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Development of an integrated pretreatment fractionation process for fermentable sugars and lignin: Application to almond (*Prunus dulcis*) shell

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ABSTRACT

An environmentally friendly pretreatment process was developed to fractionate cellulose, hemicellulose and lignin from almond (*Prunus dulcis*) shells, consisting of hot water pretreatment (HWP) coupled with organic solvent (organosolv) pretreatment of water/ethanol (OWEP). This integrated pretreatment process proved more effective on the basis of yield of fermentable sugar and lignin separation compared with HWP alone, dilute acid pretreatment (DAP), ammonia pretreatment (AP), lime pretreatment LP, organosolv water/ethanol pretreatment (OWEP), and organosolv water/acetone pretreatment (OWAP). In the coupled hot water-organosolv process, hemicellulose sugars were recovered in the first residual liquid while varying amounts of cellulose was retained in the residual solid. The lignin fraction was obtained by simply adjusting the pH from the second liquid. The optimal two-stage process consisted of first HWP stage at 195 °C for 30 min, resulting in $w_{glucose}$ = 95.4% glucose recovery yield and w_{xylose} = 92.2% xylose removal. The second organosolv OWEP stage was operated at 195 °C for 20 min, in ethanol in water mixtures of $<phi>_{ethanol}$ = 50% and resulted in nearly $w_{glucose}$ = 100% glucose recovery yield, w_{xylose} = 90% xylose and w_{lignin} = 61% lignin removal. After enzymatic hydrolysis, glucose yield was up to $w_{glucose}$ = 95%, compared to 61% yield from untreated almond. Images obtained via scanning electron microscopy (SEM) highlighted the differences in almond structure from the varying pretreatment methods during biomass fractionation.

1. Introduction

Lignocellulosic biomass is the world's most abundant feedstock. Conversion of lignocellulosic biomass to bioenergy or bioproducts is viable because of its broad availability and because it converts to fermentable sugars and added-value lignin [1]. Due to the recalcitrant nature of the lignocellulosic plant cell walls, pretreatment is required before typical lignocellulosic sources – grasses, energy crops, or woody biomass – can be converted to biofuels or bioproducts. Any effective pretreatment must provide optimized utilization of feedstock and should enhance enzymatic digestibility toward isolated useful sugars (glucose, xylose, sucrose, and arabinose) and effectively separate lignin.

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Most researchers have focused on studying individual pretreatment methods without considering the synergistic benefit of combining methods. Each pretreatment has its own effect(s) on the cellulose, hemicellulose and lignin; the three main components of lignocellulosic biomass [2]. Steam pretreatment, lime pretreatment, liquid hot water pretreatments and ammonia based pretreatments are concluded to be pretreatments with high potentials.

One could argue that these individual pretreatment methods all have limitations in how they separate the three main components in lignocellulosic materials, i.e. the cellulose, hemicellulose and lignin. Each of these is degraded to glucose, xylose and lignin, respectively. Several other teams have applied integrated methods toward lignocellulosic fractionation, such as the combination of hot water with ammonia pretreatment [3], the combination of steam explosion and alkalic deligninification [4], and steam explosion and organosolv pretreatment [1]. Each of these integrated methods has shown that the glucose, xylose and lignin in the biomass can be separated effectively. Our work has been exploring some integrated approaches with the goal of establishing effective, economical, applicable, and environmentally-friendly pretreatment methods for different specific lignocellulosic feedstocks [5,6].

Almond shells are an under-utilized lignocellulosic agriculture byproduct available in the United States and throughout the world. The FAO stats in 2009 the almond in shell production for the big 3 was 1,162,200 t, 276,100 t, 97,002 t, for the USA, Spain and Syria respectively. California is the only state that produces almonds commercially in USA. Almond shells contain $w_{glucan} = 26.8\%$, $w_{xylan} = 26.1\%$ and $w_{lignin} = 27.4\%$ [7,8]. The current uses of almond shells are limited, with reports of applications for production of xylooligosaccharides, furfural, fertilizers, animal feed, xylitol, and some specific high-end gardening uses, such as “designer” garden mulches and growth substrates.

Almond shells and husks are particularly attractive as bioenergy feedstock since they are collected at the processing plant and have thus attracted some research attention. The research group of Farriol et al. [9] combined autohydrolysis with dilute-acid hydrolysis to enhance their enzymatic digestibility resulting in a maximum yield of 97% of theoretical sugars. They also combined dilute-acid prehydrolysis and alkaline extraction to obtain 84% lignin and 66% pentose recovery. In this study, we report the results obtained using a new fractionation process with the idea of reducing chemical use and costs relative to those reported previously.

2. Materials and methods

2.1. Almond shells

Almond shell samples were from a relatively homogeneous batch of ground almond shells harvested from the Central Valley of California near the Central West Coast of the United States. The date of harvest was on the 25th, August 2007. The almonds were dried by using hot-air at 100 °C before mechanical hulling. The hulling and shelling process were detailed in this case [10]. Shell material was ground through Wiley Mill using a 1 mm sieve and had moisture of 45 g kg^{-1}, noting that all data were reported on a basis of biomass dry matter. The average compositions (with standard deviation) of six samples derived from the batch are listed in Table 1 (in percent w/w of dry lignocellulosic material).

2.2. Analytical procedures

Chemical analysis of the almond shells and the pretreated samples was conducted using the following standard methods from the NREL biomass program: Summative Mass Closure.

Laboratory Analytical Procedure (LAP) Review and Integration: Feedstocks [11]; Determination of Ash in Biomass [12]; Determination of Acid Soluble Lignin Concentration Curve by UV–Vis Spectroscopy [13]; Determination of Structural Carbohydrates and Lignin in Biomass [14]; Standard Test Method for Moisture, Total Solids, and Total Dissolved Solids in Biomass Slurry and Liquid Process Samples [15]; Preparation of Samples for Compositional Analysis [16]; Enzymatic Saccharification of Lignocellulosic Biomass [17].

2.3. Pretreatment conditions

Pretreatment protocols were one of the following, the solids concentration for the treatment was 100 g kg^{-1} in every case:

- hot water pretreatment (HWP) at 210 °C for 10 min,
- dilute acid pretreatment (DAP) in sulfuric acid in water mixtures of $w_{sulfuric\ acid} = 0.8\%$ at 160 °C for 10 min;
- ammonia pretreatment (AP) in ammonia in water mixtures of $w_{ammonia} = 15\%$ at 195 °C for 30 min;
- lime pretreatment (LP) in $Ca(OH)_2$ in water mixtures of $w_{Ca(OH)2} = 0.9\%$ at 130 °C for 60 min;
- organosolv water/ethanol pretreatment (OWEP) in ethanol in water mixtures of $<phi>_{ethanol} = 45\%$ at 180 °C for 30 min.;
- organosolv water/acetone pretreatment (OWAP) in acetone in water mixtures of $<phi>_{acetone} = 45\%$ at 180 °C for 30 min.
- two-stage combinations of these methods, as specifically noted

All pretreatments were carried out in a Parr 2L Series 4520 Pressure Reactor (Parr Instrument Co. Moline IL, USA) operated at up to 1.38 MPa. Reactor temperature curves (see Fig. 1) were controlled by the accompanying Parr 4842 controller, with stirring speed set at about 5.8 Hz.

Table 1 – Average composition of almond shells (results expressed as the dry solid basis).

Fraction	Average (g kg^{-1})	Std dev.
Ash	34	0.07
Hot water extractives (100 °C)	105	0.35
Klason lignin	237	0.53
Glucan	228	0.48
Xylan	329	0.45
Glacan	45	0.04
Others	24	0.05
Mass balance	100.2	0.11

The reactor was charged with 100 g of ground almond shell up to a volume of 1000 L (to give 100 g solids/kg water), and processed via a specific pretreatment protocol listed above. Treated samples were filtered by Whatman 1001-090 and washed three times with 10 mL deionized water every time. The liquid was collected and its pH was adjusted for compositional determination. The residual solid was air-dried for subsequent determination of moisture content, structural sugars, and lignin. Solids were imaged by scanning electron microscopy (SEM) by depositing samples on grids, coating them via gold sputtering for 45 s and then analyzing morphology using a Hitachi Scanning Electron Microscope (Model S4700, Hitachi High-Technologies, Japan) operated at a voltage of 2 kV.

2.4. Enzymatic hydrolysis

The washed water-insoluble solid fraction of pretreated almond shell was enzymatically hydrolyzed by a cellulolytic complex (Celluclast® 1.5L) kindly provided by Novozymes A/S(Denmark). Cellulase enzyme loading was 20FPU/g substrate. To supplement β-glucosi dase activity of Celluclast1.5L, fungal β-glucosidase (Novozym188, NovozymesA/S) at an enzyme loading of 15 international unit (IU)/g substrate was used. Enzymatic hydrolysis was performed in 50 mol m^{-3} sodium citrate buffer (pH4.8) at 50 °C on a rotary shaker at 2.5 Hz for 72 h and at 50 g kg^{-1} of pretreated material in liquid. Samples were taken every 24 h for glucose concentration determination. All enzymatic hydrolysis experiments were performed in duplicate.

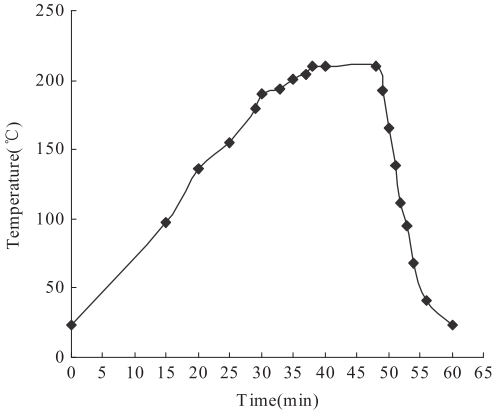

Fig. 1 – Temperature change curve (The time of raising temperature was controlled in 30 min. The cooling time was controlled in 10 min. The time of thermal retardation was in according with the requirement of every experiment.).

2.5. Calculations

The "severity" of the treatment, which is a convolution of temperature and time, was varied and monitored based on defined severity factor [18], R, as follows:

$$R = t * \exp((T - 100)/14.75) \quad (1)$$

where

T is the reaction temperature, t is the residence time, etc

Glucose recovery yield was calculated according to Eq. (2):

$$\text{Glucose recovery yield} = \frac{\text{Glucose in the pretreated almond shell}}{\text{Glucose in the raw almond shell}} \times 100\% \quad (2)$$

Xylose removal yield in the almond shell was calculated according to Eq. (3):

$$\text{Xylose removal yield} = 1 - \frac{\text{Xylose in the pretreated almond shell}}{\text{Xylose in the raw almond shell}} \times 100\% \quad (3)$$

Lignin removal yield in the almond shell was calculated according to Eq. (4):

$$\text{Lignin removal yield} = 1 - \frac{\text{Lignin in the pretreated almond shell}}{\text{Lignin in the raw almond shell}} \times 100\% \quad (4)$$

All values are expressed as the dry solid basis.

3. Results and discussion

3.1. Comparison of different pretreatment methods

Fig. 2 outlines the isolation of the three main components, glucose, xylose, and lignin, using different single-stage pretreatment protocols. First, in all cases some material was lost during processing and characterization. In order to improve precision of these analytical methods, some extractives must be removed prior to analysis for carbohydrates and lignin by 100 °C water. pretreatment. The original sample resulted in recovery of 800 g kg^{-1} of the original substrate after 100 °C hot water extraction for 1 h. All pretreatment methods differed in recovery of glucans and xylans in the almond shell. Glucans recovery was much more higher under DAP-($w_{glucose}$ = ~100%), LP($w_{glucose}$ = ~100%), OWEP($w_{glucose}$ = ~100%) than under HWP($w_{glucose}$ = 87%), OWAP ($w_{glucose}$ = 61%) and AP($w_{glucose}$ = 50%). It showed that OWAP and AP weren't suitable for treating almond shell. Xylose effectively separation with the gulcans was realized by HWP ($w_{glucose}$ = 92.5%) and DAP($w_{glucose}$ = 92.5%), only remaining xylose 75 g kg^{-1} of almond shell. All of xylose almost entered into the liquid

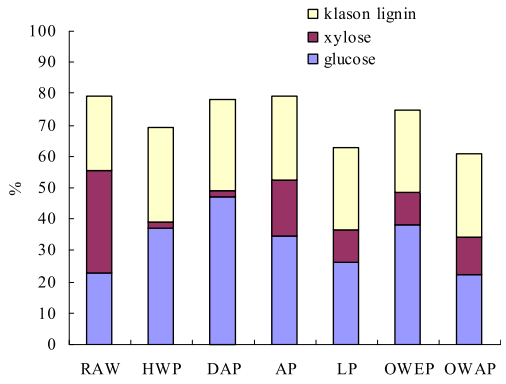

Fig. 2 – The main components change of almond shell after different pretreatment (HWP,AP,LP,OWEP, OWAP conditions see 2.3).

fraction after HWP and DAP; whereas total sugar recovery was the lowest for the organosolv water acetone pretreatment (OWAP).

Fig. 3 presents the data in terms of yield showing that; (1) the HWP and DAP methods were effective in removing xylose and obtaining glucose from the almond shell, but not relatively effective in removing lignin; (2) the ammonia and lime (alkali) methods, as well as the organosolv pretreatments were more effective at removing lignin than HWP or DAP methods; (3) organosolv treatment with water/ethanol resulted in higher glucose yield than water/acetone, with lignin recovery nearly identical for these two methods.

These discussions imply that no single method was ideal for recovery of all three components within the almond shells, thus supporting the hypothesis that combining the best of these pretreatments into a two-stage process will result in a method that, not only recovers xylose and glucose, but also effectively removes the lignin.

From Fig. 4, micrographs obtained by scanning electron microscopy (SEM), it was noted that fractionation resulted in an increase number of pores in the almond shells, with several showing a distribution of big and small dimples. An

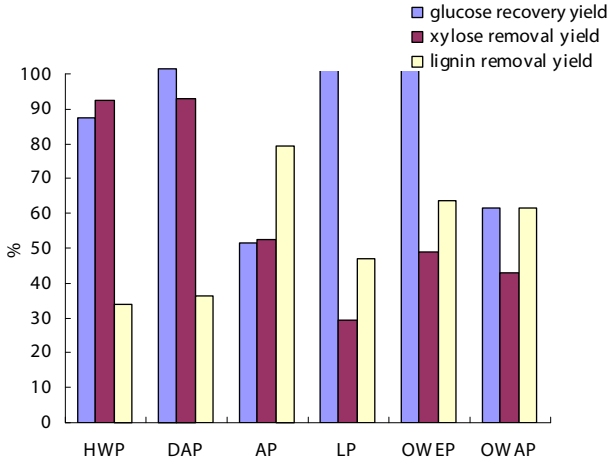

Fig. 3 – Effect of different pretreatment methods on the compositions of almond shell (HWP,AP,LP,OWEP, OWAP conditions see 2.3).

increase in pores would increase surface, which presumably would improve enzymatic hydrolysis.

3.2. *Optimization of two-stage process*

The two-stage process to treat almond-derived lignocellulosic biomass was proposed as outlined in Fig. 5. In the first step, HWP was proposed, considering that hot water was very suitable for xylose and glucans separation and is environmentally friendly, with little cost in chemicals. Organosolv methods were then explored for the second step in order to improve the accessibility of enzyme. The OWEP method was tested first in the pretreatment of the almond shell.

In the proposed two-step scheme, the first liquid after HWP mainly contained C-5 oligosaccharides with xylose the predominant sugar. Recovered xylose from this step would be utilized either in further fermentation and/or for production of value-added products (xylitol perhaps). The liquid after the second step, the OWEP liquor, mainly contained lignin. The lignin derived from organosolv treatments have been reported [19] to be of significantly higher quality than those from acid or base hydrolysis, especially lignin derived from the Kraft process. Thus, the lignin derived from the OWEP liquor could potentially be used further use in fine chemical productions if these markets open, or, more immediately, be used for co-generated energy production to power biorefinery operation. The residual solid from the second step mainly contains cellulose, which, after saccharification, can be fermented to ethanol or other non-food commodities, such as lactic acid.

3.2.1. *The 1st step; HWP optimization*

Hot water pretreatment for the lignocellulose is the effective and environmentally friendly method. The two main variables in heat treatment on correlated in the severity factor, R, as described above in Materials and Methods. The temperature and the time have interaction effects. The two main variables have significant effects on the recycling of xylose. When The temperature is very high or the time is very long, the xylose is easy to be converted to furfural. Variations in lignin, xylose and cellulose from almond shell as a function of R are shown in for HWP Fig. 6. The glucose recovery yield decreased with increasing the temperature, implying that higher temperatures do not improve hydrolysis. Xylose removal yield was highest for $R = 4.54$ (195 °C, 30 min). This correlates to an optimal condition of 195 °C for 30 min, using 100 g kg^{-1} of the solid in water resulting in recovery yields of $w_{glucose} = 95.4\%$ for glucose recovery, $w_{xylose} = 92.25\%$ for xylose and $w_{lignin} = 32.89\%$ for lignin.

3.2.2. *The 2nd step; OWEP optimization*

The organic solvent pretreatment has the ability to remove lignin in the lignocellulose. The lignin obtained from the organic solvent extraction is high equality. But the organic solvent concentration and the temperature have significant effects on the cost of the process and the yield of lignin. Figs. 7, 8 and 9 outline the effect of specific variables on recovery compositions for the 2nd processing step, the OWEP step. Fig. 7 shows the effect of temperature during OWEP on almond shell fractions. Several observations are clear. First, the glucose recovery yield remained at a high level with

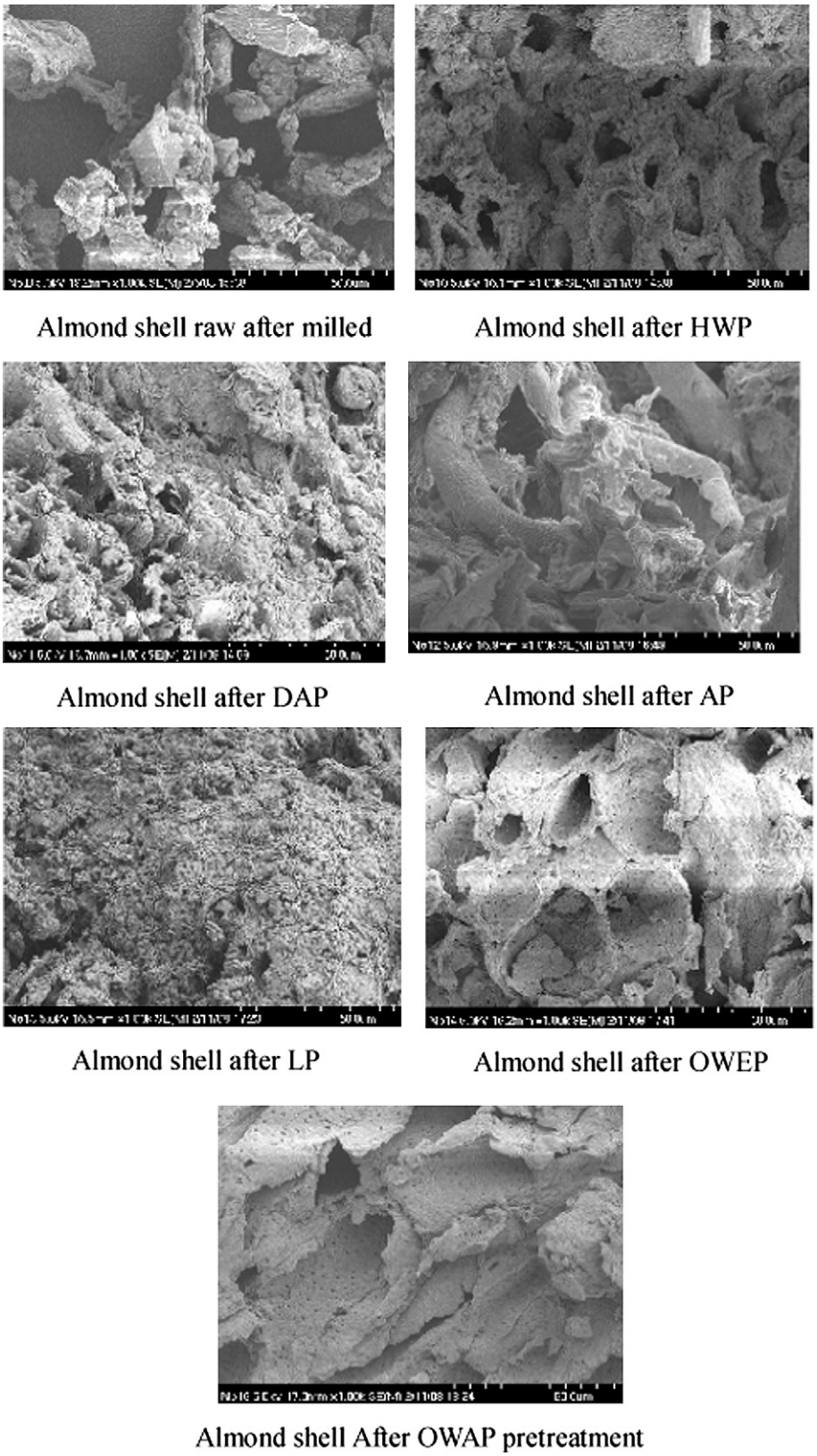

Fig. 4 – SEM images of almond shells after different pretreatment protocols.

temperature increasing from 190 °C to 195 °C. Secondly, xylose removal yield increased with increased temperature, but only up to a temperature of 230 °C, after which the almond shell was carbonized. Finally, lignin recovery was not changed significantly with changes in temperature, with lignin yield remaining at roughly 60% for all temperatures studied.

Fig. 8 shows the effect of time on the composition of almond shell in the second OWEP step. Here it was shown

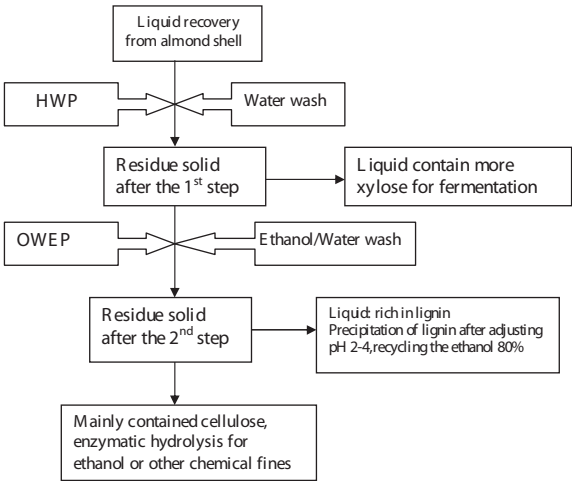

Fig. 5 – Two-stage process for almond shell pretreatment.

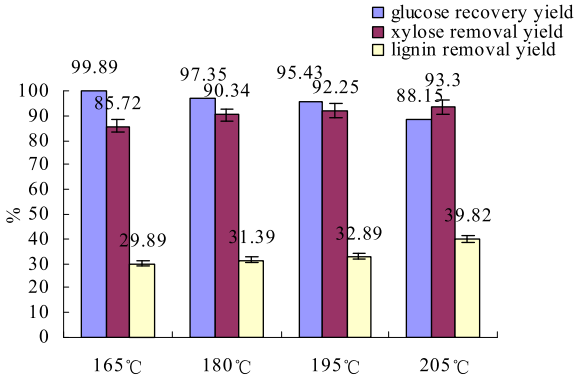

Fig. 6 – Effect of the temperature on the main components of almond shell after HWP(temperature from 165 °C to 205 °C).

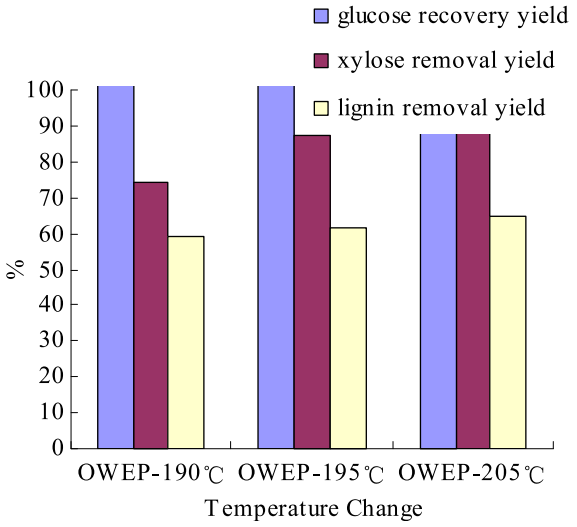

Fig. 7 – Effect of temperature on the compositions of almond shell in the second step OWEP (feedstock from the first-stage pretreatment HWP, ethanol in water mixtures of <phi>ethanol = 45% for 30 min).

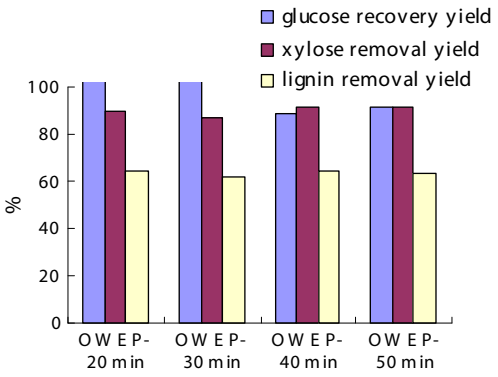

Fig. 8 – Effect of the time on the composition of almond shell in the second step of OWEP (195 °C, ethanol in water mixtures of <phi>ethanol = 45%).

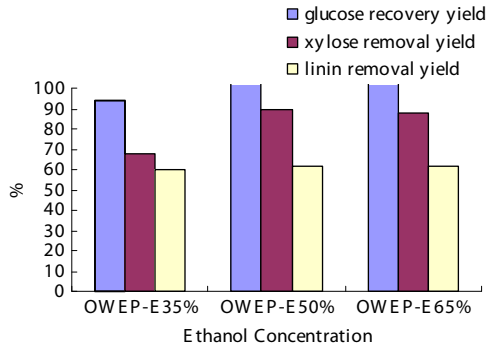

Fig. 9 – Effect of ethanol concentration on the composition of almond shell in the second step OWEP (195 °C, 20 min).

that, at 195 °C, at least (1) lignin and xylose yields did not vary significantly with increased times in the reaction vessel, (2) glucose yields decreased with increasing time after 30 min, with the most suitable time between 20 and 30 min, (3) xylose yield was about 90%, with lignin removal yield constant at about 62%.

The effect of ethanol concentration on the composition of almond shell during the second OWEP step is shown in Fig. 9. From these date, it is seen that (1) the glucose recovery yield increased with increased ethanol concentration, (2) xylose removal yield up to 89% was highest at ethanol concentrations ranging from roughly 50% (v/v), (3) again the lignin yield did not change significantly with ethanol concentration, remaining at about 60%.

4. Conclusions

From these data it can be concluded that a two-stage process can be optimized for fractionation of almond shells into lignin, xylose and cellulose. Whereas the individual pretreatment protocols such, specifically ammonia and dilute acid pretreatment, fractionate up to 84% of original material, a two-stage process consisting of a first-stage HWP (195 °C, 30 min)

and second step OWEP (195 °C, 20min, $<phi>_{ethanol}$ = 50% resulted in $w_{glucose}$ = 95.4% glucose yield and w_{xylose} = 92.2% xylose removal yield and w_{lignin} = 61% lignin yield. Thus, after enzymatic hydrolysis, the yield can reach $w_{glucose}$ = 95%, more than the untreated raw almond shells.

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