

Pretreatment of sugarcane bagasse using two different acid-functionalized magnetic nanoparticles: A novel approach

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Abstract

Purpose: Pretreatment is one of the most important steps in the production of bioethanol from renewable feedstocks like lignocellulosic biomass. However, existing pretreatment approaches have some limitation. In this context, the present study is aimed to develop novel, eco-friendly and cost-effective nanoparticles-mediated strategies for pretreatment using acid-functionalized magnetic nanoparticles (MNPs).

Methods: Initially, iron oxide MNPs (Fe_3O_4 -MNPs) were synthesized, which were further modified by applying silica coating (Fe_3O_4 -MNPs@Si) and functionalized with alkylsulfonic acid (Fe_3O_4 -MNPs@Si@AS) and butylcarboxylic acid (Fe_3O_4 -MNPs@Si@BCOOH). After, their characterization using different analytical techniques, these both acid-functionalized MNPs were evaluated for their catalytic efficacy in the pretreatment of sugarcane bagasse.

Results: Both above-mentioned acid-functionalized MNPs used for the pretreatment of sugarcane bagasse showed promising catalytic activity as compared to normal acid pretreatment. It was observed that the hydrolysis of sugarcane bagasse and release of fermentable sugars is depended on the concentrations of acid-functionalized MNPs used; increase in the concentration of acid-functionalized MNPs also increases the efficacy of pretreatment. Both Fe_3O_4 -MNPs@Si@AS and Fe_3O_4 -MNPs@Si@BCOOH at 500 mg/g of bagasse showed maximum amount of sugars (xylose) liberated i.e. 18.83 g/L and 18.67 g/L, respectively which is comparatively higher than the normal acid pretreatment (15.40 g/L).

Conclusions: The acid-functionalized MNPs used in the present study are very effective in the pretreatment of sugarcane bagasse. Hence, such nanoparticles can be used as rapid and eco-friendly alternative methods for the pretreatment of a variety of lignocellulosic materials. Moreover, reuse of these nanoparticles due to their potential magnetic nature will help to reduce the cost involved in it.

Keywords: Magnetic nanoparticles, acid functionalized magnetic nanoparticles, lignocellulosic biomass, sugarcane bagasse, pretreatment.

Introduction

The continuous depletion in the limited sources of conventional energies (fossil fuels) is a major concern across the world. Moreover, extensive consumption of these forms of energies is not accepted due to emissions of greenhouse gases in the environment [1]. Therefore, excessive dependence on fossil fuels and their limited sources forcing researchers all over the world to search for alternative renewable energy sources like biofuels. Hence, the development of novel, eco-friendly and economically viable renewable energy sources has become a very intense research area in the last few decades. In this context, utilization of a variety of lignocellulosic materials have been extensively studied as a novel renewable feedstock for the production of bioethanol during the last few years aiming to develop new strategies to deal with the huge energy crisis and environmental concerns pose due to extensive use of fossil fuels [2-4].

Nowadays it is well proven that ethanol can be used as a potential partial or complete replacement for conventional transportation fuel. Considering these possibilities, a significant rise in the production of ethanol was seen across the world during the last few years. If we look into the data related to ethanol production in almost last decade, it was reported that about 19 billion gallons of ethanol was produced worldwide in 2009 [5] which was significantly increased to 28.7 billion gallons in 2018 [6].

Currently, bioethanol is generally produced from the alcoholic fermentation of monomeric sugars obtained from sugar-based and starchy crops [5]. In addition, lignocellulosic waste (biomass) derived from forest, agriculture and various other industries can also be used for ethanol production after hydrolysis of their constitutive sugar polymers into monomers [7]. Although it was proposed in many reports that lignocellulosic biomass can be promisingly used as novel feedstocks for the production of bioethanol, there are many technological challenges due to the complex structure of these materials. The carbohydrate polymer (cellulose) which is the source of fermentable sugars is not freely available for enzyme degradation. The linear chain of monomeric units (β -1,4-linked glucopyranose) of cellulose are glued with hemicelluloses and covered with a lignin sheath which the material recalcitrant [8]. Therefore, the production of bioethanol in generally carried out in three different steps: (i) pretreatment of lignocellulosic biomass, it is used for the release of carbohydrate polymers (ii) enzymatic hydrolysis, for the conversion of carbohydrate polymers into fermentable sugars and (iii) fermentation, here fermentation of released sugars was performed using suitable microorganisms to get the bioethanol.

In the present study, we have focused on the first and most important step of bioethanol production, i.e. pretreatment of lignocellulosic biomass. Various physical, chemical and biological approaches have been proposed for the pretreatment of different lignocellulosic materials. Among the chemical pretreatment methods, mineral acids like sulfuric acid, phosphoric acid, etc. are commonly used. The basic aim of the acidic pretreatment is to solubilize part of the hemicellulose and exposing a larger area of cellulose to the enzymatic attack [9]. However, every pretreatment method including acid pretreatment has some advantages and disadvantages. Some of the methods found to be simple and rapid but at the same time these methods involved substantial capital investment due to the requirement of corrosion-resistant materials. Apart from this, the cost required for neutralization, detoxification and waste disposal is another important concern [10,11].

In this context, instead of using traditional liquid acid pretreatment, a focus has been given on the development of solid acid catalysts it has the ability to provide the catalytic properties of homogeneous acids with the advantage that they can be recovered from the reaction mixture by physical separation [12,13]. Among the solid acid catalysts, nanotechnology-based magnetic catalysts (nanobiocatalysts) have gained the tremendous attraction from the scientific community. Recently, Rai et al. [14] reviewed the possibilities of various nanobiocatalysts in the pretreatment of different biomass. For the first time, Pena et al. [15] used two different acid-functionalized magnetic nanoparticles (i.e. perfluoroalkylsulfonic (PFS) acid and alkylsulfonic acid-functionalized) for the pretreatment of wheat straw. The results obtained were significant and hence that study was proved the potential of such catalysts in the pretreatment of biomass. After that, some more attempts have been made which mainly include evaluation of catalytic efficacy of sulfonic acid-modified mesoporous silica for the hydrolysis of sucrose and starch [16]. Moreover, in one of the recent studies by Qi et al. [17] carbon-based solid catalysts functionalized with sulfonic acid were promisingly used for catalytic pretreatment of corncob. The results obtained reported the release of the high yield of xylose (78.1%).

Application of acid-functionalized magnetic nanocatalysts is advantageous in biomass pretreatment because after the reaction such catalysts can be recovered by applying an external magnetic field and reuse in subsequent cycles of pretreatments. Considering these facts, two different acid-functionalized magnetic nanoparticles (MNPs) were prepared in the study and their catalytic efficacy was evaluated for the pretreatment of sugarcane bagasse having a main aim of development of simple, rapid, eco-friendly and economically viable pretreatment approach for lignocellulosic biomass.

Materials and Methods

Reagents

Ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH), isopropanol and methanol were purchased from LabSynth Laboratory Products Ltd. (Sao Paulo, Brazil). Ethanol (96%), hydrogen peroxide (H_2O_2), D-(+)-cellobiose (98%) purchased from Chromolab Laboratory Products Ltd. (Sao Paulo, Brazil). Sulfuric acid (H_2SO_4) (98%), hydrochloric acid (HCl), 3-mercaptopropyltrimethoxysilane (MPTMS) (95%), potassium bromide (KBr) (IR grade) was purchased from Scharlab Laboratory Products Ltd (Sao Paulo, Brazil). Tetraethylorthosilicate (TEOS) (99.99%), and 4-(triethoxysilyl)-butyronitrile (CPTES) (98%) were purchased from Sigma-Aldrich Brazil Ltd. (Sao Paulo, Brazil). Moreover, SepPak C18 filter column were purchased from Water Technologies Ltd. (Brazil).

Collection of lignocellulosic biomass and processing

Sugarcane bagasse has been used as lignocellulosic biomass in the study and it was collected from Ipiranga Agroindustrial (Descalvado, SP). After collection, the biomass was sundried and grounded using milling machine followed by 10 mesh sieve. The resultant material was properly collected and particles between 20 and 30 mesh were used for the elaboration of experiments.

Methods

Synthesis and silica coating of magnetic nanoparticles (Fe_3O_4 -MNPs)

A chemical precipitation method proposed by Gaikwad et al. [18] was used with some modifications. For the synthesis of Fe_3O_4 -MNPs, a solution of 2 % ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was stirred at 80 °C on the magnetic stirrer with continuous drop wise addition of 2 M NaOH till the pH reaches to 11. Further, the mixture was heated in a microwave oven at 320 watts for 3-5 minutes. Later, the solution containing Fe_3O_4 -MNPs was centrifuged at 4000 rpm for 10-15 min and the pallet of thus synthesized MNPs was washed 3-4 times with distilled water and a wash with ethanol. Finally, the MNPs were dried in the hot-air oven at 60 °C overnight.

The dried powder of Fe_3O_4 -MNPs was later subjected for its surface silica coating using a method proposed by Rajkumari et al. [19] with some modifications. For surface silica coating of Fe_3O_4 -MNPs, the dispersion of 2 g of dried Fe_3O_4 -MNPs in a mixture of 100 mL ethanol, 15 mL distilled water and 1 mL TEOS was prepared and it was sonicated for about 3 h followed by addition of 15 mL of 2.5 M NaOH . Further, the mixture was stirred in a

magnetic stirrer for about 2 h at room temperature. Then the surface silica coated MNPs ($\text{Fe}_3\text{O}_4\text{-MNPs@Si}$) were separated by applying an external magnetic field and washed 3-4 times with each distilled water and ethanol and dried in a hot-air oven at 100 °C for 24 h.

Preparation of acid-functionalization $\text{Fe}_3\text{O}_4\text{-MNPs}$

After surface silica coating the coated $\text{Fe}_3\text{O}_4\text{-MNPs}$ were functionalized with two different acids i.e. alkylsulfonic acid ($\text{Fe}_3\text{O}_4\text{-MNPs@Si@AS}$) and butylcarboxylic acid ($\text{Fe}_3\text{O}_4\text{-MNPs@Si@BCOOH}$) according to the method proposed by Pena et al. [20] with some required changes as follows.

$\text{Fe}_3\text{O}_4\text{-MNPs@Si@AS}$ nanoparticles: 1 g $\text{Fe}_3\text{O}_4\text{-MNPs@Si}$ nanoparticles were added to a mixture of 50 mL of ethanol, 50 mL of water and 4 mL of MPTMS. The mixture was then sonicated for 1-2 h and stirred at 80 °C for 24 h. Later, the $\text{Fe}_3\text{O}_4\text{-MNPs@Si}$ with thiol groups attached ($\text{Fe}_3\text{O}_4\text{-MNPs@Si@SH}$) were magnetically separated and washed many times with distilled water. Thus recovered $\text{Fe}_3\text{O}_4\text{-MNPs@Si@SH}$ nanoparticles were further suspended in a mixture of 30 mL of each 50% H_2O_2 , distilled water, and methanol. Then the mixture was kept at room temperature for two days to oxidize the thiol groups to sulfonic acid groups. The product of the oxidation step was recovered magnetically and washed several times with a large amount of distilled water and re-acidified with 50 mL of 2 M H_2SO_4 by incubating in the shaker at room temperature for 24 h at 200 rpm. Finally, thus prepared $\text{Fe}_3\text{O}_4\text{-MNPs@Si@AS}$ nanoparticles were again washed 3-4 times with distilled water and dried in a hot-air oven at 100 °C for 24 h.

$\text{Fe}_3\text{O}_4\text{-MNPs@Si@BCOOH}$ nanoparticles: 1 g $\text{Fe}_3\text{O}_4\text{-MNPs@Si}$ nanoparticles were mixed with a mixture of 200 mL of 0.5 N HCl and 4 mL of CPTES and sonicated for 1-2 h followed mechanical stirring at 80 °C for 24 h. The nanoparticles were then magnetically recovered from the mixture and washed 3-4 times with distilled water. Later, thus obtained nanoparticles were acidified by incubating them in a 2 M H_2SO_4 solution for 24 h to oxidize the cyano groups to carboxylic acid groups. Finally, the carboxylic acid-functionalized nanoparticles ($\text{Fe}_3\text{O}_4\text{-MNPs@Si@BCOOH}$) were magnetically separated from the solution and again washed with a sufficient amount of distilled water and dried in the hot-air oven at 100 °C.

Characterization of nanoparticles

All the different types of MNPs prepared in the present study i.e. Fe₃O₄-MNPs, Fe₃O₄-MNPs@Si, Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH were analyzed for the characterization using different techniques mentioned below-

Fourier transform infrared (FTIR) spectroscopy analysis was performed to get an idea about different functional groups present on the surface of the synthesized and functionalized MNPs. For this analysis, IR grade KBr and all above MNPs were dried at 100°C overnight and then the samples were prepared by mixing 1 mg of each MNPs with 100 mg of KBr. The measurements were made for wavenumbers 400 ~ 4,000/cm, with the detector reading at 4/cm resolution and 32 scans per sample using a Perkin Elmer® Spectrum™ GX (Shelton, USA).

X-ray diffraction (XRD) analysis was performed for all the above mentioned MNPs using an X'Pert Pro PANalytical diffractometer using various parameters and conditions mentioned below: K-Alpha 1 wavelength ($\lambda = 1.54056 \text{ \AA}$), K-Alpha 2 wavelength ($\lambda = 1.54439 \text{ \AA}$), generator voltage of 40 kV, a tube current of 35 mA and the count time of 0.5 s per 0.02° in the range of 5°–90° with a copper anode.

Further, other techniques like Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) were used to determine the shape, size and elemental composition of thus synthesized Fe₃O₄-MNPs and acid-functionalized MNPs using Hitachi S520 SEM (Hitachi, Tokyo, Japan). For these analyses, all the dried samples were initially mounted on aluminum stubs, sputter-coated (JEOL JFC-1600) with a silver layer, and used for scanning and EDX analysis using X-ray detector of same SEM machine.

Pretreatment of lignocellulosic biomass

Both acid functionalized MNPs, i.e. Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH nanoparticles were evaluated for the catalytic efficacy in the pretreatment of sugarcane bagasse at different concentrations. For the pretreatment, 1 g of sugarcane bagasse was mixed with 10 ml of distilled water and effect of various concentrations (100 mg, 200 mg, 300 mg, 400 mg and 500 mg) of both the above mentioned acid-functionalized MNPs. Then the mixture was heated in simple electrical autoclave (SOC. FABBE LTDA, Sao Paulo, Brazil, capacity 70 liters) at 120 °C and 15 psi pressure for 15 min. In addition to these, normal acid-pretreatment was also performed for the comparative evaluation of normal acid-mediated pretreatment and acid-functionalized MNPs based pretreatment.

Collection of hydrolyzate and recovery of acid-functionalized MNPS

After pretreatment, the hydrolyzate (liquid fraction) was separated from a solid fraction (biomass + acid functionalized MNPs) using a 200-mesh sieve. Further, the solid fraction was mixed with a sufficient amount of distilled water and acid-functionalized MNPs used were separated from sugarcane bagasse by applying an external magnetic field. Thus recovered nanoparticles were washed 3-4 times with distilled water and dried in the hot-air oven at 60 °C overnight and reused in the second cycle of pretreatment. Moreover, the hydrolyzate obtained from normal acid-pretreatment of sugarcane bagasse was also collected for sugar concentration analysis.

Reuse of acid-functionalized MNPs for further pretreatment

The acid-functionalized MNPs recovered after first cycle of pretreatment were reused in the second cycle of pretreatment of sugarcane bagasse; the same procedures mentioned above was followed for the pretreatment of sugarcane bagasse, collection of hydrolyzate and recovery of acid-functionalized MNPs used.

High Performance Liquid Chromatography (HPLC) analysis for the determination of sugar concentration

The amount of sugars (xylose) released after pretreatment of sugarcane bagasse in the hydrolyzate (in case of both, normal acid and acid-functionalized MNPs mediated pretreatment) was determined by HPLC with a refraction index detector (Waters 410, Milford, MA, USA). The samples were diluted in a ratio of 1:1 and filtered through a SepPak C18 filter (Water Technologies Limited, Brazil). Further, samples were injected into the chromatograph, column BIO-RAD Aminex HPX-87H (7.8 × 300 mm) (Bio-Rad, Hercules, CA, USA), a temperature of 45 °C, eluent: 0.5 N sulfuric acid, flow 0.6 ml/min in a sample volume of 20 µL.

Structural analysis of pretreated sugarcane bagasse

The morphological structure of native (raw) sugarcane bagasse and the changes occur in its morphology after pretreatment with acid and both the acid-functionalized MNPs were analyzed using SEM analysis. For the analysis, initially all the different samples of sugarcane bagasse were mounted on aluminum stubs, sputter-coated (JEOL JFC-1600) with a silver layer, and used for scanning and EDX analysis using X-ray detector of same SEM machine.

Results and discussion

Synthesis of Fe_3O_4 -MNPs and preparation of silica coated and acid-functionalized MNPs

In the present study, a simple approach has been proposed for the synthesis of Fe_3O_4 -MNPs using $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and NaOH as precursor salt and reducing agent respectively. Two different visual observations, i.e. (i) the change in light yellowish colour of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (**Figure 1A**) to greenish black (**Figure 1B**) after addition of NaOH and (ii) formation of greenish black precipitate after microwave treatment preliminary indicated the formation of Fe_3O_4 -MNPs. Thus, obtained precipitated after washing and drying becomes highly magnetic Fe_3O_4 -MNPs (**Figure 1 C and D**). Considering the enormous applications of naked and functionalized MNPs in various fields, particularly in catalysis and biofuel production a great deal of attraction have been seen across the world. The synthesis approach proposed in the present study is in accordance with approached developed by Holland and Yamaura [21] and recently by Gaikwad et al. [18]. As far as the applications of MNPs in biofuel industries are concerned, some attempts have been made with the use of naked MNPs and acid-functionalized MNPs for pretreatment and enzyme functionalized MNPs for hydrolysis of biomass. Due to the potent magnetic properties, there are possibilities of repeated use of the same functionalized MNPs for more than one reaction after their recovery can help to reduce the cost involved in the process.

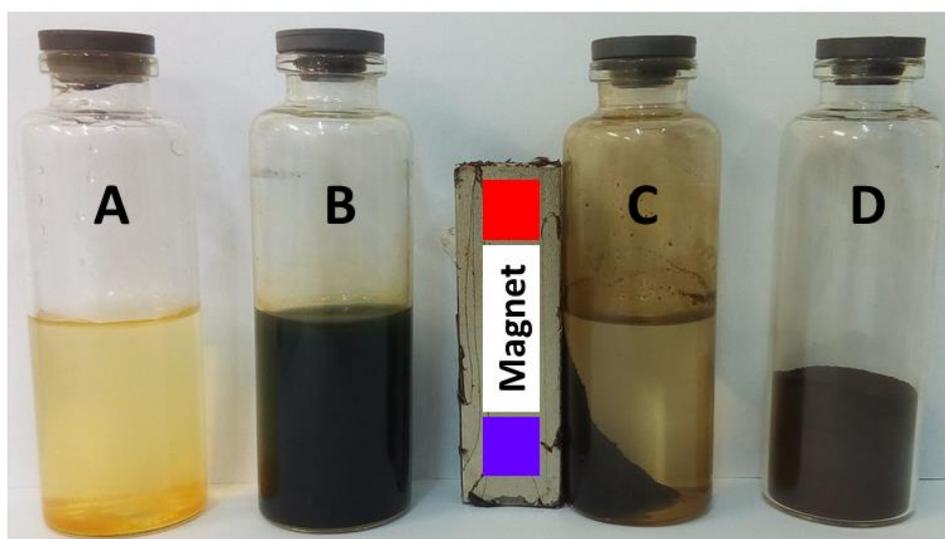


Figure 1: (A) $\text{Fe}_3\text{SO}_4 \cdot 7\text{H}_2\text{O}$ solution (B) $\text{Fe}_3\text{SO}_4 \cdot 7\text{H}_2\text{O}$ after addition of NaOH (C) Fe_3O_4 -MNPs showing magnetic property (D) Dried powder of Fe_3O_4 -MNP.

Considering these facts, the present study is aimed to develop ecofriendly and cost-effective strategies for the pretreatment of lignocellulosic biomass like sugarcane bagasse. In this context, MNPs thus synthesized were the first surface modified with silica and further functionalized with different acids to prepare two different acid-functionalized MNPs mentioned above. Pena et al. [15] demonstrated the synthesis of Fe₃O₄-MNPs and preparation of Perfluoroalkylsulfonic (PFS) and alkylsulfonic (AS) acid-functionalized MNPs for the pretreatment of wheat straw. In the same line, catalytic efficacy these nanoparticles were evaluated for the pretreatment of sugarcane bagasse in the present study as the bagasse was considered as one of the most suitable lignocellulosic feedstock in bioethanol production.

Characterization of nanoparticles

Different techniques such as FTIR, XRD, SEM and EDX were used to determine the different characteristics like surface chemistry, structure, shape, size, etc. of thus synthesized Fe₃O₄-MNPs, Fe₃O₄-MNPs@Si and both acid-functionalized MNPs (i.e. Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH).

FTIR analysis

FTIR spectra recorded for the Fe₃O₄-MNPs, Fe₃O₄-MNPs@Si both acid-functionalized MNPs (i.e. Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH) are shown in **Figure 2**. The spectra recorded for the above-mentioned MNPs showed a common peak at 3410 cm⁻¹ which is proposed to be aroused due to O-H stretching vibrations of physisorbed water and possibly surface hydroxyl groups [20]. Similarly, the appearance of other common peaks at 1632 cm⁻¹ and 1126 cm⁻¹ was considered due to the O-H deformation vibration and C-C-H bending vibration [22] respectively. Apart from these, the most important and confirmatory absorption peak was observed at 578 cm⁻¹ in all Fe₃O₄-MNPs, Fe₃O₄-MNPs@Si and acid-functionalized MNPs which was attributed due to the stretching vibration of the Fe-O bond [23], the presence of this peak is one of the confirmatory characteristics of Fe₃O₄-MNPs.

The common peaks recorded for Fe₃O₄-MNPs@Si, Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH at 811 cm⁻¹ and 973 cm⁻¹ were corresponds to the stretching vibrations of Si-O-Si and Si-O-H groups respectively [24,25]. These peaks are not found in case of Fe₃O₄-MNPs which clearly confirmed the successful surface coating in case of silica coated Fe₃O₄-MNPs and it is also seen in case of both the acid-functionalized nanoparticles. Moreover, the appearance of peak at around 1400 (i.e. 1401 cm⁻¹) is generally considered due

to carboxylic acid and presence of this peak only in case of both the acid-functionalized MNPs confirmed the effective functionalization of acids on silica coated MNPs.

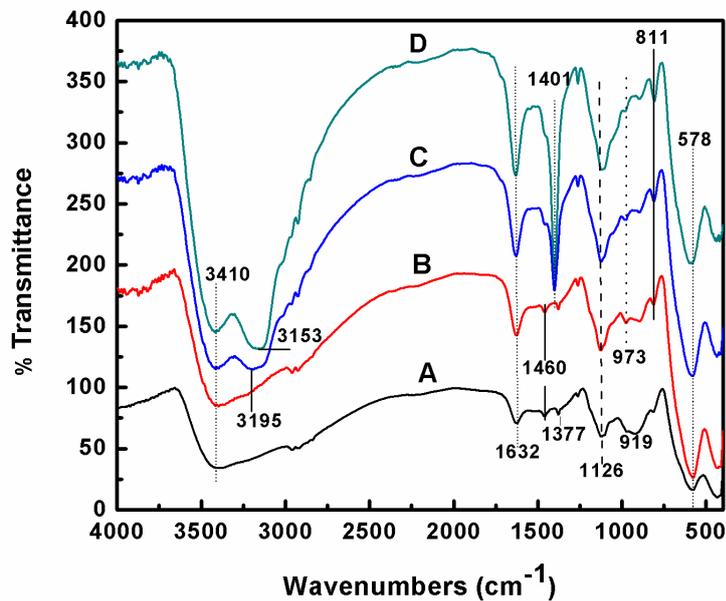


Figure 2: FTIR analysis (A) Fe₃O₄-MNPs (B) Fe₃O₄-MNPs@Si (II) (C) Fe₃O₄-MNPs@Si@AS (D) Fe₃O₄-MNPs@Si@BCOOH

XRD analysis

The XRD spectra observed for Fe₃O₄-MNPs, Fe₃O₄-MNPs@Si, Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH are shown in **Figure 3**. The spectra reported for all above mentioned MNPs (Figure 3A-D) showed common diffraction peaks at 2θ values 30.2°, 35.6°, 43.3°, 53.7°, 57.2° and 62.9° are corresponds to (220), (311), (400), (422), (511) and (440) planes. All these planes are particularly assigned to the face-centered cubic (fcc) phase of metallic iron. The appearance of all these diffraction peaks confirmed the highly crystalline nature of each MNPs. Moreover, all these peaks also showed a perfect match with the standard XRD data proposed for iron oxide nanoparticles (Fe₃O₄-MNPs). Apart from this, all the observations recorded in the present study showed resemblance with findings reported in many of previous studies proposed by Wei et al. [26], Prasad et al. [27] and Fatima et al. [28]. In spite of these peaks, the presentable peaks appearing in the range of $2\theta = 15-30$ for Fe₃O₄-MNPs@Si are proposed to be associated with amorphous silica [19,20].

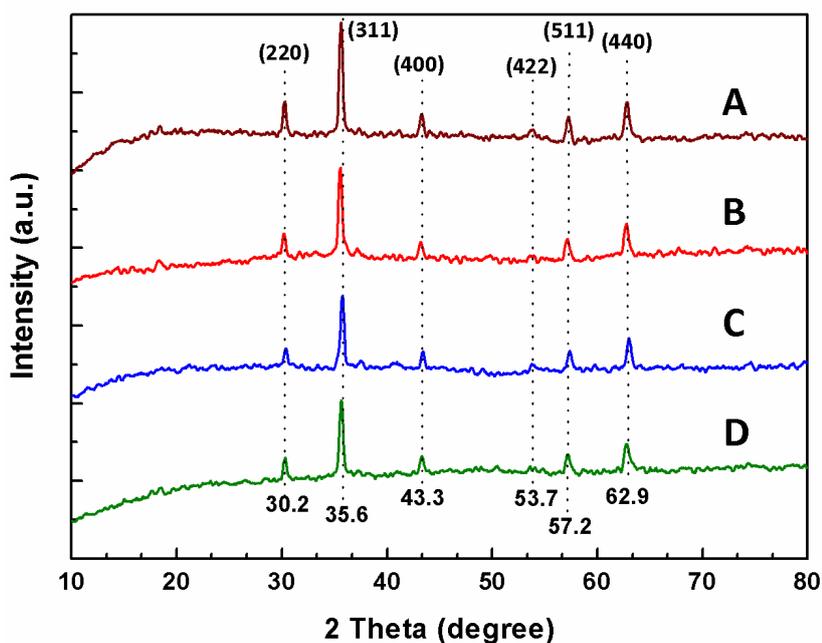


Figure 3: XRD pattern (A) Fe_3O_4 -MNPs (B) Fe_3O_4 -MNPs@Si (II) (C) Fe_3O_4 -MNPs@Si@AS (D) Fe_3O_4 -MNPs@Si@BCOOH.

EDX analysis

EDX is one of the most important techniques commonly used for the determination of elemental composition any material [29]. Therefore, various MNPs prepared in the study (i.e. Fe_3O_4 -MNPs, Fe_3O_4 -MNPs@Si and both acid-functionalized Fe_3O_4 -MNPs) were analyzed using this technique and results are shown in **Table 1**. The EDX analysis showed the presence of more than 84.26 weight % elemental iron in case of all the MNPs. The presence of elemental iron in such huge amount confirmed that thus synthesized nanoparticles are Fe_3O_4 -MNPs. Apart from this, the appearance of peaks for elemental silicon in case of Fe_3O_4 -MNPs@Si and both acid-functionalized Fe_3O_4 -MNPs indicated the successful surface modification by silica on naked Fe_3O_4 -MNPs [30]. Moreover, a significant presence of elemental sulfur in case of Fe_3O_4 -MNPs@Si@AS (i.e. 3.97 weight %) suggested the strong functionalization of acid on Fe_3O_4 -MNPs@Si. Moreover, according to Prasad et al. [27] this kind of observation indicates the spherical shape and elemental iron formed by a facile manner. These results also support the SEM analysis where the spherical shape was reported for synthesized MNPs.

Table 1: Elemental composition of various MNPs obtained from EDX analysis

Nanoparticles	Elements	Weight %	Atomic %
Fe ₃ O ₄ -MNPs	Fe	99.66	99.52
	Ca	0.34	0.48
Fe ₃ O ₄ -MNPs@Si	Fe	92.80	86.21
	Si	3.56	6.58
	Na	2.87	6.49
	Mn	0.76	0.72
Fe ₃ O ₄ -MNPs@Si@AS	Fe	86.88	77.98
	Si	8.56	15.27
	S	3.97	6.21
	Mn	0.59	0.54
Fe ₃ O ₄ -MNPs@Si@BCOOH	Fe	94.87	91.23
	Si	3.41	6.52
	S	0.82	1.37
	Mn	0.90	0.88

SEM analysis

Finally, Fe₃O₄-MNPs, Fe₃O₄-MNPs@Si and both acid-functionalized (i.e. Fe₃O₄-MNPs@Si@AS and Fe₃O₄-MNPs@Si@BCOOH) nanoparticles were subjected for SEM micrographic analysis to determine their shape and size. The presence of both spherical and irregular shaped Fe₃O₄-MNPs was recorded. The SEM micrographs recorded revealed that all these MNPs are polydispersed and have size diameter in the range of 20-80 nm (**Figure 4 A-D**). As compared to other techniques like transmission electron microscopy (TEM), high-resolution TEM, etc. SEM is considered as less promising because of its limited magnification power; however, many recent studies performed significantly used SEM for the determination of shape and size of synthesized and modified Fe₃O₄-MNPs [28,31,32].

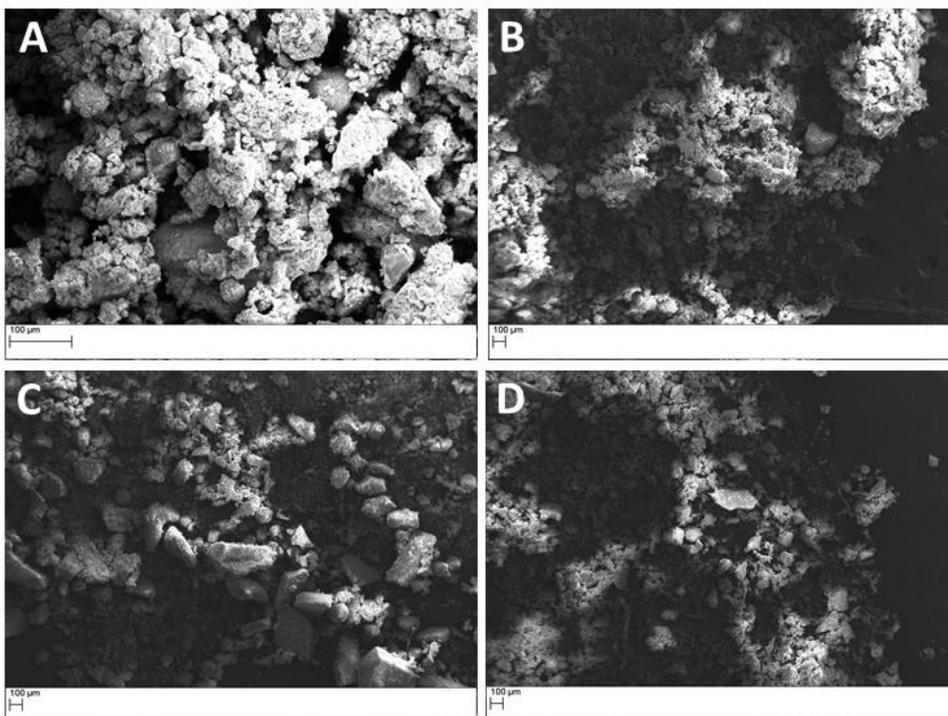


Figure 4: SEM micrographs (A) Fe_3O_4 -MNPs (B) Fe_3O_4 -MNPs@Si (C) Fe_3O_4 -MNPs@Si@AS (D) Fe_3O_4 -MNPs@Si@BCOOH

Pretreatment of sugarcane bagasse

Thus prepared both the acid-functionalized MNPs were evaluated for their catalytic in the pretreatment of sugarcane bagasse. As mentioned above, the pretreatment of sugarcane bagasse was performed at 120 °C for 15 min at 15 psi using different concentrations (i.e. 100 mg, 200 mg, 300 mg, 400 mg and 500 mg per gram of sugarcane bagasse) of both the acid-functionalized MNPs. In addition, a traditional acid pretreatment using sulfuric acid (100 mg per gram of sugarcane bagasse) was also performed and it was considered as control and further used for comparative analysis with acid-functionalized MNPs. It was observed that the hydrolysis of sugarcane bagasse and release of fermentable sugars was depended on the concentrations of acid-functionalized MNPs used; increase in the concentration of acid-functionalized MNPs also increases the release of fermentable sugars which was analyzed by using HPLC (**Table 2**). In the first cycle of pretreatment, both Fe_3O_4 -MNPs@Si@AS and Fe_3O_4 -MNPs@Si@BCOOH at 500 mg showed the maximum amount of sugars (xylose) liberated i.e. 18.83 g/L and 18.67 g/L, respectively which were comparatively higher than the normal acid hydrolysis (15.40 g/L). Moreover, after first cycle of pretreatment, these acid-functionalized MNPs were recovered from the reaction mixture by applying a suitable magnetic field and reused in the second cycle of pretreatment. A little decrease in the efficacy

was reported at each above-mentioned concentration. However, in the second cycle, the sugar released at 500 mg concentration of both Fe₃O₄-MNPs-Fe₃O₄@Si@AS and Fe₃O₄-MNPs@Si@BCOOH nanoparticles was found to be 15.98 g/L and 15.56 g/L respectively (**Table 2**). Considering these results, it is proved that acid-functionalized MNPs can be used as an alternative approach for the pretreatment of various lignocellulosic materials.

Table 2: Concentration of sugar released after pretreatment of sugarcane bagasse using various concentrations of different acid-functionalized MNPs

Pretreatment of sugarcane bagasse	Acid (H ₂ SO ₄)	Amount of sugar (xylose) liberated at different concentration of acid functionalized MNPs									
		Fe ₃ O ₄ -MNPs@Si@AS					Fe ₃ O ₄ -MNPs@Si@BCOOH				
		100	200	300	400	500	100	200	300	400	500
First cycle	15.40	5.94	8.73	13.53	15.19	18.83	3.39	10.15	13.37	14.82	18.67
Second cycle	15.40	3.23	5.98	11.49	13.73	15.98	1.94	8.35	11.23	11.86	15.56

The obtained in the present study are in agreement with the findings reported in previous studies. Pena et al. [15] studied the efficacy of perfluoroalkylsulfonic (PFS) and alkylsulfonic (AS) acid-functionalized MNPs in the pretreatment of wheat straw at two different temperatures for varied time periods, i.e. 80 °C for 24 h and 160 °C for 2 h. It was observed that PFS functionalized MNPs showed improved hydrolysis ($24.0 \pm 1.1\%$) of wheat straw hemicelluloses to soluble oligosaccharides at 80 °C for 24 h as compared with AS functionalized MNPs ($9.1 \pm 1.7\%$). However, at 160 °C for 2 h, both PFS and AS functionalized MNPs showed comparatively higher hydrolysis of hemicelluloses to oligosaccharides ($46.3 \pm 0.4\%$ and $45 \pm 1.2\%$, respectively) than the control samples ($35.0 \pm 1.8\%$). Moreover, in their another study, they evaluated the efficacy of propyl-sulfonic (PS) acid-functionalized nanoparticles for pretreatment of corn stover. The efficacy of these PS acid-functionalized nanoparticles was studied at different catalyst loads, i.e. 0.1, 0.2, and 0.3 g of catalyst per gram of biomass at three different temperatures, 160, 180, and 200 °C, for 1 h. The findings reported revealed that catalyst load did not have any effect on the glucose yield at 160 °C, and the average glucose yield obtained from hydrolysis of corn stover at this temperature was about 59.0%. Moreover, samples with a catalyst load of 0.2 g and incubated at 180 °C showed a maximum glucose yield of 90%, whereas, complete hydrolysis of corn stover was reported at 200 °C [33]. Apart from these, Lai et al. [34] demonstrated the efficacy of sulfonic acid supported silica-magnetic nanoparticle composite and Wang et al.

[35] studied the efficacy of sulfonic acid functionalized silica-coated crystalline Fe/Fe₃O₄ core/shell magnetic nanoparticles in different catalytic processes where they reported excellent catalytic activity and hence claimed that such nanocatalysts can be promisingly used for the pretreatment of lignocellulosic biomass.

It is observed that there is little decrease in the catalytic efficacy of both the acid-functionalized MNPs at all the concentrations tested when used for the second cycle of sugarcane bagasse pretreatment. It is believed this decrease in the efficacy was may be due to the loss of acid groups present on the surface of MNPs. Moreover, the possibilities proposed for decreased efficacy of acid-functionalized MNPs in the present study found to be in accordance with the reasons proposed by Pena et al. [20] where they reported a gradual decrease in cellobiose conversion in three successive cycles of hydrolysis due to the loss in acid groups present of various acid-functionalized MNPs used. In the present study, till now thus prepared acid-functionalized MNPs were used only up to two cycles of pretreatment, however, we are trying to use these nanoparticles for further more cycles of pretreatment.

Some of the studies discussed above may be showing little more efficiency as compared to present approach but most of them usually performed at very temperatures (160 °C and above for 1 h or more) and using specialized equipment's like Parr reactors. On the contrary, the approach proposed in the present study can be considered as most promising when compared with all of the above-mentioned studies because it was performed at significantly lower temperature (120 °C) and short time period (15 min) using simple equipment like conventional autoclave. Moreover, we believed that there is scope for further optimization of reaction parameters and modification of surface functional groups so as to increase the catalytic efficacy and selectivity of acid-functionalized MNPs.

Structural analysis of pretreated sugarcane bagasse

The influence of the various pretreatments on the structure of the lignocellulosic biomass can be verified using SEM analysis [36,37]. Considering these facts, in the present study, SEM approach was used to verify the effect of normal acid and both acid-functionalized MNPs mediated pretreatment on morphological features of sugarcane bagasse and it was shown in **Figure 5**. The non-treated (raw) sugarcane bagasse showed a regular and compact surface structure with fibers arranged in bundles, cell wall showed parallel stripes and waxes, extractives, and other deposits on the surface (**Figure 5A**). However, in case of sugarcane bagasse pretreated with mineral acid (H₂SO₄) (**Figure 5B**) and both the acid-functionalized MNPs (**Figure 5 C and D**) showed small pores on the surface and also

disruption in structure of fibers and pith which leads to removal the hemicellulose fraction of the cell wall. Thus obtained results are in agreement with various previous studies, Chandel et al. [38] reported structural disruption of fibers and removal of hemicellulosic fraction, waxes, and other deposit when sugarcane bagasse was subjected to oxalic acid pretreatment. Similarly, Riyajan and Intharit [39] observed that the morphology of sugarcane bagasse subjected to a combined sodium hydroxide and silane pretreatment showed the lower roughness on the surface of raw bagasse when compared with the pretreated bagasse, which was caused by the removal of fatty acids from the surface of the bagasse. Moreover, recently, Zheng et al. [40] reported that raw wheat straw showed a regular and compact surface structure with fibers arranged in bundles, however, the surface of the wheat straw samples, which is mainly composed of lignin and hemicellulose, was destroyed after applications of after acid and alkali pretreatments. The observation reported through SEM analysis clearly revealed the efficacy of acid-functionalized MNPs in the pretreatment of sugarcane bagasse. Moreover, it is believed that such acid-functionalized MNPs can be used for the effective pretreatment of a variety of lignocellulosic biomass as a novel, green and economically viable technologies.

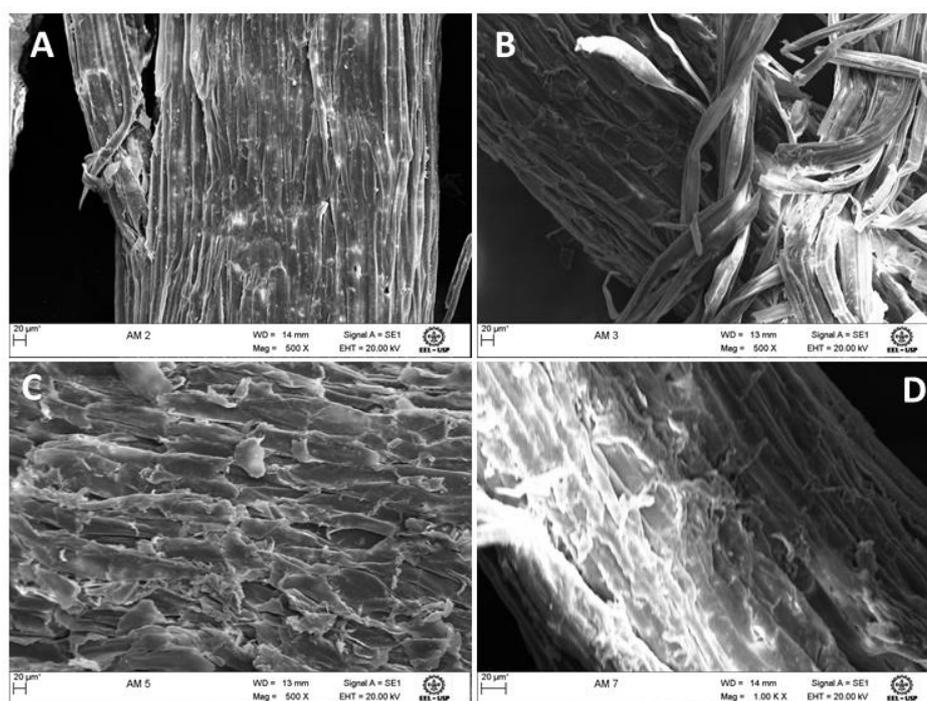


Figure 5: SEM micrographs of pretreated sugarcane bagasse after pretreatment with (A) Untreated (native) (B) Acid (H_2SO_4) (C) Fe_3O_4 -MNPs@Si@AS (D) Fe_3O_4 -MNPs@Si@BCOOH

Conclusions

The present approach proposed for the pretreatment of sugarcane bagasse using different two different acid-functionalized MNPs is found to be rapid and convenient. Considering the promising pretreatment efficacy of these acid-functionalized MNPs it is believed that such catalysts can be used in place conventional mineral acids as a novel alternative. Till date, very few reports are available on the utilization of such acid-functionalized MNPs in the pretreatment of various lignocellulosic biomasses. Among these, most of the studies were performed at very high temperatures, pressure and longer time period using some specialized equipment's like Parr reactor. However, approach proposed in the present study, was performed at a significantly lower temperature and short time period which makes this approach more convenient. In addition, reuse of acid-functionalized MNPs for cycles of pretreatment will help to reduce the substantial cost involved in the process and makes the process cost-effective.

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