

RESEARCH ARTICLE

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Pretreatment of garden biomass using Fenton's reagent: influence of Fe^{2+} and H_2O_2 concentrations on lignocellulose degradation

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Abstract

Garden biomass (GB) is defined as low density and heterogeneous waste fraction of garden rubbish like grass clippings, pruning, flowers, branches, weeds; roots. GB is generally different from other types of biomass. GB is mostly generated through maintenance of green areas. GB can be processed for bio energy production as it contains considerably good amount of cellulose and hemicellulose. However, pretreatment is necessary to delignify and facilitate disruption of cellulosic moiety. The aim of the present investigation was to pretreat GB using Fenton's reagent and to study the influence of Fe^{2+} and H_2O_2 concentrations on degradation of lignin and cellulose. The data were statistically analyzed using ANOVA and numerical point prediction tool of MINITAB RELEASE 14 to optimize different process variables such as temperature, concentration of Fe^{2+} and H_2O_2 . The results of the present investigation showed that Fenton's reagent was effective on GB, however, concentration of Fe^{2+} and H_2O_2 play crucial role in determining the efficiency of pretreatment. An increase in H_2O_2 concentration in Fenton's reagent significantly increased the rate of cellulose and lignin degradation in contrast to increasing concentration of Fe^{2+} ion which led to a decrease in lignocellulosic degradation.

Keywords: Fenton's reagent, Pretreatment, Lignin, Cellulose, Garden biomass

Introduction

Biomass, in general, fourth largest energy source in the world, provides about 13% of world energy consumption [1]. Globally, biomass has an annual primary production of 220 billion oven dry ton [2]. Many cities, large or small, have developed gardens and recreational parks. The number of parks and other recreational centers, home gardens etc. contribute to the sizable quantum of garden biomass (GB) generation. Maintenance of green areas produces significant amount of waste in the form of GB [3]. GB is generally different from other types of biomass, and it is defined as low density and heterogeneous waste fraction of garden rubbish like grass clippings, pruning, flowers, branches, weeds, roots. The disposal of garden biomass is mainly through open burning, dumping and composting in India. Although these methods of disposal are

universally applicable they neither recover energy nor eco-friendly except for composting.

GB contains recalcitrant or complex compounds such as cellulose and lignin, and relatively small amounts of saccharides, amino acids, proteins, aliphatic compounds and carbohydrates [3,4]. As GB is rich in cellulose, it can be used as a raw material for bio energy production after suitable pretreatment. Pretreatment is necessary to delignify and facilitate the disruption of lignocellulosic moiety. Pretreatment alters the structure of cellulose and making it more accessible to the enzyme that convert carbohydrate polymer into fermentable sugar [5,6].

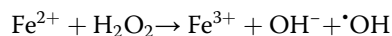
There are different methods of pretreatment available for various substrates. However, it is necessary to evaluate every pretreatment process as the efficiency of pretreatment differs from substrate to substrate. Generally, pretreatment methods are either physical or chemical. Some methods incorporate both effects [7]. However, it is necessary to evaluate pretreatment processes for different substrates. Fenton's reagent defined as a mixture of hydrogen peroxide and

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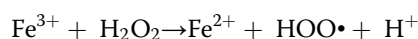
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ferrous ion is one of the most effective methods for the oxidation of organic compounds.

Fenton process is a reaction between hydrogen peroxide (H_2O_2) and ferrous ion (Fe^{2+}), producing the hydroxyl radical ($\bullet\text{OH}$). $\bullet\text{OH}$ radical is a strong oxidant capable of oxidization and degradation of various organic compounds into carbon dioxide and water. Thus, the degradation process could be increased with increasing $\bullet\text{OH}$ concentration and vice versa [8-12].



The ferric ions produced during the reaction further react with hydrogen peroxide regenerating ferrous ions, thus continuing the process [13].



However, the efficiency of Fenton's reaction depends mainly on H_2O_2 concentration, $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ratio, pH and reaction time [14].

In the present study we aimed at evaluating the effectiveness of Fenton's reaction for pretreatment with a major emphasis on the influence of Fe^{2+} and H_2O_2 concentrations on degradation of lignin and cellulose.

Materials and methods

Preparation of the feedstock

Garden biomass (GB) consisting of grass cuttings, fallen leaves, flowers, roots, twigs etc. were collected from the garden area of National Environmental Engineering Research Institute (NEERI). After initial screening, GB was air-dried for 24 hours followed by 3 days of sun drying. The dried material was pulverized using a pulveriser to the size of 1 to 5 mm for further experiments and stored in an air tight container.

Pretreatment by Fenton's reagent

Fenton's reagent was prepared by mixing $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and H_2O_2 in distilled water in different proportions. The $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ concentration varied from 250 ppm to 1000 ppm and H_2O_2 concentration varied from 1000 ppm to 10000 ppm. Every time Fenton's reagent was prepared fresh and used in the experiment. All the experiments were carried out with 5 g of GB and 100 mL of Fenton's reagent of different composition. All the reactions were initially carried out at 30°C and repeated at 50 and 80°C . The reaction was carried out in a shaker and reaction time was varied from 60 min to 180 min. The reaction mixture was filtered and then the treated GB was thoroughly washed and dried at 60°C for 2 days. The concentration of lignin and cellulose was estimated as described in section 2.3.

Analytical methods

The dried sample of GB was ground to powder for chemical analysis. The organic carbon content of GB was estimated by combustion method according to Nelson & Sommers, 1982 [15]. Known quantity (mg) of substrate (GB) and its hydrolysed residue after pretreatment was taken and analyzed for cellulose by HNO_3 -ethanol method. Lignin content of samples was estimated by 72% (w/w) H_2SO_4 method and hemicellulose by Liu method [16]. The total nitrogen (TN) content of the sample was estimated using LECO Protein-Nitrogen Analyzer (Model FP528).

Evaluation of cellulose and lignin degradation

Degradation of cellulose and lignin was evaluated on the basis of solid recovery [17,18] and actual degradation was calculated on the basis of residual concentration after pretreatment.

Cellulose recovery

Actual degradation (g) and actual degradation (%) of cellulose and solid recovery was calculated according to following formula:

$$\text{Solid recovery (\%)} = \frac{\text{Dry weight of sample after pretreatment}}{\text{Initial weight of sample (g)}} \times 100$$

Recovered cellulose (g)

$$= \frac{\text{Conc. (\%)} \text{ of cellulose after pretreatment} \times \text{Solid recovery}}{\text{Material taken for pretreatment}}$$

Actual degradation (g) and Actual degradation percentage (%) of cellulose was calculated by following formula:

$$\text{Actual degradation (g) of cellulose} = \text{Initial conc. of cellulose} - \text{Recovered cellulose}$$

Actual degradation (%) of cellulose

$$= \frac{100 \times \text{Actual degradation (g) of cellulose}}{\text{Initial conc. of cellulose}}$$

Lignin recovery

Actual degradation (g), actual degradation (%) of lignin and solid recovery was calculated according to following formula:

$$\text{Solid recovery (\%)} = \frac{\text{Dry weight of sample after pretreatment}}{\text{Initial weight of sample (g)}} \times 100$$

Recovered lignin (g)

$$= \frac{\text{Conc. (\%)} \text{ of lignin after pretreatment} \times \text{Solid recovery}}{\text{Material taken for pretreatment}}$$

Actual degradation (g) and Actual degradation percentage (%) of lignin was calculated by following formula:

$$\begin{aligned} \text{Actual degradation (g) of lignin} &= \text{Initial conc. of cellulose} \\ &\quad - \text{Recovered cellulose} \\ \text{Actual degradation (\% of lignin)} &= \frac{100 \times \text{Actual degradation (g) of lignin}}{\text{Initial conc. of lignin}} \end{aligned}$$

Statistical guided experimental design and procedure

The Fenton’s pretreatment was statistically evaluated by applying statistical methodology viz. analysis of variance (ANOVA) followed by response surface methodology for process optimization [19,20]. The experimental runs were designed to cover variables that assess impact of pretreatment on cellulose and lignin degradation. The effects of Fe²⁺ concentration (X1), Hydrogen peroxide concentration (X2) and Temperature (X3) on lignin and cellulose degradation were described statistically. The regression analysis was performed to estimate the response function as a second-order polynomial:

$$Y = \beta_0 + \sum_{i=1}^K \beta_i X_i^2 + \sum_{i=1}^K \beta_{ij} X_i^2 + \sum_{i=1}^{k-1} \times \sum_{j=2}^k \beta_{ij} X_i X_j \tag{1}$$

Where Y is the predicted response, β_i, β_j, β_{ij} are coefficients estimated from regression, they represent the linear, quadratic and cross-products of X₁, X₂, X₃ on response.

A statistical program package MINITAB RELEASE 14, was used for regression analysis of the data obtained and to estimate the coefficient of regression equation. The equations were validated by analysis of variance (ANOVA) analysis. The significance of each term in the equation is to estimate the goodness of fit in each case. Response surfaces were drawn to determine the individual and interactive effects of test variable on degradation of respective components.

Table 1 Initial characterization of garden biomass

Parameter	Concentration (%)
Total organic matter	94.10
Organic carbon	49.12
Cellulose	38.54
Hemicellulose	26.24
Lignin	25.68
Nitrogen	1.65

Table 2 Levels of process variables in un-coded form for Fenton pre-treatment

Process variables	Levels of process variables		
Fe ²⁺ concentration ppm (X ₁)	250	500	1000
Hydrogen Peroxide concentration (ppm) (X ₂)	1000	5000	10000
Reaction temperature (°C) (X ₃)	30	50	80

Results & discussion

Initial characterization of GB

GB was analyzed to find out concentration of various constituents such as lignin, cellulose, hemicellulose, organic matter, organic carbon etc. (Table 1).

Table 3 Design matrix along with predicted and experimental values for cellulose degradation (%) by Fenton’s pretreatment

Runs	Fe ²⁺ (ppm)	H ₂ O ₂ (ppm)	Reaction temperature (°C)	Cellulose degradation (%)	
				Observed value	Predicted value
250	1000	30	26.433	24.591	
250	1000	50	27.337	28.535	
250	1000	80	20.000	19.662	
250	5000	30	30.767	30.552	
250	5000	50	31.340	34.495	
250	5000	80	27.000	25.622	
250	10000	30	43.067	41.901	
250	10000	50	47.230	45.844	
250	10000	80	35.000	36.971	
500	1000	30	13.367	16.098	
500	1000	50	*	*	
500	1000	80	10.000	11.168	
500	5000	30	24.267	22.058	
500	5000	50	*	*	
500	5000	80	20.000	17.129	
500	10000	30	31.267	33.408	
500	10000	50	40.790	37.351	
500	10000	80	26.000	28.478	
1000	1000	30	16.933	15.496	
1000	1000	50	17.487	19.440	
1000	1000	80	14.000	10.567	
1000	5000	30	19.467	21.457	
1000	5000	50	*	*	
1000	5000	80	15.000	16.527	
1000	10000	30	32.800	32.806	
1000	10000	50	38.230	36.749	
1000	10000	80	27.000	27.876	

*Outliers removed.

A perusal of results showed that GB contained 94.10% of total organic matter, 49.12% of organic carbon, 38.54% of cellulose, 25.68% of lignin and 26.24% of hemicellulose. The total nitrogen content of GB was found to be 1.65%.

Model fitting

The Levels of process variables, design of experiment along with experimental and predicted responses is given in Table 2, 3 and 4, respectively.

Full quadratic multiple regression analysis of experimental data yielded the following regression equations for the degradation of cellulose and lignin achieved through Fenton’s pretreatment:

Table 4 Design matrix along with predicted and experimental values for lignin degradation (%) by Fenton’s pretreatment

Runs	Fe ²⁺ (ppm)	H ₂ O ₂ (ppm)	Reaction temperature (°C)	Lignin degradation (%)	
				Observed value	Predicted value
1	250	1000	30	43.000	41.940
2	250	1000	50	52.000	52.634
3	250	1000	80	39.800	36.885
4	250	5000	30	46.500	45.032
5	250	5000	50	55.113	55.725
6	250	5000	80	39.340	39.976
7	250	10000	30	47.630	48.821
8	250	10000	50	57.390	59.515
9	250	10000	80	43.520	43.766
10	500	1000	30	33.580	36.155
11	500	1000	50	45.210	46.848
12	500	1000	80	28.660	31.099
13	500	5000	30	41.563	39.246
14	500	5000	50	48.560	49.940
15	500	5000	80	36.220	34.190
16	500	10000	30	42.900	43.036
17	500	10000	50	55.230	53.729
18	500	10000	80	40.300	37.980
19	1000	1000	30	35.940	32.680
20	1000	1000	50	44.317	43.374
21	1000	1000	80	26.733	27.625
22	1000	5000	30	33.580	35.772
23	1000	5000	50	47.437	46.466
24	1000	5000	80	28.750	30.716
25	1000	10000	30	37.550	39.562
26	1000	10000	50	53.230	50.255
27	1000	10000	80	33.420	34.506

Cellulose degradation

$$Y_1 = 16.7866 - 0.0667432 * X_1 + 0.000970293 * X_2 + 0.985852 * X_3 + 0.0000436 * X_1 * X_1 + 0.0000000866 * X_2 * X_2 - 0.00985 * X_3 * X_3 \tag{2}$$

Lignin degradation

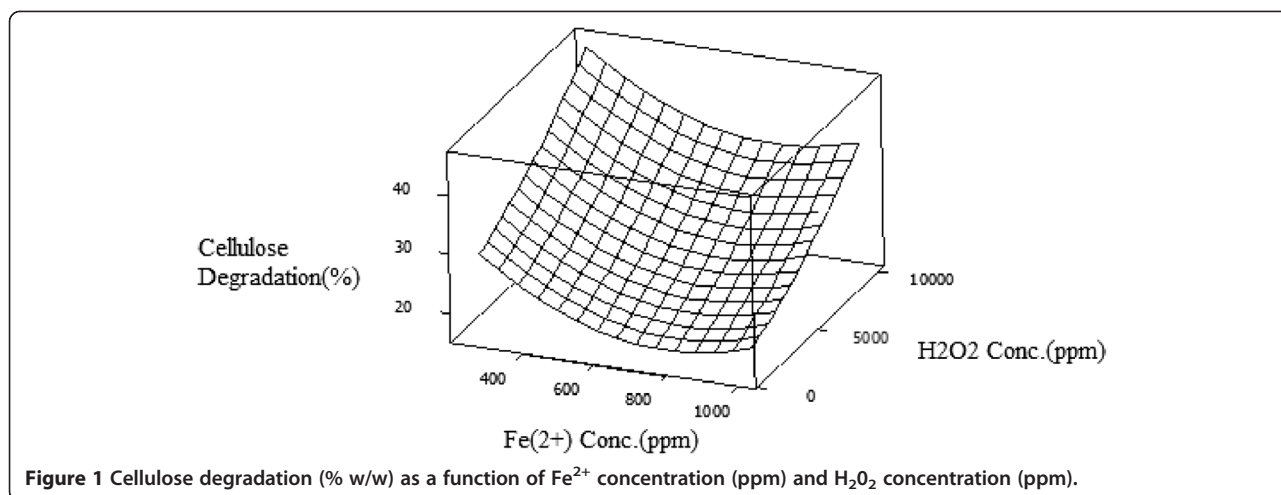
$$Y_2 = 1.81315 - 0.0393363 * X_1 + 0.000782833 * X_2 + 2.23014 * X_3 + 0.0000216 * X_1 * X_1 - 0.00000000166 * X_2 * X_2 - 0.02119 * X_3 * X_3 \tag{3}$$

Where Y₁ is the % cellulose degradation achieved by Fenton’s pretreatment, Y₂ is % lignin degradation by Fenton’s pretreatment, X₁ is Fe²⁺ concentration, X₂ and X₃ are hydrogen peroxide concentration (ppm) and reaction temperature, respectively.

Tables 3 and 4 show degradation of cellulose and lignin at different concentrations of Fe²⁺ and H₂O₂ in Fenton reagent. A perusal of results indicated that Fenton’s reagent is effective on GB. The degradation of cellulose and lignin responded positively to the concentration of H₂O₂ and reaction temperature. Whereas increasing concentration of Fe²⁺ decreased the rates of

Table 5 Analysis of variance (ANOVA) of model parameters

Terms	Coefficient	F	P
Cellulose degradation (%)			
Constant	16.7866		
Fe (X ₁)	-0.0667432	61.47	0.000
H ₂ O ₂ (X ₂)	0.000970293	222.65	0.000
Reaction temperature (X ₃)	0.985852	19.48	0.000
Fe * Fe (X ₁ * X ₁)	4.36930E-05	24.77	0.000
H ₂ O ₂ * H ₂ O ₂ (X ₂ * X ₂)	8.66348E-08	2.55	0.129
Reaction temperature * Reaction temperature (X ₃ * X ₃)	-0.00985858	25.89	0.000
R-Sq = 95.79% R-Sq(pred) = 91.36% R-Sq(adj) = 94.31%			
Lignin degradation (%)			
Constant	1.81315		
Fe (X ₁)	-0.0393363	86.45	0.000
H ₂ O ₂ (X ₂)	0.000782833	47.74	0.000
Reaction temperature (X ₃)	2.23014	25.77	0.000
Fe * Fe (X ₁ * X ₁)	2.15921E-05	0.006	0.006
H ₂ O ₂ * H ₂ O ₂ (X ₂ * X ₂)	-1.66049E-09	0.970	0.970
Reaction temperature * Reaction temperature (X ₃ * X ₃)	-0.0211932	0.000	0.000
R-Sq = 95.20% R-Sq(pred) = 91.26% R-Sq(adj) = 93.76%			

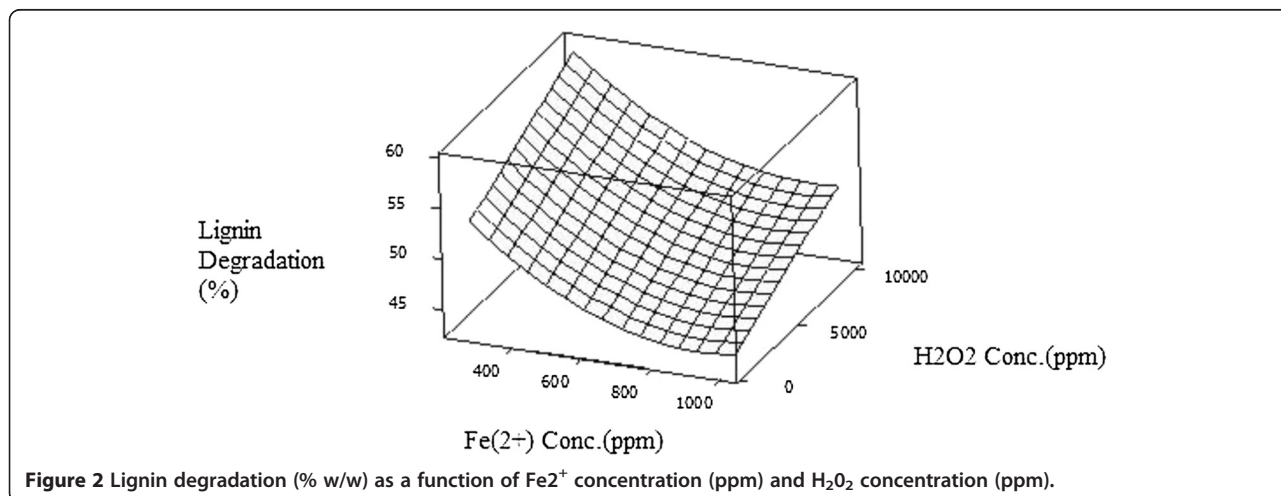


lignin and cellulose degradation. The best effective concentration (BEC) of Fe^{2+} and H_2O_2 was found to be 250 ppm and 10000 ppm, respectively at a temperature of 50°C. Though the degradation of lignin and cellulose was significant at this BEC, compared to the other conventional methods such as alkali or H_2O_2 oxidation tried on other lignocellulosic biomass, Fenton's pretreatment pronounced only a low level of delignification [21,22]. However, there is no such report to our search which exclusively deals with the effects of Fenton's reagent on lignin and cellulose degradation in GB. The cellulose reduction rates as observed (47.23%) in the present investigation are slightly higher than that of Liu and Cheng [23] who reported maximum of 20.28% removal of cellulose and 20.09% of lignin using acid pretreatment on herbal residue. However, Ayeni et al. [24] reported 17% lignin removal by alkaline peroxide assisted wet air oxidation with no loss of cellulose. The effect of H_2O_2 alone on wood waste was also studied by Ayeni et al.

[24] who reported 11% lignin removal without loss of cellulose.

The regression coefficients values for Fenton pretreatment with respect to cellulose and lignin removal is close to one ($R^2 > 95\%$), indicating the aptness of second order polynomial in predicating the response in terms of the chosen independent values, moreover the predicted values were found to be in close agreement with the experimental results (Table 2). The adjusted R^2 value (94.31% and 93.76% respectively) obtained by correcting the R^2 value for sample size and number of terms for cellulose removal is indicative of high significance of the model. The ANOVA model for the degradation of lignin and cellulose is shown in Table 5.

The ANOVA demonstrates that the model is more significant. This is evident from the calculated F -values 64 and 66 for effect of Fenton's pretreatment on cellulose and lignin removal respectively ($P = < 0.05$). The ANOVA results also indicate that the coefficients for linear



effects are significant ($P = <0.01$) for cellulose degradation and for lignin removal. The positive linear effect for H_2O_2 concentration and temperature indicate an increase in cellulose and lignin removal with increase in peroxide concentration and temperature in contrast to the observed negative linear effect for Fe^{2+} concentration. The concentration of Fe^{2+} ions present in the pretreatment solution should be in catalytic amounts as over dosage leads to adsorption on the substrate which may lead to subdued processing activity after treatment.

Many studies reported in literature have revealed that the use of a much higher concentration of Fe^{2+} could lead to the self-scavenging of $\bullet OH$ radical by Fe^{2+} and induce the decrease in degradation rates [25-27]. According to Neyens & Baeyens, 2003, when the amount of Fe^{2+} employed exceeds that of H_2O_2 , the treatment tends to have the effect of chemical coagulation. When the two amounts are reversed, the treatment tends to have the effect of chemical oxidation [28].

The effects of Fe^{2+} ion and H_2O_2 concentration on lignin and cellulose degradation when temperature was set at their centre point are shown in Figures 1 and 2. An increase in H_2O_2 concentration during pretreatment lead to considerable increase in lignin and cellulose degradation in contrast to increasing Fe^{2+} ion concentrations which lead to a decrease in cellulose removed from biomass. For example the cellulose and lignin removal increased from 13% to 31% & from 33% to 42% respectively at 500 ppm Fe^{2+} ion concentration when the H_2O_2 concentration was increased from 1000 to 10000 ppm.

The interactive effect of reaction time was however insignificant in Fenton pretreatment for lignin and cellulose removal and hence omitted from the regression analysis.

Overall, there is a predominance of the linear effects over the quadratic and interactive effects for both lignin and cellulose removal from the biomass. Higher peroxide concentrations lower Fe^{2+} concentrations higher reaction temperatures favour cellulose and lignin removal from the biomass.

Conclusion

Effect of Fenton's pretreatment on lignin and cellulose degradation of GB was studied. The results showed that Fenton's reagent was effective on GB, however, concentration of Fe^{2+} and H_2O_2 play crucial role in determining the effectiveness of lignin and cellulose degradation. An increase in H_2O_2 concentration in Fenton's reagent significantly increased the rates of cellulose and lignin degradation in contrast to increasing Fe^{2+} ion concentrations which led to a decrease in lignin and cellulose degradation. Further studies are necessary to compare and contrast Fenton's pretreatment with other pretreatments and to understand the compatibility of Fenton's pre-treated biomass for bioenergy production.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

VB and SPMPW are the main investigators, performed all experimental work and paper writing, JG carried out data analysis, AS carried out statistical analysis, ANV and SRW provided all essential supports. All authors read and approved the final manuscript.

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