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An Evaluation of British Columbian Beetle-Killed Hybrid Spruce for Bioethanol Production

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Abstract

The development of bioconversion technologies for production of fuels, chemicals, and power from renewable resources is currently a high priority for developed nations such as the United States, Canada, and the European Union as a way to improve national energy security and reduce greenhouse gas emissions. The widespread implementation of such technologies will require a sustainable supply of biomass from forestry and agriculture. Forests are a major source of feedstocks for biofuels production in Canada. Woody biomass includes residues from logging and forest thinning, and from wood processing and pulp production.

More recently, damaged wood caused by beetle infestations has become available on a large scale in Western Canada. This study evaluates beetle-killed British Columbian hybrid spruce (HS) (*Picea glauca* × *P. engelmannii*) as a feedstock for the production of bioethanol. In the past 30 yr, attack by the beetle *Dendroctonus rufipennis* and associated fungi has resulted in estimated losses of more than three billion board feet in British Columbia alone. Here we describe the chemical and some physical characteristics of both healthy (HHS) and beetle-killed (BKHS) British Columbian HS and evaluate the technical feasibility of using these feedstocks as a source of biomass for bioethanol production. Untreated HHS and BKHS did not differ significantly in chemical composition except for the moisture content, which was significantly lower in BKHS (approx 10%) compared with HHS (approx 18%). However, the yields of carbohydrates in hydrolyzable and fermentable forms were higher at mild pretreatment conditions (H-Factor <1000) for BKHS compared with HHS. At medium (H-Factor 1000–2000) and severe (H-Factor >2000) pretreatment conditions HHS and BKHS behaved similarly.

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Organosolv pretreated HHS and BKHS demonstrated good ethanol theoretical yields, approx 70 and 80%, respectively.

Index Entries: Cellulase; enzyme; ethanol; hydrolysis; lignocellulose; fermentation.

Introduction

The expansion of the use of biomass as an energy source is seen by developed nations and many developing countries as a way to reduce the need for fossil oil and gas to secure national energy supply. Furthermore, this principle is considered a feasible approach to support sustainable development of rural economies based on agriculture and forestry. It is also recognized that in order to provide a continuous supply of renewable sources of biomass for bioenergy production, bioethanol in particular, biomass feedstocks from both forest and agricultural sources will be required *(1)*. Forest residues, mainly softwood, are particularly abundant in Northern countries such as Canada. This includes residues from logging and forest thinning, and from wood processing and pulp production.

More recently, damaged wood resulting from insects' attacks, such as beetles, and associated fungi have become available as potential substrates for biomass conversion. These outbreaks of infestation are interpreted as a consequence of global warming trends, which make forests more vulnerable to pest infestations. Moreover, proliferation of global trading is likely to introduce further insect pests; for example, the establishment of brown spruce longhorn beetle *Tetropium fuscum* (Fabricius) in Nova Scotia, Canada. Therefore, it is expected that these types of woody residues will continue to be available for several decades. A representative example of an extensively affected softwood species is the British Columbian hybrid spruce (HS) (*Picea glauca* × *P. engelmannii*).

Spruce is an important component of boreal forests and occupies more than 75% of Canada's forested land *(2)*. Microbial pathogens and outbreak species of insects are the major biotic disturbance agents of spruce forests *(3,4)*. In Canada, the annual volume loss because of growth reduction and mortality caused by insects and pathogens averaged 102.8 million cubic metres between 1982 and 1987, which is equivalent to 70% of the volume harvested nationwide *(5)*. Bark beetles (*Coleoptera*: *Curculionidae*: *Scolytinae*) and their associated fungi currently cause the greatest amount of mortality in Canada and the rest of the world's coniferous forests. Total forest losses as a result of bark beetle–fungal interactions are difficult to estimate but are considered to be greater than those caused by all other insects, pathogens, and fire combined, amounting to millions of dollars every year in British Columbia *(6)*. *Dendroctonus rufipennis* (Kirby) and *Ips typographus* (Linnaeus) are examples of highly aggressive spruce-colonizing bark beetles that have destroyed millions of hectares of spruce forests in North America and Eurasia, respectively *(7–10)*. The capacity of aggressive bark

beetles to overcome the resistance of healthy trees is usually mediated by associated fungi.

Most fungi associated with bark beetles are blue stainers: the filamentous ascomycetes generally referred as Ophiostomatoid fungi *(11)*. These fungi are responsible for significant economic losses to wood and forest industries worldwide. Some of them, for example, *Ophiostoma ulmi* (Buisman), are among the most virulent pathogens and many others are the causal agent of wood discoloration *(11–13)*. The main goal of this study was to perform a preliminary evaluation of the feasibility of utilizing beetle-killed British Columbian HS for bioethanol production.

Materials and Methods

Sampling and Sample Preparation

Representative samples of approx 125-yr old HHS and BKHS, respectively, were collected from a single stand located in the University of British Columbia (UBC)/Alex Fraser Research Forest (Williams Lake, BC, Canada) at the biogeoclimatic subzone ICHmk3. After harvesting, logs were debarked, split, chipped, and milled to a chip size of approx $\leq 10 \times 10 \times 3$ mm^3.

Substrate Pretreatment

Healthy HS and BKHS chips were organosolv-pretreated in aqueous ethanol (50%, w/w), with sulfuric acid as catalyst (1.2%, w/w oven-dried wood) at 7 : 1 liquor : wood ratio in a custom-built, four-vessel, rotating digester (Aurora Products, Ltd., Savona, BC, Canada). Two hundred-gram (oven-dry weight) batches of HHS and BKHS chips were cooked in each 2-L vessel under 12 different conditions, chosen (Table 1) depending on cooking extents described by H-factors *(14)* varying from approx 440 to 5800, corresponding to approx 168°C for about 34 min to approx 195°C for about 50 min, respectively.

The experimental design was performed with the help of SAS v. 9.00 software package (SAS Institute, Inc., Cary, NC). The range of conditions was defined based on our previous experience in organosolv pretreatment of softwoods *(15,16)*. After cooking, vessels were cooled to room temperature in a water bath. Solids and liquor were then separated using a nylon mesh. Solids (pulp and rejects) were homogenized and separated from rejects (recalcitrant, poor-pretreated substrate fraction) by using a standard vibrating screen (Voith, Inc., Appleton, WI) fed with tap water. The resultant fibers, so-called "accepts" or "pulps," were subjected to enzymatic hydrolysis and subsequent fermentation. Carbohydrates dissolved in the spent liquor (liquid fraction) or present in form of rejects were considered as losses for the purpose of this study.

The liquid fractions after ethanol organosolv (OS) pretreatment were diluted with four volumes of tap water to precipitate the dissolved lignin.

Table 1
Ethanol Organosolv Pretreatment Conditions of HHS and BKHS Wood Samples

	Set variables			Observed variables			
Pulp sample	*T* (°C)	*t* (min)	H-Factor[a]	*T* (°C)	*t* (min)	H-Factor	P (psi)
HHS1	168	34	516	168 ± 2	34	510	190
HHS2	192	46	4293	192 ± 2	46	3985	310
HHS3	172	40	836	172 ± 2	40	911	210
HHS4	188	40	2945	188 ± 2	40	3456	300
HHS5	180	36	1447	180 ± 2	36	1238	235
HHS6	180	44	1724	180 ± 2	44	1505	240
HHS7	180	40	1585	180 ± 2	40	1880	270
HHS8	180	40	1585	180 ± 2	40	1687	255
HHS9	180	40	1585	180 ± 2	40	1390	260
HHS10	195	50	5978	195 ± 2	50	5816	340
HHS11	175	45	1185	175 ± 2	45	1139	230
HHS12	185	36	2125	185 ± 2	36	2090	260
BKHS1	168	34	516	168 ± 2	34	437	180
BKHS2	192	46	4293	192 ± 2	46	3960	330
BKHS3	172	40	836	172 ± 2	40	738	210
BKHS4	188	40	2945	188 ± 2	40	2645	310
BKHS5	180	36	1447	180 ± 2	36	1265	250
BKHS6	180	44	1724	180 ± 2	44	1510	230
BKHS7	180	40	1585	180 ± 2	40	1406	240
BKHS8	180	40	1585	180 ± 2	40	1515	250
BKHS9	180	40	1585	180 ± 2	40	1390	230
BKHS10	195	50	5978	195 ± 2	50	5190	325
BKHS11	175	45	1185	175 ± 2	45	1043	220
BKHS12	185	36	2125	185 ± 2	36	1909	275

[a]H-Factor values calculated based on Vroom's single variable model *(14)* using the online tool at www.knowpulp.com.

The lignin precipitate, so-called "ethanol organosolv lignin," was collected on a Whatman No. 1 filter and air-dried for 3 d at room temperature.

Characterization of Untreated and Pretreated Substrates

The carbohydrate composition and lignin content of untreated and OS HHS and BKHS were determined using a modified Klason lignin method derived from the Technical Association of the Pulp and Paper Industry (TAPPI) standard method T222 om-88 *(17)*. Acetone extractives were analyzed by Soxhlet extraction according to the standard TAPPI procedure T 280 pm-99 *(17)*. Ash content was determined according to the standard TAPPI method T211 om-02 *(17)*. In addition, high-resolution fiber quality (FQ) analysis (determination of fiber length, width, and percent content of short fibers or so-called "fines") of OS HHS and BKHS was performed as previously described *(18)*.

Batch Separate Enzymatic Hydrolysis and Fermentation

Twenty four OS HHS and BKHS samples (HHS1-12, BKHS1-12) (Table 2) were hydrolyzed using a *Trichoderma reesei* cellulase preparation Celluclast 1.5 L (60.4 filter paper units [FPU]/mL; Novozymes, NC) supplemented with a β-glucosidase preparation (1 : 2 FPU : cellobiase units [CBU] ratio) Novozym 188 (269 CBU/mL; Novozymes, NC) at 2% (w/w) washed substrate consistency, 40 FPU/g glucan, 72 h, pH 5.0, 50°C, 150 rpm. Reactions were run in foam-plugged 125-mL Erlenmeyer flasks in total reaction volume 60 mL. Samples were taken at 3, 6, 12, 24, 48, and 72 h and analyzed by high-pressure liquid chromatography for glucose content as it is described elsewhere *(15)*. The sugar hydrolyzates resulting from the enzymatic hydrolysis were used for the subsequent fermentation.

Fermentation of enzymatic hydrolyzates was performed with the *Saccharomyces cerevisiae* strain Y-1528 (Agricultural Research Service, US Department of Agriculture, Peoria, IL). Fermentations were performed in foam-plugged 125-mL Erlenmeyer flasks containing approx 45 g of sugar hydrolyzates adjusted with 10% NaOH (w/v) to a starting pH of 5.50 in an orbital shaker for 48 h at 36°C and 150 rpm. The hydrolyzates were inoculated to achieve an initial yeast cell concentration of 6.0 g per DCWL-1 (dry cells weight per liter). Samples were taken at 0, 0.25, 2, 4, 6, 8, 12, 18, 24, 30, 36, and 48 h for ethanol analysis.

Results and Discussion

Characteristics of Untreated and Treated HHS and BKHS

Untreated HHS and BKHS showed no significant differences in carbohydrate composition content (Table 2). However, BKHS moisture content was significantly lower (approx 10%) compared with HHS (approx 18%) leading to formation of a larger amount of small chips during chipping in the case of BKHS. Pretreated HHS and BKHS samples showed higher glucan content, relative to untreated samples (approx 53–84% and approx 62–90%, respectively) and lower xylan content (approx 0.2–4.5% and approx 0.1–3.4%, respectively). Almost all arabinan and galactan was solubilized or degraded. Mannan in pretreated samples was also significantly lower compared with untreated HHS and BKHS (0.1–4.8%). Lignin content in HHS and BKHS was 22–34% and 23–30%, respectively. Ash content in pulped samples was lower or similar to the untreated wood (0–0.3%).

Detailed results of OS BKHS and HHS chemical composition are contained in Table 2. FQ analysis showed that OS BKHS fibers had significantly lower FQ than OS HHS (lower average fiber length and width, and a larger amount of fines). This indicates that BKHS would be a poor source of high quality fiber for papermaking (Fig. 1).

Table 2
Chemical Composition of Untreated and Treated (Pulp) HHS and BKHS (dry weight [%])[a]

Sample	Arabinan (%)	Galactan (%)	Glucan (%)	Xylan (%)	Mannan (%)	AIL (%)	ASL (%)	Ash (%)	Extractives (%)
HHS	1.11	1.77	48.22	5.53	12.64	28.21	0.20	0.36	2.59
HHS1	0.16	0.23	52.79	4.51	4.78	34.29	0.22	0.32	n/a
HHS2	0.06	0.05	83.95	0.25	0.22	22.60	0.46	0.15	n/a
HHS3	0.15	0.06	72.15	2.20	1.41	25.55	0.41	0.20	n/a
HHS4	0.06	0.03	82.38	0.31	0.23	23.90	0.61	0.33	n/a
HHS5	0.01	0.00	82.23	1.36	0.87	22.23	0.48	0.14	n/a
HHS6	0.06	0.05	83.64	1.19	0.91	21.85	0.52	0.11	n/a
HHS7	0.08	0.03	78.77	0.96	0.69	25.46	0.52	0.21	n/a
HHS8	0.00	0.00	81.56	1.18	0.77	21.99	0.44	0.19	n/a
HHS9	0.00	0.00	81.73	0.78	0.62	24.47	0.62	0.00	n/a
HHS10	0.00	0.00	77.42	0.20	0.10	31.85	0.31	0.22	n/a
HHS11	0.00	0.00	78.18	1.22	0.84	28.20	0.38	0.13	n/a
HHS12	0.14	0.04	76.57	1.15	1.21	24.08	0.19	0.07	n/a
BKHS	1.23	2.50	48.81	7.03	11.89	28.50	0.20	0.36	2.72
BKHS1	0.10	0.04	62.16	3.38	2.19	26.18	0.22	0.13	n/a
BKHS2	0.06	0.05	78.08	0.14	0.16	26.83	0.65	0.06	n/a
BKHS3	0.10	0.04	69.10	2.85	1.57	27.01	0.25	0.11	n/a
BKHS4	0.07	0.04	82.84	0.32	0.29	26.64	0.55	0.12	n/a
BKHS5	0.06	0.06	90.08	1.14	0.82	24.09	0.32	0.08	n/a
BKHS6	0.00	0 00	78.86	0.96	0.89	23.25	0.33	0.06	n/a
BKHS7	0.07	0.06	81.66	1.07	0.78	23.40	0.31	0.13	n/a
BKHS8	0.06	0.06	77.36	0.48	0.41	28.28	0.41	0.14	n/a
BKHS9	0.07	0.06	80.98	0.79	0.63	25.51	0.33	0.11	n/a
BKHS10	0.06	0.04	76.29	0.12	0.13	29.49	0.67	0.10	n/a
BKHS11	0.06	0.06	78.57	1.59	1.17	25.74	0.28	0.11	n/a
BKHS12	0.10	0.04	68.15	2.14	0.93	26.00	0.43	0.06	n/a

AIL, acid-insoluble lignin; ASL, acid-soluble lignin; n/a, not analyzed.
[a]Carbohydrate, lignin, and ash contents untreated HHS and BKHS are based on extractives-free samples.

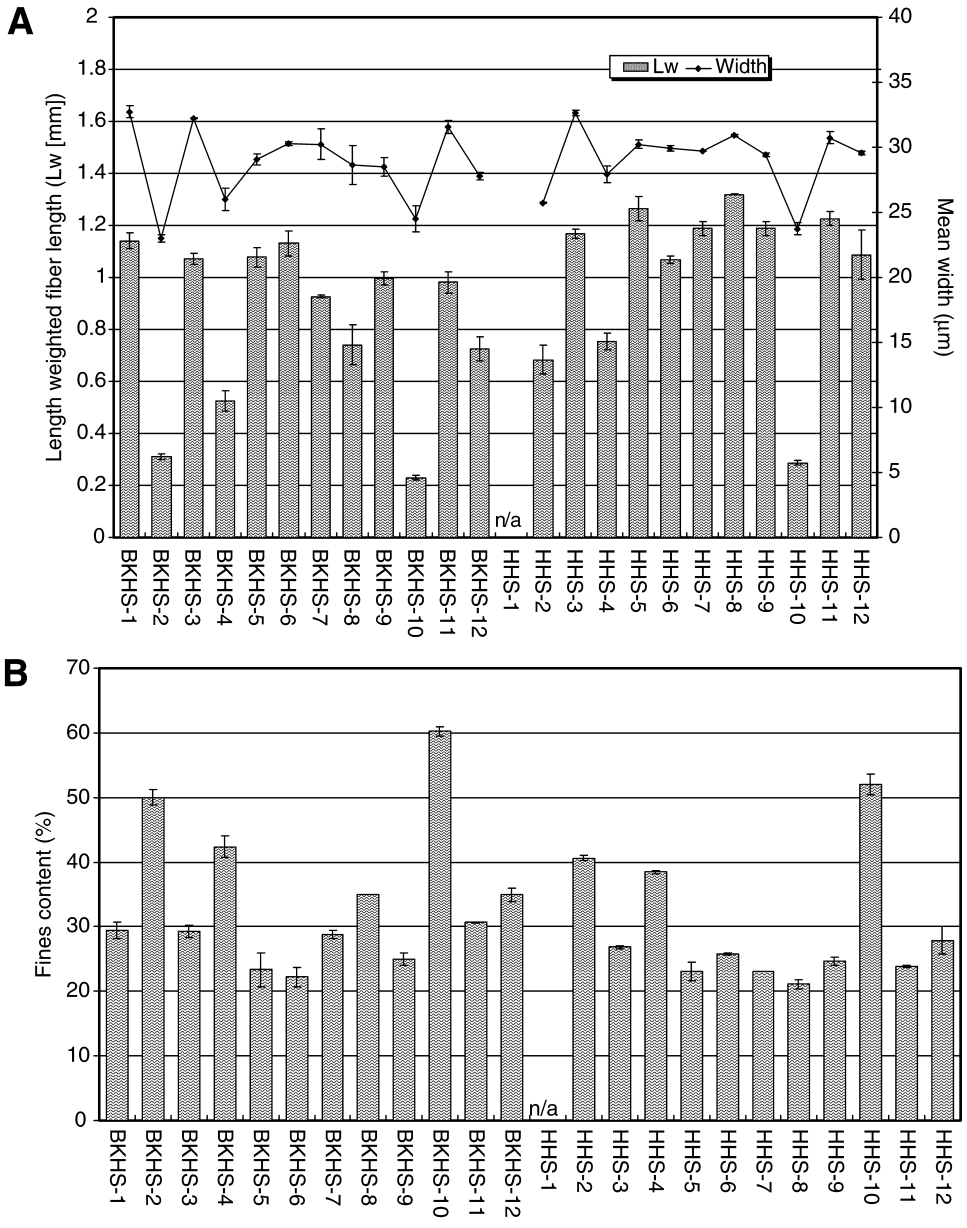

Fig. 1. Fiber quality analysis of OS HHS and OS BKHS fibers. **(A)** fiber length and width **(B)** % fines content.

The mass balance of OS HHS1-12 and BKHS1-12 expressed as yields of each component (g) per 100 g (oven-dried weight) untreated HHS and BKHS chips is shown in Table 3. The yields of pretreated carbohydrates (carbohydrates in pulps and liquid fractions) for OS HHS was significantly lower than those for OS BKHS when wood samples were prepared under mild pretreatment conditions (H-Factor <1000) (Table 3). HHS1

Table 3
Mass Balances for Organosolv Pretreatment of HHS and BKHS at a Range of Severities (H-factors)[a]

Sample	H-factor	Solids (pulp)								Water-soluble fraction						
		Ara	Gal	Glu	Xyl	Man	AIL	ASL	Ash	Ara	Gal	Glu	Xyl	Man	Rejects	OS lignin
HHS1	510	0.01	0.01	1.71	0.15	0.15	1.01	0.01	0.01	0.32	0.39	0.54	0.58	2.28	68.99	3.41
HHS2	3985	0.03	0.02	41.05	0.12	0.11	10.05	0.20	0.07	0.25	0.38	2.78	1.01	2.69	0.09	10.92
HHS3	911	0.06	0.02	29.04	0.90	0.57	9.36	0.15	0.07	0.24	0.36	1.03	1.06	2.67	16.87	5.94
HHS4	3456	0.03	0.01	40.66	0.16	0.11	10.73	0.27	0.15	0.01	0.02	0.54	0.06	0.21	0.00	10.24
HHS5	1238	0.01	0.00	44.51	0.75	0.47	10.95	0.24	0.07	0.57	0.86	2.89	2.46	6.11	4.48	8.20
HHS6	1505	0.04	0.03	48.10	0.70	0.52	11.43	0.27	0.06	0.56	0.90	2.92	2.71	6.71	0.88	8.90
HHS7	1880	0.04	0.02	42.83	0.53	0.38	12.59	0.26	0.10	0.05	0.09	1.62	0.43	0.82	1.13	8.20
HHS8	1687	0.00	0.00	43.64	0.64	0.41	10.71	0.21	0.09	0.06	0.13	1.96	0.50	1.01	2.53	9.00
HHS9	1390	0.00	0.00	46.47	0.45	0.35	12.66	0.32	0.00	0.53	0.90	3.50	2.85	6.98	0.63	9.71
HHS10	5816	0.00	0.00	35.14	0.09	0.05	13.15	0.13	0.09	0.01	0.02	2.29	0.03	0.25	0.00	10.79
HHS11	1139	0.00	0.00	40.17	0.64	0.43	13.18	0.18	0.06	0.26	0.57	3.27	1.36	3.60	6.39	8.36
HHS12	2090	0.07	0.02	40.15	0.61	0.63	11.49	0.09	0.03	0.12	0.34	3.44	0.73	1.91	0.15	10.07
BKHS1	437	0.03	0.01	17.23	0.95	0.61	6.60	0.06	0.03	0.65	1.40	1.75	2.96	6.05	47.03	8.10
BKHS2	3960	0.03	0.02	35.14	0.06	0.07	10.99	0.27	0.02	0.19	0.64	5.64	0.90	2.39	0.00	11.11
BKHS3	738	0.06	0.02	37.95	1.60	0.86	13.50	0.12	0.05	0.58	1.26	2.00	3.09	5.93	8.02	9.27
BKHS4	2645	0.04	0.02	42.27	0.17	0.15	12.37	0.26	0.06	0.39	0.90	4.41	2.05	4.18	0.03	10.44
BKHS5	1265	0.03	0.03	47.82	0.62	0.44	11.64	0.15	0.04	0.54	1.12	2.82	2.97	5.41	1.46	10.26
BKHS6	1510	0.00	0.00	43.65	0.54	0.49	11.71	0.17	0.03	0.54	1.15	2.94	2.95	5.18	1.19	8.92
BKHS7	1406	0.04	0.03	45.38	0.61	0.43	11.83	0.16	0.07	0.55	1.21	2.94	3.17	5.74	0.54	10.06
BKHS8	1515	0.03	0.03	38.78	0.25	0.21	12.90	0.19	0.06	0.39	0.96	3.25	2.02	3.86	0.20	9.01
BKHS9	1390	0.04	0.03	40.03	0.40	0.31	11.47	0.15	0.05	0.51	1.14	3.21	3.01	5.53	0.25	10.11
BKHS10	5190	0.03	0.02	33.05	0.05	0.06	11.63	0.26	0.04	0.13	0.45	4.48	0.62	1.62	0.00	9.65
BKHS11	1043	0.03	0.03	41.53	0.86	0.62	12.38	0.13	0.05	0.57	1.35	2.75	3.42	6.23	4.80	8.39
BKHS12	1909	0.06	0.02	37.73	1.21	0.51	13.10	0.22	0.03	0.47	1.15	4.02	2.78	5.04	0.10	7.94

All data are yields of each component (g) per 100 g (oven-dried weight) untreated HHS and BKHS chips (*see* untreated HHS and BKHS composition in Table 2). The content of carbohydrate degradation products and low-molecular lignin in liquid fractions was not evaluated.

[a]Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; Man, mannose.

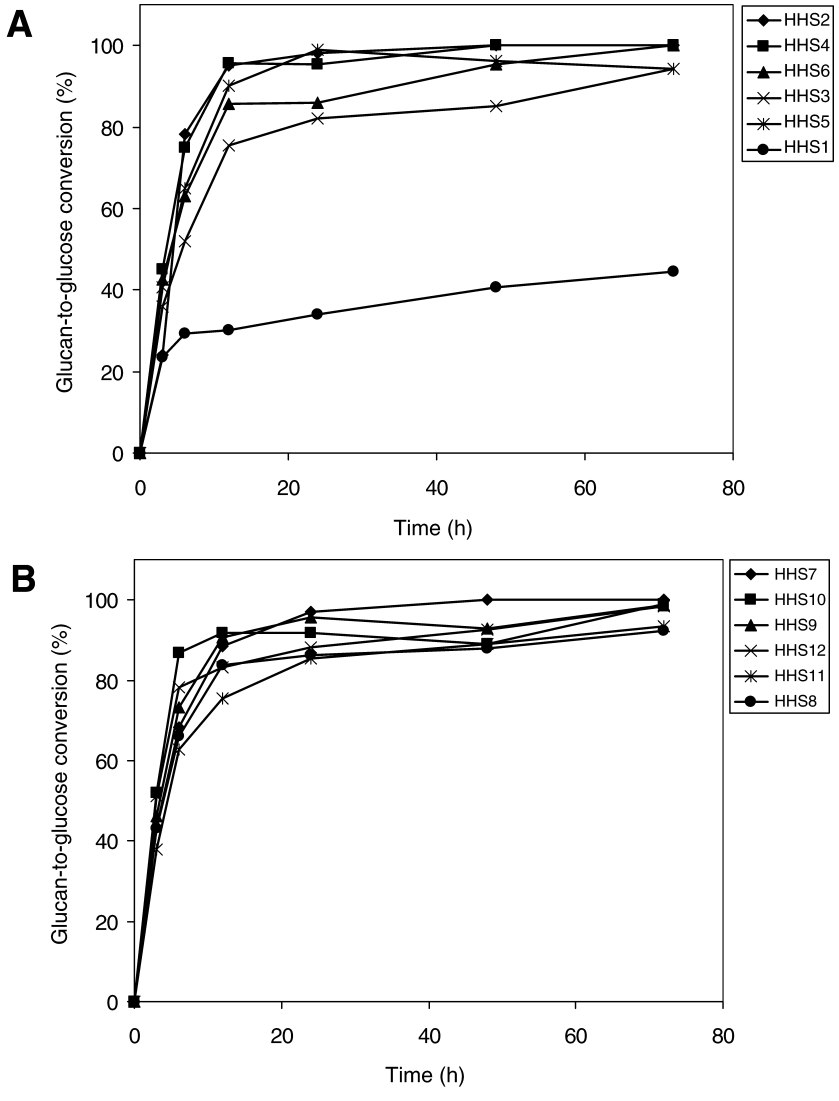

Fig. 2. *(Continued)*

(H-Factor –510) and HHS3 (H-Factor –911) yielded approx 6 and 36% carbohydrates, respectively, whereas BKHS1 (H-Factor –437) and BKHS3 (H-Factor –738) yielded approx 32 and 53% carbohydrates, respectively. Furthermore, as it is shown in Table 3 HHS pretreated under mild pretreatment conditions yielded higher rejects content (approx 69 and 17% for HHS1 and HHS3, respectively) compared with BKHS (approx 47 and 8% for BKHS 1 and BKHS3, respectively). These results indicate that BKHS was easier to pretreat than HHS under mild pretreatment conditions. However, under mild or severe pretreatment conditions (H-Factor approx 1000–5000) HHS and BKHS, in general, behaved similarly (Table 3).

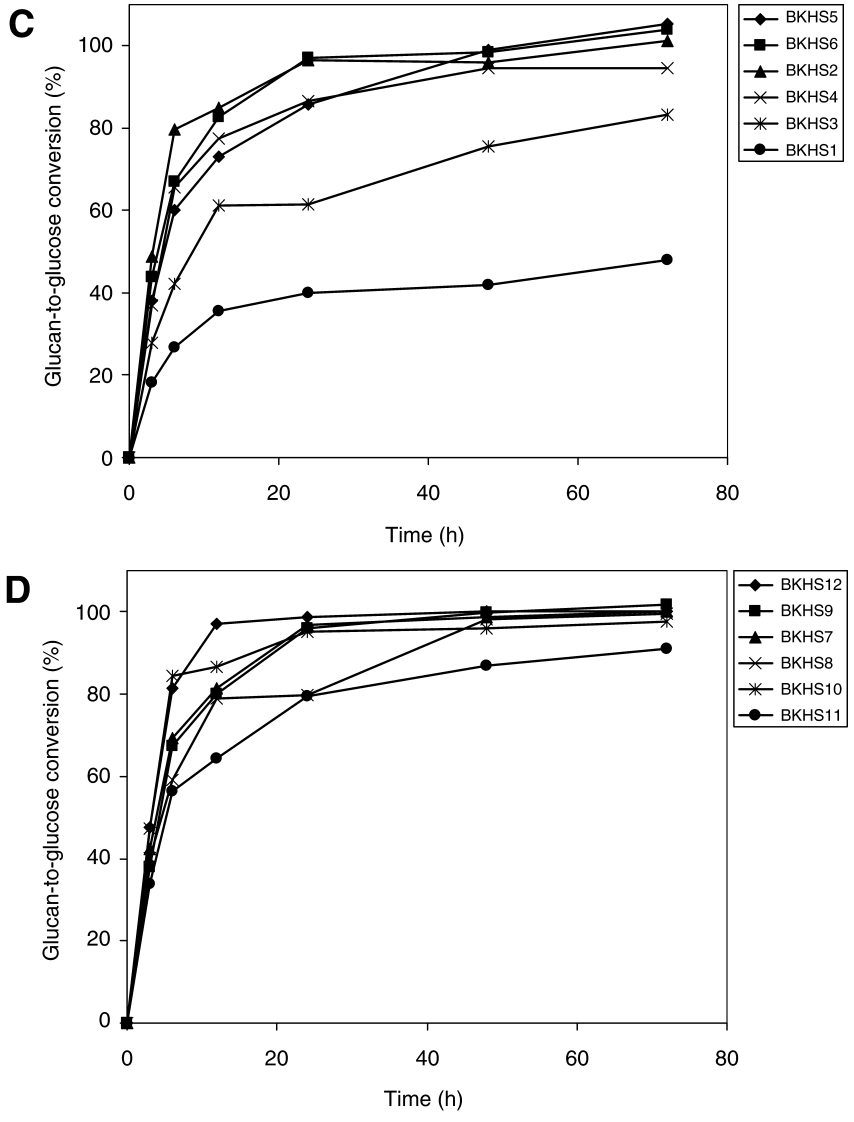

Fig. 2. Enzymatic hydrolysis of OS HHS **(A,B)** and OS BKHS **(C,D)**.

Enzymatic Hydrolysis and Fermentation

OS HHS and BKHS showed similar enzymatic hydrolyzability. HHS2, HHS4, HHS5, HHS7, HHS10 and BKHS2, BKHS6-7, BKHS9-10 pulp samples reached more than 90% conversion in 12–24 h; HHS3, HHS6, HHS8-9, HHS11-12 and BKHS3-5, BKHS8, BKHS11–12 yielded more than 90% conversion in 48–72 h. HHS1 and BKHS1 showed significantly lower glucan-to-glucose conversion compared with the other OS-pretreated samples (45 and 48%, respectively). Results of OS HHS and OS BKHS enzymatic hydrolysis are shown in Fig. 2A–D. Fermentation of OS HHS and BKHS

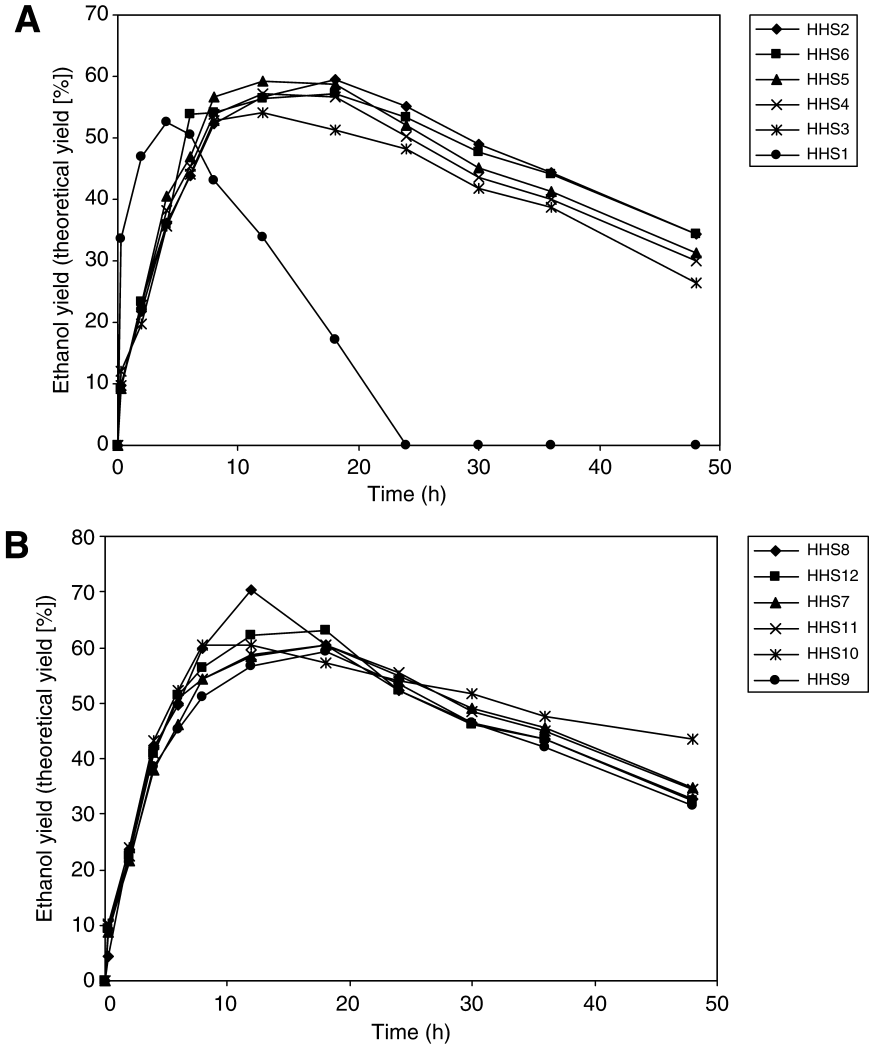

Fig. 3. *(Continued)*

hydrolyzates yielded 53–80% theoretical ethanol yield of carbohydrates in pulps in 4–24 h (Fig. 3A–D, Table 4). The best fermentation was observed for hydrolyzates HHS8 (70%, 12 h) and BKHS12 (80%, 24 h). The lowest ethanol yield was shown for HHS1 (53%, 4 h), HHS3 (54%, 12 h), and BKHS5 (53%, 12 h), apparently because of poor substrate hydrolyzability. Relatively low or moderate fermentability was observed for the other substrates (56–64%).

Significant catabolic oxidation of ethanol for all substrates was observed after reaching maximal ethanol yields. After 48 h fermentation, ≥40% reduction in maximum ethanol yield was observed (Fig. 3A–D). This effect can be attributed to the so-called "diauxic shift." characteristic in ethanologenic yeasts fermenting at low sugar concentrations *(19)*.

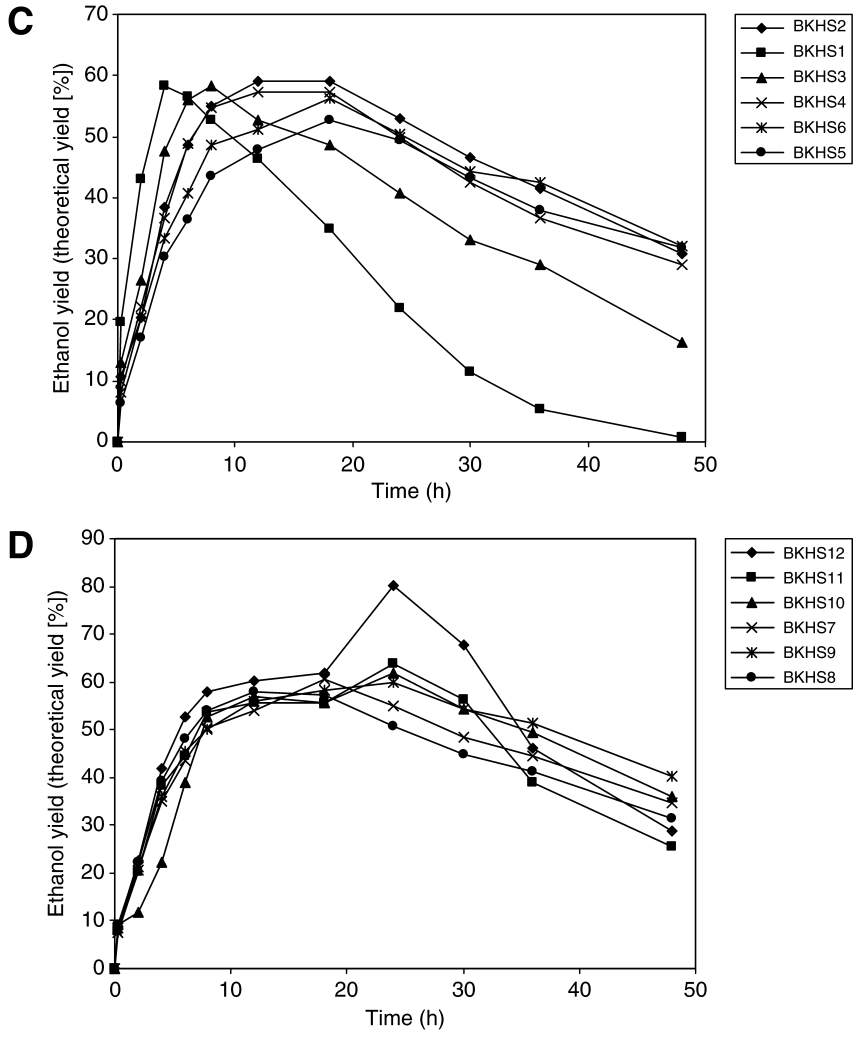

Fig. 3. Ethanol fermentation of OS HHS **(A,B)** and OS BKHS **(C,D)** hydrolyzates.

Conclusion

Beetle-killed HS and HHS pretreated by ethanol organosolv were shown to be good substrates for bioethanol production achieving approx 80 and 70% theoretical ethanol yield, respectively, during relatively brief incubation (Fig. 3, Table 4). Untreated HHS and BKHS did not differ significantly in chemical composition except for the moisture content, which was significantly lower in BKHS (approx 10%) compared with HHS (approx 18%). However, the FQ of OS BKHS pulps was significantly lower than OS HHS pulps (Fig. 1) indicating a poor suitability of BKHS OS pulps for high-quality papermaking. Furthermore, the yields of carbohydrates obtained in hydrolyzable and fermentable forms was higher at mild

Table 4
Maximal Observed BKHS and HHS Ethanol Yields

Pulp sample	Time (h)	Ethanol yield (%)
HHS1	4	52.5
HHS2	18	59.6
HHS3	12	54.1
HHS4	12	57.2
HHS5	12	59.3
HHS6	18	57.3
HHS7	18	60.5
HHS8	12	70.3
HHS9	18	59.4
HHS10	8	60.3
HHS11	18	60.4
HHS12	18	62.9
BKHS1	4	58.2
BKHS2	12	59.0
BKHS3	8	58.2
BKHS4	12	57.3
BKHS5	18	52.8
BKHS6	18	56.4
BKHS7	18	60.7
BKHS8	12	57.8
BKHS9	24	60.1
BKHS10	24	62.0
BKHS11	24	63.8
BKHS12	24	80.1

Percent of ethanol theoretical yield of carbohydrates in pretreated solids.

pretreatment conditions (H-Factor <1000) for BKHS compared with HHS. At medium (H-Factor 1000–2000) and severe (H-Factor >2000) pretreatment conditions HHS and BKHS behaved similarly (Table 3).

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References

1. Perlack, R. D., Wright, L. L., Turhollow, A. F., Graham, R. L., Stokes, B. J., and Erbach, D. C. (2005), In: Biomass as feedstock for a bioenergy and bioproducts industry: the technical

feasibility of a billion-ton annual supply, *US Department of Energy*, US Department of Agriculture, pp. 1–60. Available electronically at: http://www.osti.gov/bridge. Last accessed date: December 20, 2006.
2. Henry, J. D. (2002), Canada's Boreal Forest, ed. Smithsonian Institution Press, Washington.
3. Armstrong, J. A. and Ives, W. G. H. (1995), Forest insect pestes, In: Natural Resources Canada, Canadian Forest Service, Ottawa, Canada.
4. Malmström, C. M. and Raffa, K. F. (2000), *Global Change Biol*. **6,** 35–48.
5. Hall, J. P. and Moody, B. (1994), Report ST-X-8, Natural Resources Canada, Canadian Forest Service, Ottawa, Canada.
6. Wilson, J., Bell, P., Nettleton, P., Mackey, D., (2001), http://www.governmentcaucus.bc.ca/media/pinebeetle.pdf/2007_update.html. Last date website accessed on January 22, 2007.
7. Schmid, J. M. and Frye, R. H. (1997), Report RM–49, U.S. Department of Agriculture, Colorado.
8. Christiansen, E. and Bakke, A. (1988), In: Dynamics of Forest Insect Populations: Patterns, Causes, Implications. Berryman, A. A. (ed.), Plenum Press, New York: pp. 479–503.
9. Safranyik, L. and Linton, D. A. (1988), *Can. Entomol.* **120,** 85–94.
10. Day, K. R. and Leather, S. R. (1997), In: Forests and Insects. Watt, A. D., Stork, N. E., and Hunter, M. D. (eds.), Chapman and Hall, London, pp. 177–205.
11. Seifert, K. A. (1993), In: *Ceratocystis and ophiostoma:* Taxonomy, Ecology and Pathogenicity. Wingfield, M. J., Seifert, K. A., and Webber, J. F. (eds.), APS Press, St. Paul Minnesota. pp. 141-172.
12. Brasier, C. (1991), *Mycopathologia* **115,** 151–161.
13. Harrington, T. C. (1993), In: *Ceratocystis and Ophiostoma:* Taxonomy, Ecology and Pathogenicity. Wingfield, M. J., Seifert, K. A., and Webber, J. F. (eds.), APS Press, St. Paul Minnesota. pp. 161–172.
14. Vroom, K. E. (1957), *Pulp Pap. Mag. Can.* **58,** 228–231.
15. Kurabi, A., Berlin, A., Gilkes, N, et al. (2005), *Appl. Biochem. Biotechnol.* **121–124,** 219–230.
16. Berlin, A., Gilkes, N., Kilburn, D., et al. (2005), *Enzyme Microb. Technol*. **37,** 175–184.
17. TAPPI (2004), TAPPI test methods in CD–Rom, TAPPI Press, GA.
18. Berlin, A., Maximenko, V., Bura, R., Kang, K., Gilkes, N., and Saddler, J. (2005), *Biotechnol. Bioeng*. **93,** 880–886.
19. Costa, V., Amorim, M. A., Reis, E., Quintanilha, A., and Moradas-Ferreira, P. (1997), *Microbiology* **143,** 1649–1656.