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Research Article

IMMOBILIZATION OF PECTINASE ON IRON OXIDE NANOPARTICLES AND ITS POTENTIAL APPLICATION IN BIOMETHANOL PRODUCTION

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ABSTRACT

Fossil fuel depletion resulted in growing energy prices have made bio diesel an interesting alternative that is being introduced to the transport sector in many countries. Alternative fuel produced from agricultural waste using microbial enzymes has considerably gaining interest especially sugar beet pulp, a by-product and waste that released from sugar industry which rich in pectin residues. Pectinase from *Aspergillus niger* was commercially procured and it was immobilized onto chemically synthesized iron oxide nanoparticles using co-precipitation method and characterized using scanning electron microscope showed 85 nm and 136 nm in size and spherical in shape. Functional groups were determined using Fourier Transform Infrared spectroscopy that ensured pectinase immobilization. Enzyme immobilized nanoparticles were subjected into sugar beet pulp thereby methanol produces. Methanol from water was separated and purified using distillation process. Presence of methanol was confirmed by Schiff's reagent test. Biomethanol production from agricultural waste with the help of pectinase enzyme bounded on iron oxide nanoparticles found as novel one. In conclusion, current work has taken step towards production of non-renewable fossil fuel which builds an eco-friendly atmosphere.

Keywords: Biomethanol, sugarbeet pulp, Pectinase, iron oxide nanoparticles, Scanning Electron Microscope, Fourier Transform Infrared spectroscopy.

INTRODUCTION

In recent years the oil and natural gas prices were increased and the fuels source would depleted in the future. In 2012, 19 billion tons of fossil fuels were consumed over all the worldwide that resulted in acid rain, photochemical smog, an increase of atmospheric carbon dioxide (CO_2). Many scientists were in research to found out the alternative fuels from the renewable sources. Renewable methanol could reduce the carbon dioxide emission in the atmosphere from 65% to 95%. Alternative fuel was being introduced into transport sectors in many countries that were a successful displacement for petrol, diesel, and gasoline. In the world, China still maintained its top position in the methanol production as well as consumer. Since India was known for its agricultural wastes and the bio fuels were produced from agricultural wastes was encouraged[1]. Pectin was the major component constituted in the plants and fruits such as apple, sugar beet, citrus fruits etc., since these vegetables and fruits were an important healthy diet for human energy, hence we should not use them for a methanol production process[2].

Sugar beet pulp, by-product were used as animal feed or thrown as waste having 15% of pectin-rich compounds that would be a good resource for bio methanol production. Pectin residues in this waste would employed for methanol production by means of esterification process that done by Pectinase enzyme. Nowadays, enzymes have been used remarkably in industrial applications to simplify the work with added advantages such as minimum quantity of initial product, huge amount of end product in industrial purposes which also saves energy, chemicals, water, money and raw materials. Here Pectinase in the industrial applications have been limited since it's very costlier, less stability and difficult to recover from reactor solution at end of the process. It could be overcome by immobilizing the Pectinase in iron oxide nanoparticles. Nanoparticles as carrier in immobilization was rapidly mushrooming technique especially iron oxide nanoparticles because of its high stability, easier separation from the solution using magnet, prevents the loss of excess enzyme that could be reused later tends to be cost-effective in nature retain their residual activity after many cycles[3].

The current study was designed with the main objective to novel synthesis and structural study of iron oxide nanoparticles as a support for immobilization of Pectinase enzyme that would apply in methanol production from the plant source for. In conclusion, current work has taken steps towards the production of non-renewable fossil fuel using microbial enzymes, which builds an eco-friendly atmosphere.

MATERIALS AND METHODS

Procurement of enzyme

Pectinase from *Aspergillus niger* (Hi-media) was acquired from Jayam Scientific Company, Coimbatore, India.

Synthesis of iron oxide nanoparticles

Ferric salt (0.1M $FeCl_3$) and Ferrous salt (0.2M $FeSO_4$) was mixed and added into heated basic solution (0.5M NaOH) at 75°C until it turned into black in colour would indicate iron oxide nanoparticle formation. The solution was stirred vigorously for 30 min and centrifuged for 15 min at 5000 rpm. The pellet was rinsed several times with deionised water followed by centrifugation at 3000 rpm for 10 min to remove excess irons in the suspension. Finally, the particles were dispersed in distilled water. The particles were collected and dried overnight in an oven at 80°C[4].

Characterization of iron oxide nanoparticles

UV-Visible spectroscopy analysis

The optical absorption characteristics of Fe_3O_4 nanoparticles were analysed by dispersing the solids in the methanol for the length of 1 cm under the UV-Visible region ranging from 400 to 1100 nm by UV-1700 series spectrophotometer with the slit width of 1 nm[4].

Scanning electron microscope (SEM) analysis

The size and shape of the nanoparticles were characterized by Scanning Electron Microscopy. The specimens were prepared as thin film in double-sided carbon tape. The surface of the particles was visualized using FEI Quanta FED 200[4].

Fourier transform infrared spectroscopy

The prepared sample was also investigated for MIR region using Schimadzu make FTIR 8400 S series spectrophotometer at the scan rate of 25 scans/ min with 4 cm^{-1} resolution. To analyse, 1 mg of Fe_3O_4 nanoparticles were mixed with 100 mg of KBr (Infrared grade) and pelletized under vacuum environment and the sample was examined in the range between 400 and 4000 cm^{-1}[4].

Immobilization Pectinase on the iron oxide nanoparticles

Iron oxide nanoparticles were added with 4 ml of 10% glutaraldehyde solution to the magnetic nanoparticles (MNP) solution with continues stirring for 60 minutes. Centrifuged was done to remove excess reagent at 10000 rpm for 15 minutes at 4°C. Activated MNPs were stored in 0.1 M potassium phosphate buffer, pH 6.0 at 4°C overnight. After separation of MNPs, 50 ml of phosphate buffer, pH 6.0 containing Pectinase enzymes (5 U /ml) was added to activate MNOs and the mixture was incubated under shaking condition for 24 hours. The Pectinase bound MNPs were then decanted using a permanent magnet and washed several times by deionised water[4].

Application of Pectinase for biomethanol production

Collection of sugar beet pulp

Sugarbeet pulp was collected from the sugar industry in Sathyamangalam, Tamil Nadu, India.

Methanol production from sugar beet pulp

Sugar beet pulp was washed with running tap water to remove dust, dirt and impurities and blot dried with tissue paper. It was sterilized with addition of water in the ratio of 1:2 for 121°C for 15 mins to ensure the quality of the sample. After sterilization, 1% immobilized Pectinase enzymewas added and kept for incubation for 36 hours at pH 7. Enzyme was added every 2 hours in the first 6 hours for quicker production. After incubation period was over, it was subjected for distillation for methanol separation[5].

Separation and purification of the methanol

Sugar beet pulp residue after completion of process was removed by filtration followed by centrifugation at 5000 rpm for 15 mins. Residual enzyme immobilized iron oxide nanoparticles were decanted using permanent magnet. Methanol – water mixture was subjected for distillation process using alcohol distillation unit with a fixed temperature of 78.4°C (Boiling point of methanol). The evaporator steam from the fermented sample was condensed with the help of a condenser attached to it. The condensed distillate sample was then collected through the outlet and measured the methanol concentration using Schiff's reagent test[6].

Qualitative analysis of methanol using Schiff reagent test

About 4 ml of distillate, 0.5 ml of ethyl alcohol, 2 ml of 3% potassium permanganate solution and 0.2 ml of phosphoric acid were added in a test tube and kept aside for 10 minutes. Then 1 ml of 10% oxalic acid was added followed by 1 ml of concentrated sulphuric acid and the contents were cooled to room temperature. Now, 5 ml of Schiff's reagent was added and the colour was observed after 30 minutes[7].

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is reliable technique to identify the functional groupsof the alcohol from the distillate that represents the methanol. The spectrum was generated than analysed by the IR solution software. The sample transmittance and reflectance of the infrared rays at different frequencies were translated into an IR absorption plot which was then analysed and matched with known signatures of identified materials in the FTIR library[6].

RESULT AND DISCUSSION

In the current research, the chemical synthesis of iron oxide nanoparticles was done and acquired nanoparticles as black colour precipitates and magnetic adsorption were shown in Figure 1[4] Obtained as a black coloured precipitate. Characterization of nanoparticles was done. The absorption band at 229 nm indicates the formation of nano sized particles. The two bands observed at 256 nm, 275 nm and 352 nm are attributed to the local vacancies present in the Fe_3O_4 nanoparticle lattice. The broad peak at 267 nm indicates the polydispersity of the nanoparticles in Figure 2. The high resolution scanning electron microscopy (HRSEM) was performed to visualize the synthesized Fe_3O_4 nanoparticles morphology. Acquired nanoparticle showed that hexagonal and spherical in nature. The microscopy observation shows the Fe_3O_4 nanoparticles not appear as discrete particles but form much larger dendritic flocks whose size could have reached 86 nm shown in Figure 3(a).[8] Described that the obtained iron oxide nanoparticles showed that hexagonal and spherical in nature however the percentage of nanoparticles beyond 100 nm is very less. The SEM image of Pectinase immobilized on Fe_3O_4 nanoparticles is also shown in same Figure 3(b). The result shows that the size of the nanoparticles has increased to 138 nm which is because of Pectinase immobilization on iron oxide nanoparticles. The increase in size of nanoparticles after immobilization with Pectinase is due to the crosslinking of glutaraldehyde to the amino functionalized Fe_3O_4 nanoparticles. It was found that the functionalized Fe_3O_4 nanoparticles.[9] Reported that the functionalized Fe_3O_4 nanoparticles have number of OH groups. The functional group analysis has been performed by using Fourier Transform Infrared Analysis (FTIR). The bands observed from the spectra at 1050 and 1006 cm^{-1} in iron oxide nanoparticles were attributed to the tether of hydroxyl groups onto the surface of iron oxide nanoparticles. The characteristic vibration peaks at 265 and 405 cm^{-1}, did not be detected in our experiments since the former was beyond the detection limit, and the latter is too weak to be resolved. Figure 4 shows FTIR spectra of iron oxide nanoparticles. The above findings suggested that some groups of Pectinase enzyme got attached on the surface of the nanoparticles.

Sugar beet, a by-product from sugar industry as a plant waste and animal feed stock was used as a substrate for the production of methanol in this study. Since, it could be served as a pectin sources as well as various scientific publications resulted in the usage of sugar beet for biofuel production, it has been selected for the production of biomethanol. The methyl-ester group in pectin component which was present in the sugar beet were cleaved by the enzyme that was immobilized on iron oxide nanoparticles released the methanol in the liquor solution.[5] Conveyed that trans esterification process had been carried out in the production process for the methanol production. Sugar beet pulp should be separated from the methanol-water mixture by filtration or centrifuged. The liquid portion supernatant was collected which constitutes the methanol and water. Distillation process was carried out for the separation of methanol and water. During distillation process, the temperature was maintained at the boiling point of the methanol not exceeded water boiling point as to prevent the vaporization of water content. This could helped out to get methanol more concentrated by lowering the water content still remains in the bottom of the flask had been depicted in Figure 5.[6] Had revealed that the distillation process could be widely used in the purification of biofuel production from the plant waste.[5] Had described that temperature could be maintained at the boiling point of the suspected product might get the product more concentrated.

Schiff reagent test could be used for the detection of alcoholic and aldehyde groups. Schiff reagent could have reacted with the methanol and produced violet colour.[7] Described that this test was widely used to detect the methanolic group among other alcoholic groups. According to findings of Schiff, Schiff's reagent was a red solution of rosaniline hydrochloride dissolved in water that reacts with the alcoholic groups, there by magenta colour produced shown in Figure 6. The separated distillate product was subjected to Fourier transform infrared spectroscopy (FTIR) for characterization in order to identification of the alcoholic group representing the presence of methanol in the given sample. The bands observed from the spectra were found to be strong absorption peaks at 3672.47, 3495.01, 3194.12 and 3101.54 cm^{-1} were attributed to the presence of alcoholic groups in the distillate confirmed the presence of methanol showed in Figure 7.

Figure 1: Separation of Magnetic Nanoparticles

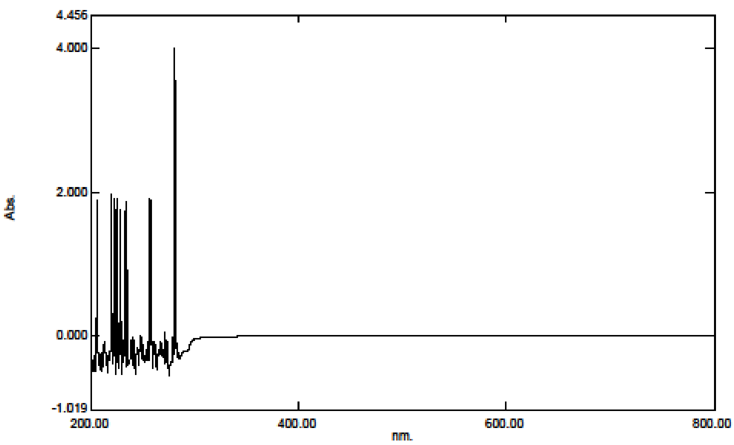

Figure 2: UV-Visible spectroscopy analysis of iron oxide nanoparticles

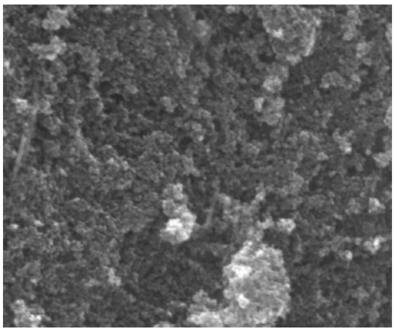

Figure 3(a): Naked MNPs (86 nm)

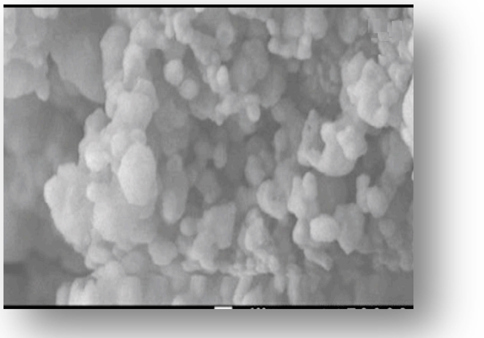

Figure 3(b): Pectinase bound MNPs (138 nm)

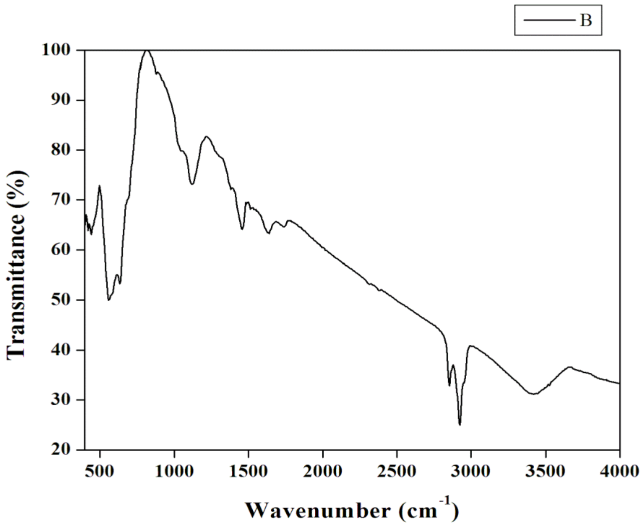

Figure 4: FT-IR Spectrum of immobilized iron oxide nanoparticles

Figure 5: Methanol separation using simple handmade distillation apparatus

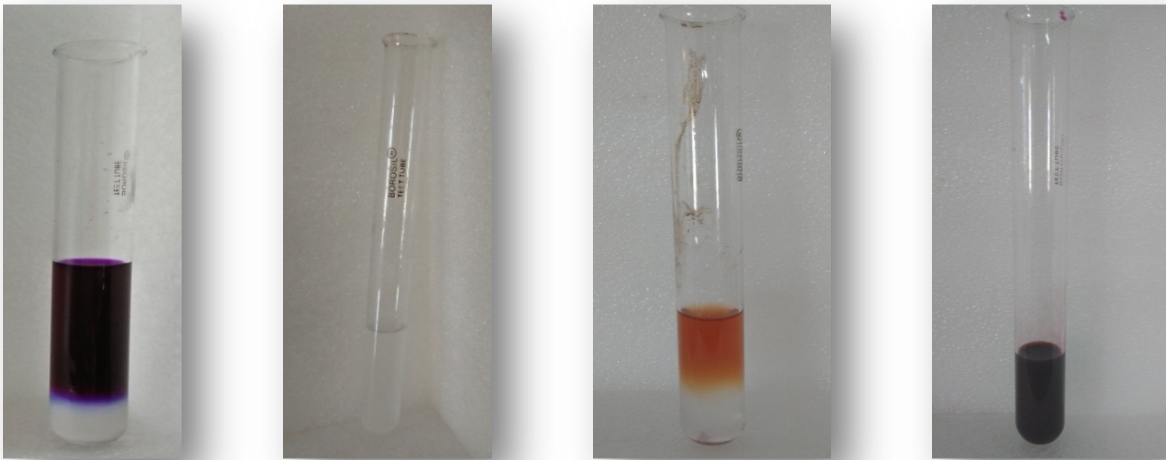

Figure 6: Qualitative analysis of biomethanol production

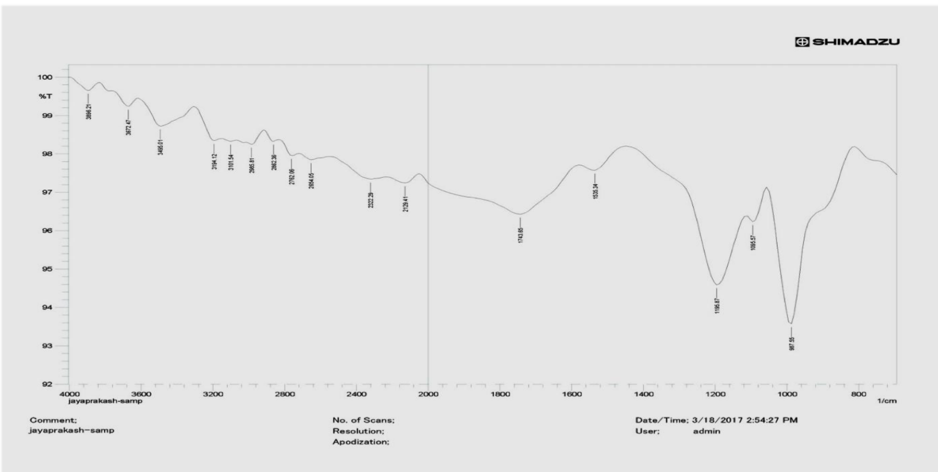

Figure 7: The functional groups (FTIR spectra) of methanol

CONCLUSION

The current study gave the step taken towards the replacement of fossil fuels by biofuel produced from plant waste. Though the production of methanol for biofuel purposes has already existed the production of methanol from the cheaper sources such as plant waste was found to be novel in this field that reduces the cost and higher energy production. Biomethanol production has a considerable interest for present and future technologies due to non-toxic and eco-friendly in nature.

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