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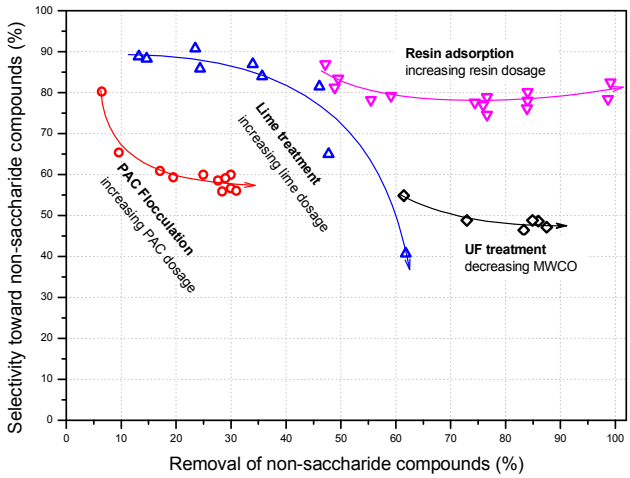


Selectivity comparison for saccharides separation from wood hydrolysate

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ARTICLE TYPE

# Saccharides separation from wood prehydrolysis liquor: comparison of selectivity toward non-saccharide compounds with separate techniques

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Pre-pulping extraction of hemicellulose from wood produces a prehydrolysis liquor (PHL) rich in monosaccharides and oligosaccharides (OS). However, PHL also contains non-saccharide compounds (NSC), mainly lignin-derived byproducts. A promising usage of PHL is to separate OS from NSC as value-added products. In this work, NSC selectivity was defined as the ratio of NSC removal over the sum of NSC and saccharides removal, and applied to evaluate the performance of several separation techniques. Ultrafiltration (UF) of PHL with molecular weight cut-off (MWCO) from 50 kDa to 1 kDa showed no selectivity toward NSC because of the equal retention of saccharides and NSC. Polymer flocculation by using polyaluminium chloride (PAC) was infeasible due to the conflict between NSC selectivity and NSC removal. Lime treatment showed remarkable selectivity up to 90% due to the specific removal of phenolic lignin derivatives. Adsorption by macroporous resin attained nearly complete removal of NSC with 78.9% saccharides recovery, but at the expense of massive resin consumption. The comparison of NSC selectivity suggested the combination of lime treatment and resin adsorption as an economic and practical process for saccharides separation from PHL.

## 1. Introduction

Oligosaccharides (OS) are polymeric carbohydrates that are widely applied in pharmaceuticals, agricultural, agrochemistry and food industry. OS can either be extracted from plants, such as chicory root for fructo-oligosaccharides,[1] or can be synthesized by enzymes with transfructosylation activity.[2, 3] In recent years, lignocellulose has been used as a feedstock for OS production because of the low production costs and wide availability, *e.g.*, xylooligosaccharides (XOS) are usually produced from xylan-rich lignocellulosic materials (hardwood and agricultural byproducts) by autohydrolysis in hot water or steam, and chemical treatments in dilute mineral acid.[4] In the pulp and papermaking industry, prehydrolysis of wood is performed prior to kraft cooking to remove the majority of hemicelluloses for dissolving pulp production. Therefore, prehydrolysis liquor (PHL) is identified as a potential feedstock for OS production and receives great attention due to its contribution to the industrial biorefinery.[5-8] However, PHL also contains various non-saccharide compounds (NSC) that are released during the prehydrolysis step. NSC includes wood resins, water soluble aromatic compounds from lignin depolymerization,[9, 10] organic acids and furans from carbohydrate degradation.[11] Lignin-derived byproducts accounted for nearly 50% of NSC. These contaminants have to be removed effectively and specifically to obtain pure saccharides suitable for food or pharmaceutical applications.

Several techniques for the separation of saccharides from PHL have been presented.[12-18] Flocculation was used to remove lignin derivatives from lignocellulosic hydrolysate by using alum, poly ethylene imine (PEI), poly-diallyl dimethyl ammonium chloride (p-DADMAC), and cationic poly acrylamide (CPAM). However, these cationic-polymers were not quite specific to lignin as indicated by the removal of lignin (38.1%-70.3%) and saccharides (17.1%-36.8%).[19] The study from Duarte et al.[20] showed that p-DADMAC-induced flocculation was selective toward lignin as indicated by saccharides removal (3%) and lignin removal (36%). However, lignin removal was too low for saccharides separation. A similar study from Chen et al.[21] demonstrated that polyaluminium chloride (PAC)-induced precipitation was highly specific to large lignin molecules in wood hydrolysate, but lignin removal of 25.1% was not enough for practical application. Diafiltration with1 kDa MWCO membrane was reported to be effective in removing monosaccharides from the autohydrolysis liquor of *Pinus pinaster*, but lack of selectivity toward lignin.[12] Preparative gel filtration chromatography was applied for OS purification from the autohydrolysis liquor of olive tree prunings by Cara et al.[22] OS yields in the range 80-90% were obtained for fractions with an average degreee of polymerization between 25 and 7. A similar study showed that gel filtration chromatography was an efficient purification method for the recovery of interesting categories of OS from hydrolysates of rice straw.[12] However, information about saccharides purity was not provided in both publications relating to gel filtration chromatography. Montané et al.[23] studied the separation of xylo-oligosaccharides from the autohydrolysis liquor of almond shells by using commercial activated carbons (AC). The selective adsorption of lignin-

derived impurities over saccharides was observed at low concentrations of AC. However, when the dosage of AC was increased for a higher impurities removal, the adsorption of saccharides dramatically increased synchronously. Similar to AC treatment, lime treatment was observed to be very selective toward phenolic lignin derivatives [24] and non-carbohydrate impurities [15] for OS separation from wood PHL with a maximum lignin removal of 48.9%. However, an overdose of lime destroyed the selectivity toward lignin due to significant OS loss as a result of alkaline degradation and binding to calcium ions. Resin adsorption has been employed to purify OS-containing liquors,[25] whereas ion-exchange resins showed remarkable selectivity toward non-carbohydrate compounds.[26] However, a disadvantage of resin adsorption in OS separation is the high cost due to massive resin consumption [13, 27], and the short life caused by osmotic shock and organic fouling.

Previous work done in this field provided basic information of several promising techniques on saccharides separation from wood hydrolysates and PHL. However, information is still lacking on how these existing methods should be operated in the most optimal way in order to reduce saccharides loss during processing. This work is focused on selectivity toward NSC among several existing techniques of saccharides separation, including ultrafiltration, flocculation by polymers, lime treatment, and resin adsorption. For the purposes of this work, lignin selectivity was defined as the ratio of lignin removal over the sum of lignin removal and saccharides removal, and then applied to evaluate the performance of these techniques.

## 2. Experimental

### 2.1 Materials

PHL of poplar wood (*Populus×Euramericana 'Neva'*) was prepared in a laboratory at 170 °C for one hour with wood to water ratio of 1:6 (w/w) in a sealed digester as detailed in a previous study [24]. The collected PHL was filtered through 0.45 μm membrane to remove particulates and suspended particles. The structural materials of poplar wood contain 0.38% arabinan, 0.71% glactan, 40.7% glucan, 16.4% xylan, 3.8% mannan, and 23.5% lignin (20.5% Klason lignin and 3.0% acid soluble lignin) . The components of PHL after filtration are listed in Table 1. Regenerated cellulose membranes with MWCO of 1, 3, 10, 30 kDa were provided by Merck Millipore, Billerica, MA. PAC was purchased from Aspirit Chemical Co., Ltd., Qingdao, P.R. China. Calcium oxide was provided by Tianjin Damao Chemical Reagent Factory. Macroporous adsorption resins (CAD-40, D101, DM301, S-8, and X-5) and strong-base anion exchange resin (D201) were generally provided by Huizhu Resin Co., Ltd., Shanghai, P.R. China. The properties of macroporous adsorption resins are listed in Table 2. D201 is a highly efficient and durable, macroporous anion exchange resin with quaternary ammonium as the functional group on the matrix of styrene cross-linked with divinylbenzene. Dextran with weight average molecular weight ($M_w$) from 0.18 to 36.3 kD (American Polymer Standards Corporation, Mentor, OH) was used as standard samples for the size exclusion chromatography (SEC) analysis.

### 2.2. Remove NSC by various separation techniques

#### 2.2.1 Ultrafiltration

Table 1 Components of PHL from poplar wood (g/L)

| Oligosaccharides | Monosaccharides | NSC |
|---|---|---|
| AOS [a] 1.08 | Arabinose 0.61 | Lignin derivatives 7.12 |
| GalOS [b] 1.44 | Galactose 0.39 | Formic acid 2.23 |
| GluOS [c] 1.68 | Glucose 0.27 | Acetic acid 4.10 |
| XOS [d] 13.32 | Xylose 0.99 | Furfural 1.64 |
| MOS [e] 1.36 | Mannose 0.20 | HMF [f] 0.25 |

[a] arabinooligosaccharides, [b] galactooligosaccharides, [c] glucooligosaccharides, [d] xylooligosaccharides, [e] mannooligosaccharides, [f] 5-hydroxymethyl furfural

Table 2 Properties of macroporous resins employed in present study

| Resins | CAD-40 | D101 | DM301 | S-8 | X-5 |
|---|---|---|---|---|---|
| Structure | PS* | PS | PS | PS | PS |
| Polarity | Moderate | None polar | Moderate | Polar | None polar |
| Micropore area ($m^2$/g) | 450~500 | 500~550 | 330~380 | 100~120 | 500~600 |
| Micropore volume (mL/g) | 0.73~0.77 | 1.18~1.24 | 1.3~1.4 | 0.72~0.82 | 1.20~1.24 |
| Pore diameter (nm) | 7~8 | 9~10 | 13~17 | 28~30 | 29~30 |

* polystyrene

UF of PFL was performed using a stirred ultrafiltration cell (Model 8200, Amicon, MA) at a stirring rate of 100 rpm, ambient temperature, and 0.3 Mpa by compressed nitrogen. Membranes having an area of 40 $cm^2$ and MWCO of 30, 10, 3 and 1 kDa were employed. The starting volume of PHL was 150 mL. In each UF step, permeate stream was collected and filtered through a membrane with smaller MWCO. Samples were prepared for saccharides and NSC analysis by taking a small aliquot of each retentate stream during UF process.

#### 2.2.2 Flocculation

Flocculation experiments were conducted in a beaker with 200 mL PHL using PAC as flocculant. Various dosages of PAC from 0.08 g/L to 7 g/L were investigated to determine optimal conditions. Flocculant was added while string at 300 rpm. After flash mixing for one min. and gentle mixing for 5 min., PHL containing fine floc was processed for sedimentation in a 200 mL graduated glass cylinder without any disturbance for 40 min. Supernatant was then taken and filtered through 0.45 μm membranes for saccharides and NSC analysis.

#### 2.2.3 Lime treatment

Lime milk was added gradually to an Erlenmeyer flask filling 500 mL PHL and mixed by a magnetic stirrer at 150 rpm. Once desired pH was attained, aliquots of PHL, approximately 2 mL, were sampled and then filtered through 0.45 μm membranes for saccharides and NSC analysis. To minimize the alkaline degradation of saccharides, treatments were conducted at ambient temperature and finished within half an hour.

#### 2.2.4 Resin adsorption

Five kinds of macroporous resins with different properties (Table 2) were used as absorbent to remove NSC from PHL. Adsorption experiments were performed in batch mode in a beaker placed on a magnetic stirrer at 200 rpm. Resin dosage varies from 2% to 10% on PHL. Two-step adsorption was attempted to improve adsorption efficiency. The resin dosage was 2% or 10% in the first step. Once equilibrium was achieved, PHL was separated from the saturated resin by filtration and subjected to the next step of adsorption at 2% resin dosage. On each equilibrium state of two-step adsorption, samples were taken for saccharides and NSC analysis.

### 2.3 Analytical methods

A spectrophotometric method was used for the determination of lignin derivatives according to standard method TAPPI UM-250 by using a spectrophotometer (Agilent 8453, Agilent Technologies, CA) at 275 nm with extinction coefficient of 8.69 $L \cdot g^{-1} \cdot cm^{-1}$. Formic acid, acetic acid, furfural and hydroxymethyl furfural (HMF) were analyzed by HPLC system equipped with a Waters C18 symmetry column (4.6×150 mm, 5 μm) and a UV-vis detector at 210 nm (fromic acid and acetic acid) and 275 nm (furfual and HMF) at 30 ºC with 0.1% $H_3PO_4$ (v/v) as eluent at 0.5 mL/min. NSC in PHL also includes inorganic elements, and extractives but at low level of concentration, and were not taken into account in this work. Saccharides in PHL consist of OS and monosaccharides. OS was determined by first hydrolysing oligomers to monomeric saccharides and then analyzing the monosaccharides using high-performance anion-exchange chromatography coupled with pulsed amperometric detector (HPAEC-PAD). Acid hydrolysis was performed according to the NREL standard method [28]. An HPAEC-PAD system (ICS-5000, Thermo Fisher, Sunnyvale, CA) equipped with a CarboPac PA20 analytical column and a guard column was used for MS determination. Gradient was set as 2 mM NaOH isocratic with a step to 200 mM NaOH at 15 min. to regenerate the column at a flow rate of 0.5 mL/min and 25 °C. The concertration of OS was reported on an monosaccharide basis. Reported results are the average of duplicates with an average relative standard deviation of about 2%. Monosaccharides L-arabinose, D-galactose, D-glucose, D-xylose, and D-mannose were used as standards. The molecular weight distribution of the saccharides and NSC in PHL was analyzed by SEC using an HPLC system (Shimadzu LC-20T) equipped with a SB-803 HQ column (8×300 mm, 6μm), a UV detector, and a refraction index (RI) detector with pure water as mobile phase at 0.5 mL/min and 35 °C.

### 2.4. Equation

NSC selectivity was defined as the ratio of NSC removal over the sum of NSC removal and saccharides removal after treatment (Eq. 1). NSC was used to evaluate the performance of separation processes.

$$NSC\ selectivity\ (\%) = \frac{NSC\ removal}{NSC\ removal\ +\ saccharides\ removal} \times 100\% \quad (1)$$

## 3 Results and discussion

### 3.1 Inability of UF in saccharides separation from PHL

Membranes with MWCO from 50 kDa to 1 kDa were used in UF process to divide compounds in PHL into two streams: a retentate and a permeate stream. The saccharides and NSC in each UF retentate stream were determined for calculation of saccharides and NSC retention. The plot of saccharides retention against NSC retention (Figure 1A) suggested the inability of UF in saccharides separation from PHL. First, the maximum saccharides retention of 49% obtained by 1 kDa membrane indicated poor recovery of saccharides for UF process. Secondly, the difference between

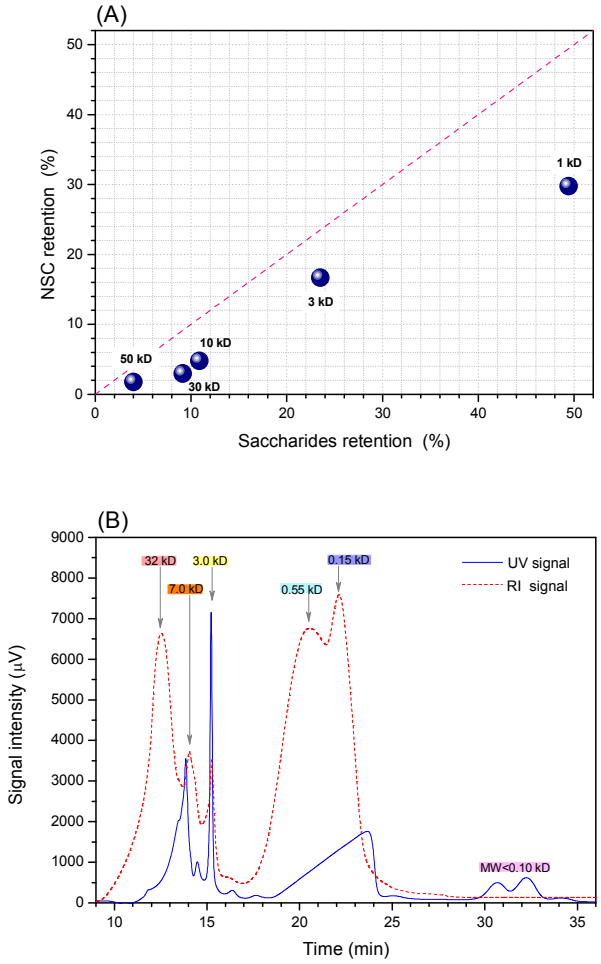


Figure 1 (A) Relation between the retention of saccharides and NSC for each UF retentate stream, (B) Comparison of SEC elution profiles of PHL obtained by the UV (275 nm) and RI detectors.

saccharides retention and NSC retention was not big enough to get an acceptable separation selectivity. According to Eq. (1), NSC selectivity ranged from 50.6% to 58.1%, which confirmed the poor selectivity of UF process. Compared to saccharides, less NSC was retained for each membrane from 50 kDa to 1kDa, which suggested that the average size of NSC was smaller than that of saccharides. For a better understanding of the performance of UF, the molecular weight distribution of saccharides and NSC in PHLwas analyzed by SEC. Fig. 1B gives the elution profiles obtained by the RI and UV detectors, respectively. The UV-detected elution profile reflects the molecular weight distribution of NSC, while the RI-detected elution profile reflects the molecular weight distribution of saccharides. The elution profiles showed the overlapping of molecular weight for saccharides and NSC, especially in the regions around 7.0 kDa, 3 kDa and 0.15 kDa. This information explains the incapability of UF in saccharides and NSC separation. Further, comparison of elution profiles in Fig. 1B suggested the majority of NSC was smaller than saccharides in molecule size. This is consistent with the study of Liu et al. [17]

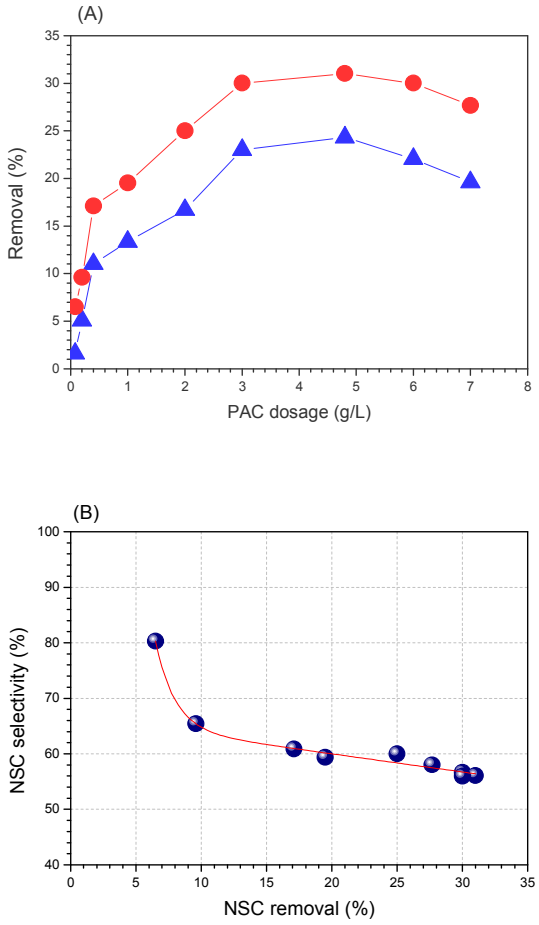


Figure 2 (A) Removal of NSC (-●-) and saccharides (-▲-) obtained at different PAC dosages, (B) relation between NSC removal and NSC selectivity for PAC-induced flocculation of PHL. Flocculation experiments were conducted at the original pH 3.6, and at ambient temperature. The red line only indicates trends.

### 3.2 Conflict between NSC selectivity and NSC removal for PAC-induced flocculation

Flocculation experiments were conducted at pH 3.6, the original pH of PHL. Along with PAC dosage, both NSC removal and saccharides removal showed a gradual increase to maximum value at 4.8 g/L of PAC dosage, then a gradual decrease (Figure 2A). NSC selectivity was determined according to Eq.(1), and then plotted against NSC removal to evaluate the performance of PAC flocculation. It was observed that the increase of NSC removal resulted in gradual drop of NSC selectivity toward 50%, implying the loss of separation selectivity. This also means a conflict between NSC selectivity and NSC removal for PAC flocculation of PHL. Because lignin-derived phenolic compounds are the majority of NSC [24], optimization of flocculation conditions, especially pH, could promote the NSC removal and selectivity. PAC flocculation conducted at an elevated pH 8.9 resulted in a remarkable lignin selectivity of 94% with 25% lignin removal as reported in a previous study [21]. This can be explained by the ionization of phenolic lignin at an alkaline condition and the subsequent binding to PAC polymers.

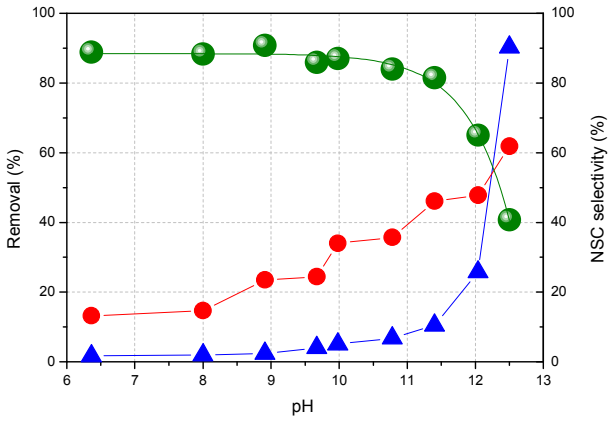


Figure 3 NSC Removal (-●-), saccharides removal (-▲-), and NSC selectivity (-●-) at different pH during lime treatment.

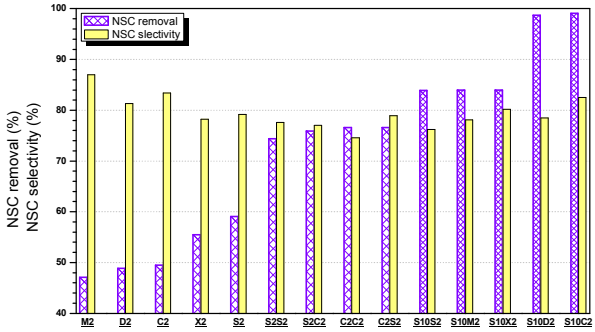


Figure 4 Bar graph of NSC removal and NSC selectivity for adsorption experiments with different resins at various dosages. NSC removal were organized in ascending order for comparison.

### 3.3 Remarkable NSC selectivity obtained by lime treatment at mild alkaline conditions

Lime treatment of PHL was performed by gradual adding of calcium hydroxide milk into PHL at ambient temperature. Saccharides and NSC removal were plotted against pH values (Figure 3). It was observed that lime treatment at mild alkaline conditions (pH < 9) led to remarkable NSC selectivity up to 90% due to the specific NSC removal. However, further adding of lime initiated saccharides removal with a rapid raise at pH 12. This means significant loss of saccharides, and can be interpreted by the ionization of hydroxyl groups in saccharides molecules and the subsequent binding to calcium ions. In addition, alkaline degradation also contributed to saccharides loss at strong alkaline conditions. In order to improve NSC selectivity and minimize saccharides loss, pH of lime treatment should be carefully controlled. In another experiment, lime treatment was conducted at a stable pH of 11 by bubbling carbon dioxide into PHL. NSC removal increased to 50% with NSC selectivity of 83.3% at total lime dosage of 1.35%. The enhancement of NSC removal and selectivity may derive from the adsorption of lignin-derived compounds on calcium carbonate particles.

Table 3 NSC and saccharides removal (%) as well as NSC selectivity (%) for resin adsorption of PHL at various conditions.

| Sample labels * | Resin and dosage | | | | NSC removal | Saccharide removal | NSC selectivity |
|---|---|---|---|---|---|---|---|
| | First step | | Second step | | | | |
| Single-step adsorption | | | | | | | |
| S2 | S-8 | 2% | | | 59.1 | 15.5 | 79.2 |
| X2 | X-5 | 2% | | | 55.5 | 15.5 | 78.2 |
| M2 | DM301 | 2% | | | 47.1 | 7.0 | 87.0 |
| D2 | D101 | 2% | | | 48.9 | 11.3 | 81.3 |
| C2 | CAD-40 | 2% | | | 49.5 | 9.9 | 83.4 |
| Two-step adsorption | | | | | | | |
| C2C2 | CAD-40 | 2% | CAD-40 | 2% | 76.6 | 26.1 | 74.6 |
| C2S2 | CAD-40 | 2% | S-8 | 2% | 76.6 | 20.5 | 78.9 |
| S2S2 | S-8 | 2% | S-8 | 2% | 74.4 | 21.5 | 77.6 |
| S2C2 | S-8 | 2% | CAD-40 | 2% | 75.9 | 22.7 | 77.0 |
| S10C2 | S-8 | 10% | CAD-40 | 2% | 99.1 | 21.1 | 82.5 |
| S10D2 | S-8 | 10% | D101 | 2% | 98.7 | 27.1 | 78.5 |
| S10M2 | S-8 | 10% | DM301 | 2% | 84.0 | 23.6 | 78.1 |
| S10S2 | S-8 | 10% | S-8 | 2% | 83.9 | 26.2 | 76.2 |
| S10X2 | S-8 | 10% | X-5 | 2% | 84.0 | 20.8 | 80.2 |

* The letter for the label represents the type of resin employed, and the following value is the corresponding dosage (w % on PHL)

### 3.4 Highest NSC removal by resin adsorption

Single step adsorption and two step adsorption were conducted in batch mode with various dosages to remove NSC from PHL by using five kinds of macroporous resin as listed in Table 3. To compare the adsorption performance, NSC removal in Figure 4 were organized in ascending order. For single step adsorption, resin with larger pore diameter seems to be more proficient in NSC adsorption, as indicated by the highest and second highest NSC removals obtained by S2 (resin S-8: pore diameter 28~30 nm) and X2 (resin X-5: pore diameter 29~30), respectively. In order to achieve complete removal of NSC, two-step adsorption was performed with total resin dosage of 4% or 12%. Based on the results presented in Table 3, it was observed that double resin dosage does not obtain double NSC removal. For two-step adsorption with total resin dosage of 12%, NSC removal varied from 83.9% to 99.1% depending on resin type employed. Notably, almost all NSC was removed by the cooperation of S-8 and CAD-40 (S10C2) with 82.5% NSC selectivity. However, much attention should be paid to the huge consumption of resin when production cost is concerned.

### 3.5 Performance comparison among separate techniques

To compare the separation performance, NSC selectivity was plotted against NSC removal for all separate techniques employed in this study (Figure 5). As mentioned above, when the retentate stream is used as product, NSC selectivies varied from 50.6% to 58.1%. This information implied the disability of UF in saccharides separation despite the high NSC removal. Actually, same conclusion will be made if the permeate streams of UF are used as products. Compared to PAC flocculation, lime treatment showed much higher NSC selectivity at the same NSC removal. However, pH should be controlled carefully as discussed above. Otherwise, NSC selectivity will decline rapidly as a result of saccharides loss when lime is overdosed. Clearly, resin

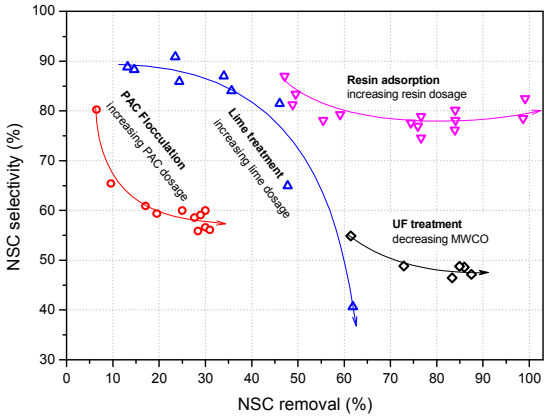


Figure 5 Comparison of NSC selectivity among UF, PAC flocculation, lime treatment, and resin adsorption based on NSC removal. The lines only indicate trends.

adsorption showed the highest NSC removal with acceptable NSC selectivity, but with the disadvantage of massive resin consumption up to 12%. In this context, the combination of lime treatment and resin adsorption is supposed to be effective in resin reduction. In a previously study [24], the combination of lime treatment and resin adsorption (anion exchange resin D201) at just 3% resin dosage resulted in 95.2% lignin removal with 78.8% saccharides recovery yield, corresponding to 81.8% lignin selectivity. This means great reduction of resin consumption, and the feasibility of the combined treatment in saccharides separation from PHL. The resin reduction may be ascribed to the enhanced NSC diffusion into the pores of resin as a result of the removal of large NSC molecules by lime treatment.

## 4. Conclusion

UF, flocculation by PAC, lime treatment, and adsorption by macroporous resin were used to separate NSC from PHL for saccharides production. Particular emphasis was made on the separation selectivity toward NSC. The results showed that UF was non-selective due to equal retention of NSC and saccharides. NSC selectivity of PAC flocculation declined with increasing NSC removal, but could be improved via elevating pH to a mild alkaline condition. Compared to PAC flocculation, lime treatment showed much higher NSC selectivity up to 90% as a result of specific removal of lignin-derived compounds. In addition, lime dosage should be carefully controlled because overdose of lime with pH higher than 11 would result in substantial loss of saccharides. Notably, resin adsorption achieved 99.1% NSC removal with 82.5% NSC selectivity, but at the expense of 12% resin dosage. Therefore, integration of lime treatment and resin adsorption is proposed as a feasible process for saccharides separation from PHL due to the great reduction of resin consumption.

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## Notes

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