IMPACTS OF LIGNIN CONTENTS AND YEAST EXTRACT ADDITION ON THE INTERACTION BETWEEN SPRUCE PULPS AND CRUDE RECOMBINANT *PAENIBACILLUS* ENDOGLUCANASE

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Crude recombinant *Paenibacillus* endoglucanase was employed to investigate its ability to gain access into and to degrade spruce pulps having different lignin and pentosan contents. Since yeast extract is commonly present in the simultaneous saccharification and fermentation processes as a nitrogen source, its effect on the accessibility and degradability of crude endoglucanase was examined. Pulps with more lignin contents adsorbed more overall proteins. More protein impurities other than the recombinant *Paenibacillus* endoglucanase were found to be preferentially adsorbed on the surfaces of pulp with higher lignin contents. The addition of yeast extracts further enhanced the above trends, which might reduce the non-productive binding by pulp lignin. Pulps with more lignin contents were more difficult to be degraded by the crude endoglucanase; the reductions of degree of polymerization (DP) for pulps were more sensitive to the dosage of endoglucanase applied. The presence of yeast extracts increased the DP degradation rate constants, but decreased the release of reducing sugars during hydrolysis for pulp with higher lignin contents.

Keywords: Accessibility; Adsorption; Degree of polymerization; Endoglucanase; Hydrolysis; Lignin

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INTRODUCTION

Utilization of cellulosic materials for biofuel can provide environmental, economic, and strategic benefits on a global perspective (Lynd et al. 2008). Bioconversion of cellulose to soluble sugars and glucose is catalyzed by a group of enzymes referred to as cellulases (Bhat 2000); among them, the endoglucanase randomly hydrolyzes the β-1,4 bonds within the amorphous region of cellulose molecule (Lynd et al. 2002; Zhang et al. 2006). The viability of bioethanol production is highly dependent on the efficient conversion of lignocellulosic biomass to fermentable sugars (Himmel et al. 2007). Among the components of cellulase system, endoglucanase plays the central role in the saccharification process (Zhang et al. 2006; Himmel et al. 2007).

Lignin, the major component of lignocellulosic biomass, forms a physical barrier surrounding cellulose microfibrils (Fengel and Wegener 1983); and its negative impacts on biomass digestibility have been well demonstrated (Chang and Holtzapple, 2000; Yang et al. 2002). Hence, a variety of thermochemical pretreatments have been devel-

oped to increase accessibility of lignocellulosic materials (Kumar and Wyman 2009; Zhu et al. 2009).

A mostly negative correlation between lignin contents of pretreated biomass and the accessibility and degradability of cellulase had been found by using commercial cellulase preparations (Boussaid and Saddler 1999) or cellobiohydrolase (CBH) (Jeoh et al. 2007; Kumar and Wyman 2009). It has also been argued that lignin content lower than 5% (g g^{-1}) results in a dramatic reduction in cellulose digestibility (Ishizawa et al. 2009). Although the adsorption process has been usually combined in models of enzymatic hydrolysis (Andrić et al. 2010), correlation between the accessibility onto pretreated biomass and degradability of various cellulase components still has not been completely clarified (Gerber et al. 1997; Kumar and Wyman 2009). The unit cost of activities for the enzyme could significantly impact the economics of the pretreatment (Sheehan and Himmel 1999); 10-fold of enzyme specific activity could save more than 0.6 $USD per gallon bioethanol. More than 30 times of cost increase per enzyme activity unit by two steps of purification were reported (Ladisch et al. 1977).

Non-productive adsorption between lignin and cellulase is also generally regarded as a major factor to impede the degradability by the cellulases (Boussaid and Saddler 1999; Ishizawa et al. 2009; Várnai et al. 2010). In order to alleviate the non-productive adsorption by lignin, extraneous materials have been brought into the saccharification processes. Additions of Tween 20 (Zheng et al. 2008), bovine serum albumin (BSA) (Yang and Wyman 2006), and various polyethylene glycols (Ouyang et al. 2010) were shown to raise the hydrolysis yields.

In this study, the crude recombinant *Paenibacillus* endoglucanase (Ko et al. 2007; Ko et al. 2010a) were employed to investigate its accessibility and degradability for pulps with different concentration. Kraft pulping, oxygen delignification, and chlorine dioxide bleaching were employed to prepare spruce pulps for emulating pretreated lignocellulosics. Degradability was evaluated by the changes of molecular weight reduction and reducing sugar released.

Yeast extract is commonly present as a nitrogen source for the yeasts in simultaneous saccharification and fermentation processes (Ballesteros et al. 2004). Hence, its effects on the accessibility and degradability of the crude endoglucanase were also examined.

EXPERIMENTAL

Fiber Preparation and Chemical Analysis

The original kraft pulps samples were produced from spruce chips by using an M/K digester (Peabody, MA, USA). The cooking liquor consisted of NaOH and Na_2S, with 25% sulfidity and 20 and 25 % active alkali. Cooking temperature was raised from 25 to 165^{o}C at 1.5 ^{o}C per minute, then maintained isothermally for the next 150 minutes. Oxygen delignification was conducted with 1.2 % (w/w) NaOH per gram oven-dried pulp (g. o. d. p.) under 4 kg/cm^2 (0.4 M Pa) oxygen pressure raised to 99 ^{o}C within 60 minutes, then maintained isothermally for next 15 minutes. Fully bleached pulps were prepared from oxygen-bleached pulps by using a common commercial DEDD bleaching sequence,

as described previously (Ko et al. 2010b). Holocellulose, pentosans, Klason lignin, and acid-soluble lignin of the pulps were analyzed by using TAPPI standard test methods T 203 um-09, T 223 cm-01, T 222 cm-06, and T 250 um-91, respectively. The viscosity of pulp dissolved in a cupriethylene-diamine solution was determined according to TAPPI standard (T 230 om-99). The measurements were conducted in triplicate. These values were then converted into degrees of polymerization (DP) with a regression equation by Sihtola et al. (1963).

Chemical compositions of the pulps are listed in Table 1. Pulp samples were coded with their total lignin contents as follows: LIG 12.4, LIG 8.3, LIG 6.2, and LIG 1.2.

Table 1. Chemical Properties of Four Spruce Pulps

Chemical properties	Sample code			
	LIG 12.4	LIG 8.3	LIG 6.2	LIG 1.2
Pulp lignin content (%)	12.43 ± 0.13	8.29 ± 0.14	6.22 ± 0.12	1.24 ± 0.03
Holocellulose (%)	83.69 ± 0.10	89.50 ± 0.08	93.45 ± 0.05	98.72 ± 0.01
α-cellulose (%)	69.81 ± 0.09	70.54 ± 0.09	78.71 ± 0.02	83.73 ± 0.04
Pentosan (%)	8.83 ± 0.11	8.53 ± 0.13	6.65 ± 0.09	5.98 ± 0.08
DP	3876 ± 85	3226 ± 63	2462 ±37	2135± 26

Enzyme Preparation

Crude recombinant *Paenibacillus* endoglucanase, the proteins in the supernatant obtained after rupturing transformed *E. coli* cell, was prepared as described (Ko et al. 2010a). The CMCase activity of crude recombinant *Paenibacillus* endoglucanase was measured at 35.2 IU/mg at pH 7 and 40 ^{o}C. None of cellobiohydrolase (CBH, Avicelase), β-glucosidase, or xylanase activities were detected. The activities for all the carbohydrate hydrolases were measured by the dinitrosalicylic acid (DNSA) method (König et al. 2002). β-glucosidase activity was defined by the release of 1 mmol of 4-nitrophenol from 4-nitrophenyl-β-D-glucoside (4-NPG) per minute (Hrmova et al. 1996).

Enzyme Accessibility and Digestibility

To determine enzyme accessibility, adsorption on the pulps was performed in 2 mL 0.5 M Tris buffer at pH 7, 4 ^{o}C to avoid hydrolysis. The substrate concentration was 0.1 % (w/v), with the dosages for 10 to 100 mg protein per gram oven dried pulp. The mixtures were loaded into 2.5 mL centrifuge tubes. The loaded tubes were then turned end-over-end on a home-made rotator. Tubes in triplicates were removed over 1 h and then centrifuged at 5,000 rpm. The adsorbed enzymes were determined by the difference between the amounts of initially added protein and free protein in the supernatant assayed by the Bradford method. Yeast extract (Bionovas, Toronto, Canada) was derived from *Saccharomyces cerevisiae* and added at 25 % (total dried weight vs. weight of oven dried pulp). The trace interferences by yeast extract with the Bradford method and DNSA method were corrected for the measurements.

Adsorption parameters (maximum adsorption capacity [σ] and equilibrium constant [K_d]) were determined by non-linear regression of the adsorption data to the Langmuir expression (Kumar and Wyman 2009) as Eq. 1, using SigmaPlot software (ver

10.0, SPSS Inc., Chicago). In equation 1, [*CE*] is the amount of adsorbed protein in mg/mL, [E_f] is the free protein concentration in mg/mL. σ is the maximum adsorption capacity in mg/mg substrate. [S_t] is the substrate concentration in mg protein/mL. [*C*] is the amount of free binding site in mg/mL.

$$[CE] = \frac{\sigma[S_t][E_f]}{K_d + [E_f]} \quad (1)$$

In Eq. 1, K_d is the equilibrium constant = [*C*][*E*]/[*CE*] in mg of protein/mL (Kumar and Wyman 2009).

The digestibility was analyzed by measuring the release of reducing sugars and the change of DP. The dosages of 5 to 50 IU CMCase activity per g. o. d. p. were employed at 10 % (w/v) pulp consistency at pH 7, 40°C. Samples were placed and sealed in a PE bag, and thorough hand kneading was conducted every 20 minutes to ensure sufficient mixing. The released reducing sugars were measured by the dinitrosalicylic acid (DNSA) method. Kinetics of hydrolytic DP reduction was examined by fitting experimental data using the Eq. 2 (Calvini 2005). DP^o was defined as original degree of polymerization, LODP was defined as leveling-off degree of polymerization, and *k* was the rate constant for degradation. The parameters (LODP and k) were determined by non-linear regression of the hydrolysis data to Eq. 2, using SigmaPlot software (ver 10.0, SPSS Inc., Chicago).

$$1/DP - 1/DP^0 = \left(1/LODP - 1/DP^0\right)\left(1 - e^{-kt}\right) \quad (2)$$

RESULTS AND DISCUSSION

Effects on the Adsorption of Proteins

The effect of yeast extract addition and lignin contents on the adsorption isotherms of the crude endoglucanase onto spruce pulps at 4°C is shown in Fig. 1. The results at 4°C demonstrate that the pulp with more lignin contents accommodated more proteins, as shown Fig. 1A.

Although many studies have found that the biomass with more cellulose favors cellulase adsorption using purified cellulases (Chernoglazov et al. 1988), commercial cellulase formulations (Ooshima et al 1990; Boussaid and Saddler, 1999), purified CBH (Ishizawa et al. 2009), different cellulases, and hemicellulases might have different binding affinity to cellulose, hemicellulose, and lignin. The interaction depends on the nature of the enzyme proteins, as well as the nature of the substrate used. Kumar and Wyman (2009) did not find clear correlations between maximum adsorption capacities and affinity constants and compositions of biomass after different pretreatment methods.

CMCase activity of the purified and crude recombinant *Paenibacillus* endoglucanase was measured at 250 IU/mg (Ko et al. 2010a) and 35.2 IU/mg at pH 7 and 40 °C in the present study. So it could be assumed that the masses of protein impurities were 6.1 times more than the purified endoglucanase. Figure 1A describes the impact of

pulp lignin contents on the adsorption behavior of the ensemble of less endoglucanase and much more protein impurities derived from the rupture of cells. The addition of yeast extract facilitated more protein adsorption onto pulps with more lignin contents, as shown in Fig. 1B. Yeast extract, mainly composed of amino acid and peptides, also can be regarded as a mixture of polyelectrolytes.

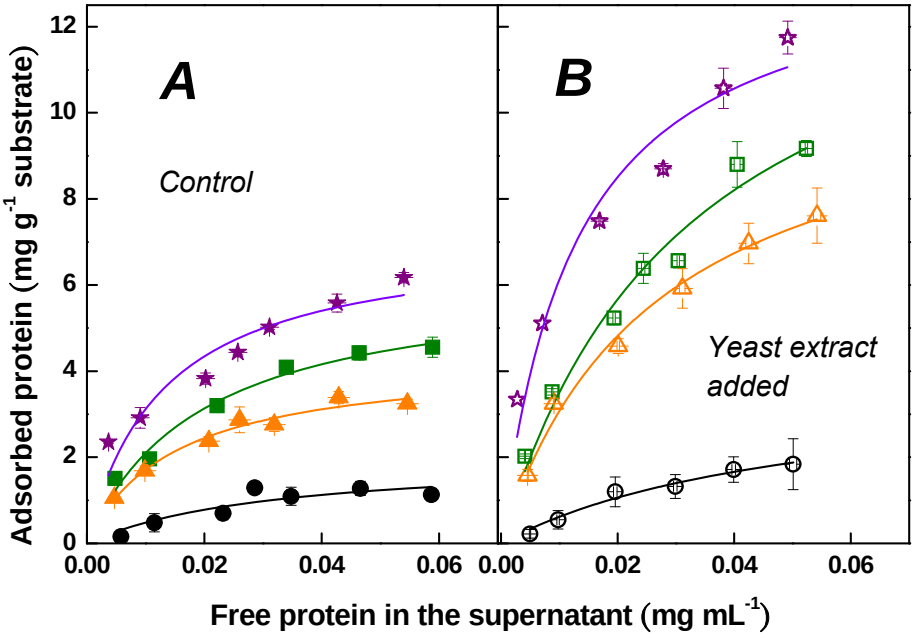

Fig. 1. Effect of yeast extract addition on the adsorption isotherms of the crude recombinant *Paenibacillus* endoglucanase on four spruce pulps at 4°C. Panel A: Controls with closed symbols. Panel B: Yeast extract with open symbols. Legends of pulps with different lignin contents: LIG 12.4 (★), LIG 8.3 (■), LIG 6.2 (▲), LIG 1.2 (●).

The adsorption parameters were also estimated by nonlinear regression of adsorption data for the protein mixtures containing endoglucanase and protein impurities and four spruce pulps using the Langmuir equation. As illustrated in Table 2, the adsorption data of the protein well followed the Langmuir relationship. The results of Table 2 showed that the addition of yeast extract significantly increased the maximum adsorption capacities of the protein onto all four pulps. The maximum adsorption capacities [σ] in Table 3 were much lower than published values of treated biomass by leading treatments (Kumar and Wyman 2009), although in same order of magnitude. The above observation may due to much smaller specific surface areas of longer and more complete softwood kraft fibers used in the study, when compared with much shorter and smaller fibers obtained by leading pretreatments (Kumar and Wyman 2009).

In the present study, the maximum adsorption capacities [σ] increased with pulps of increasing lignin contents. The above trend matched increasing equilibrium constants [K_d] with pulps of decreasing lignin contents. The protein mixtures in the present study contained less endoglucanase and much more protein impurities, and their adsorption

dependence on pulp lignin content was shown to be different from those of purified cellulases (Chernoglazov et al. 1988), commercial cellulase formulations (Ooshima et al. 1990; Boussaid and Saddler 1999; Sipos et al. 2010), and purified CBH (Eriksson et al. 2002; Ishizawa et al. 2009; Sipos et al. 2010). Adsorption parameters demonstrated the dominance by the interaction between protein impurities and pulp lignin in the present study. The discrepancy between parameters of LIG 8.3 and LIG 6.2 may due to two competing interactions for protein impurities and pure endoglucanase with overall pulp surfaces. Addition of yeast extract increased the maximum adsorption capacities [σ] and equilibrium constants [K_d] of all four pulps. The interaction between yeast extract components and pulp surface might create more available binding sites for the proteins, especially for the protein impurities.

Table 2. Effects of Pulp Lignin Contents and Yeast Extract Addition on the Maximum Adsorption Capacity (σ) and Equilibrium Constants (K_d) of the Proteins onto Four Spruce Pulps

Sample code	*σ		**K_d	
	Control	With Y. E.	Control	With Y. E.
LIG 12.4	7.14	13.93	0.0129	0.0128
LIG 8.3	6.19	14.87	0.0195	0.0325
LIG 6.2	4.29	11.20	0.0150	0.0265
LIG 1.2	2.02	3.82	0.0317	0.0519

Effects on the Preferential Adsorption of Protein Impurities

To further verify the different adsorption of endoglucanase and of irrelevant proteins, the specific endoglucanase (CMCase) activities of supernatant portion during adsorption were separately measured at pH 7 and 40°C. Yeast extract hardly interferes with the Bradford assay, or with the DNSA assay. Since the CMCase activity of crude recombinant *Paenibacillus* endoglucanase was measured at 35.2 IU/mg at pH 7 and 40°C, the preferential adsorption of protein impurities on to the pulp could be indicated by the specificity activity, more than 35.2 IU/mg of proteins in the supernatant. The effect of yeast extract addition on specific endoglucanase activity of proteins in supernatants is shown in Fig. 2.

Figure 2A shows specific endoglucanase activity of proteins in supernatants without yeast extract addition. With sample LIG 12.4, the specific endoglucanase activities of proteins in supernatants were higher than 35.2 IU/mg. The above trend was more so at lower total proteins loading. However, an opposite trend was shown for LIG 1.2: the specific endoglucanase activity of proteins in supernatants was slightly lower than 35.2 IU/mg at low protein loading. But interestingly, the specific endoglucanase activities of proteins in supernatants for all pulps more or less converged at around 35.2 IU/mg. It could be deduced that the interaction between cellulose and endoglucanase was dominant for the low lignin pulp (LIG 1.2) at low protein loading. On the other hand, the interaction between pulp surface and protein impurities dominated for the high lignin pulps at low protein loadings. Protein concentrations were quite dilute at low protein

loadings, and the collisions between endoglucanase, proteins impurities onto pulp surface were fewer. There were competitions between endoglucanase and protein impurities being adsorbed onto fiber surface. Figure 2 showed that lignin favored the adsorption by protein impurities, especially at low protein loading. However, the competition between endoglucanase and protein impurities was diminished at higher protein loading, since there were too many collisions between both endoglucanase onto pulp surfaces, leading to successful adsorption.

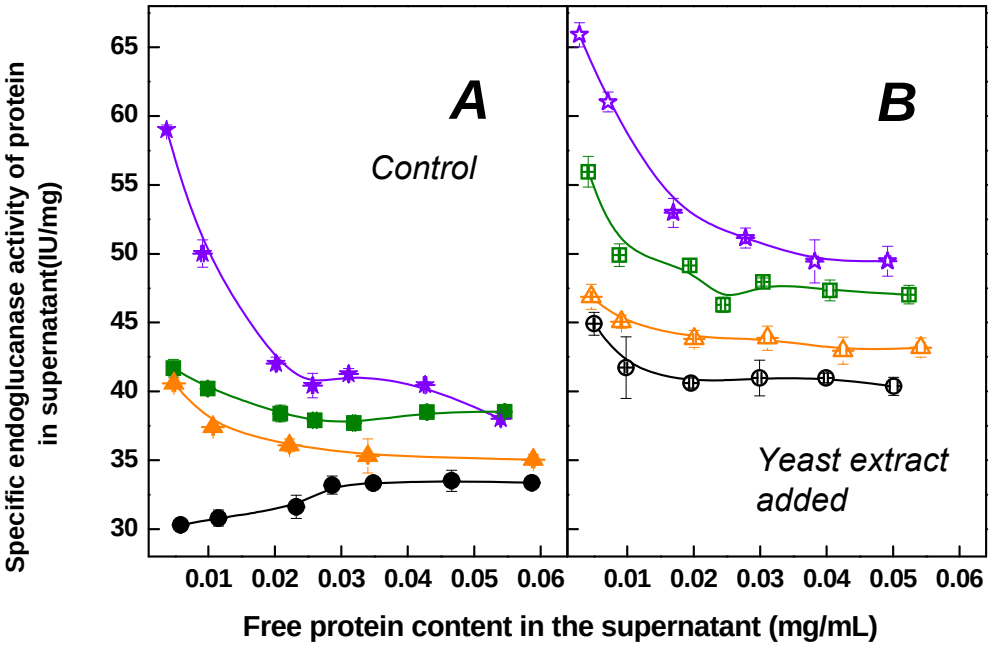

Fig. 2. Effect of yeast extract addition on specific endoglucanase activity of proteins in supernatants. Panel A: Controls with closed symbols. Panel B: Yeast extract with open symbols. Legends of pulps with different lignin contents: LIG 12.4 (Star), LIG 8.3 (Square), LIG 6.2 (Triangle), LIG 1.2 (Circle).

Figure 2B shows the specific endoglucanase activity of proteins in supernatants with yeast extract addition. Additional amino acid and peptide (with much smaller molecular weights) was brought to the system by yeast extract. Since the surfaces of all pulps were further attached by yeast extract to facilitate the increased adsorption for protein impurities, more than 10% of increased specific endoglucanase activities were found for the proteins in the supernatants. Again, it was demonstrated that the specific endoglucanase activities of proteins in supernatants converged at increasing levels in proportion to increasing pulp lignin contents. Since the higher specific endoglucanase activities of proteins in supernatants were exhibited, it could be assumed that more endoglucanase was still un-adsorbed at pulps with higher lignin contents at the presence of yeast extract.

Effects of Pulp Lignin Contents on Hydrolysis

Effects of pulp lignin contents on the reduction of pulp DP by crude recombinant *Paenibacillus* endoglucanase are shown in Fig. 3 for samples LIG 12.4 and LIG 1.2. Table 3 lists degradation rate constants (k) and leveling-off degree of polymerization (LODP) during hydrolysis with Eq. 2. As indicated by Table 1, DP values of LIG 12.4 and LIG 1.2 were 3876 and 2135 prior to hydrolysis. 5 IU and 50 IU p.g.o.p. degraded DP of LIG 12.4 to LODP at 2732 and 2020, which is equivalent to 70.5 % and 52.1 % of the original LODP values. 5 IU and 50 IU p.g.o.d.p. degraded DP of LIG 1.2 to LODP at 1489 and 1297, which is equivalent to 69.7 % and 60.7 % of the original LODP values. A higher lignin content of LIG 12.4 made it more difficult to be degraded by *Paenibacillus* endoglucanase than LIG 1.24. Since there was no CBH and β-glucosidase in this system, LODP values could not be reduced further.

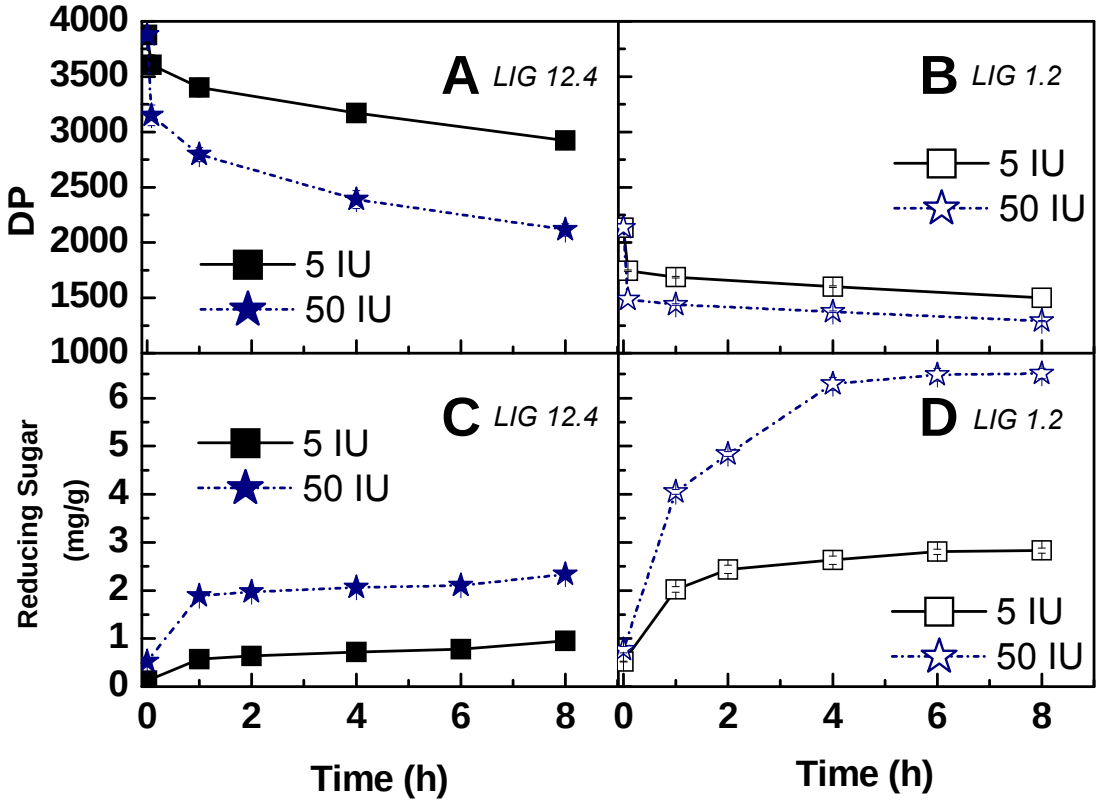

Fig. 3. Effect of pulp lignin contents on the digestibility by crude recombinant *Paenibacillus* endoglucanase at different dosages during hydrolysis at pH 7, 40°C. DP reductions are shown by panel A (LIG 12.4) and panel B (LIG 1.2). Releases of reducing sugars are shown by panel C (LIG 12.4) and panel D (LIG 1.2).

Figure 3C and 3D shows the effect of pulp lignin contents on the release of reducing sugars during hydrolysis. Two different patterns of reducing sugar release are shown by the comparison of Figs. 3C and 3D. The total crude *Paenibacillus* endoglucanase was added at 0.14 mg protein per gram oven dried pulp during hydrolysis. As shown by Fig. 2A, the very low concentration of the loaded proteins caused more endoglucanase presented in solution from LIG 12.4 pulps. But more endoglucanase was adsorbed onto LIG 1.2 pulps in this low range of protein loading.

Table 3. Effects of Pulp Lignin Contents and Dosages of Endoglucanase Applied on Degradation Rate Constants (*k*) and Leveling-Off Degree of Polymerization (LODP) during Hydrolysis of Four Spruce Pulps

Sample code	Enzyme dosage (IU/gram oven dried pulp)							
	5		10		20		50	
	k	LODP	k	LODP	k	LODP	k	LODP
LIG 12.4	0.2132	2732	0.2514	2683	0.2958	2364	0.3250	2020
LIG 8.3	0.3141	1887	0.3287	2148	0.3736	1809	0.3856	1722
LIG 6.2	0.2518	1736	0.2913	1949	0.3179	1550	0.3352	1290
LIG 1.2	0.1815	1229	0.1841	1441	0.1863	1132	0.1940	1037

So it could be assumed that the scenario of Fig. 3C was "minimized un-productive adsorption by lignin, with more un-adsorbed endoglucanase in solution". On the other hand, the scenario of Fig. 3D was "maximized productive endoglucanase adsorption by cellulose, with more irrelevant proteins in solution". The above assumption could be verified by similar DP reduction of LIG 12.4 and of LIG 1.2 pulps. More reducing sugars releases shown in Fig. 3D suggests that the more degraded cellulose fragments were further degraded during hydrolysis of LIG 1.2 pulps.

Effects of pulp lignin contents and dosages of endoglucanase applied on degradation rate constants (*k*) and leveling-off degree of polymerization (LODP) during hydrolysis of four spruce pulps are listed in Table 3. Relations between *k*, LODP values and enzyme dosages were as expected, but *k* values of degradations for LIG 8.3 were the highest for all four dosages of endoglucanase applied. The following four complicating factors in this system contributed to the above observation: endoglucanase, proteins, lignin, and cellulose. The above observation also suggested that a higher degree of delignification might not provide additional benefit for a complicated system, such as simultaneous saccharification and fermentation. From a practical perspective, the purest enzymes and the lowest lignin contents of lignocellulosic materials may be not mandatory for the most cost-effective bio-ethanol conversion process.

Effects of Yeast Extract Addition on Hydrolysis

Effects of yeast extract addition on the reduction of pulp DP and reducing sugar release during hydrolysis are shown in Figs. 4 and 5. As shown by Fig. 4, the trends of pulp DP reduction were slightly altered by yeast extract addition with faster initial decreases and higher final DP.

Although there was more un-adsorbed endoglucanase in solution (as shown by Fig. 2) by yeast extract addition, the reduced non-productive adsorption by pulp lignin was negated by the presence of yeast extract to interfere with endoglucanase action. The excess yeast extract presented might reduce the collision frequencies between endoglucanase and fiber surfaces in the later stages of hydrolysis.

Figure 5 shows that the trends of reducing sugar release were altered by yeast extract addition. The excess yeast extract present in this system might compete with hydrolyzed cellulose fragments to interact with endoglucanase; hence the reducing sugar releases were decreased. The above finding was more evident in the case of high enzyme dosages with low lignin pulp, LIG 1.2. The degradation rate constants (k) and leveling-

off degree of polymerization (LODP) obtained by regression with Eq. 2 of spruce pulps LIG 12.4 and LIG 1.2 are summarized in Table 4.

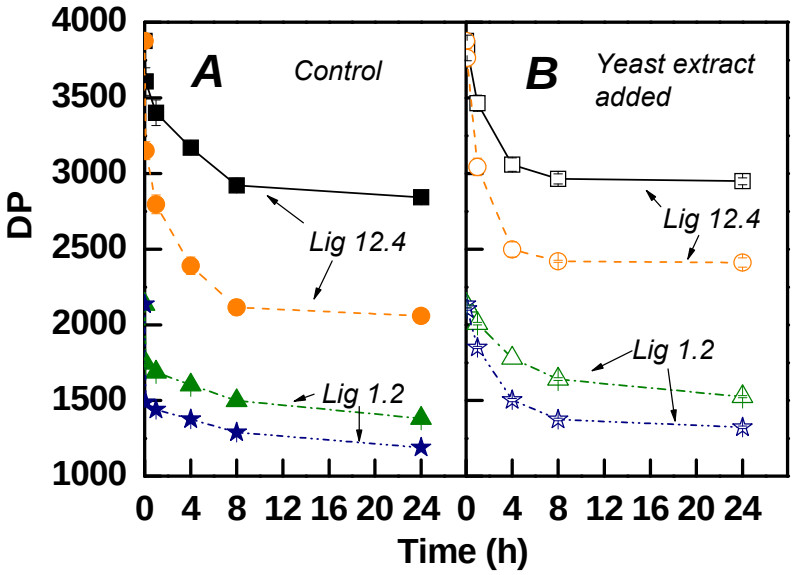

Fig. 4. Effect of yeast extract addition on the reduction of pulp DP (degree of polymerization) during hydrolysis at pH 7, 40°C. Panel A: Controls with closed symbols. Panel B: Yeast extract with open symbols. Legends: LIG 12.4 with 5 IU (Square), LIG 12.4 with 50 IU (Round), LIG 1.2 with 5 IU (Triangle), LIG 1.2 with 50 IU (Star). Protein is applied as IU per gram oven dried pulp.

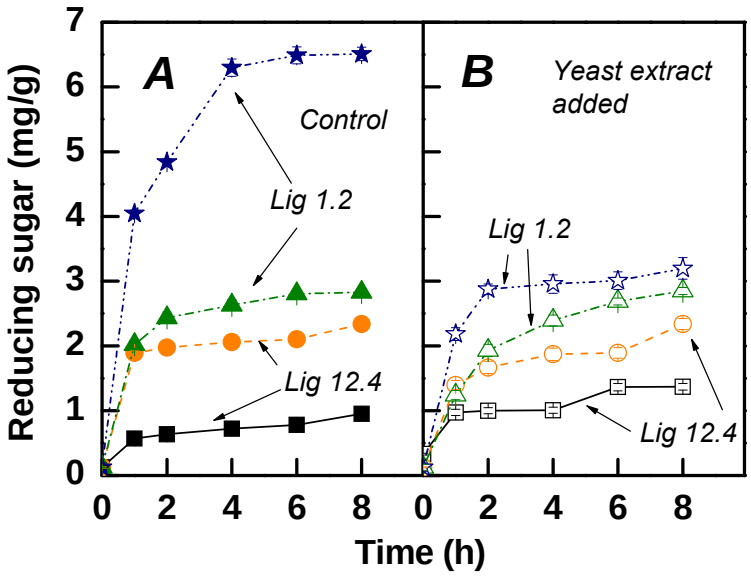

Fig. 5. Effect of yeast extract addition on the release of reducing sugars during hydrolysis at pH 7, 40°C. Panel A: Controls with closed symbols. Panel B: Yeast extract with open symbols. Legends: LIG 12.4 with 5 IU (Square), LIG 12.4 with 50 IU (Round), LIG 1.2 with 5 IU (Triangle), LIG 1.2 with 50 IU (Star). Protein is applied as IU per gram oven dried pulp.

Table 4 shows that the yeast extract addition increased degradation rate constants (*k*) for high lignin and high enzyme dosage cases. Fig. 2 shows that the endoglucanase was preferentially adsorbed while being loaded in the low protein concentration onto the low lignin pulp (LIG 1.2). However, the protein impurities were preferentially adsorbed in the cases for high protein loadings and high lignin pulps. The above observation corresponded well with the parameters listed in Table 4. In Table 4, the degradation rate constant (*k*) of the low enzyme dosage applied on low lignin pulp was not increased by yeast extract addition. The addition of yeast extracts caused slightly higher leveling-off degree of polymerization (LODP) values. Despite the presence of protein impurities and yeast extract, the basic mechanism of endoglucanase action remained. The excess yeast extract presented might reduce the collision frequencies between cellulose fragments and endoglucanase at the later stages of hydrolysis. The yeast extract addition significantly reduced the release of reducing sugars during hydrolysis of low lignin pulps.

Table 4. Effects of Yeast Extract Addition and Dosages of Endoglucanase Applied on Degradation Rate Constants (*k*) and Leveling-Off Degree of Polymerization (LODP) during Hydrolysis of Spruce Pulps LIG 12.4 and LIG 1.2.

Sample code	5 IU per gram oven dried pulp		50 IU per gram oven dried pulp	
	k	LODP	*k*	LODP
LIG 12.4	0.4739	2689	0.5987	2152
LIG 1.2	0.1683	1257	0.2886	1063

CONCLUSIONS

1. Protein impurities preferentially adsorbed on pulp surfaces with higher lignin contents to a more pronounced extent than in the case of endoglucanase adsorption, and the above trend was enhanced by the addition of yeast extracts.
2. Complicating factors impacted the endoglucanase hydrolysis rates of the pulps: the presence of protein impurities, lignin, and yeast extract addition.
3. Addition of yeast extract increased the hydrolysis rates by endoglucanase with the presence of protein impurities for pulps with higher lignin contents and for higher enzyme dosages applied.
4. Addition of yeast extract altered the mode of hydrolysis by the decrease of releasing reducing sugars and slightly different LODP values.

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