Optimization of Microwave-Assisted Pretreatment of Rice Straw with $FeCl_3$ in Combination with H_3PO_4 for Improving Enzymatic Hydrolysis

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Abstract

Pretreatment is a key step to alter the recalcitrance structure of lignocellulosic biomass for enhancing enzymatic hydrolysis. Rice straw is an agricultural residue which is one of the potential substrate for ethanol production. In the present work, optimization of microwave-assisted pretreatment of rice straw in $FeCl_3$ solution with H_3PO_4 was performed. The effect of concentration of $FeCl_3$ and H_3PO_4 along with pretreatment time was evaluated. The optimal pretreatment condition was found as follows: 250mM $FeCl_3$, 3%H_3PO_4, 155°C, and 20 minutes. The pretreated pulp was subjected to enzymatic hydrolysis using commercial cellulase for assessing effectiveness of pretreatment system. The maximum saccharification per pulp and per biomass was observed as 98.9% and 66.4%, respectively, under enzyme load of 3 FPU/g of substrate after incubation for 48 h.

Keywords

Microwave · Enzymatic hydrolysis · Lignocellulosic biomass · Delignification

7.1 Introduction

The rapid increase in energy demand, fast depletion of fossil fuel reservoirs, and environment pollution caused by use of fossil fuels have forced the government and scientific community to search for alternative sources of energy generation that are inexpensive, eco-friendly, and renewable and can efficiently replace conventional fossil fuels [1]. Lignocellulosic biomass is regarded as one of the most promising alternative to fossil fuel. It can be transformed into biofuel and various

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value-added products which can offer sustainable system to help meet up this global necessity. Rice is one of staple crop of the world with annual production of 481.54 million metric tons of which India contributes 106.50 million metric tons (2016–2017) [2]. Each kilogram of rice obtained after harvesting generates 1–1.5 kg of the straw [3]. Based on this it can be estimated that about 106.5–159.8 million metric tons of rice straw was produced in India (2016–2017) of which a part goes as cattle feed. However, the remaining part is waste and disposal of which is a major concern. Most often these residues are burnt resulting in pollution. Thus, utilization of rice straw for its conversion to biofuel is one approach that can help in lignocellulosic waste management. The bioconversion of lignocellulosic biomass to ethanol involved three major steps: first pretreatment for the opening of the crystalline structure of cellulose by breaking down of lignin, second conversion of cellulose to glucose by hydrolysis with combination of enzymes, and third fermentation of sugars to ethanol [4]. Pretreatment is one of the most essential steps in the cost-effective conversion of lignocellulosic biomass to bioethanol or other bio-based products. The major goal of the pretreatment is to improve the accessibility of cellulose to the hydrolytic enzymes. Pretreatment breaks the physical barrier by disrupting the cell wall, removing hemicellulose or lignin fractions, reducing the cellulose crystallinity, improving porosity in the biomass structure, and increasing the accessible surface area. As a consequence, hydrolytic enzymes can easily access the cellulose fibers and act with higher efficiency. Previously various physical methods such as ball milling [5], irradiation [6], and chemical pretreatment methods such as ammonia [7], alkali [8], dilute acids [9], and organosolv process [10] have been utilized frequently which can help to modify the structural framework of lignocellulosic biomass and improve the saccharification of the cell wall carbohydrates [11]. However, the existing pretreatment technologies have certain limitations such as formation of inhibitory compounds which hinder fermentation, inadequate separation of cellulose and lignin, and considerable production of wastes [12].

In order to disrupt the recalcitrance structures of lignocelluloses, microwave heating has been used [13]. Unlike conventional heating microwave irradiation has offered advantage over conventional heating methods due to nonthermal or thermal effects [14]. Microwave irradiation has increased reaction rate and reduced reaction time. Verma and Chaturvedi [15] have carried out studies using microwave pretreatment of lignocellulosic biomass especially for the efficient enzymatic hydrolysis of woody biomass. Similar approach has also been used by Sindhu et al. [16] where they used microwave-assisted surfactant pretreatment of chilli postharvest residue for the production of bioethanol and biopolymer. On the other hand, some microwave sensitizer chemicals can enhance the effect of irradiation, which will result in improved delignification with negligible or no carbohydrate degradation [17]. However only few studies have been carried out especially for the enzymatic hydrolysis of rice straw using microwave system and microwave pretreatment. Liu et al. [18] demonstrated effect of $FeCl_3$ pretreatment on corn stover. In the present study, optimization of microwave pretreatment of rice straw with $FeCl_3$ in combination with H_3PO_4 at different pretreatment time was evaluated. Enzymatic hydrolysis of the pretreated pulp was performed to assess the impact of the microwave-assisted $FeCl_3$ pretreatment on rice straw.

7.2 Materials and Methods

7.2.1 Materials

The chemicals used were purchased from HiMedia, India, and Merck, India. The chemicals are of analytical grades. Rice straw was collected from local farms of Rajasthan, India. They were washed with water in order to remove dirt and mud, followed by oven drying at 50 °C. They were grounded in an electric grinder, sieved to 2–5 mM mesh size, and stored in airtight polythene bags until use.

7.2.2 Microwave Pretreatment of Rice Straw Using $FeCl_3$

Rice straw (2g) was soaked in aqueous solution of $FeCl_3$ of different concentrations 50–400 mM for 24 h. The solid to liquid ratio was maintained at 20:1 (w/w) in all the experiment .The treatment was performed in a microwave reactor with a 700W magnetron "Microwave Reaction system SOLV, Multiwave Pro" (Make: Anton Paar, Austria). The program was set up as heating as fast as possible with high stirring during attaining of the desired temperature and holding temp for desired time with fast stirring, then followed by cooling the system to 70 °C with slow stirring before opening the system. After the reaction, the pulp fraction was separated by vacuum filtration and washed three times with 150 ml of distilled water. The pulp fraction or pulp yield was calculated by using equation

$$\text{Pulp Yield}\left(\%\right) = \frac{Wi - Wf}{Wi} * 100$$

where Wi and W_f are weight of substrate before and after pretreatment, respectively.

7.2.3 Effect of H_3PO_4 Concentrations

Rice straw (2g) was treated with different concentrations of H_3PO_4 (0.5–5%) at 155 °C for 30 min to study the effect of H_3PO_4 concentration on saccharification obtained from pulp fractions.

7.2.4 Effect of Pretreatment Time

Rice straw (2g) samples soaked in 250 mM $FeCl_3$ for 24 h and 3%H_3PO_4 were added just before start of reaction which were subjected to microwave treatment at 155 °C for 10, 20, and 30 min in order to study effect of pretreatment time. The pretreated pulp was subjected to enzymatic hydrolysis using commercial cellulase.

7.2.5 Enzymatic Saccharification of Pulp

The enzymatic saccharification of pulp was performed as method suggested by Verma et al. [17]. The wet pulp fraction was hydrolyzed with a commercial cellulase preparation, "ONOZUKA R-10" from *Trichoderma viride* (HiMedia). The cellulase enzyme loading was 1FPU/g substrate. Enzymatic hydrolysis was performed at a substrate concentration of 2% in 0.05 M sodium acetate buffer (pH 4.5) containing 0.02% sodium azide at 50 °C in rotary shaker water bath (Tempo, India) at 140 rpm for 48 h. The saccharification ratio per pulp was calculated according to the NREL LAP-009 procedure[19]. After enzymatic hydrolysis, 1ml sample was collected from each tube; the samples were placed in 1.5 ml eppendorf tubes, and then the solutions were centrifuged at 5000 rpm for 5 min. The appropriate dilutions were made to estimate sugar yield using dinitrosalicylic assay [20].

The saccharification ratio per pulp was evaluated based on how much pulp fraction was susceptible to the enzymatic hydrolysis. The saccharification per biomass was based on the weight percentage of reducing sugar to the original biomass. All enzymatic hydrolysis experiments were performed in duplicate.

7.2.6 Effect of Enzyme Load on Saccharification Yield on Pretreated Pulp

Rice straw (2g) was pretreated with 250 mM $FeCl_3$ in combination with 3% H_3PO_4 at 155 °C for 20 min, and then the obtained pretreated pulp fractions were hydrolyzed with different concentrations i.e. 1, 3, and 5 FPU/g (filter paper unit per gram of substrate) of commercial cellulase "ONOZUKA R-10" in order to examine maximum saccharification yield for evaluating the optimum enzyme dose. The control set was incubated without addition of enzyme.

7.3 Results and Discussion

7.3.1 Microwave Pretreatment of Rice Straw Using $FeCl_3$

In the present work, we compared the effect of different concentrations of $FeCl_3$ (50–400 mM) on microwave treatment of rice straw at 155 °C for 30 min. The maximum pulp yield of 90.2% was observed for control (soaked in distilled water) which may be because of minimum breakdown of lignocellulosic constituents of rice straw. With increase in concentration of $FeCl_3$, pulp yield gradually decreased which may be due to loss of lignin (data not shown) and breakdown of carbohydrate polymer. The obtained pulp was subjected to enzymatic hydrolysis using commercial cellulase. The maximum saccharification per pulp and per biomass of 43.1% and 25.3%, respectively, (Fig. 7.1a) was obtained with 250 mM $FeCl_3$.

The results clearly show that with an increase in $FeCl_3$ concentration from 50 to 250 mM, the saccharification yield gradually increased; however above 250 mM decrease in the saccharification yield was observed which may be due to loss of cellulosic part. The saccharification yield per biomass was 10.1-fold higher than the control, which was better than the results obtained by Lü and Zhou [21]. However, they pretreated rice straw in microwave at 140 mM $FeCl_3$, 160 °C for 19 min followed by utilization of *Trichoderma viride* and *Bacillus pumilus* for the production of reducing sugars.

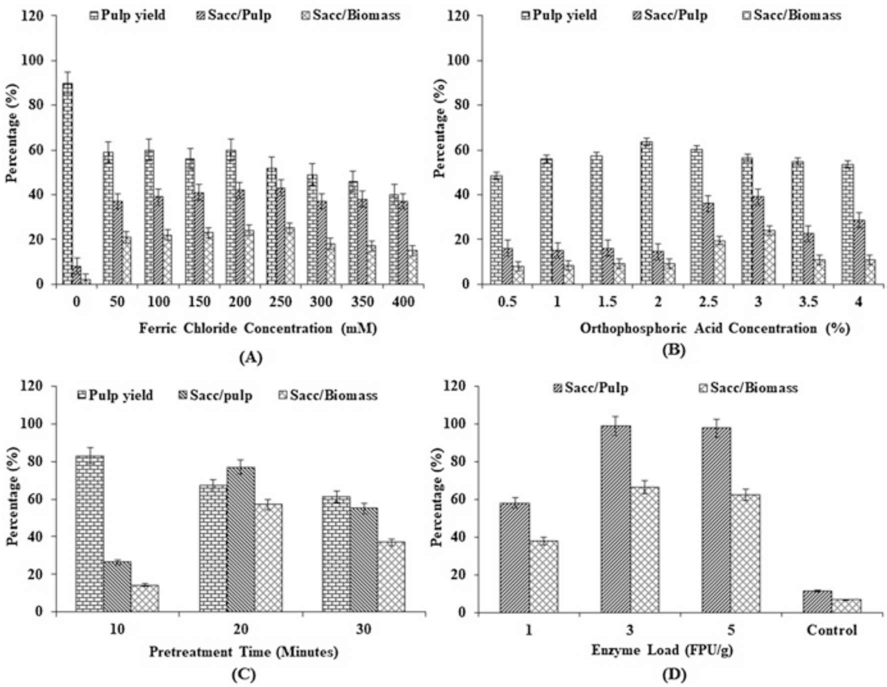

Fig. 7.1 (**a**) Pulp yield and saccharification yields of microwave pretreated rice straw in different concentrations (50–400 mM) of $FeCl_3$ at 155 °C for 30 min. (**b**) Pulp yield and saccharification yields of microwave pretreated rice straw in different H_3PO_4 concentrations (0.5–4%) at 155 °C for 30 min. (**c**) Pulp yield and saccharification yields of microwave pretreated rice straw in 250 mM $FeCl_3$ with 3% H_3PO_4 at 155°C for different time intervals. (**d**) Effect of enzyme dose on saccharification yield of the pretreated pulp obtained after microwave pretreatment of rice straw with 250 mM $FeCl_3$ and 3% H_3PO_4 at 155 °C for 20 min

7.3.2 Effect of H_3PO_4 Concentrations

The rice straw was subjected to addition of different concentrations (0.5–4%) of H_3PO_4 for microwave treatment at 155 °C for 30 min. The maximum pulp yield of 63.7% was obtained by addition of 2% H_3PO_4. The maximum saccharification per pulp and per biomass obtained was 39.01% and 24.03%, respectively, (Fig.7.1b) for the pretreatment under 3% acid concentration.

7.3.3 Effect of Pretreatment Time

The rice straw was soaked in 250 mM $FeCl_3$ for 24 h; 3% H_3PO_4 was added with just before start of pretreatment. The pretreatment was performed at 155 °C for different pretreatment time, i.e., 10, 20, and 30 min. The maximum pulp yield of 82.97% was obtained after pretreatment of 10 min, whereas pulp yield of 67.13% and 61.43% for 20 and 30 min, respectively. Maximum saccharification per pulp and per biomass of 77.03% and 57.09%, respectively (Fig. 7.1c), was obtained for pretreatment for 20 min which was much higher as compared to saccharification yields for pretreatment for 10 and 30 min. This can be explained as low pretreatment time (10 min) caused low delignification of lignocellulosic biomass due to inefficient breakdown of lignin carbohydrate complexes that resulted in less accessibility of cellulase enzyme to cellulosic part. The pulp yields for 20 and 30 min of pretreatment are in comparable range, but the saccharification yield for 30-min pretreatment is relatively low. It can be due to loss of cellulosic component with long pretreatment time (30 min) which finally affects the overall saccharification yield. The optimum pretreatment time obtained was 20 min, which is comparable to the results obtained by Lü and Zhou [21] where the optimum irradiation time was obtained as 19 min.

7.3.4 Effect of Enzyme Dose on Saccharification Yields

The enzymatic hydrolysis was performed for pulp obtained by microwave pretreatment of rice straw in 250 mM $FeCl_3$ and 3% H_3PO_4 at 155 °C for 20 min with different cellulase enzyme load, i.e., 1, 3, and 5 FPU/g of substrate. The maximum saccharification per pulp and per biomass of 98.9% and 66.4%, respectively, was obtained at enzyme load of 3 FPU/g after 48 h of incubation (Fig. 7.1d). The saccharification per pulp and per biomass by enzymatic hydrolysis of pretreated rice straw with enzyme load of 5 FPU/g was obtained as 97.8 and 62.3, respectively, which are comparable to the saccharification yields obtained for enzyme load of 3 FPU/g. The enzyme load higher than 3 FPU/g did not result in any increase in saccharification yield; it may be due to its adsorption onto the substrate that restricted the diffusion process through the structure as explained by Martín et al. [22] where enzyme loading higher than 15 FPU/g did not result in any increase in initial rate.

7.4 Conclusion

The conditions for microwave-assisted $FeCl_3$ in combination with H_3PO_4 pretreatment of rice straw were optimized. The optimal conditions were found as follows: 250 mM $FeCl_3$ concentration, 3% H_3PO_4 concentration, 155 °C pretreatment temperature, and 20-min pretreatment time. Enzymatic hydrolysis of pretreated pulp with enzyme load (3 FPU/g) for 48 h resulted in maximum saccharification per pulp and per biomass as 98.9% and 66.4%, respectively. The pretreatment step and cost of enzyme are rate-limiting step of the commercial biofuel production. The present work gives an efficient pretreatment strategy resulting in high amount of reducing sugars at low enzyme load. It will lead to develop cost-effective lignocellulosic biorefinery concept.

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