Fuel 106 (2013) 187–194

Contents lists available at SciVerse ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Hydrogen peroxide and lime based oxidative pretreatment of wood waste to enhance enzymatic hydrolysis for a biorefinery: Process parameters optimization using response surface methodology

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HIGHLIGHTS

- Hydrogen peroxide and lime based oxidative pretreatment of wood waste.
- Response surface methodology was used to optimize the process parameters.
- Optimum conditions of 150 °C, 1% H_2O_2, and 45 min were predicted and validated.
- 59% (w/w) cellulose content was retained in the solid fraction.
- Reducing sugars yield from pretreated material was up to 263.49 mg g^{-1} dry biomass.

ARTICLE INFO

Article history:
Received 8 July 2011
Received in revised form 18 December 2012
Accepted 19 December 2012
Available online 8 January 2013

Keywords:
Response surface methodology
Lignocellulose
Vitellaria paradoxa
Central composite design
Optimization

ABSTRACT

Response surface methodology (RSM) was adopted for the optimization of process variables in the alkaline peroxide oxidation (APO) pretreatment of *Vitellaria paradoxa* sawdust based on central composite design (CCD) experiments. A 2^3 five level CCD with central and axial points was used to develop a statistical model for the optimization of process variables. Maximum response for the pretreatment was obtained when applying the optimum values for temperature (150 °C), time (45 min), and 1% (v/v) H_2O_2. At the optimum conditions, up to 70% of the initial hemicellulose was removed in treatments, which also caused some delignification (up to 11% of the initial lignin was removed), whereas cellulose was almost quantitatively retained in the solid phase. Alkaline peroxide assisted wet air oxidation (APA-WAO) pretreatment at the optimum conditions resulted in enrichment up to 60% cellulose content along with solubilization of 80% hemicellulose and 17% of lignin initially present in the raw sawdust. Reducing sugars yield after 72 h enzymatic hydrolysis of pretreated biomass at optimized APO conditions was 177.89 mg equivalent glucose g^{-1} dry biomass. Addition of 10 bar air pressure at the optimized pretreatment conditions increased the sugars yield to 263.49 mg equivalent glucose g^{-1} dry biomass.

1. Introduction

Ethanol production from lignocellulosic residues has potential to significantly improve sustainability of biofuels for transport by avoiding land-use competition with food crops and reducing impacts related to agricultural inputs. However, high production costs remain bottleneck for large scale development of this pathway [1]. Cellulose is recalcitrant to biodegradation and needs to be hydrolyzed in an initial pretreatment step into its constituent cellobiose units and into simpler D-glucose units in order to be liable to biochemical conversion. The rate and extent to which cellulose in lignocellulosic materials can be enzymatically saccharified is limited by two important factors: the close physical and chemical association between lignin and the cell wall polysaccharides, and the degree of crystallinity within the cellulose polymer itself [2]. In order to hydrolyze lignocellulosic biomass with enzymes successfully, it is also important to apply a suitable pretreatment that can effectively disrupt linked lignin and crystalline cellulose. Certain kinds of chemical, physical and/or biological pretreatments remove or disrupt lignin sheath, reduce the degree of cellulose crystallinity, remove or separate hemicellulose from cellulose and increase the accessible surface area of biomass, resulting in an enhancement of lignocellulosic substrate digestibility.

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http://dx.doi.org/10.1016/j.fuel.2012.12.078

Hydrogen peroxide and alkaline oxidative pretreatment (alkaline peroxide oxidation (APO)) is known to decrystallize cellulose [3]. It is also known that under proper conditions hydrogen peroxide (H_2O_2) will react readily with lignin and related phenolics [4,5] to yield an array of low molecular weight, water-soluble oxidation products [6]. Natural degradation of lignin can occur through a variety of different organisms. Hydrogen peroxide excreted by the organism plays an important role in the degradation [7,8]. The mechanism by which alkaline peroxide pretreatment enhances enzymatic saccharification appears to involve both a release of lignin from the lignocellulosic matrix and a dramatic increase in the degree of hydration of the cellulose polymer [9]. The APO process (an alternative oxidative treatment to air or oxygen delignification) has been shown to be effective in increasing the digestibility of crop residues [3]. Extensive studies exist on the APO process pretreatment of agricultural residues [9–12].

The conventional technique for the optimization of a multi-factorial system is to deal with one-variable at a time (OVAT). However, this type of method is time-consuming and also does not reveal the interactive and square effects hence; response surface methodology (RSM) which is based on statistical principles was employed as a strategy to study individual as well as interactive effects of the parameters and also to generate significant information regarding the optimum sets of levels of the pretreatment process.

In the present study attempts were made to find out whether the APO process could be used for delignification (lignin removal) and to enhance the digestibility of high lignin wood waste (sawdust). Also investigated was if lime can be used as a viable alternative source of alkali for sodium hydroxide (a conventional alkali source of the APO process), thereby reducing the cost of treatment. RSM based on central composite design (CCD) of experiments was adopted to investigate the optimum parameters of APO pretreatment such that the cellulose content, the hemicellulose solubilization, and delignification will enhance enzymatic hydrolysis. The effects of three process parameters (reaction temperature, reaction time, and percent hydrogen peroxide) on the pretreatment step were studied. The most suitable APO conditions to obtain enriched solid fraction for enzymatic digestibility were selected after optimizing and validating the pretreatment conditions.

Further variations at the optimized conditions; alkaline wet air oxidation (WAO), and alkaline peroxide assisted wet air oxidation (APAWAO) were compared with the APO process. The enzymatic hydrolysis of biomass was also investigated to ascertain the efficiencies of pretreatments.

2. Material and methods

2.1. Raw material

Vitellaria paradoxa sawdust raw biomass was carefully collected from a sawmill in Ifo town, south west Nigeria. Samples were sieved to yield fine particles. The fraction which passes through BSS 14 and retained by BSS 80 mesh sizes was used in all the experiments. The average particle sizes therefore varied between 0.09 and 0.51 mm, which made up 73% of the harvested raw material. Samples were dried in a convectional oven at 105 °C for 3 h to a dry matter content of 88%. The sieved and dried samples were stored in rubber bottles capped tightly and kept in a locker. The materials were used shortly after.

2.2. Alkaline peroxide oxidation pretreatment

Table 1
Statistical 2^3 – central composite design for APO experiments.

Factor	Low level	High level
Reaction temp., X_1 (°C)	120	150
Reaction time, X_2 (min)	20	40
H_2O_2, X_3 (%)	1	1.5

All pretreatment experiments were carried out in a Parr reactor model 4578. This 1.8 L batch reactor fitted with double six-blade turbine impellers was equipped with an external heating element sheathed with a jacket and internal stainless steel loops for cooling. 30 g of dry substrate were mixed with 500 mL distilled water containing 30%(w/v) H_2O_2 (0.84%, 1.00%, 1.25%, 1.50%, 1.66%) and adjusted to pH 11.5 with respective lime loadings (9.0, 13.7, 17.5, 20.3, 30.0 g). Slurries were pretreated at different temperatures, and at different time intervals (Table 1).

The reaction was controlled by a Parr PID controller model 4857. A solenoid valve adjusted the water flow through the internal coil, and regulated the temperature at the set point (±2 °C) with constant stirring at 21 rad s^{-1}. Each reaction was terminated by running cold water through the internal loops. There was constant stirring while cooling, thereby maintaining a relatively homogenous environment. After the specified reaction time, the reactor and slurry were allowed to cool to ambient temperature. Specific lime consumption (g $Ca(OH)_2$ g^{-1} dry biomass) for each reaction time was determined through pH titration (neutralizing slurry with 5 N HCl) [13]. The pretreated slurry was separated into the solid and liquid fractions by vacuum filtration, and the solid fraction was washed with water. The solid fraction was dried and weighed. The solid fraction was used to determine the optimum conditions for pretreatment.

2.3. Analysis of the solid fraction

The compositional analysis of the raw, pretreated, and specific lime consumption of biomass is given below (Table 2).

2.3.1. Extractives

Extractives were determined by means of the Soxhlet extractor. Acetone (300 mL) was used as the solvent for extractives (5 g of dry biomass, w_o, g) with residence times for the boiling and rising stages equal to 70 °C and 25 min respectively for a 4 h run period. The sample was air dried for few minutes at room temperature. It was then dried at 105 °C in a convectional oven until a constant weight (w_1, g) was obtained. The extractives weight percent, % (w/w) is calculated as given in Eq. (1) [14–16]. Mineral components were determined by ashing at 575 °C for 6 h.

$$\%(\mathrm{w/w}) = \{(w_o - w_1)/w_o\} \times 100\% \qquad (1)$$

2.3.2. Hemicellulose

One gram of dried biomass from the extractive analysis was transferred into a 250 mL Erlenmeyer flask and then 150 mL NaOH solution (0.5 mol L^{-1}) was added. The mixture was boiled for 3.5 h with distilled water so as to increase the heating effect and minimize lime scales. It was filtered after cooling through vacuum filtration and washed (until pH value of solution approached 7). The residue was dried to a constant weight at 105 °C. The residue was then cooled in a desiccator and weighed. The difference between the sample weight before and after this treatment is the hemicellulose [15,16].

2.3.3. Lignin

Three hundred milligrams of dry biomass was weighed in glass test tubes and 3 mL of 72% H_2SO_4 was added. Acid hydrolysis was made to occur by keeping the samples at room temperature for 2 h with mixing of samples every 30 min. 84 mL of distilled water was