydrothermal method. The image indicated that the particles were uniformly regular spherical sponge like in the shape of Co and fall in the size range of 2 – 90 nm. This is comparable to the crystallite size calculated from XRD by applying Sherrer formula. The XRD has given prominent peaks for the metallic nano particles of cobalt. The XRD pattern of fresh cobalt nano particles was studied and from the data, cubic structure for cobalt nano particles was obtained using the standard



**Figure 1.** Schematic representation of the biofuel production from spent tea.

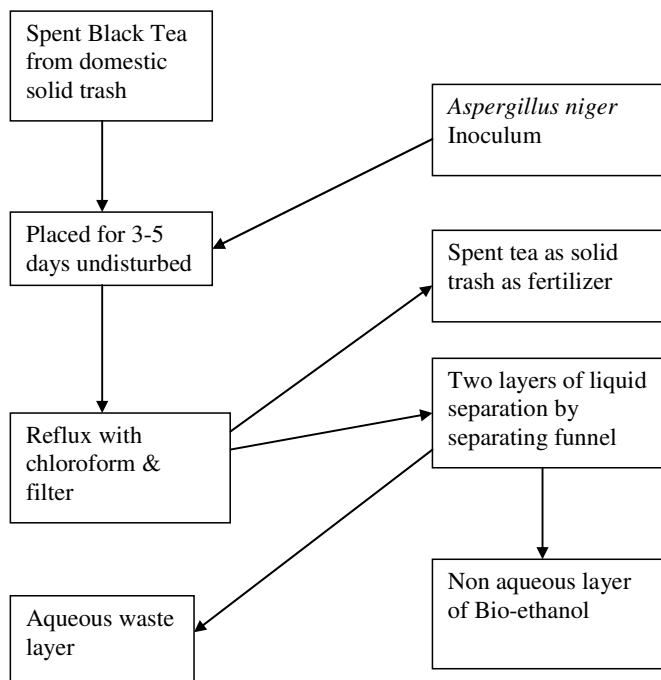
ASTM XRD files. The XRD peaks corresponds to the indices (111) and (200). It could be concluded that the nano particles prepared through this method were pure with a controlled phase of FCC structure.

Various studies reported that Co nano particles are used in catalytic gasification of biomass. The temperature, particle size, particle morphology, pressure, surface area, nature of nano particles and nature/ chemistry of biomass were responsible for product percentage, nature of product and reaction rate (Aye and Yamaguchi, 2006; Davidian et al., 2007; Kimura et al., 2006; Svoboda et al., 2007; Swierczynski et al., 2007; He et al., 2008). The nano particles act as green catalyst for selective reduction of the aldehydic group in the presence of other functional groups. For example,  $\text{NO}_2$ , CN and alkenes gave the corresponding alcohols in excellent yields (Kidwai et al., 2006) (Tables 1 and 2).

These results were supported by previous studies (Bran et al., 2003). The liquid extract of tea containing

small quantity of tar showed acidic pH (4.12). The FTIR spectrum (Figure 3) showed that first percentage transmittance peak was at  $1641.38 \text{ cm}^{-1}$  while the other was at  $3321.38 \text{ cm}^{-1}$ . First is showing C=O linkage of ester. Voort et al. (2008) described that the peak between 1500 to 2000 showed ester linkage but between 3000 to 3500 is of -OH or hydrocarbons. According to Lima et al. (2008) FTIR spectrum of biodiesel peaks between 3000 and 4000 and showed C-OH bending. Alkanes are oxidized to alcohols and later to carboxylic acids by catalyst. Alcohols are reduced to alkanes by catalyst. The alcohols react with carboxylic acid to give esters as shown in FTIR spectrum (Figure 3).

The liquid extract of tea was transesterified with ethyl alcohol and NaOH at low temperature. Esters presence was verified from GC-MS analysis of ethyl ester (biodiesel). Table 3 shows that 40.79% ester was produced (Table 3). Fragments and their production patron



**Figure 2.** Bio-ethanol productions from spent tea by *A. niger*.

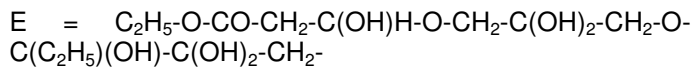
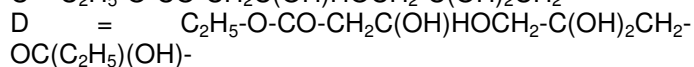
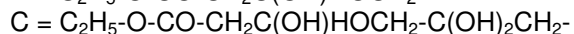
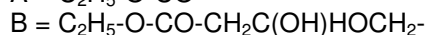
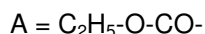
**Table 1.** Experimental conditions for gasification.

Parameters	Conditions
Spent tea	100 g
Co nano particles	0.15 g
Temperature	300 °C
Gaseous product	28%
Liquid product	60%
Solid product	12%

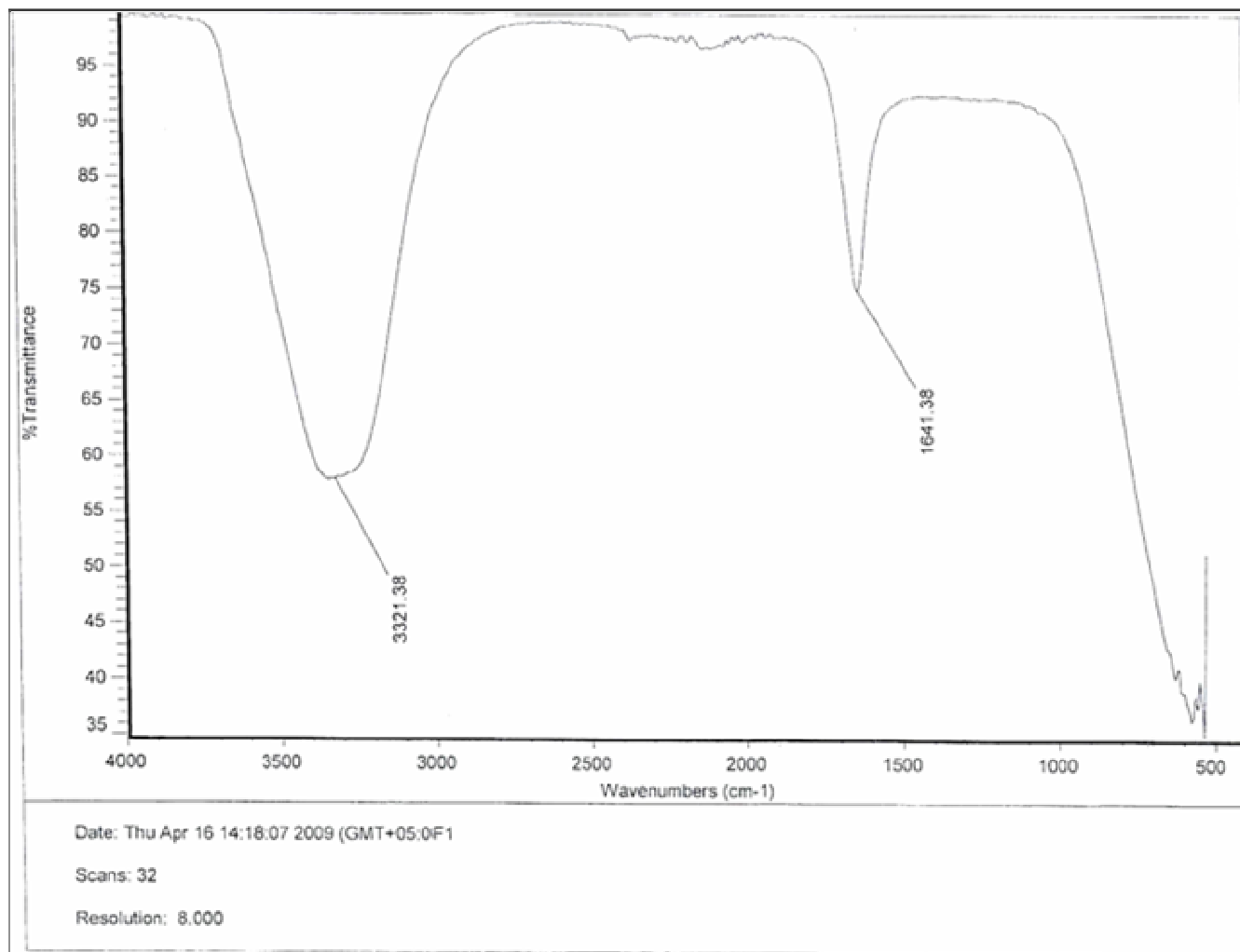
**Table 2.** Results of GC-MS of gaseous sample taken at 300 °C.

No of Peaks	Fragments	m/z	Abundance	Percentage (%)
1	Methane (CH <sub>4</sub> )	16	607744	4.21
2	Ethene (C <sub>2</sub> H <sub>4</sub> )	28	8388096	58.17
3	Methanol/(CH <sub>3</sub> OH)	32	4255232	29.51
4	Propyne (C <sub>3</sub> H <sub>4</sub> )	40	256512	1.77
5	Propane (C <sub>3</sub> H <sub>8</sub> )	44	911616	6.32

are given below.



These results showed that it is a good source of ethyl ester like compounds. Synthesis of esters or biodiesel was reported by Lestari et al. (2008). They synthesized biodiesel/esters via deoxygenation of carboxylic acids over supported Pd/C catalyst. The tea extract contained traces of Co nano particles. Table 3 shows that biodiesel



**Figure 3.** FTIR spectrum of liquid extract of tea after gasification.

**Table 3.** Results GC-MS of transesterified tea extract by using NaOH and C<sub>2</sub>H<sub>5</sub>OH.

No of Peaks	Fragments	m/z	Abundance	Percentage (%)
1	A	73	139456	40.79
2	B	147	28408	8.30
3	C	207	104904	30.66
4	D	281	39160	11.44
5	E	341	30160	8.81

production from spent tea is greater than the production from spent coffee as studied by Kondamudi et al. (2008). They extracted 10 - 15% oil while 60% of liquid extract was gotten. On transesterification, coffee oil produced diesel while spent tea produced 40.79% ethyl ester as biodiesel. The results of GC-MS showed that biodiesel obtained from spent tea extract is a strong candidate as an alternative to diesel. Currently, the use of spent tea is limited to gardens as compost for rose plants. After

recovery of the catalyst, the charcoal left behind could still be used as a source of carbon for gardens, fertilizer for crops and adsorbent for metal pollutant's removal as in previous studies (Razmovski and Sciban, 2008). Suarez et al. (2009) suggested that biodiesel obtained by thermal pyrolysis is better than petroleum diesel and alcoholysis based biodiesel, in sulfur content and in lubricity. So the present study diesel shall be low in sulfur and nitrogen and its lubricity shall be better than other

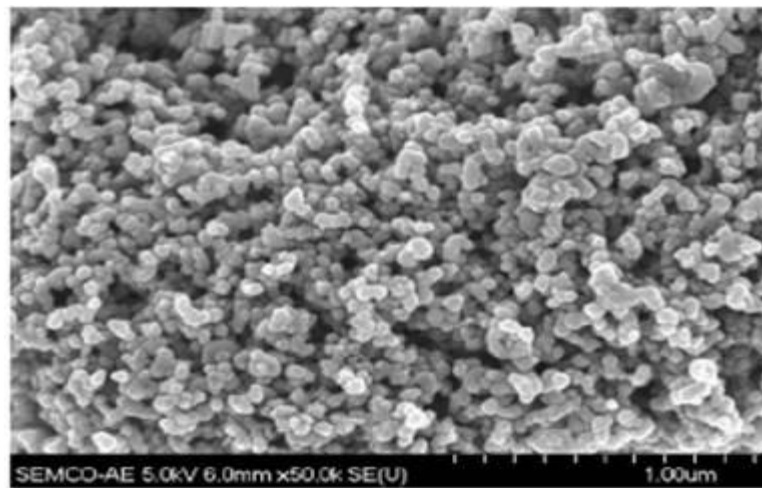


Figure 4. SEM of Co-nano particles.

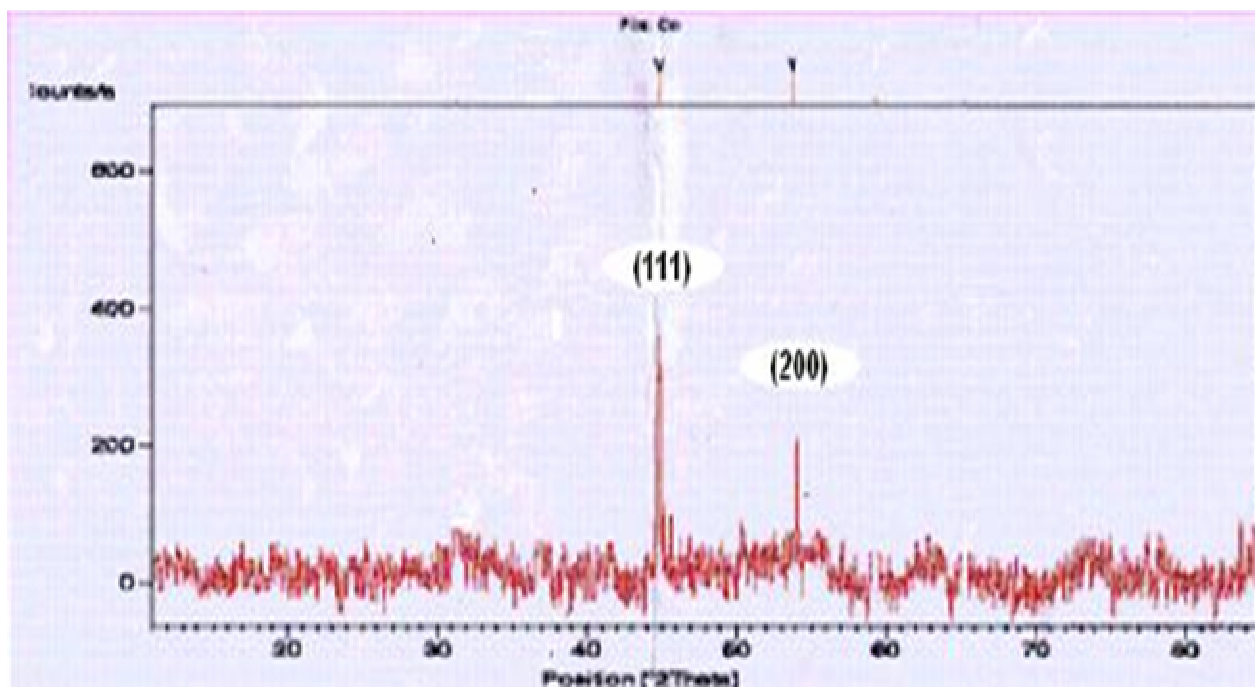


Figure 5. X-ray diffraction patterns of Co nano particles.

type of diesels. Therefore it shall be more environmentally friendly. Huge availability of raw material, environment friendly and useful byproduct makes this technology cheap. The spent tea can be used as a potential source to produce quality biodiesel and fuel hydrocarbon gases. 60 ml liquid extract after gasification gives more than 24.4 5 ml biodiesel (Figure 4).

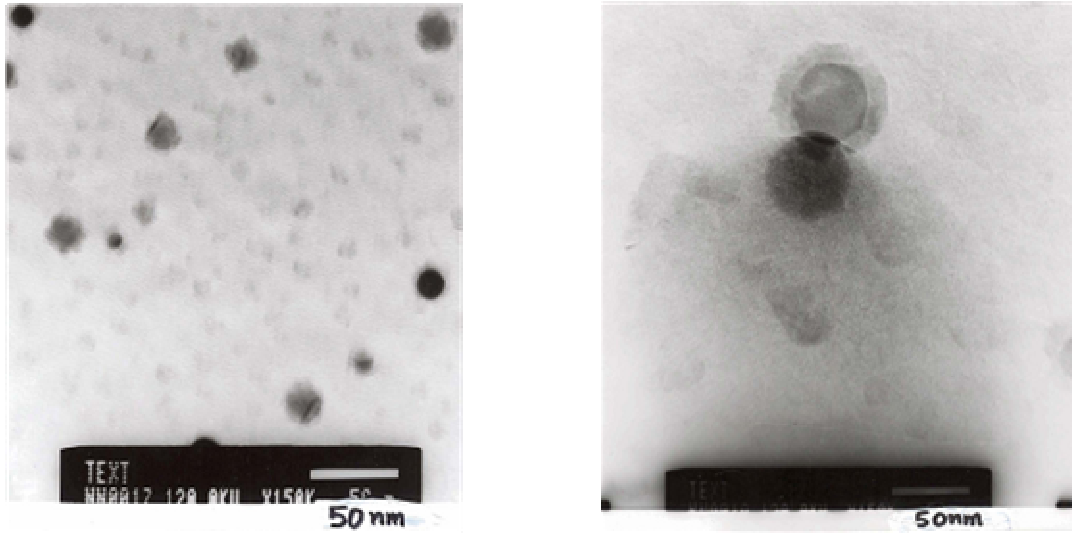
The Co nano particles and particles of charcoal's morphology and size were studied by SEM, TEM and XRD. Figure 4 shows the SEM images of Co nano

particles. The morphology of particles seemed to be single crystals of nearly uniform size for Co particles. Cobalt particles show directional linkages due to magnetic interaction (Figures 5, 6 and 7).

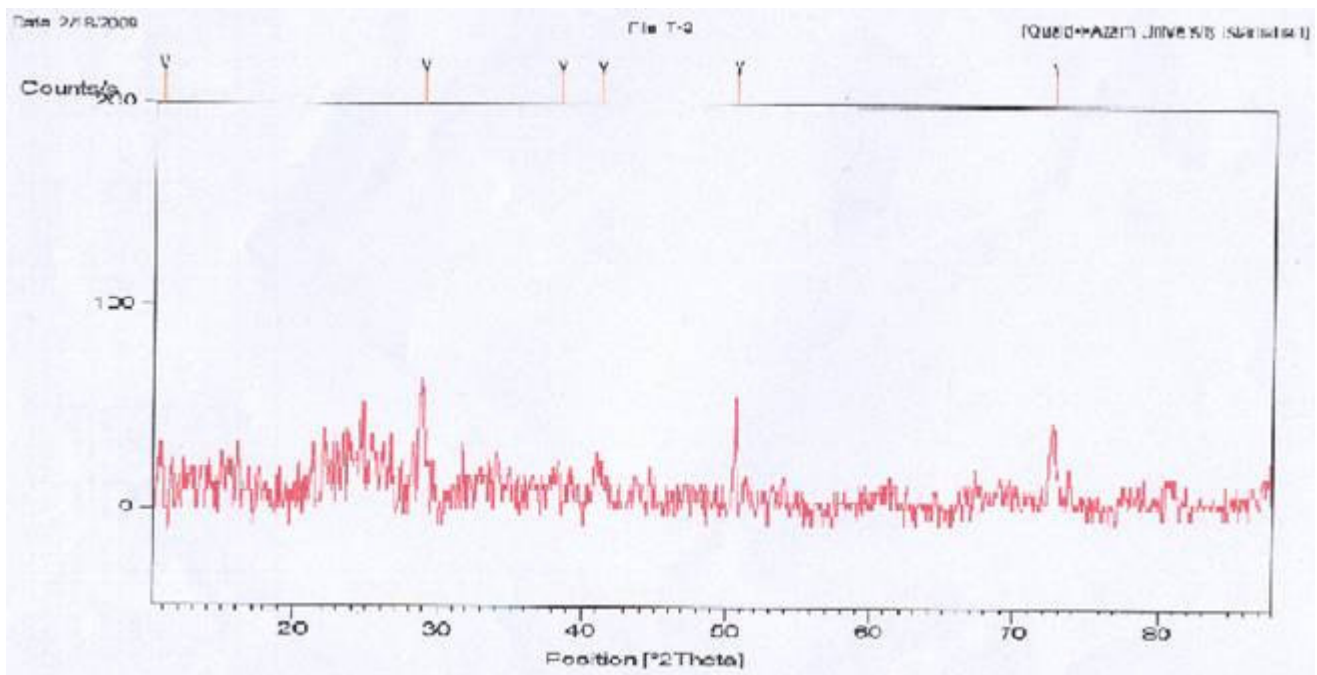
Figure 6 shows TEM of charcoal of spent tea produced. The morphology of particles of charcoal showed that they are spherical in shape.

The X-ray diffraction pattern of spent tea charcoal after gasification by Co nano particles is shown in Figure 7. The particle sizes ranged from 9 to 122.15 nm. The XRD





**Figure 6.** TEM images of tea residue/charcoal particles at low magnification with scale bar: 50 nm synthesized from spent tea.



**Figure 7.** X-ray diffraction patterns of tea residue/charcoal particles.

pattern for six peaks (y-1 to y-6) showed a structure that is hexagonal and cubic. Some peaks were clearly broadened and some were not. This may be due to reduced particle size. Using Scherrer formula, based on line broadening, the mean crystal sizes of the powders were determined to be 16.88, 34.82, 30.50, 9.0, 122.15 and 25.6 nm, respectively.

For bioethanol production *A. niger* was used. The non aqueous filtered sample was analyzed on GC-MS for

ethanol. The results of GC-MS showed ethanol (m/z 45, abundance 1513472 and ethanol was 57.49%). There were some strong evidences for the use of fermentation for bioethanol production (Ali and El-Dein, 2008; Zakpaa et al., 2009). Table 4 shows results of identification, confirmation of growth temperature, shape, colony morphology, mobility (Kausar, 2004), spore formation, H<sub>2</sub>S test (Tabassum, 2003; Kausar, 2004) and gram staining, (Pandey, 1994) of *A. niger*. It showed that *A. niger* is



**Table 4.** Results of identification and confirmation tests of *A. niger*.

Name of test	Results
Growth Temperature	30 °C
Shape and Colony morphology	Colony consists of compact yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidiophores were long, smooth-walled and terminate in pale-brown colored globose vesicles
Mobility	-ve
Spore formation	+ve
H <sub>2</sub> S Test	-ve
Gram staining	-ve

excellent for bioethanol production from spent tea. Spent tea contains sugar in sufficient quantity. Skory et al. (1997) examined 19 *Aspergillus* and 10 *Rhizopus* strains for their ability to ferment sugars. An appreciable level of ethanol has been produced by them. *A. niger* can easily grow on milk and sugar already present in spent tea. Hence, it can easily produce ethanol. Cellular hemicellulases are enzymes that can be used in fermentation. The sucrose was enzymatically converted into ethanol (Gray et al., 2006). The ethanol producing enzymes are cellulose and hemicellulose-degrading. These were isolated from *A. niger* (Thygesen et al., 2003; Villena and Gutierrez-Correa, 2006). *A. niger* can be isolated from different sources for bioethanol production (Li et al., 2009). The sample containing ethanol was subjected to distillation. The pure sample was analyzed on GC (Gas Chromatograph Model Varian CP-3800). Result showed that when minimum time was 2.358 min, peak area was 199986.96  $\mu$ Vs, height was 35341.77  $\mu$ V, area percentage was 100% and BL area /height (s) was 5.6587. The results of GC-MS and GC showed that spent tea can produce bioethanol on large scale. The biomass polysaccharide part has been considered as safe source of energy for the production of fuel ethanol. The enzyme based application is advantageous over chemical treatments due to its higher conversion efficiency, the absence of substrate loss due to chemical modifications and the use of more moderate and non-corrosive physical-chemical operating conditions, such as lower reaction temperatures, "neutral" pH and the use of biodegradable and non-toxic reagents. Biomass degradation is a complex multi-enzymatic process. Cellulose digestibility by cellulases alters the organization and the chemical interactions amongst the cellulose, hemicellulose and lignin. Pending on process conditions, there is formation of significant sugars and lignin degradation products that are inhibitory to ethanol fermentation. Bon and Ferrara (2007) studied bioethanol production via enzymatic hydrolysis of cellulosic biomass (Bon and Ferrara, 2007). They reported that the rate of saccharification is directly proportional to substrate concentration up to optimal substrate concentration. This is because random collisions between the substrate and

enzyme active sites happen more frequently. Beyond the optimum, the active sites are saturated so that higher substrate concentration has no effect on rate of saccharification. Saccharification increase with substrate concentration and also increase in reducing sugars with increase in substrate. Temperature has a complex effect on enzyme activity. It affects the speed of molecules, the activation energy of the catalytic reaction and thermal stability of the enzyme. Generally, saccharification increase with temperature up to the optimum after which it declines. The increase in temperature is due to corresponding increase in kinetic energy and the decline after the optimum due to enzyme denaturation.

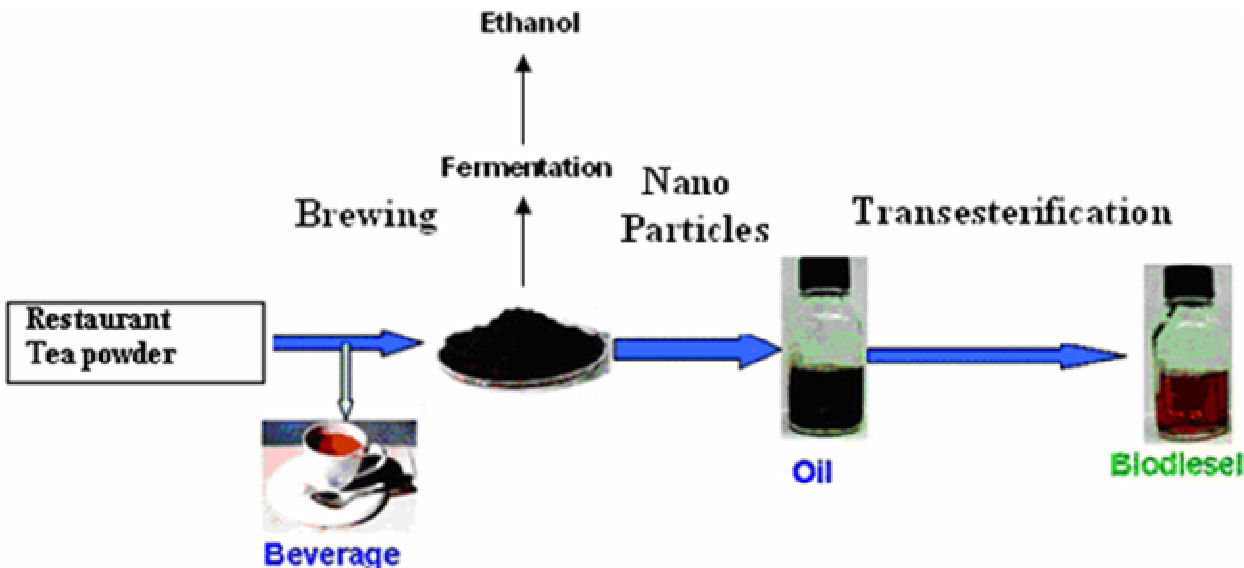
The pH of a solution has several effects on the structure and activity of enzymes and hence saccharification and fermentation. Enzymes are amphoteric molecules containing a large number of acidic and basic groups, mainly situated on their surface. The charges on these groups are different. The variation is according to their acid dissociation constants, with the pH of the solution. Thus pH affects the reactivity of the catalytically active groups. The relative low pH provides acidic condition which prevents bacterial contamination during fermentation (Zakpaa et al., 2009).

The fermentation waste can be used as fertilizer. Bioethanol technology is very useful because it can be used to produce 4 billion gallons of alcohol fuel enough to replace 460 million barrels of oil. The graphical summary is given in Figure 8.

## Conclusions

The following conclusions were reached

1. Spent tea (*Camellia sinensis*) is a potential resource that can be used for cheap industrial production of biofuels.
2. After fermentation of spent tea by *A. niger*, solid residue can be used as fertilizer.
3. Gasification waste of spent tea is a best source of carbon for soil fertility.
4. Spent tea is an excellent source of environmentally friendly hydrocarbon fuel gases.



**Figure 8.** Graphical summary of biofuels produced from spent tea.

5. The use of this solid waste as a source of biofuel will add green energy technology in the existing environment. The process would be ideal for countries where tea is produced and consumed.
6. This study gives a basis for further research.

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