효소 당화를 위한 Softwood 계 섬유소 폐기물의 전처리 공정

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Pretreatment of Softwood-based Lignocellulosic Wastes to Enzymatic Hydrolysis

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INTRODUCTION

During alkaline delignification of biomass, Kraft pulping being one example, lignin is predominantly degraded by cleavage of lignin-hemicellulose bonds (1). It also brings about changes in the structure of cellulose fiber (2). As an alkaline reagent, ammonia has a number of characteristics suitable for processing of lignocellulosic materials (3). Pursuing this concept, a pretreatment method termed as the ammonia recycled percolation (ARP) process was developed in our laboratory. It uses aqueous ammonia as the pretreatment reagent. The reaction is carried out in a flow-through packed-bed reactor. The process stream is continuously fed into and withdrawn from the reactor. Under this operation mode, the lignin and other extraneous components are separated from the biomass structure preventing recondensation of lignin. Ammonia is then recovered at the end of the process. This process has been proven to be very effective for pretreatment of hardwood and herbaceous substrates (4,5,6). In contrast to these type of substrates substrates, the literature information on biological conversion of softwood is scarce. Perhaps it is due to the fact that softwood is one of the most difficult substrates to degrade biologically. The softwood based feedstocks have high lignin content and dense structure between cellulose fiber and lignin. They exhibit extremely low enzymatic digestibility. Hydrogen peroxide is one of the few delignifying reagents that are environmentally benign. Currently it is widely used in the bleaching process of pulp and paper industry. Hydrogen peroxide in alkaline condition promotes rapid oxidative depolymerization of the lignin molecule in lignocellulosic materials (7,8). It has been used in the pretreatment of biomass (9-15). It has also been tested in our laboratory as a supplementary reagent in the ARP system (5). The feedstocks used in these studies, however, have been limited to hardwood and herbaceous feedstocks. The purpose of this investigation is to find an effectiveness pretreatment method for softwood based feedstocks, specifically the primary sludge from a Kraft mill and newsprint waste paper. Our focus was placed on the conventional ARP and its variation utilizing hydrogen peroxide, concurrently and in succession with aqueous ammonia.

MATERIALS AND METHODS

Paper mill sludge was obtained from a Kraft-Mill, Mead-Beit, Inc. (Columbus, GA). Newsprint waste paper feedstock was supplied from Korea Institute of Energy Research (KIER), Taejon, KOREA. The composition of newspaper feedstock was 53.3 wt.% cellulose, 11.2 wt.% hemicellulose, and 26.3 wt.% klason lignin. The composition of pulp mill sludge was 58.4 wt.% cellulose, 12.4 wt.% hemicellulose, and 19.72 wt.% klason lignin. The cellulase enzyme, Spezyme-CP (Lot. No. 41-95034-004) was supplied from National Renewable Energy Laboratory (NREL). The average enzyme activity was 84.7 FPU/mL. An experimental apparatus for pretreatment is shown in Fig 1. The reactor was constructed out of SS316 with the volume of 175 cm^3. In the ARP-H (H indicating hydrogen peroxide), aqueous ammonia and hydrogen peroxide were pumped simultaneously to a packed-bed reactor through a preheating coil. Alternatively, the treatment was done in two stages: hydrogen peroxide followed by ARP. At the completion of a run, wash water was put through the reactor to remove residual sugar and ammonia trapped in the treated substrates. The wet solids discharged from the reactor were separated into two portions. One was oven-dried at 105 ^{O}C overnight for measurement of weight loss and further subjected to composition analysis. The other was used in the enzymatic digestibility test. The cellulase enzyme loading was kept at 60 IFPU/g glucan. The initial glucan concentration was 1% (w/v). Sample were taken periodically and analyzed for glucose and cellobiose content using HPLC. Glucose and cellobiose content taken for calculation of the enzymatic digestibility. Solid biomass samples were analyzed for sugars, Klason lignin, acid soluble lignin, and ash according to the procedures described in the NREL Chemical Analysis & Testing Standard Procedure (No. 002 and 010). Oligomeric sugars in the liquor and rinsate were converted to monomer using 4% H_2SO_4 hydrolysis at 121 ^{O}C for 1 h. Sugars were determined by HPLC using Bio-Rad Aminex HPX-87P column.

RESULTS AND DISCUSSION

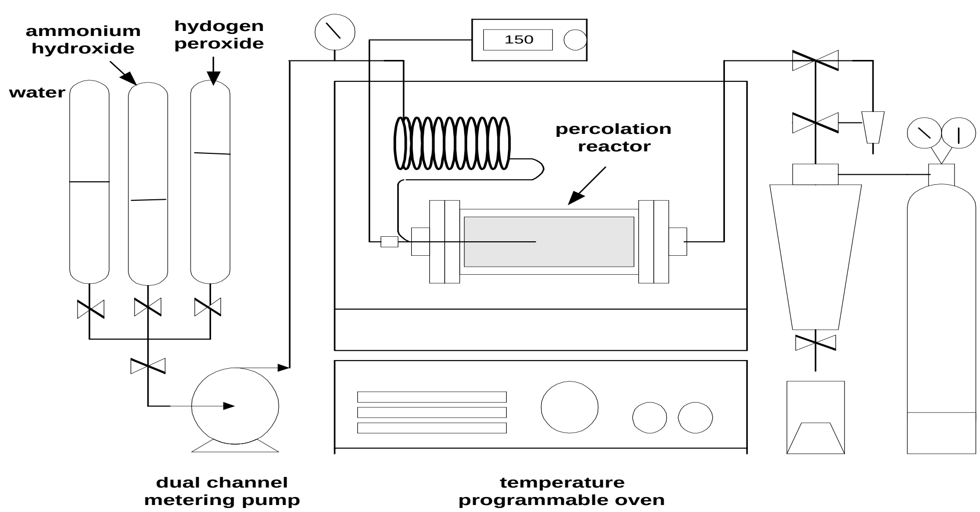
The feedstocks were first put through a series of ARP process using 17 wt.% aqueous ammonia solution. The temperature was varied over the range of 150-220^{O}C. The weight remaining after the treatment averaged to 88% for newspaper and 72% for the pulp mill sludge. The treated solid samples were then analyzed for sugars and Klason lignin. The results are summarized in Table 1. The cellulose fraction after the ARP treatment increased slightly as temperature was raised from 150 to 170 ^{O}C. The cellulose retention at these temperatures was near 100% for newspaper, 78% for the pulp mill sludge. Further increase in temperature decreased the cellulose fraction of the treated biomass. The hemicellulose content and Klason lignin remained relatively constant over the entire range of the temperature. About 30% of the lignin and 50% of hemicellulose is removed form biomass by the ARP process. The ARP renders 40-60% delignification for hardwood or herbaceous feedstocks (4,5,6). This again proves our contention that delignifcation is more difficult for softwood based biomass than it is for hardwood or herbaceous biomass. Because of high retention of cellulose and relatively high delignification, 170 ^{O}C is deemed as the optimum operating temperature for the ARP. The ARP-H (ammonia and hydrogen peroxide are put into the reactor simultaneously) was then attempted for the treatment of the aforementioned biomass feedstocks. It was run at 170^{o}C. The concentration of hydrogen peroxide concentration was varied from 0 to 10 wt.% keeping ammonia concentration constant at 17 wt.% (Table2). The tendency shown in the table is that the H_2O_2 increases the overall weight loss and the extent of delignification. Thus the cellulose fraction within the treated biomass actually increases as the hydrogen peroxide concentration was raised from 2.5 to 5.0 wt.%. With the hydrogen peroxide concentrations at 7.5 and 10 wt. %, the percent weight remaining and cellulose composition decreases dramatically. The extent of delignification is also quite high being in the range of 40-60%. This is a clear indication that H_2O_2, at or above 7.5 wt. %, reacts not only with lignin but also attacks cellulose causing substantial degradation. From observation of Table 2, hydrogen peroxide at the level of 5.0 wt. % was selected in further experiments involving ARP-H.

Further refinement of the ARP-H operation was sought, this time with regard to the hydrogen peroxide through put (flow rate). Its effect on the ARP-H performance is shown in Table 3. Again taking the percent weight remaining and cellulose retention into consideration, the flow rate of 0.5 mL/min was taken to be the optimum. With this flow rate and the concentration of H_2O_2 fixed at 5 wt. %, the performance test of ARP-H was conducted varying the temperature from 150 to 190 ^{O}C. The results on the ARP-H were similar to that of the ARP. Considering the three main factors collectively (weight loss, delignification, and cellulose retention), 170 ^{O}C was chosen as the optimum temperature for the ARP-H.

Additional variation of the ARP-H was investigated. In this case, the H_2O_2 and aqueous ammonia were put through the percolation reactor in succession applying different temperature and duration at each stage. In the first stage, 5 wt.% aqueous hydrogen peroxide was delivered into reactor operated at various temperatures with the flow rate of 0.5 mL/min. In the second stage, the regular ARPs were run with 17 wt. % ammonia solution at 170 ^{O}C with 3.2mL/min of flow. The results are summarized in Table 4. The data indicate that the low temperature hydrogen peroxide runs, especially 60 and 80 ^{O}C, provide high cellulose retention, and high degree of delignification. For example, with H_2O_2(80 ^{O}C, 120min)-ARP, the cellulose retention is 48.52/53.30 (91%) for newspaper feedstock. The delignification was 40% (from

26.30 to 15.70). For the pulp mill sludge, the results were much different, yielding only 80% of cellulose retention and 39% of delignification. The poor performance indexes for the pulp mill sludge in comparison to the newspaper feedstock was observed in all of the ARP runs and its variations. The data presented to this point dealt with the changes in the composition of biomass brought about by various pretreatments. The composition of biomass is an important factor in the process economics. It can also

serve as an indirect index for the digestibility, the lignin content for example. However, the true yardstick for a pretreatment must come from the direct measurement of digestibility. All of the solid samples obtained from the ARP, ARP-H, and two-stage treatment were therefore subjected to the standard enzymatic digestibility test. The data are summarized in Figures 2-5. The digestibility data after ARP treatment for both substrates are presented in Figures 2 and 3. The data indicate that ARP alone is not an efficient pretreatment method for these feedstocks. After ARP treatment, the digestibility of newspaper and the pulp mill sludge is improved only by 5% (from 40 to 45% for the former, and from 68 to 73% for the latter). This is despite a substantial degree of delignification occurring after the ARP process. Apparently the lignin content is not a prime factor controlling the digestibility for these substrates. The measured digestibility shown here is a drastic departure from hardwood and herbaceous materials where the digestibilities in the range of 70-90 % are easily attainable by the ARP (5,6). The enzymatic digestibility of pulp mill sludge after ARP-H treatment is shown in Fig. 4. After the ARP-H run, the digestibility of pulp mill sludge is only slightly improved over that of ARP going form 73% to 76%. However, the gain made in the digestibility is offset by the loss of cellulose due to interaction with H_2O_2. Although the absolute value of the digestibility is comparable to that of α-cellulose (83%), the improvement made by ARP or ARP-H is no more than 10% for the pulp mill sludge. Among the various schemes we applied, the one that showed most promise is the successive treatment by H_2O_2 and ARP. The digestibility data for newspaper coming form this series runs are shown in Fig. 5. The run that gave the highest digestibility is the one made with H_2O_2, 80 ^{O}C, 120min, followed by 90 min ARP. The digestibility of newspaper feedstock coming from this run was measured to be 75%. It is a significant improvement over that of the untreated substrate (41%). It is certainly at a level comparable to α-cellulose (83%). Whether the digestibility of this level is acceptable from a process viewpoint is debatable. For the two softwood based waste materials, however, this is the best one can do at this time with the known pretreatment technology employing environmentally safe pretreatment chemicals. There is also a positive note on this. When a biomass substrate is subjected to a SSF process, the overall digestibility of improves considerably over the enzymatic digestibility.



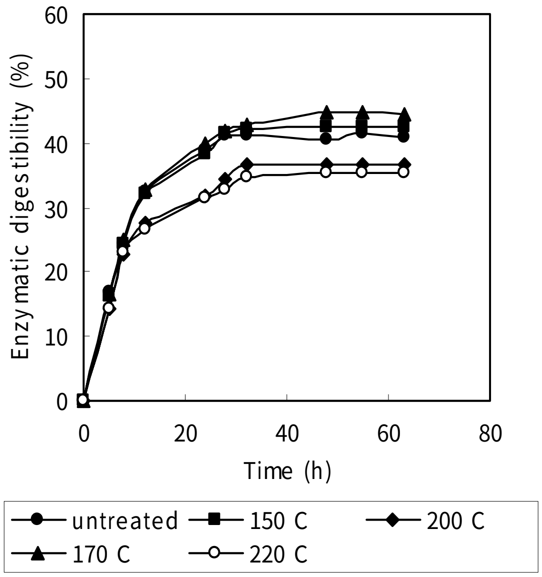


Fig.2. Enzymatic digestibility of newspaper after ARP treatment.

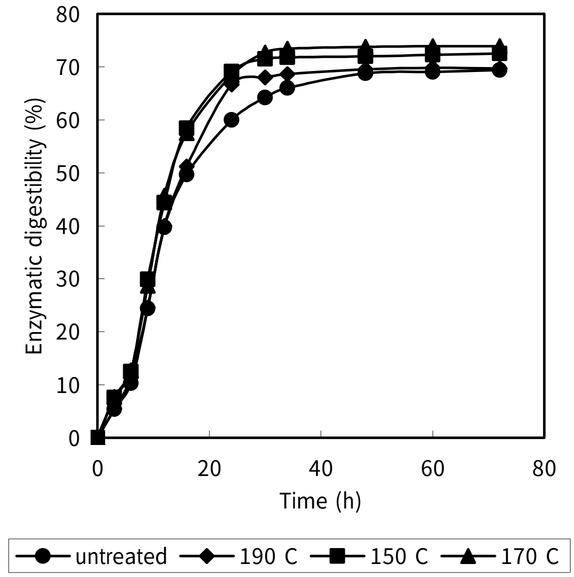


Fig.3. Enzymatic digestibility of pulp mill sludge after ARP treatment.

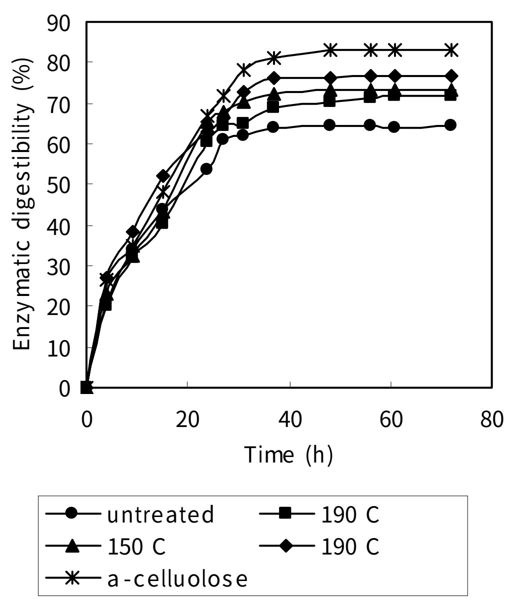


Fig.4. Enzymatic digestibility of pulp mill sludge after ARP-H2O2 treatment.

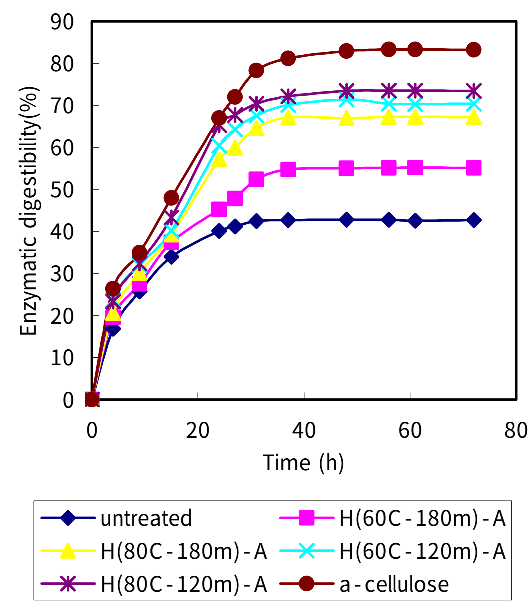


Fig.5. Enzymatic digestibility of newspaper after successive treatment with H2O2 and ARP.