Pretreatment of garden biomass using Fenton’s reagent: influence of Fe\(^{2+}\) and H\(_2\)O\(_2\) 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\(_2\)O\(_2\) 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\(_2\)O\(_2\). The results of the present investigation showed that Fenton’s reagent was effective on GB, however, concentration of Fe\(^{2+}\) and H\(_2\)O\(_2\) play crucial role in determining the efficiency of pretreatment. An increase in H\(_2\)O\(_2\) 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
ferrous ion is one of the most effective methods for the oxidation of organic compounds.

Fenton process is a reaction between hydrogen peroxide (H$_2$O$_2$) and ferrous ion (Fe$^{2+}$), producing the hydroxyl radical (•OH). •OH radical is a strong oxidant capable of oxidation and degradation of various organic compounds into carbon dioxide and water. Thus, the degradation process could be increased with increasing •OH concentration and vice versa [8-12].

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}$$

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

$$\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HOO}^\bullet + \text{H}^+$$

However, the efficiency of Fenton’s reaction depends mainly on H$_2$O$_2$ concentration, Fe$^{2+}$/H$_2$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$_2$O$_2$ 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 FeSO$_4$.7H$_2$O and H$_2$O$_2$ in distilled water in different proportions. The FeSO$_4$.7H$_2$O concentration varied from 250 ppm to 1000 ppm and H$_2$O$_2$ 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$_2$SO$_4$ 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:

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

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

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

Actual degradation (g) of cellulose = Initial conc. of cellulose – 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:

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

$$\text{Recovered lignin (g)} = \frac{\text{Conc.} (%) \text{ of lignin after pretreatment} \times \text{Solid recovery}}{\text{Material taken for pretreatment}}$$
Actual degradation (g) and Actual degradation percentage (%) of lignin was calculated by following formula:

Actual degradation (g) of lignin = Initial conc. of cellulose - Recovered cellulose

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

**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 Fe$^{2+}$ 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:

\[
Y = \beta_0 + \sum_{i=1}^{K} \beta_i X_i^2 + \sum_{i=1}^{K} \sum_{j=1}^{K} \beta_{ij} X_i X_j
\]

Where $Y$ is the predicted response, $\beta_0$, $\beta_i$, $\beta_{ij}$ are coefficients estimated from regression, they represent the linear, quadratic and cross-products of $X_i$, $X_j$, $X_2$ 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  | 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:

\[
Y_1 = 16.7866 - 0.0667432 \times X_1 + 0.000970293 \times X_2 + 0.985852 \times X_3 + 0.0000436 \times X_1 \times X_3 + 0.000000866 \times X_2 \times X_2 - 0.00985 \times X_3 \times X_3
\]

(2)

\[
Y_2 = 1.81315 - 0.0393363 \times X_1 + 0.000782833 \times X_2 + 2.23014 \times X_3 + 0.0000216 \times X_1 \times X_1 - 0.0000000166 \times X_2 \times X_2 - 0.02119 \times X_3 \times X_3
\]

(3)

Where \(Y_1\) is the % cellulose degradation achieved by Fenton's pretreatment, \(Y_2\) is % lignin degradation by Fenton's pretreatment, \(X_1\) is Fe\(^{2+}\) concentration, \(X_2\) and \(X_3\) are hydrogen peroxide concentration (ppm) and reaction temperature, respectively.

Tables 3 and 4 show degradation of cellulose and lignin at different concentrations of Fe\(^{2+}\) and H\(_2\)O\(_2\) 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 H\(_2\)O\(_2\) and reaction temperature. Whereas increasing concentration of Fe\(^{2+}\) decreased the rates of

### 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             | 1000              | 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         |                 |

### 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| 0.00 |
| H\(_2\)O\(_2\) (X\(_2\)) | 0.000970293 | 222.65| 0.00 |
| Reaction temperature (X\(_3\)) | 0.985852 | 19.48| 0.00 |
| Fe * Fe (X\(_1\) * X\(_1\)) | 4.369308E-05 | 24.77| 0.00 |
| H\(_2\)O\(_2\)* H\(_2\)O\(_2\) (X\(_1\) * X\(_1\)) | 8.66348E-08 | 2.55| 0.12 |
| Reaction temperature * Reaction temperature (X\(_1\) * X\(_1\)) | -0.00985858 | 25.89| 0.00 |
| 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| 0.00 |
| H\(_2\)O\(_2\) (X\(_2\)) | 0.000782833 | 47.74| 0.00 |
| Reaction temperature (X\(_3\)) | 2.23014 | 25.77| 0.00 |
| Fe * Fe (X\(_1\) * X\(_1\)) | 2.15921E-05 | 0.06| 0.006 |
| H\(_2\)O\(_2\)* H\(_2\)O\(_2\) (X\(_1\) * X\(_1\)) | -1.66049E-09 | 0.970| 0.970 |
| Reaction temperature * Reaction temperature (X\(_1\) * X\(_1\)) | -0.0211932 | 0.00| 0.00 |
| 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\(_2\)O\(_2\) 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\(_2\)O\(_2\) 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 tried 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\(_2\)O\(_2\) 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

![Figure 1](image1.png)  
**Figure 1** Cellulose degradation (% w/w) as a function of Fe\(^{2+}\) concentration (ppm) and H\(_2\)O\(_2\) concentration (ppm).

![Figure 2](image2.png)  
**Figure 2** Lignin degradation (% w/w) as a function of Fe\(^{2+}\) concentration (ppm) and H\(_2\)O\(_2\) concentration (ppm).
effects are significant \( (P < 0.01) \) for cellulose degradation and for lignin removal. The positive linear effect for \( \text{H}_2\text{O}_2 \) 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 \( \text{Fe}^{2+} \) concentration. The concentration of \( \text{Fe}^{2+} \) 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 \( \text{Fe}^{2+} \) could lead to the self-scavenging of \( \cdot \text{OH} \) radical by \( \text{Fe}^{2+} \) and induce the decrease in degradation rates [25–27]. According to Neyens & Baeyens, 2003, when the amount of \( \text{Fe}^{2+} \) employed exceeds that of \( \text{H}_2\text{O}_2 \), 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 \( \text{Fe}^{2+} \) ion and \( \text{H}_2\text{O}_2 \) concentration on lignin and cellulose degradation when temperature was set at their centre point are shown in Figures 1 and 2. An increase in \( \text{H}_2\text{O}_2 \) concentration during pretreatment lead to considerable increase in lignin and cellulose degradation in contrast to increasing \( \text{Fe}^{2+} \) 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 \( \text{Fe}^{2+} \) ion concentration when the \( \text{H}_2\text{O}_2 \) 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 \( \text{Fe}^{2+} \) 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 \( \text{Fe}^{2+} \) and \( \text{H}_2\text{O}_2 \) play crucial role in determining the effectiveness of lignin and cellulose degradation. An increase in \( \text{H}_2\text{O}_2 \) concentration in Fenton’s reagent significantly increased the rates of cellulose and lignin degradation in contrast to increasing \( \text{Fe}^{2+} \) ion concentrations which lead 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 SPM PW 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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**References**

1. Hall DO, House J, Scrase I. Overview of biomass energy, in industrial uses of biomass Energy—The example of Brazil In: Rosili Calle F, Bajaj S, Rothman H, editors. Taylor & Francis, London, 2000. p.26.

2. Hall DO, Rosili CF. Biomass Resources other than wood. London: World Energy Council; 1998.

3. Tai HS, He WH. A novel composting process for plant wastes in Taiwan military barracks. Resour Conserv Recycl. 2007;51:408–17.

4. Coffie Olufunke, Adams Bradford A. Organic Waste Reuse for Urban Agriculture, Case study of Ouagadougou city in Burkina Faso. 2006.

5. Bak JS, Ko JK, Han YH, Lee BC, Choi IG, Kim KH. Improved enzymatic hydrolysis yield of rice straw using electron beam irradiation. J Ferment Technol. 2009;62:377–84.

6. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol. 2005;96:673–86.

7. Hsu TA. Pretreatment of Biomass, Handbook of Bioethanol, production and utilization. Washington, DC: Taylor and Francis; 1996.

8. Lin SH, Lo CC. Fenton process for treatment desizing wastewater. Water Res. 1997;31:2050–6.

9. Tang WZ, Huang CP. 2,4-Dichlorophenol oxidation kinetics by Fenton’s reagent. Environ Technol. 1996;17:1371–7.

10. KangYW HKY. Effect of reaction conditions on the oxidation efficiency in the Fenton process. Water Res. 2000;34:2786–90.

11. Huseh CL, Huang YH, Wang CC, Chen CY. Degradation of azo dyes using low iron concentration of Fenton and Fenton-like system. Chemosphere. 2005;58:409–1414.

12. Munuganandham M, Swaminathan M. Decolourisation of reactive orange by Fenton and photo-Fenton oxidation technology. Dyes Pigments. 2004;63:315–21.

13. Prateek J, Nandanathangam V. Effect of Fenton’s pretreatment on cotton cellulosic substrates to enhance its enzymatic hydrolysis response. Bioresour Technol. 2012;103:219–26.

14. Barbusiński K. Fenton reaction-controversy concerning the chemistry. Ecol Chem Eng. 2009;16:3.

15. Nelson DW, Sommers LE. Total carbon, organic carbon and organic matter. In: Page AL, editor. Methods of Soil Analysis Part II. Madison: American Society of agronomers; 1982. p. 539–79.

16. Liu S. Analysis and Measurement in Papermaking Industry. Beijing, China: Chemical Industry Press; 2004.
17. Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D. Determination of Extractives in Biomass, National Renewable Energy Laboratory, Midwest Research Institute, Golden, CO. NREL/TP-510-42619. 2008.
18. Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J Agric Food Chem. 2010;58:9045–53.
19. Montgomery DC, Anderson W, Russell S, Aiello E. Design and Analysis of Experiments. New York: John Wiley, and sons Publishers; 2001. p. 427–92.
20. Mathews P. Design of experiments with MINITAB. India: Pearson Education (Singapore) Pte. Ltd; 2005. p. 437–74.
21. Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res. 2009;48:3713–29.
22. Mosier N, Hendrickson R, Ho N, Sedlak N, Ladish MR. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioreourc Technol. 2005;96:1986–93.
23. Liu C-Z, Cheng X-Y. Microwave-assisted acid pretreatment for enhancing biogas production from herbal-extraction process residue. Energy Fuels. 2009;23:152–5.
24. Ayeni AO, Hymore FK, Mudliar SN, Deshmukh SC, Satpute DB, Omoleye JA, et al. Hydrogen peroxide and lime based oxidative pretreatment of wood waste to enhance enzymatic hydrolysis for a bio refinery: Process parameters optimization using response surface methodology. Fuel. 2013;106:187–94.
25. Chen R, Pignatello JJ. Role of quinone intermediates as electron shuttles in Fenton and photo assisted Fenton oxidations of aromatic compounds. Environ Sci Technol. 1997;31:2399–406.
26. Joseph JM, Destaillets H, Hung HM, Hoffmann MR. The sonochemical degradation of azobenzene and related azo dyes: rate enhancements via Fenton’s reactions. J Phys Chem A. 2000;104:301–7.
27. Hameed BH, Lee TW. Degradation of malachite green in aqueous solution by Fenton process. J Hazard Mater. 2009;164:468–72.
28. Neyens E, Baeyens J. A review of classic Fenton’s peroxidation as an advanced oxidation technique. J Hazard Mater. 2003;98:33–50.