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# BioResources

A peer-reviewed Online Journal Devoted to the Science  
and Advanced Applications of Lignocellulosic Resources

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**Cover image: Oil palm – see article by Law et al., page 351**

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*BioResources*, a peer-reviewed journal devoted to the science of lignocellulosic materials, chemicals, and applications

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## WHEN IS A TREE NOT A RESOURCE?

Martin A. Hubbe

Although this journal mainly considers the study of cellulosic materials as sources of structural wood, fibers, chemicals, energy, and products such as paper, it would be short-sighted to view all trees as existing in order to meet such needs. An individual tree may have multiple roles, from a human perspective. The point of this essay is that different groups of trees ought to be managed in one of four ways – as crops, as natural habitat, as an awe-inspiring heritage, as in the case of national parks, and as dear friends in our yards and along our boulevards.

*Keywords: Forests, Trees, Natural Resources, Habitat, Intensive forestry*

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## TREES AND DREAMERS

Trees hold a special place in the popular imagination. Maybe it's because the lifespan of an individual tree can rival and often exceed our own. Maybe it is due to their persistence in reaching continually toward the sky without complaint. Few people admit to feeling sad when a lawnmower cuts down a dandelion or a blade of grass. But the absence of a tree, felled by lightning or a chainsaw, can weigh upon the heart of one who has grown up in its presence.

Some attribute a spiritual beauty to trees. As Joyce Kilmer wrote, "I think that I shall never see a poem as lovely as a tree; a tree whose hungry mouth is prest; against the Earth's sweet flowing breast; a tree that looks at God all day; and lifts her leafy arms to pray; a tree that may in summer wear; a nest of robins in her hair; upon whose bosom snow has lain; who intimately lives with rain; poems are made by fools like me; but only God can make a tree."

Others may be nostalgic for a simpler time. A visit to a national park sometimes can make both us and our problems feel small, awed by the sheer grandeur of nature. The white pines of Acadia Park in Maine and the redwood trees of the Pacific Northwest can remind us of our humble place in the natural order of the world.

## TREES AS A CROP

According to my dictionary, a resource is something that is "available to be used." Since the dawn of civilization humans have become adept at the planting of crops, which can be thought of as a way to make ensure that food, as well as fibers for cloth, will be available at the time of harvest. The planters also learned of opportunities to select seeds from the more delicious fruits or the more productive fiber-bearing plants, such as cotton.

Presently, most of the food that we eat comes from intensively planted and fertilized fields. Not surprisingly, there have been recent trends towards increasing dependence on plantation forests, especially in the tropics, as a source of fiber for pulp and paper. Tropical plantations can offer a favorable combination of fiber uniformity and high rate of production per acre.

## TREES AS HABITAT

Though plantation forests appear to be well suited to meet many of our needs for cellulosic materials, there are some needs that they do not fulfill. First and foremost is the need for biodiversity. From a strictly utilitarian sense, biodiversity can be viewed as a kind of safety net, a buffer of self-regulating biomass that contains a rich pool of genes, capable of responding to a wide range of climactic and environmental stresses, including fires and floods. Natural forests also can be a delight to visit, for instance, when we hunt, fish, and collect wild berries – returning to our pre-civilized roots as hunter-gatherers.

## FARM ANIMAL, SHOW ANIMAL, WILD ANIMAL, AND PET

In many respects, the previous sections of this essay describe incompatible roles for the respective trees. A management style well suited for trees in a natural habitat (by analogy to the wild animal) is unlikely to be ideal for implementation in an intensively harvested forest (by analogy to the farm animal). A management style well suited for a national park (by analogy to a show animal) is unlikely to be ideal for implementation in the subdivision where you live (by analogy to the pet animal). The argument is sometimes heard that intensive forestry, using only a fraction of the available land, can make it possible to preserve natural areas, having a rich biodiversity. Others are fearful that modified species in an intensively managed forest may have potential to invade so-called natural areas. Conversely, hardy trees from the natural forest may act like “weeds,” invading and disrupting the uniformity of monoculture stands. Issues such as these suggest that well-planned management practices, rather than merely a hopeful attitude, is most likely to help meet our future needs for trees.

Trees will remain prominent in people’s imaginations. We will look upon their grandeur, their beauty, their utility, and their integration into a natural web of life. We will need to employ different kinds of management styles to make the optimum usage of this natural resource. And yes, some forests deserve to be considered as part of our national heritage or as our friends; those trees may be placed off limits, *i.e.* not considered as a “resource” for materials, chemicals, or fuels. But they can be a resource for our spiritual well-being and the health of our natural environment.

## REACTIONS OF LIGNIN CHROMOPHORES OF THE ENAL AND ENONE TYPES WITH SULFITE

Knut Lundquist,<sup>a</sup> Jim Parkås,<sup>a\*†</sup> Magnus Paulsson,<sup>a‡</sup> and Cyril Heitner<sup>b</sup>

In NaHSO<sub>3</sub>-solutions of coniferaldehyde and its methyl ether (models for lignin chromophores of the cinnamaldehyde type) a dynamic equilibrium between the aldehydes and their hydrogen sulfite adducts is set up. A comparatively slow addition of hydrogen sulfite to the double bond leading to 1,3-disulfonic acid derivatives occurs; coniferaldehyde reacts slower than its methyl ether. In Na<sub>2</sub>SO<sub>3</sub>-solution both aldehydes are rapidly converted to 1,3-disulfonic acid derivatives. The results suggest that in both NaHSO<sub>3</sub>-solution and Na<sub>2</sub>SO<sub>3</sub>-solution the free cinnamaldehydes and sulfite ion are the reactants in the formation of disulfonic acid derivatives. Alkaline treatment of the 1,3-disulfonic acids leads to regeneration of the cinnamaldehydes. On prolonged storage in Na<sub>2</sub>SO<sub>3</sub>-solution, the 1,3-disulfonic acid derivative of the methyl ether of coniferaldehyde undergoes reactions leading to colored products and regeneration of the cinnamaldehyde on alkaline treatment fails. A model compound representative of lignin chromophores of enone type (*trans*-3,3',4,4'-tetramethoxychalcone) rapidly undergoes addition to the double bond with formation of a sulfonic acid derivative in Na<sub>2</sub>SO<sub>3</sub>-solution. Treatment of a second model of enone type, 2,6-dimethoxy-*p*-quinone, with NaHSO<sub>3</sub>/Na<sub>2</sub>SO<sub>3</sub>-solution results in formation of a sulfonic acid derivative. The reactions of lignin chromophores of the enone and enal types with hydrogen sulfite/sulfite are discussed.

*Keywords:* Cinnamaldehyde, Chalcone, Chromophore, Lignin, Model compounds, Pulping, Sulfonation

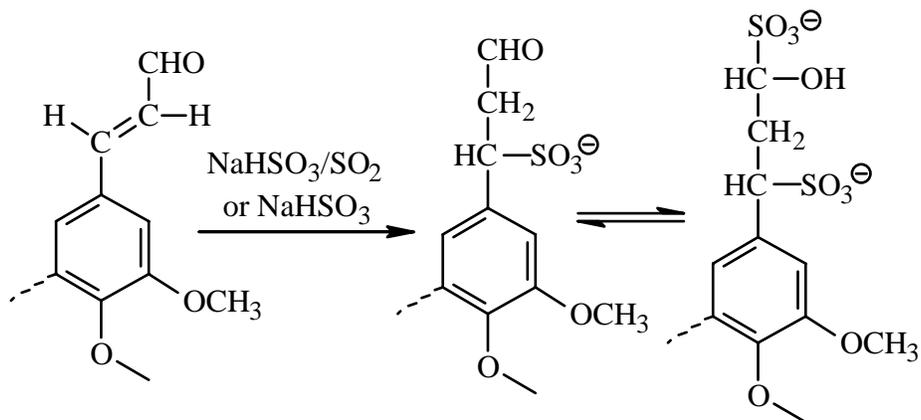
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## INTRODUCTION

Several pulping processes are based on delignification effected by sulfurous acid and/or salts of sulfurous acid (Rydholm 1965). Salts of this acid are also used as impregnation chemicals in certain chemithermomechanical pulping processes in order to facilitate pulping and improve pulp quality (Lindholm and Kurdin 1999). Of interest in these contexts are the reactions of salts of sulfurous acid with lignin chromophores of the enal [e.g. cinnamaldehyde units (Adler 1977; Lundquist 1992)] and enone types (e.g. quinone units). Cinnamaldehyde groups not only contribute to the color of the pulp but also may play a role in the light-induced yellowing of the pulp (Gellerstedt and Pettersson 1975). It is known that lignin units of the cinnamaldehyde type are sulfonated on acid sulfite pulping or bisulfite pulping (Fig. 1) (Adler and Elmer 1948; Mothershead and Glennie 1964; see also Kratzl 1948). Experiments with the model compounds

coniferaldehyde (**1**) and its methyl ether (**2**) show that sulfonation occurs on treatment with acid sulfite (Aulin-Erdtman 1953), and experiments with compounds of type **2** show that this also occurs on treatment with bisulfite and neutral sulfite (Suckling 1991) [see also the studies of the sulfonation of cinnamaldehyde by Tiemann (1898) and Kratzl and Däubner (1944)].



**Fig. 1.** Formation of sulfonic acid derivatives of lignin units of the cinnamaldehyde type on acid sulfite and bisulfite pulping.

Noteworthy also is the isolation of small amounts of the disulfonic acid derivative **6a** from the reaction mixture obtained on neutral sulfite cooking of **2** (Gellerstedt et al. 1977). According to Heitner and Min (1987) the effect of sulfite treatment on increasing the brightness of chemithermomechanical pulp can be attributed to sulfonation of cinnamaldehyde groups.

In connection with studies of the light-induced yellowing of mechanical pulps (Parkås et al. 2001) we found it was of interest to obtain a more detailed knowledge about the sulfonation reactions of lignin chromophores of the enal and enone types. To elucidate the reaction sequences leading to sulfonation and other modifications of chromophores of enal type, we have studied the reactions of the lignin-related cinnamaldehydes **1** and **2** in NaHSO<sub>3</sub>-solution and Na<sub>2</sub>SO<sub>3</sub>-solution. The sulfonation of a model compound representative of lignin chromophores of the enone type, *trans*-3,3',4,4'-tetramethoxychalcone (**12**), in a Na<sub>2</sub>SO<sub>3</sub> solution was examined. Sulfonation experiments with a quinone (**17**), representative of a second type of enone chromophore, were also carried out. To study the initial reactions and the course of the reactions we have carried out the experiments with model compounds at low temperature (22 °C). The reactions of lignin chromophore models with salts of sulfurous acid result in decolorization or color changes. We have followed these changes by UV/VIS spectroscopy. It was found that this technique could also with advantage be applied in studies of the course of the reactions, particularly in the case of models of the cinnamaldehyde type. The reactions lead to changes of the conjugated systems, which in turn result in dramatic changes of the spectral properties. The thorough and comprehensive studies of the UV properties of lignin models (including compounds of the type examined in this paper) by Aulin-Erdtman (Aulin-Erdtman 1953; Aulin-Erdtman

and Hegbom 1956) and other studies of the UV properties of lignins and model compounds served as a basis for our investigations (for reviews see Goldschmid (1971), Lin (1992), see also Silverstein et al. 1991).

## EXPERIMENTAL

### Chemicals and Starting Materials

(*E*)-3-(4-hydroxy-3-methoxyphenyl)propenal (coniferaldehyde) (**1**) (Iliefski et al. 2003), (*E*)-3-(3,4-dimethoxyphenyl)propenal (**2**) (Iliefski et al. 1998), (*E*)-3-(3,4-dimethoxyphenyl)-2-propen-1-ol (**7**) (Li and Lundquist 1995), *trans*-3,3',4,4'-tetramethoxychalcone (**12**) (Li et al. 1993), deoxyveratrolin (**14**) (Kubiczek 1946) and sodium 1-(4-hydroxy-3-methoxyphenyl)propane-1-sulfonate (Adler et al. 1962) were prepared according to methods described in the literature. Buffer solution at pH 12 was prepared by adding 0.1 M NaOH (26.9 ml) to 0.05 M Na<sub>2</sub>HPO<sub>4</sub> (50 ml). Na<sub>2</sub>SO<sub>3</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> were purchased from Merck. Hydroquinonesulfonate (**16**) (potassium salt) and 2,6-dimethoxy-*p*-benzoquinone (**17**) were purchased from Aldrich.

**Wiesner Reaction** (Nakano and Meshitsuka 1992; see also Sarkanen and Ludwig 1971)

Three drops of the samples were added to 5 ml of a mixture of equal amounts of 0.1 M phloroglucinol in 60% ethanol and 4 M hydrochloric acid in ethanol-water 2:1. A red or violet color appearing within 3 min was taken as evidence for the presence of cinnamaldehydes (**1** or **2**).

### Thin Layer Chromatography (TLC)

TLC was performed on silica gel plates (Merck, Kieselgel 60 F<sub>254</sub>) with methylene chloride-ethyl acetate (10:1) (*R<sub>f</sub>* values: sulfonic acid salts, 0.00; **1**, 0.28; **17**, 0.30; **2**, 0.37; veratraldehyde, 0.45), butanol-propanol-water-acetic acid (22:18:10:1) (Glennie 1966) (*R<sub>f</sub>* values: disulfonic acid derivatives (**4a** and **6a**), 0.0-0.1; **13**, 0.38; sodium 1-(4-hydroxy-3-methoxyphenyl)propane-1-sulfonate, 0.39; hydroquinone-sulfonate (**16**) (potassium salt), 0.43; veratraldehyde, 0.63; **2**, 0.65; **1**, 0.68). Spots were made visible with UV light and by spraying with formalin-H<sub>2</sub>SO<sub>4</sub> (1:9) and subsequent heating. Cinnamaldehydes were also detected by spraying with a mixture of equal amounts of 0.1 M phloroglucinol in 60% ethanol and 4 M hydrochloric acid in ethanol-water 2:1.

### UV Spectroscopy

UV spectra were recorded with a Varian Cary 4 UV-Visible Spectrophotometer. Absorption maxima for reference compounds (solvent, 95% ethanol): **1**,  $\lambda_{\max}$  342 nm ( $\epsilon_{\max}$  24000),  $\lambda_{\max}$  242 nm ( $\epsilon_{\max}$  10700); **2**,  $\lambda_{\max}$  338 nm ( $\epsilon_{\max}$  22200),  $\lambda_{\max}$  247 nm ( $\epsilon_{\max}$  11600); 3-(3,4-dimethoxyphenyl)-2-propen-1-ol (**7**),  $\lambda_{\max}$  262 nm ( $\epsilon_{\max}$  16900); **12**,  $\lambda_{\max}$  364 nm ( $\epsilon_{\max}$  24900),  $\lambda_{\max}$  239 nm ( $\epsilon_{\max}$  13800); **14**,  $\lambda_{\max}$  306 nm ( $\epsilon_{\max}$  8900),  $\lambda_{\max}$  277 nm ( $\epsilon_{\max}$  13000).

### Sulfonation of Coniferaldehyde (1) and its Methyl Ether (2) by Treatment with 1 M NaHSO<sub>3</sub>

Coniferaldehyde (1) (55 mg) or its methyl ether (2) (56 mg) was dissolved in 20 ml 1 M NaHSO<sub>3</sub> (magnetic stirring) at 22 °C. Complete dissolution required 1-2 h. The UV spectra of samples of the reaction mixtures diluted with water (1:100 and 1:500) were recorded after different periods of time. The reaction mixtures were also examined by TLC. The Wiesner reagent was used for the detection of cinnamaldehydes in the reaction mixtures.

### Sulfonation of Coniferaldehyde (1) and its Methyl Ether (2) by Treatment with 1 M Na<sub>2</sub>SO<sub>3</sub>

Coniferaldehyde (1) or its methyl ether (2) (about 70 mg) was dissolved in 20 ml 1 M Na<sub>2</sub>SO<sub>3</sub> (magnetic stirring) at 22 °C. To facilitate dissolution the aldehydes were ground in a mortar. Complete dissolution required about 2 h. UV spectra of samples of the solutions diluted with water (1:100 and 1:500) were recorded after different periods of time. To suppress ionization the samples from experiments with coniferaldehyde were acidified (2 ml of 0.4 M HCl were added to a 1 ml sample) prior to dilution and recording of the UV spectra. The reaction mixtures were also examined by TLC. The Wiesner reagent was used for the detection of cinnamaldehydes in the reaction mixtures.

### Regeneration of 2 Reacted with 1 M Na<sub>2</sub>SO<sub>3</sub> Solution

A sample (1 ml) of a solution of 2 in 1 M Na<sub>2</sub>SO<sub>3</sub> was acidified with 2 M hydrochloric acid (1 ml). The solution was concentrated to dryness and the residue dissolved in 100 ml pH 12 buffer. UV spectra of the solution were recorded after different periods of time.

### Sulfonation of *Trans*-3,3',4,4'-tetramethoxychalcone (12)

A mixture of *trans*-3,3',4,4'-tetramethoxychalcone (12) (102 mg), dioxane (2 ml) and 10 ml 1 M Na<sub>2</sub>SO<sub>3</sub> was stirred at room temperature (22 °C). Complete dissolution of the chalcone required 4 h. TLC showed that, excepting traces of the starting material, only one compound was present in the solution. The UV spectrum of the solution, recorded after dilution with water (1:500), similarly showed that 12 was essentially absent. A portion of the solution (5 ml) was acidified with 4 M hydrochloric acid (1.75 ml), and the solvents were removed by film evaporation. The <sup>1</sup>H NMR spectra [recorded at 400 MHz with a Varian Unity 400 instrument (temperature, ≈293 K)] of the residue and a fraction of the residue (30 mg, obtained by leaching of the residue with butanol) dissolved in D<sub>2</sub>O were recorded (internal reference, the sodium salt of 3-(trimethylsilyl)propane sulfonic acid). The two spectra did not differ significantly. Based on the examinations it was concluded that the product consisted of the sulfonic acid salt 13. <sup>1</sup>H NMR spectrum of the fraction obtained by leaching with butanol: δ 3.67 (3H, s, OCH<sub>3</sub>), 3.78 (3H, s, OCH<sub>3</sub>), 3.79 (3H, s, OCH<sub>3</sub>), 3.82 (3H, s, OCH<sub>3</sub>), 3.70 (1H, dd, J= 4.2 and 16.4 Hz, CH<sub>2</sub>), 3.87 (1H, dd, J= 10.6 and 16.4 Hz, CH<sub>2</sub>) 4.60 (1H, dd, J= 4.2 and 10.6 Hz, >CH-SO<sub>3</sub>Na), 6.69 (1H, d, J= 8 Hz, H-Ar), 6.94 (1H, d, J= 8 Hz, H-Ar), 7.04 (1H, dd, J= 2 and 8 Hz, H-Ar), 7.10 (1H, d, J= 2 Hz, H-Ar), 7.11 (1H, d, J= 2 Hz, H-Ar), 7.41 (1H, dd, J= 2 and 8 Hz, H-Ar).

### Treatment of 2,6-dimethoxy-*p*-benzoquinone (**17**) with 1 M Na<sub>2</sub>SO<sub>3</sub> and 1 M Na<sub>2</sub>SO<sub>3</sub>-1 M NaHSO<sub>3</sub> (1:1)

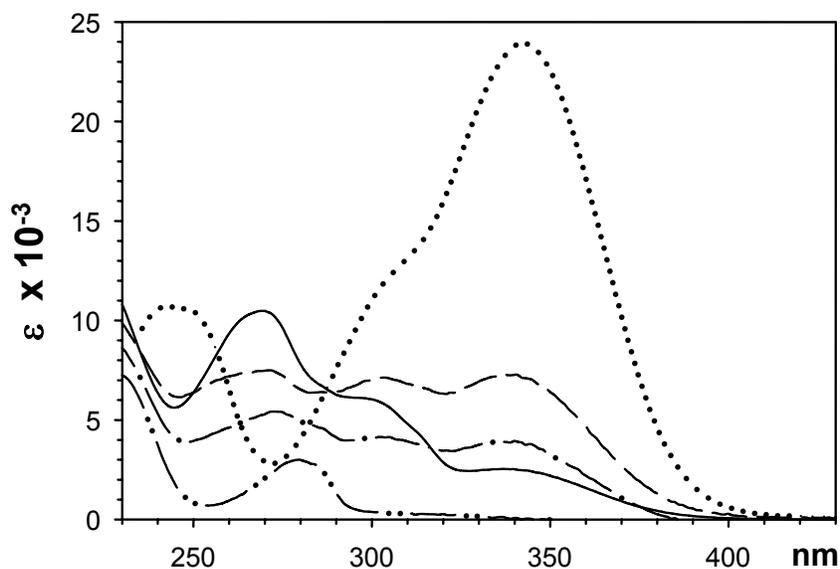
a. 2,6-Dimethoxy-*p*-benzoquinone (**17**) (ca. 50 mg) was dissolved in 10 ml 1 M Na<sub>2</sub>SO<sub>3</sub>-1 M NaHSO<sub>3</sub> (1:1). Dissolution occurred almost immediately and the solution was colorless. No starting material was present in the solution (TLC). TLC [eluent, butanol-propanol-water-acetic acid (22:18:10:1)] exhibited only one spot (*R<sub>f</sub>* value 0.38). Based on comparisons with *R<sub>f</sub>* data published by Glennie (1966) and the *R<sub>f</sub>* value for hydroquinonesulfonate (**16**) (potassium salt) we attribute the spot to **18**.

b. 2,6-Dimethoxy-*p*-benzoquinone (**17**) (ca. 50 mg) was dissolved in 10 ml 1 M Na<sub>2</sub>SO<sub>3</sub>. Dissolution required 1-2 h. The solution acquired a yellow color. No starting material was present in the solution (TLC). TLC [eluent, butanol-propanol-water-acetic acid (22:18:10:1)] showed only materials with very low *R<sub>f</sub>* value (< 0.1). Based on comparisons with *R<sub>f</sub>* data published by Glennie (1966) disulfonic acids may be present in the product.

## RESULTS AND DISCUSSION

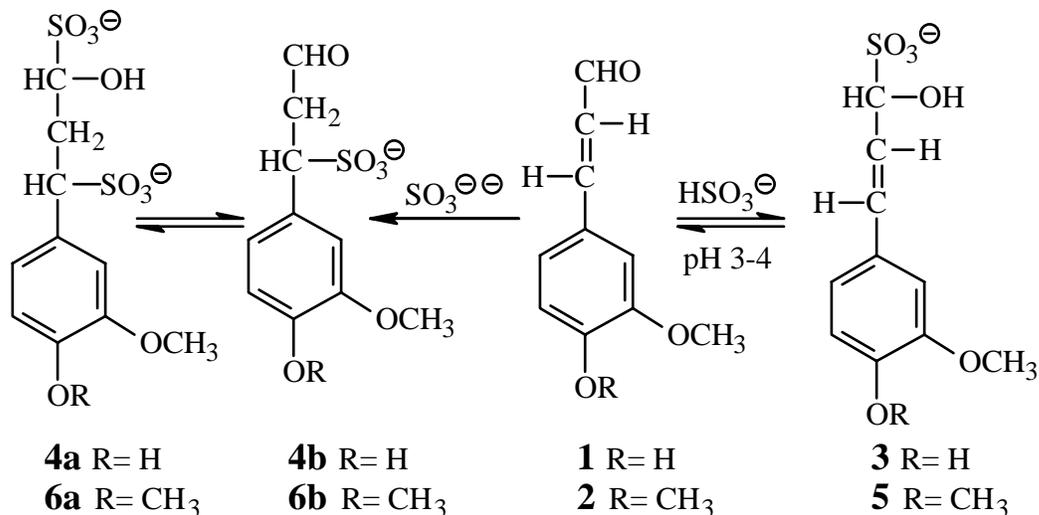
### Reactions of Coniferaldehyde (**1**) and its Methyl Ether (**2**) in 1 M NaHSO<sub>3</sub>

Solutions of cinnamaldehydes **1** and **2** in 1 M NaHSO<sub>3</sub> were examined by UV spectroscopy and TLC after different periods of time. UV spectra of a solution (diluted with water) obtained in an experiment with **1** are shown in Fig. 2.

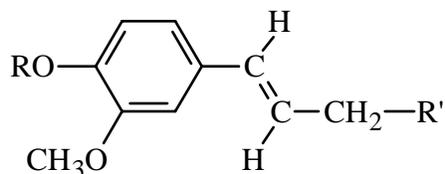


**Fig. 2.** The UV spectrum of a solution of coniferaldehyde (**1**) in 1 M NaHSO<sub>3</sub> after 5 h (----), 25 h (- · - · -) and 100 h (- - - - -). The solution was diluted with water 1:500 before the recording of the spectra. The solution stored for 5 h was also diluted with water 1:100 (——). The UV spectrum of **1** in 95% ethanol (· · · · ·) is also shown.  $\epsilon$  is calculated from the initial concentration of **1**.

The presence of starting material in the solution could be demonstrated by TLC and the Wiesner reaction. Even after several days the solution showed a weak but distinct Wiesner reaction.



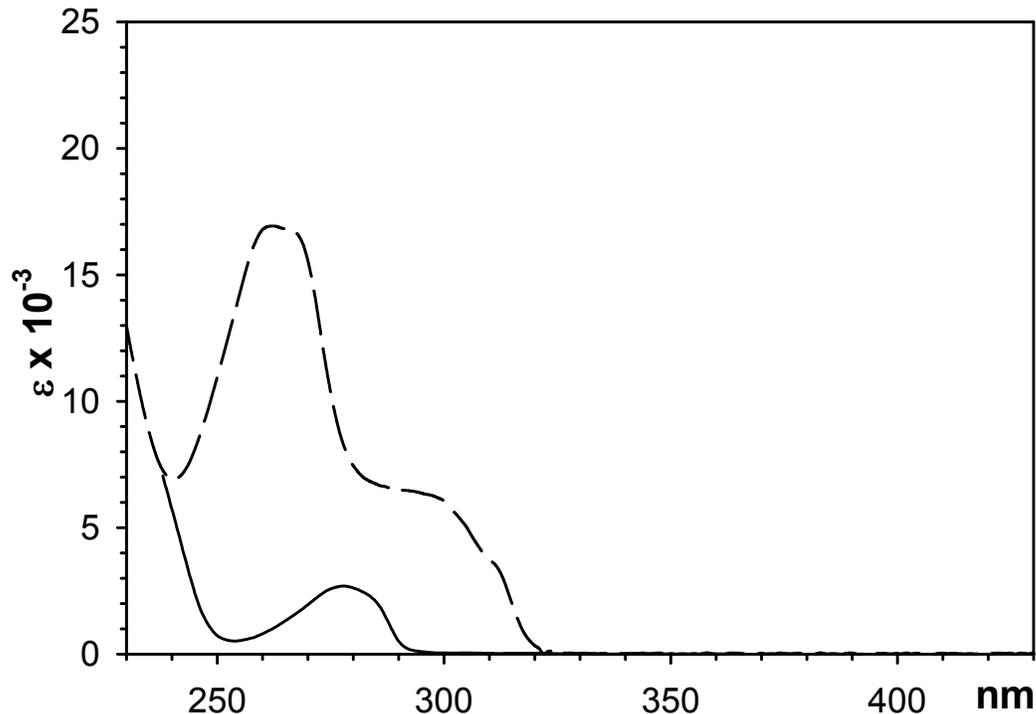
**Fig. 3.** Reactions of coniferaldehyde (**1**) and its methyl ether (**2**) in 1 M NaHSO<sub>3</sub>. A dynamic equilibrium is set up between the aldehydes and the corresponding hydrogen sulfite adducts (**3** and **5**). A slow conversion to **4** and **6** occurs.



- 7** R= CH<sub>3</sub>, R'= OH  
**8** R= H, R'= OH  
**9** R= H, R'= SO<sub>3</sub>Na

**Formula Fig. 1**

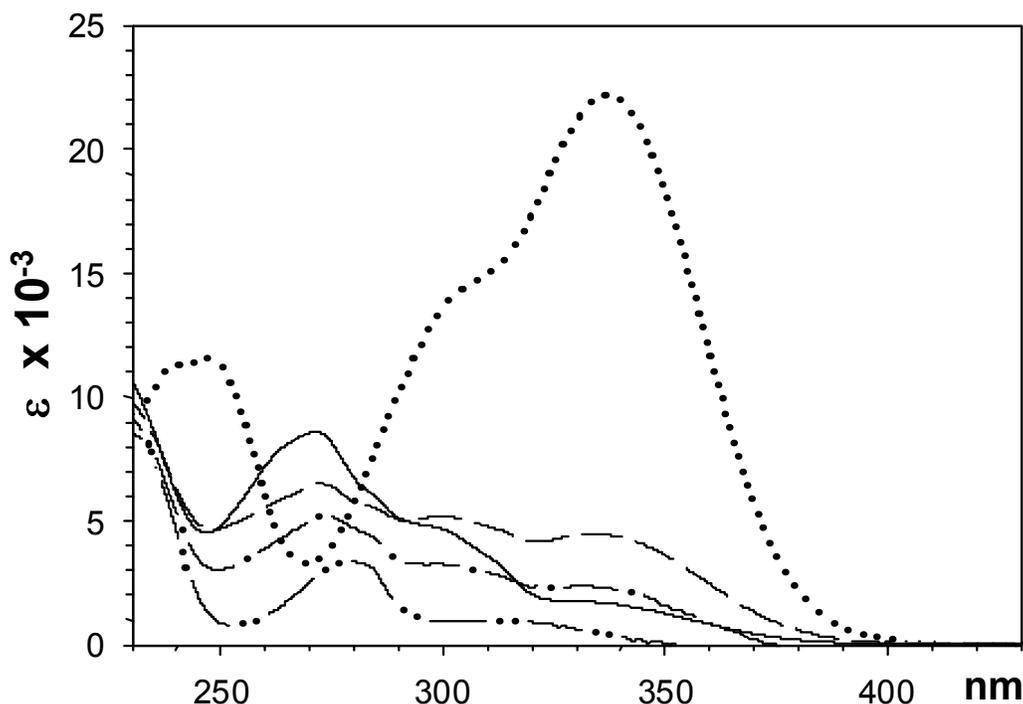
The reactions leading to the spectral changes are shown in Fig. 3. From comparisons of the UV spectra (Fig. 2) with the spectra of **1**, the sulfonic acid derivative **6** prepared according to Aulin-Erdtman (1953), 3-(3,4-dimethoxyphenyl)-2-propen-1-ol (**7**) (Fig. 4), coniferyl alcohol (**8**) [solvent 95% ethanol:  $\lambda_{\max}$  = 266 nm,  $\epsilon_{\max}$  = 15100, aqueous solution:  $\lambda_{\max}$  = 261 nm,  $\epsilon_{\max}$  = 15100 (Aulin-Erdtman and Hegbom 1957)] and salts of 3-(4-hydroxy-3-methoxyphenyl)-2-propene-1-sulfonic acid (e.g. **9**) ( $\lambda_{\max}$  = 267 nm,  $\epsilon_{\max}$  = 15600 [Schubert et al. 1967]), it could be concluded that an equilibrium is initially set up between the starting material and the hydrogen sulfite adduct **3** (Fig. 3).



**Fig. 4.** UV spectra of **6** [2 sulfonated according to Aulin-Erdtman (1953)] (—) and 3-(3,4-dimethoxyphenyl)-2-propen-1-ol (**7**) (---). The spectrum of **6** closely resembles that of **4** (Aulin-Erdtman 1953).

That an equilibration rapidly occurs is evident from comparisons of the spectra obtained on dilution of the solution with water 1:100 and 1:500 (Fig. 2, cf. Fig. 4). As judged from UV spectral examinations, equilibration after dilution required  $< 20$  min. On the basis of comparisons with the spectra in Fig. 4, it can be concluded from the spectra recorded after different periods of time (Fig. 2) that a slow conversion to the sulfonic acid derivative **4** occurs (Fig. 3). A weak Wiesner reaction suggests that traces of **1** were present in the solution even after 100 h. TLC [eluent: butanol-propanol-water-acetic acid (22:18:10:1)] showed a spot with very low  $R_f$  value and streaking ranging from  $R_f$  0.1 to  $R_f$  0.5. Based on literature data (Glennie 1966) and the use of the sodium salt of 1-(4-hydroxy-3-methoxyphenyl)propane-1-sulfonic acid as a reference compound ( $R_f$  0.39), we attribute the low  $R_f$  value spot to the 1,3-disulfonic acid salt **4a** and the streaking to monosulfonic acid salts (**3** and **4b**).

UV spectra of a solution of **2** in 1 M  $\text{NaHSO}_3$  (diluted with water) recorded after different periods of time are shown in Fig. 5.



**Fig. 5.** The UV spectrum of a solution of the methyl ether of coniferaldehyde (**2**) in 1 M NaHSO<sub>3</sub> after 5 h (----), 25 h (- · - · -) and 100 h (- - - - -). The solution was diluted with water 1:500 before the recording of the spectra. The solution stored for 5 h was also diluted with water 1:100 (——). The UV spectrum of **2** in 95% ethanol (· · · · ·) is also shown.  $\epsilon$  is calculated from the initial concentration of **2**.

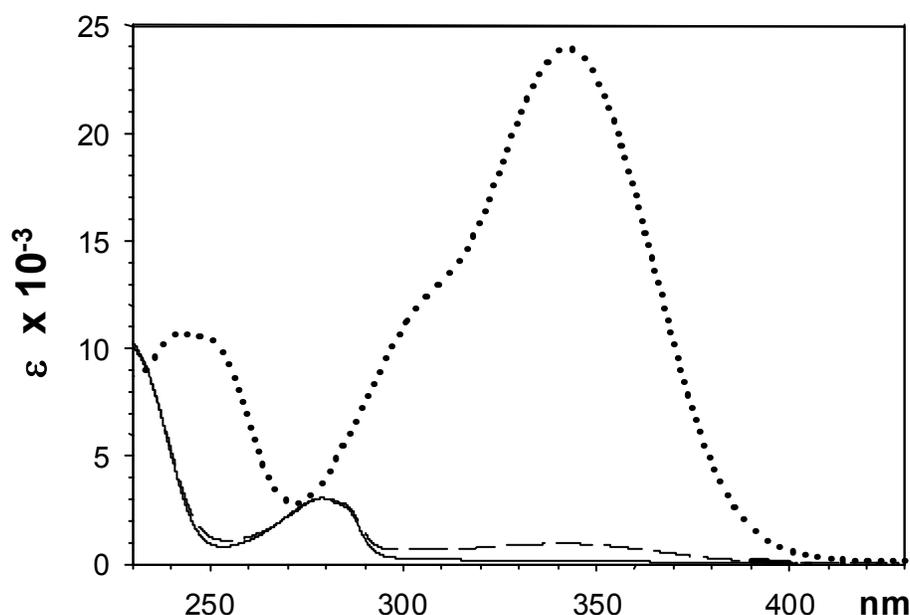
Comparisons with the spectra in Figs. 2 and 5 suggest that **1** and **2** react with 1 M NaHSO<sub>3</sub> in an analogous manner (Fig. 3). However, the rate of addition to the double bond differs. The non-phenolic cinnamaldehyde **2** adds hydrogen sulfite to the double bond more rapidly than does the phenolic cinnamaldehyde **1**. This is evident from comparisons of the absorbance at 260-270 nm (attributed to **3/5**) in the UV spectra in Figs. 2 and 5 and is also in accordance with results from examinations by TLC and the Wiesner reagent. Thus the solution of **2** in 1 M NaHSO<sub>3</sub> gave a very weak Wiesner reaction after 72 h, and TLC showed that an only trace of **2** was present in the solution. After 100 h **2** could not be detected by the Wiesner reaction or TLC. TLC with the eluent butanol-propanol-water-acetic acid (22:18:10:1) showed spots and streaking that can be attributed to sulfonic acids (cf. the results from the TLC examination of the solution of **1** described above). The UV spectra of the solutions of **2** stored for a long period of time (e.g. 100 h) show some absorbance in the region above 300 nm (Fig. 5) that cannot be attributed to the sulfonic acids formed or the starting material. This UV absorbance is attributed to formation of condensation products (Fig. 6) (cf. Suckling 1991).



Lowering of the pH of the reaction mixtures led to slower formation of **4** and **6**. This is consistent with the assumption that the free aldehyde and sulfite ion are reactants in the formation of **4** and **6** (Fig. 3). To further elucidate this point we have studied the reactions of **1** and **2** in 1 M Na<sub>2</sub>SO<sub>3</sub> (see below).

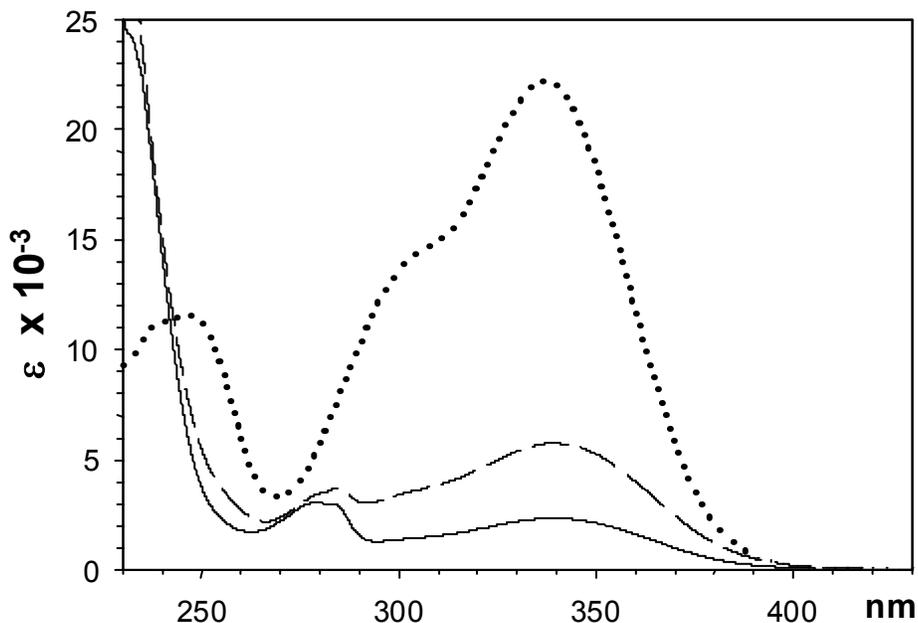
### Reactions of Coniferaldehyde (**1**) and its Methyl Ether (**2**) in 1 M Na<sub>2</sub>SO<sub>3</sub>

Experiments with cinnamaldehydes **1** and **2** of the aforementioned type in which 1 M NaHSO<sub>3</sub> had been replaced by 1 M Na<sub>2</sub>SO<sub>3</sub> were carried out. UV spectra of the solutions (diluted with water, in the case of **1** after acidification) recorded after different periods of time are shown in Figs. 8 and 9.

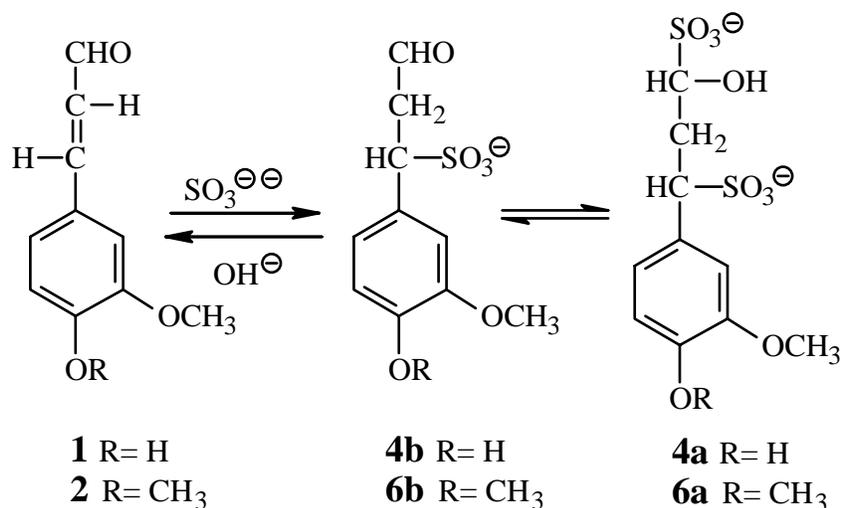


**Fig. 8.** The UV spectrum of a solution of coniferaldehyde (**1**) in 1 M Na<sub>2</sub>SO<sub>3</sub> after 1.5 h (—) and 24 h (---). The solution was diluted with water (1:100) before the recording of the spectrum. The UV spectrum of **1** in 95% ethanol (· · · · ·) is also shown.  $\epsilon$  is calculated from the initial concentration of **1**.

The spectra suggest that a rapid addition of sulfite to the double bonds occurs with formation of **4** and **6** (Fig. 10) (cf. Fig. 4). Free cinnamaldehydes could not be detected in the solutions by TLC. Only the solution of coniferaldehyde (**1**) gave a weak Wiesner reaction, and on long term storage this reaction became even weaker. The UV absorbance in the region above 300 nm increases on storage of the solutions. This increase is particularly pronounced in experiments with **2** (Fig. 9). Formation of condensation products (cf. Suckling 1991) probably explains the increase of the absorbance above 300 nm (Fig. 6). The formation of **11** on sulfite treatment of **2** has been reported by Suckling (1991).



**Fig. 9.** The UV spectrum of a solution of the methyl ether of coniferaldehyde (**2**) in 1 M Na<sub>2</sub>SO<sub>3</sub> after 2 h (—) and 29 h (---). The solution was diluted with water (1:100) before the recording of the spectrum. The UV spectrum of **2** in 95% ethanol (····) is also shown.  $\epsilon$  is calculated from the initial concentration of **2**.



**Fig. 10.** Reactions of coniferaldehyde (**1**) and its methyl ether (**2**) in 1 M Na<sub>2</sub>SO<sub>3</sub>. Regeneration of the aldehydes could be accomplished by treatment with pH 12 buffer.

Aulin-Erdtman (1953) showed that the aldehydes **1** and **2** are regenerated when **4** and **6** are treated with pH 12 buffer. We found that **1** was regenerated when a sample of

the Na<sub>2</sub>SO<sub>3</sub> solution was diluted (1:100) with pH 12 buffer (even if the Na<sub>2</sub>SO<sub>3</sub> solution had been kept at room temperature for several days). Similar treatment of the Na<sub>2</sub>SO<sub>3</sub> solution of **2** did not result in a regeneration of **2**. However, acidification followed by evaporation to dryness and dissolution of the residue in pH 12 buffer led to regeneration of **2**. An explanation for the different results obtained with **1** and **2** may be that an equilibrium involving sulfite ion, free cinnamaldehyde, and its sulfonic acid derivative is set up in the buffer solution and that this equilibrium is much more in favor of the sulfonic acid derivative in the case of cinnamaldehyde **2** (Fig. 10). Regeneration of **2** could only be accomplished if the solution was fairly fresh. Attempts to regenerate **2** from Na<sub>2</sub>SO<sub>3</sub> solutions that had been stored for several days at 22°C failed. Evidently, prolonged storage of **2** in Na<sub>2</sub>SO<sub>3</sub> solution leads to formation of products that are not converted to **2** on alkaline treatment. A plausible explanation would be that reversed aldol condensation occurred in the slightly alkaline sulfite solution (**6** → **2** → veratraldehyde + acetaldehyde). It was found that veratraldehyde actually formed, but very slowly. The formation of veratraldehyde is in accordance with observations made by Suckling (1991). The conversion to veratraldehyde contributes to the failure to regenerate **2** on alkaline treatment to a small extent; the main reason for the failure is probably formation of condensation products (Fig. 6) (Suckling 1991).

Aulin-Erdtman (1953) prepared the sulfonic acid derivatives **4** and **6** by subjecting **1** and **2** to conditions similar to those of acid sulfite pulping. Treatment of the obtained sulfonic acid derivatives with pH 12 buffer resulted in regeneration of **1** and **2** but the regeneration was not complete (in particular in the case of **2**) [Aulin-Erdtman (1953)]. The above described studies of the regeneration of the cinnamaldehydes from **4** and **6** provide an explanation of the incomplete regeneration of the cinnamaldehydes observed by Aulin-Erdtman (1953). Acid treatment did not lead to regeneration of the cinnamaldehydes (**1** and **2**) from the sulfonic acid derivatives **4** and **6**. This is in accordance with the literature dealing with sulfonic acids of cinnamaldehydes (see e.g. Mothershead and Glennie 1964).

### Sulfonation of *trans*-3,3',4,4'-tetramethoxychalcone (**12**) by Treatment with Na<sub>2</sub>SO<sub>3</sub>

*Trans*-3,3',4,4'-Tetramethoxychalcone (**12**) was dissolved in 1 M Na<sub>2</sub>SO<sub>3</sub>-dioxane (5:1). Examinations of the solution (TLC and UV) showed that a practically quantitative conversion to the sulfonic acid salt **13** had occurred (Fig. 11). The UV spectrum of the solution illustrates the completeness of the reaction (Fig. 12). The structure of **13** was confirmed by <sup>1</sup>H NMR spectroscopy.

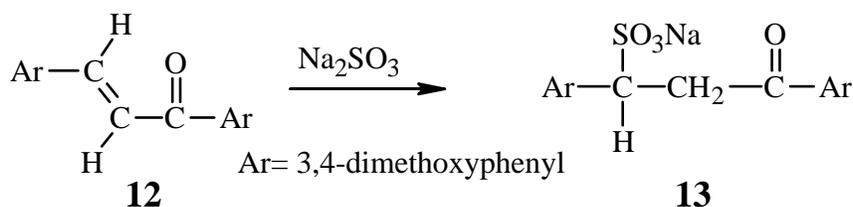
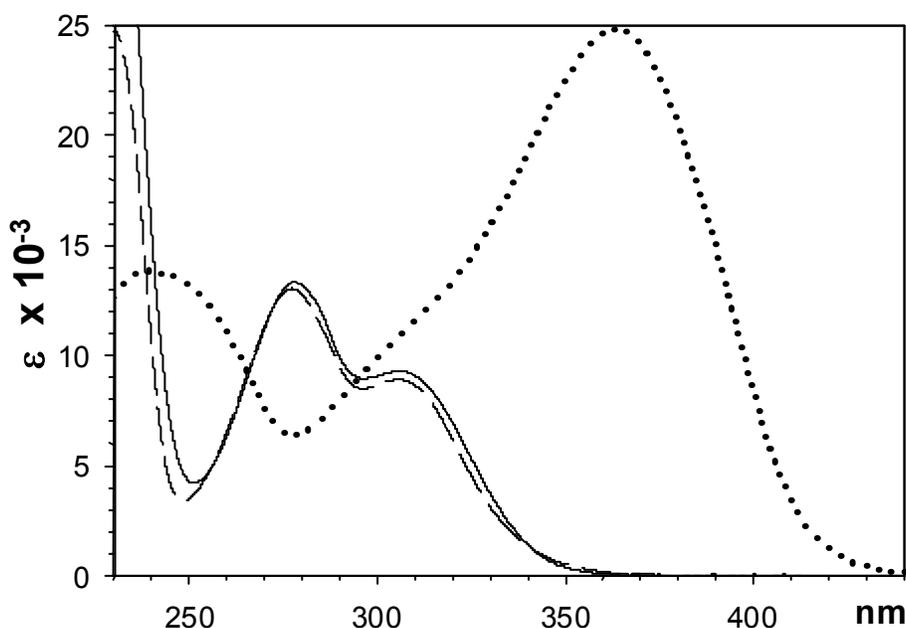
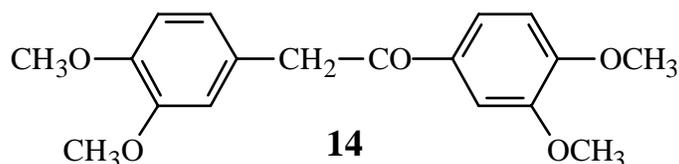


Fig. 11. Sulfonation of *trans*-3,3',4,4'-tetramethoxychalcone (**12**) in 1 M Na<sub>2</sub>SO<sub>3</sub>-dioxane (5:1).



**Fig. 12.** UV spectrum of *trans*-3,3',4,4'-tetramethoxychalcone (**12**) treated with Na<sub>2</sub>SO<sub>3</sub> solution (—). For comparisons the UV spectra of **12** (·····) and deoxyveratrin (**14**) (---) in 95% ethanol are shown.  $\epsilon$  is calculated from the initial concentration of **12**.



**Formula Fig. 2**

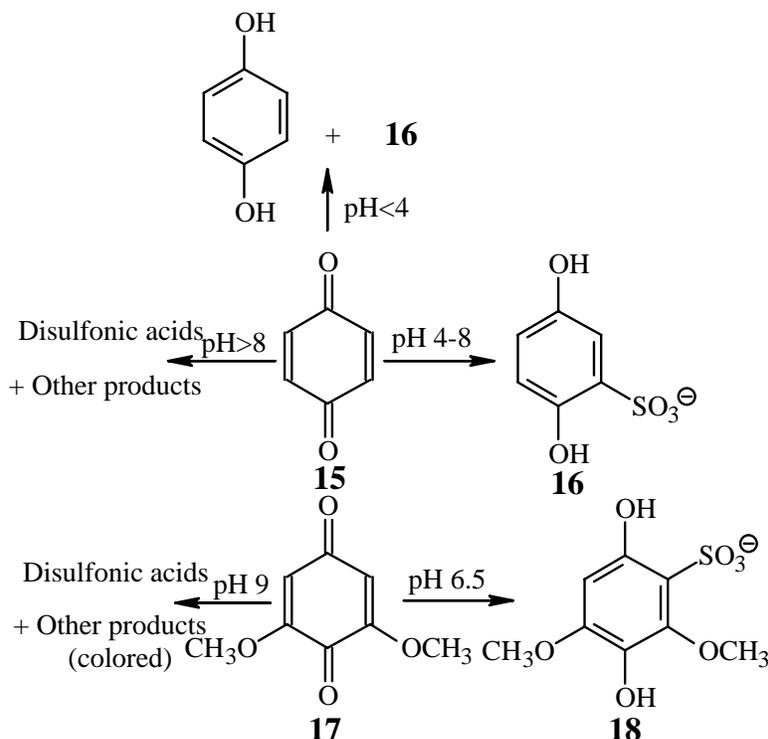
### Reactions of Lignin Chromophores of the Enal and Enone Types with Sulfite

Cinnamaldehydes **1** and **2** are appropriate model compounds for lignin chromophores of the enal type. Compound **2** is representative of the major part of the chromophores of this type, since most of the lignin units are non-phenolic. We think that it is justified to assume that the sulfonation reactions of cinnamaldehyde end groups in lignins are the same as those of the model compounds **1** and **2**.

Based on the results from the cinnamaldehyde study we considered it of interest to examine whether lignin chromophores of the arylconjugated enone type could be expected to react with 1 M Na<sub>2</sub>SO<sub>3</sub> at low temperatures. The chalcone derivative **12** was selected as a model compound representative of such chromophores. It was found that **12** reacted rapidly with formation of the sulfonic acid salt **13** (Fig. 11) under such conditions. Kratzl and Däubner (1944) have demonstrated that sulfonic acid derivatives

structurally related to **13** are formed on acid sulfite cooking of chalcone derivatives and several other compounds of the enone type.

Quinone units represent a second type of lignin chromophores of the enone type. It has been shown that the outcome of reactions of *p*-benzoquinone (**15**) with sulfite is strongly pH-dependent (Fig. 13) (Youngblood 1986). We have made some experiments with the methoxylated quinone **17**. The results (see Experimental) suggest that the reactions of **17** are analogous to those of **15** (Fig. 13).



**Fig. 13.** Reactions of quinones with sulfite.

Arylconjugated enals (such as cinnamaldehydes) and conjugated enones (such as chalcones and quinones) are in all probability important chromophoric groups in pulps. Such groups could also play a role as sensitizers in connection with the photochemical “yellowing” of paper produced from mechanical and chemimechanical pulps. The model compound studies presented in this paper suggest that these types of chromophoric groups can be eliminated by treatment with sulfite under suitable conditions. Sulfite concentration and pH are important factors. Treatment with sulfite at pH about 9 can be expected to generate new chromophoric groups from cinnamaldehyde units and perhaps also from quinone units. It is notable in this context that the optical properties of chemithermomechanical pulp manufactured from Black spruce wood (*Picea mariana*) pretreated with sulfite at pH 4.2 are better than those of such pulp manufactured from wood pretreated with sulfite at pH 8.6 (Argyropoulos and Heitner 1991). It is also of interest to note that formation of chromophores, possibly of quinoid type, was lessened when sulfite was present during refining of *Pinus radiata* wood (Suckling 1995).

## CONCLUSIONS

1. The course and outcome of the reactions of lignin chromophores of the enal and enone types with sulfite are strongly pH-dependent.
2. Based on the experiments with model compounds it can be concluded that in both NaHSO<sub>3</sub>-solution and Na<sub>2</sub>SO<sub>3</sub>-solution the free cinnamaldehyde units and SO<sub>3</sub><sup>2-</sup>-ion are the reactants in the formation of disulfonic acid derivatives. (On treatment with NaHSO<sub>3</sub>-solution an equilibrium is initially set up between cinnamaldehydes **1** and **2** and their sulfite adducts. This is followed by a comparatively slow conversion to disulfonic acid derivatives. Increased acidity leads to slower formation of such derivatives. On treatment with Na<sub>2</sub>SO<sub>3</sub>-solution **1** and **2** are rapidly converted to disulfonic acid derivatives).
3. Cinnamaldehyde units are regenerated on alkaline (but not acid) treatment of their disulfonic acid derivatives, provided the sulfite concentration is low. Prolonged treatment with Na<sub>2</sub>SO<sub>3</sub>-solution irreversibly leads to new types of chromophoric groups.
4. Lignin chromophores of the chalcone type are eliminated on treatment with Na<sub>2</sub>SO<sub>3</sub>-solution.
5. On sulfite treatment quinoid units may be reduced, form sulfonic acids, or give rise to complex, colored products dependent on pH.

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## MORPHOLOGICAL AND CHEMICAL NATURE OF FIBER STRANDS OF OIL PALM EMPTY-FRUIT-BUNCH (OPEFB)

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In this work we examined the morphological and chemical characteristics of the fibrous strands of oil palm empty-fruit-bunch which were left behind after being stripped of their fruits used for oil production. The empty-fruit-bunches were mechanically loosened to yield the fibrous strands, which can be used in paper and board making. We found that the fibrous strands had unique structure by having several large-diameter, long vessel elements in their core region, surrounded by vascular fibers. They had numerous silica-bodies attached to craters on their surfaces; the craters were perforated at the bottom. Many other minerals were also present in the strands. Our microscopic observations suggested that the silica-bodies are connected to a network of siliceous pathway within the fibrous matrix, and minerals tend to concentrate adjacent to the silica-bodies. Our findings could be useful in identifying suitable techniques for processing the oil palm fiber strands into value-added products.

*Keywords:* *Elaeis guineensis*, *Oil palm empty-fruit-bunch*, *Fiber morphology*, *Chemical constituents*, *Silica*, *Minerals*

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### INTRODUCTION

Oil palm, *Elaeis guineensis* (Fig. 1), is one of the most economical perennial oil crops for its valuable oil-producing fruits in tropical regions such as West Africa (Khozirah and Khoo 1991) and Southeast Asia. In Malaysia alone large amounts of oil palm biomass are generated by the palm oil industry, for example 5000 million tonnes (green) of felled trunk in 2000 (as projected by Husin et al. 1986), 36 million tonnes (odmt) per year of fronds from pruning and replanting (Wan Zahari et al. 2004) and 5.2 million tonnes (odmt) per annum of empty-fruit bunches (EFB) in 2002 (Tanaka and Yamamoto, 2004).

In the oil extraction process the fruits or nuts are first stripped from fruit bunches, leaving behind the empty-fruit bunches as waste. The abundance of oil palm empty-fruit-bunches (OPEFB) has created an important environmental issue such as fouling and attraction of pests. However, valuable fiber can be obtained from the OPEFB for manufacturing board and paper, for example. The OPEFB, nonwoody lignocellulosic material, is rather complicated in terms of cell type, morphology and chemical composition. Albeit its approximate chemical constituents have been reported before (Khoo, et al., 1991; Mansor and Ahmad, 1991; Jalil et al., 1991; Law and Jiang, 2001),

the physical and chemical nature of the fibrous strands prepared from OPEFB has not been investigated in detail. Such knowledge on this biomass is of great importance relative to its industrial processing and potential utilization in value-added products, mitigating the environmental concerns mentioned earlier.

The objective of this work was to characterize the physical nature of the fibrous strands of OPEFB, quantify the organic and inorganic components in OPEFB and examine techniques that can remove the problematic constituents in view of possible use of OPEFB in paper and other products.

## EXPERIMENTAL

### Material

The fibrous strands of OPEFB (Fig. 1, right) used in this study were obtained from an oil palm mill in Malaysia. The OPEFB was mechanically treated to loosen the fibrous strands, which were washed, cleaned, sorted, and air-dried.



Fig. 1. Photographs showing oil palm tree (left), fruit bunch (center), and fibrous strands (right)

### Methods

#### *Fiber morphology measurements*

Physical properties such as fiber diameter and cell-wall thickness were determined using 40- $\mu\text{m}$ -thick cross-sections of the fibrous strands and a transmission light microscope (Zeiss Photomicroscope III). The cross-sections were prepared from the fibrous strands embedded in paraffin prior to microtoming. Some fibrous strands were macerated in a solution containing equal volumes of hydrogen peroxide and acetic acid, at 70 °C, for about 3 h or until the fibers became readily separable by a gentle mechanical stirring. Fiber morphology data were obtained by means of a Fibre Quality Analyzer (FQA, OpTest Inc., Canada).

The chemical characteristics were determined using TAPPI test methods: lignin (T 222), holocellulose (T 222), hot-water solubility (T 207), 1% NaOH solubility (T 212), alcohol-benzene solubility (T 204), dichloromethane (DCM) solubility (T 204), and ash (T 211, T 266).

#### *Elemental analyses and microscopy*

Analysis of the elements in samples was carried out by means of LEO SUPRA 55VP scanning electron microscope (SEM) interfaced with energy dispersive analysis of X-ray (EDAX).

## RESULTS AND DISCUSSION

### Physical Characteristics

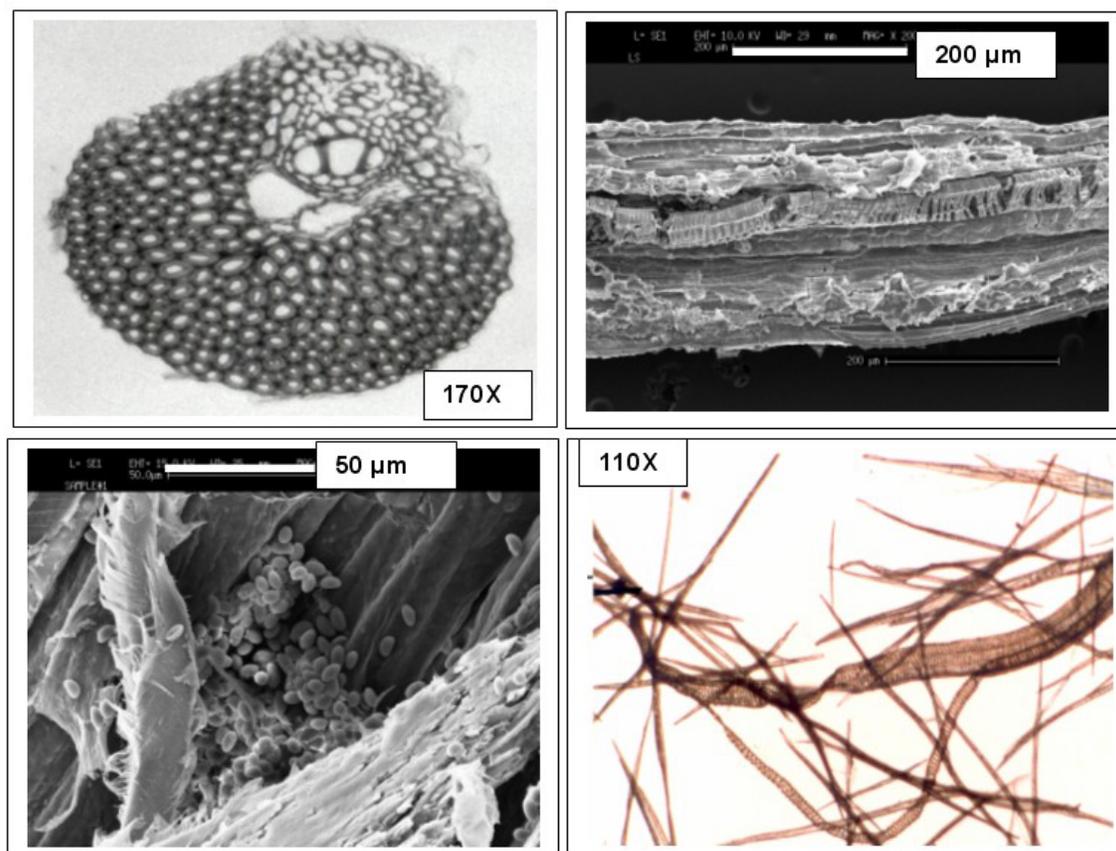
In cross-sectional view one can observe usually three large vessel elements in the core region of a vascular bundle (Fig. 2, left, top). These highly pitted vessel elements are clearly shown in longitudinal view (Fig. 2, right-top). Fibers and vessels of various sizes are presented in the bottom-right photograph of Fig 2. Physical characteristic of the fibrous elements are shown in Table 1. In comparison with those from a Canadian trembling aspen, the OPEFB fibers have similar length cell diameter but much thicker cell wall and, as a result, higher coarseness and rigidity index. Consequently, they yield paper with higher bulk or lower sheet density and inferior tensile strength when compared to paper made from aspen fibers (Law and Jiang, 2001). However, due to the presence of the particularly long vessel elements, they give considerably high tear index. Noteworthy, granules of starch are found in the interior of the vascular bundle (Fig. 2, left, bottom); their presence was identified using an iodine solution.

**Table 1.** Fiber Properties

Property	OPEFB fiber	Aspen fiber
Length-weighted fiber length, mm	0.99	0.96
Fiber diameter ( <i>D</i> ), $\mu\text{m}$	19.1	20.8
Cell-wall thickness ( <i>T</i> ), $\mu\text{m}$	3.38	1.93
Fiber coarseness, mg/m	1.37	1.01
Fines (<0.2 mm), % (arithmetic mean)	27.6	18.1
Rigidity index, $(T/D)^3 \times 10^{-4}$	55.43	7.99

### Chemical Constituents

The chemical constituents in OPEFB are presented in Table 2. Note that differences in magnitudes exist in comparison with the findings of other researchers. These variations could arise depending on the sources of raw material and on the historic treatments the material had received prior to laboratory analyses. The extent of pre-treatment such as washing, a standard process step, could influence significantly water-soluble and 1% NaOH-soluble components. The content of acid-insoluble lignin, which has poor solubility in water, is comparable to those reported by other workers (Khoo and Lee, 1991, Law and Jiang, 2001) and is similar to that of most common hardwood species (Berzins, 1966).



**Fig. 2.** Cross-section view of a fibrous strand (left, top, 170X), longitudinal view (right, top), interior view showing starch granules (left, bottom), and fibrous elements (right, bottom, 110X).

In addition to its organic component the OPEFB is rich in inorganic elements (Table 2). The presence of silica and other metals could complicate pulping and bleaching of this raw material. Silica is harsh on treatment equipment and complicates the recovery of spent liquor (Pan and Learly, 2000; Kulkarni et al., 2005; Tutus and Eroglu, 2003). Metals in raw material can interfere with pulping and bleaching chemicals, particularly in processes where hydrogen peroxide is used (Prasakis et al., 1996; Heikkila and Vuorinen, 2000; Zhao et al., 1992; He et al., 2003).

### Surface Characteristics of OPEFB Strand

Silica-bodies are found in great number on OPEFB strand (Fig. 3, left). They attach themselves to circular craters which are spread relatively uniformly over the strand's surface. The round-shaped spiky silica-bodies measure about 10-15 µm in diameter. These bodies are not detectable with EDAX or seen under SEM at the interior (Fig. 3, right). However, minute pit-like conduit-openings are observable at the interior; they might probably be the siliceous pathways connecting the interior to the surface. The silica-bodies (Fig. 4, top) are hard but can be dislodged mechanically, e.g. hammering the OPEFB strands in a Ziplock LDPE bag with a Staccato Hammer.

The shape, size and distribution pattern of silica bodies observed on fiber strands of OPEFB of *Elaeis guineensis* are strikingly similar to those in the epidermis of oil palm leaf (*Syagrus coronata*) as reported by Lins et al (2002). These researchers also reported that silicon and oxygen are two elements present in the silica bodies.

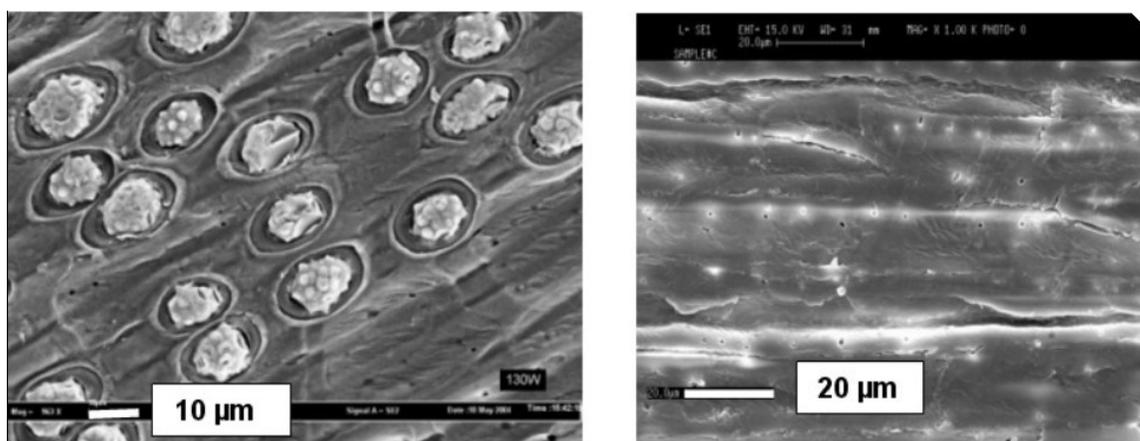
The deposition of silica bodies in biologically well engineered craters is a unique feature, contrasting with the usual extra- and intra-cellular silica deposition (Neumann and De Figueiredo, 2002; Richmond and Sussman, 2003). The presence of well engineered craters with perforated bottoms (Fig. 4) suggests that the deposition of silica on fiber strand surfaces is a predetermined biological process, not a random occurrence. Such genetic arrangement underscores the biological need of oil palm trees, the purpose of which, speculatively, might be multifunctional and beyond the nutritional requirement,

Table 3 shows the changes in silica content after mechanical treatment (hammering) and washing. Hammering reduced the silica content by 11.1% or 12.9% when adjusted to the yield of 98%. The action of hammering and washing combined gave greater loss in silica, 22.2% or 26.6% when the final yield of 94% was taken into account. Note that, for the hammered sample, the EDAX gave higher, about two fold, silica content than the TAPPI method did. This difference is suspected to come from variation in sample homogeneity, since no such difference occurred in the hammered and washed sample, as shown in Table 3.

**Table 2.** Chemical Constituents in OPEFB

Constituent	% of dry OPEFB	Reference	
		Khoo & Lee 1991	Law & Jiang 2001
Extractives	3.7 ± 0.3	0.9	2.8
Acid-insoluble lignin	18.8 ± 0.3	17.2	17.6
Ash-free acid-insoluble lignin	17.8 ± 0.2	-	-
Ash	1.3 ± 0.2	0.7	3.8
Hot-water soluble	7.5 ± 0.8	2.8	9.3
1% NaOH soluble	14.5 ± 2.7	17.2	29.9
Holocellulose	82.4 ± 1.4	70.0	86.3
Cellulose	62.9 ± 2.0	42.7	-
Hemicellulose	28.0	32.5 (Leh, 2002)	-
Arabinose	2.5 ± 1.1	-	-
Xylose	33.1 ± 2.6	-	-
Mannose	1.3 ± 0.01	-	-
Galactose	1.0 ± 0.0	-	-
Glucose	66.4 ± 3.7	-	-
		Singh et al.,	1999
Silica (EDAX)	1.8 (atomic)	-	
Silica (TAPPI method)	0.9 ± 0.1		
Copper	0.8 ± 0.7 g/g	23 mg/L	
Calcium	2.8 ± 0.1 g/g	0.25% (CaO)	
Manganese	7.4 ± 0.4 g/g	48 mg/L	
Iron	10.0 g/g*	473 mg/L	
Sodium	11.0 ± 0.4 g/g	-	

\* Only found in one sample



**Fig. 3.** Surface of OPEFB strand with silica bodies (left) while the interior is lack of visible silica bodies (right).

The removal of silica-bodies revealed that the bottom of the silica-crater is perforated (Fig. 4, bottom), which could suggest that the formation of silica might have originated from the interior of the fibrous strand through the probable siliceous pathways mentioned above. It is, however, unclear at this point whether the perforations are intra-cellular or extra-cellular in nature. However, the removal of silica-bodies would enhance chemical penetration in pulping.

**Table 3.** Effect of Mechanical Treatment on Silica Content

Silica	Amount in treated o.d. OPEFB	*Change %	
		Raw	Corrected**
TAPPI method	0.8 %	- 11.1	- 12.9
EDAX	1.3 % (Atomic)	- 27.8	- 29.2
Yield	98 %		

\* Relative to untreated OPEFB

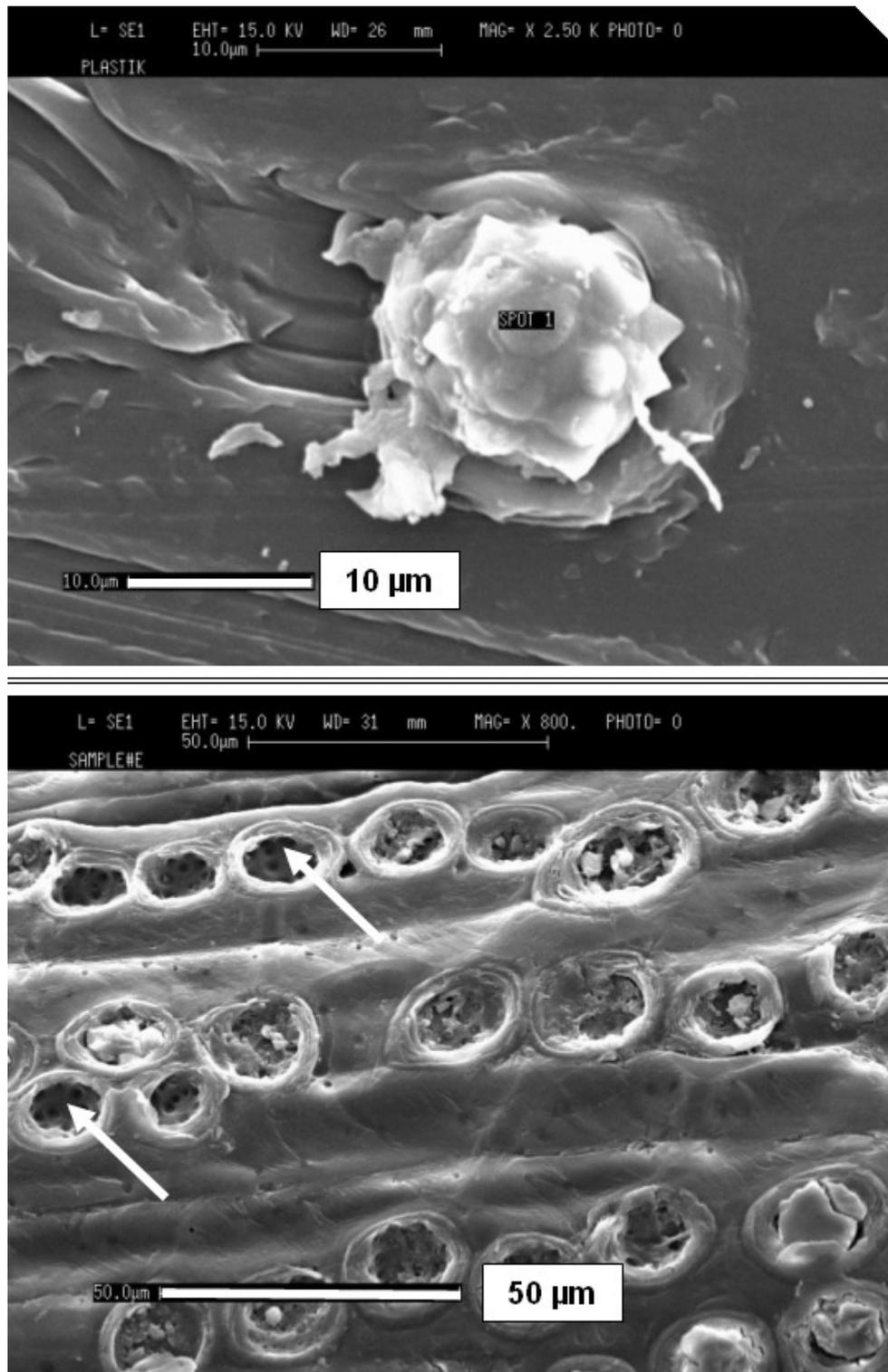
\*\* Corrected for yield

**Table 4.** Combined Effect of Hammering and Washing on Silica Content

Silica	Amount in treated o.d. OPEFB	*Change %	
		Raw	Corrected**
TAPPI method	0.7 %	- 22.2	- 26.6
EDAX	0.7 % (Atomic)	- 61.1	- 63.3
Yield	94 %		

\* Relative to untreated OPEFB

\*\* Corrected for yield



**Fig. 4** . A dislodged silica-body attached to plastic film (top). Perforations at bottom of silica-body craters (bottom, arrows).

### Minerals in OPEFB Strand

As stated earlier, hydrogen peroxide can be decomposed by the minerals present in the raw material to be processed, deactivating its chemical efficiency. Hence, in pulping or bleaching that involves the use of hydrogen peroxide, it is essential to sequester these minerals beforehand. Here, we studied the combined effect of hammering-washing-DTPA (diethylenetriaminepentaacetic acid) treatment on mineral content in OPEFB strand. The idea of DTPA treatments was to remove soluble minerals from OPEFB strand and to deactivate the non-acid-soluble minerals, as these were potential hindrance of efficient peroxide pulping or bleaching system. The results are presented in Table 5. Most of the elements evaluated were efficiently reduced, except for calcium, whose removal rate was comparatively poor, about 54%. The silica removal as determined by gravimetric technique showed low removal (28%). However, the EDAX method showed an 83% reduction.

The hammered-washed and DTPA-treated OPEFB strand was further treated with alkali peroxide by compression-decompression impregnation using a liquor-to-OPEFB ratio of 10. The chemical composition of the treatment liquor was similar to that used by Law et al. (1994). The treated OPEFB strand was heated at 70 °C for 60 min. The resulting surface of the strand was free of silica-bodies, exposing the craters (Fig. 5).

**Table 5.** Effect of Hammering-washing and DTPA Treatment on Mineral Content

Mineral	Amount in Treated OPEFB	Change*, %	
		Raw	Corrected**
Metals by AAS:			
Iron	0.5 g/g	-	- 94.5
Manganese	0.4 ± 0.0 g/g	- 95.0	- 95.0
Calcium	1.4 ± 0.1 g/g	- 49.3	- 54.1
Copper	0 g/g	- 100	- 100
Sodium	0.8 ± 0.6 g/g	- 93.3	- 93.6
Silica (TAPPI gravimetry)	0.7 ± 0.1 g/g	- 14.1	- 28.6
Silica (EDAX)	0.4 % (atomic)	- 79.6	- 83.3
Yield	92 %		

\* Relative to untreated OPEFB

\*\* Corrected for yield

### Morphology of OPEFB Ash

Burning of OPEFB strand yielded fascinating feature of the residue. The ash was in close resemblance to fiber, in shape and length. This might indicate a high affinity of lignin towards metals and thus, the shape of the ash of lignin coated fiber. The observation might also explain the association of metals with cellulose as claimed by Mirshokraie and Abdulkhani (2004).

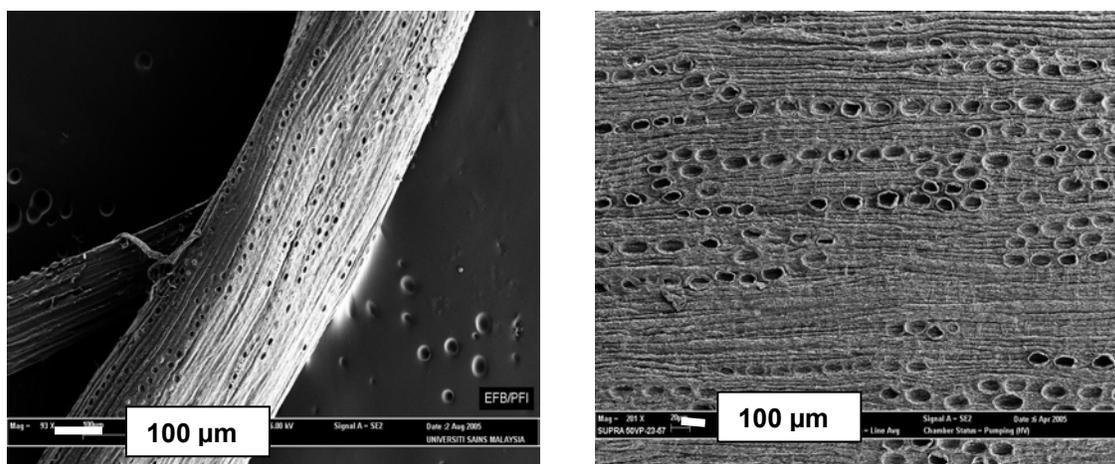


Fig. 5. Surface characteristics after alkali peroxide treatment.

Figure 6 (left, top) represents the ash of untreated natural OPEFB strand. Amazingly, the residue appeared as a perfect reflection of the original fibrous strand. In addition to the fiber-like residue of minerals one can observe the spherical-shaped silica-bodies. The right-top SEM micrograph shows the residue of a handsheet prepared from fibers produced in a simulated alkali-peroxide pulping process. On this micrograph one can easily identify several large vessel elements. A close-up view of the pitted-structure is shown in the left-bottom micrograph. One might also encounter a massive layer of silica-bodies atop a layer of minerals, as shown in the right-bottom micrograph.

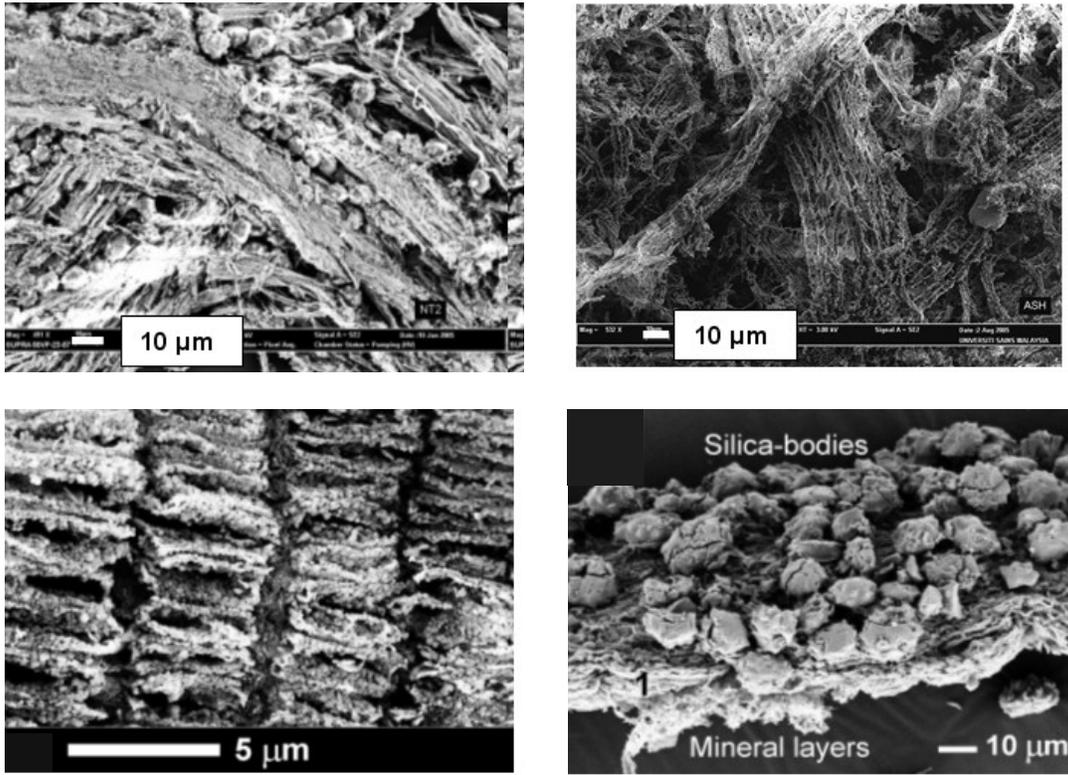
The principal elements of a mixed ash residue determined by EDAX are listed in Table 6, which shows that the main components are carbon and oxygen.

Table 6. Elements in mixed ash of OPEFB

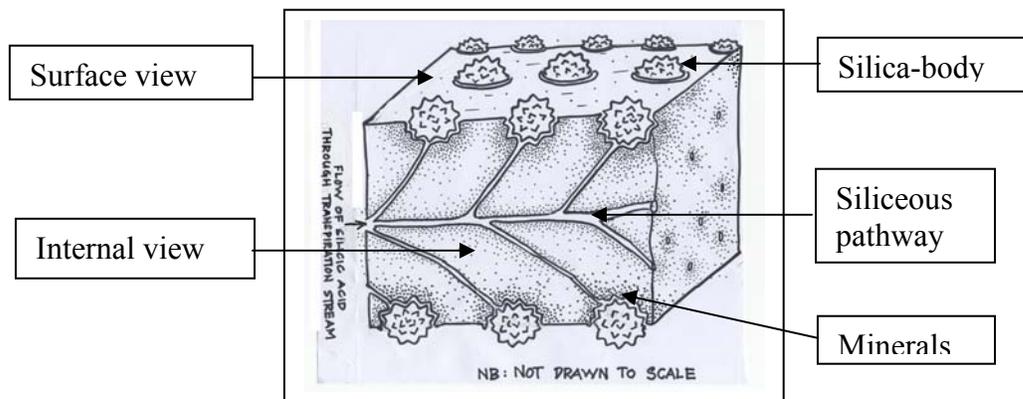
Elements	Atomic %
Carbon	46.5
Oxygen	45.2
Magnesium	1.3
Silica	5.5
Sulfur	-
Calcium	0.7
Potassium	-
Copper	0.3

### Organization of Minerals and Silica in OPEFB Strand

Setting aside the morphological aspects of an OPEFB strand, and based on the observed physical and chemical characteristics, a hypothesized scheme of the organization of silica-bodies and other minerals is depicted in Fig. 7. Our hypothesis suggests that there exists a network of siliceous pathways within the fibrous strand matrix and that during the growth season basic silica element is transported through this pathway and deposited onto the craters on the surface of the strand. The hypothesis also implies that minerals are distributed along the pathway and concentrated adjacent to the silica-bodies.



**Fig. 6.** Fiber-like ash residue of untreated OPEFB (left, top) and handsheet made from alkali peroxide treated fibers (right, top). Ash resembling pit structure of DPTA treated vessel (left, bottom). Silica-bodies encountered atop mineral layer (right, bottom).



**Fig. 7.** Hypothesized organization of minerals and silica-body in the matrix of an OPEFB strand: Minerals are indicated by dots.

## CONCLUSIONS

1. The vascular strand of OPEFB is unique in structure; it has several large-diametered and long vessel elements in its core region surrounded by vascular fibers.
2. It has large number silica-bodies attached to craters on its surface; the craters have perforated bottoms.
3. The silica-bodies are hard but can be dislodged mechanically from their craters.
4. Large amounts of the minerals including silica can be removed by combined actions of hammering, washing, and DPTA treatment.
5. The principal elements of mixed ash residue determined by EDAX are carbon and oxygen.

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## THE INFLUENCE OF NATURAL AROMATIC COMPOUNDS ON THE DEVELOPMENT OF *LYCOPERSICON ESCULENTUM* PLANTLETS

Anca Bălaș\* and Valentin I. Popa

The influences of flax lignin and phenolic compounds obtained from spruce bark on the development of *Lycopersicon esculentum* plantlets were evaluated. Depending on the applied treatment and the concentrations used, the natural aromatic compounds had stimulatory effects on the germination capacity and the plantlets height and leaf area. The influence of lignin on *Lycopersicon esculentum* was lower compared to the phenolic extract.

*Keywords:* Spruce bark, Polyphenolic compounds, Flax lignin, *Lycopersicon esculentum*

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### INTRODUCTION

In recent years, a special concern has been manifested towards “green chemistry.” Some of the effort has been based on the use of new waste sources, with the aim to obtain biologically active compounds which can be applied in different fields. These natural compounds are compatible with the environment and could provide the sources for specialty chemicals (Popa et al. 2006). In this context wood bark, which represents an important part of the tree, resulting in large amounts after the processing of logs for the pulp and paper industry, could be taken into account as a valuable raw material. The majority of this raw material is used as fuel, since its processing is expensive due to transport and maintenance costs (Hemingway 1997). Based on the present knowledge of the bark components, this raw material can be used as a source of various separated extractives with important application potential. Thus we proposed a procedure which allows turning to good account the wood bark after a scheme of complex processing (Simionescu et al. 1987) assuming the separation of each component. In the first stage, it was possible to separate polyphenols, which can be used as substitutes for phenols or phenolic resins in the wood processing industry (Simionescu et al. 1988a). Lignocelluloses resulting from extraction may represent products which can be introduced in composite structures, after different treatments (Simionescu et al. 1988b). We have also demonstrated that crude spruce extract may have a positive influence on seed germination and plants growth, manifesting similar effects to the endogenous hormones auxins and cytokinins (Simionescu et al. 1991, Popa et al. 1996, Popa et al. 2002, Bălaș et al. 2005).

On the other hand, lignin resulting from the chemical processing of wood or from lignocelluloses after polyphenols extraction is known to be involved in a series of chemical transformations modifying the soil fertility. In this process, the soil

microorganisms have important roles in these processes, due to their capability to modify and metabolize these compounds, with the formation of humic acids (Popa 1983). We observed that the introduction of lignin in sandy soil had a favorable influence on the growth of *Phaseolus vulgaris* plantlets, stimulating the growth speed and the quantity of green and dry biomass, as well as the productivity of obtained seeds (Dumitru et al. 2003).

In this paper, the results concerning the influence of spruce bark polyphenols and flax lignin on *Lycopersicon esculentum* plantlets are presented. The effects of polyphenolic extract were tested using different addition times: at the beginning of the experiment and in constant doses. The percentages of established plantlets, the variation of height, and leaf area during the experimental time were evaluated.

## EXPERIMENTAL

### Obtainment of Phenolic Compounds

The phenolic extract was obtained according to Simionescu et al. (1989). Spruce bark, provided by a Romanian pulp and paper company, was air-dried and ground in a mill. The lipophilic compounds were extracted in a Soxhlet apparatus with ethyl ether. The phenolic compounds were separated with 1.5% NaOH, for three hours at 90°C, and at a liquor-to-wood ratio of 10. The liquid fraction was passed over an ion-exchange resin (Vionit SR3) in order to obtain a neutral pH for the extract and to eliminate the sodium ions. After filtration, the neutralized extract was dried under vacuum at 40°C, obtaining a brown colored powder.

Lignin used in the experiments was obtained from flax alkaline delignification and contained 3.07 % hemicelluloses (Granit - Lignin, Switzerland, type PF 30-35 Flax Soda Pulping).

### Bioassay

Seeds of *Lycopersicon esculentum* L. cv. A106/25, collected during 2004, were commercially available products. The seeds were planted in vegetable pots in sand, 20 variants per sample. The plantlets growth was followed using aqueous solutions of the spruce extract at different concentrations: 0.04, 0.08, 0.16, and 0.32 g/L and diverse additions of bioactive compounds:

- Single treatment with crude extract and every 5 days with distilled water.
- Repeated treatment with phenolic extract.

The influence of lignin on plantlets growth was tested at different dosages: 100, 150, 200, and 250 kg/ha. In both cases, control tests were performed under the same conditions using distilled water.

Plantlets development was observed for 30 days from seedling, and the number of established plants, their height, and leaf area were followed. The leaf area was determined according to Schwarz et al. (2001).

In the graphics, data are reported as percentage differences from control. Thus, zero represents the control; positive values represent stimulation of the parameter studied,

and negative values represent inhibition. The characteristics for the control samples are presented in table 1.

**Table 1.** Parameters of the control samples

Period (days)	15	20	25	30
Height (cm)	2.29	2.65	3.23	3.53
Leaf area(cm <sup>2</sup> )	0.05	0.09	0.14	0.15

### Statistical Analysis

Statistical analysis was performed using ANOVA one way test and the level of significance was set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### The Influence of Spruce Extract on Plant Growth

The global extract had different influences on the *L. esculentum* plantlets, depending on the concentrations of bioactive compounds and the treatment applied during the experimental period.

Single treatment with spruce extract at a concentration of 0.04 g/L stimulated the number of established plants by 10% (Fig. 1 A). The concentration of 0.08 g/L induced a strong inhibitory effect on the number of established plantlets, but increasing the concentration of bioactive compounds reduced the studied characteristic.

Repeated treatment with phenolic extract had lower influence on the germination capacity in comparison to the first experimental lot (Fig. 1 B). In this case, the increase of concentration was correlated with the inhibition of the established plantlets.

The single treatment with phenolic compounds at concentration 0.04 g/L increased the plantlets' height by a factor of 20 %, while the other concentrations tested had slight effects (Fig. 2 A). The continuous treatment with spruce extract had strong stimulatory effects at concentrations 0.04 g/L and 0.08 g/L, and inhibitory influence at a concentration 0.16 g/L (Fig. 2 B).

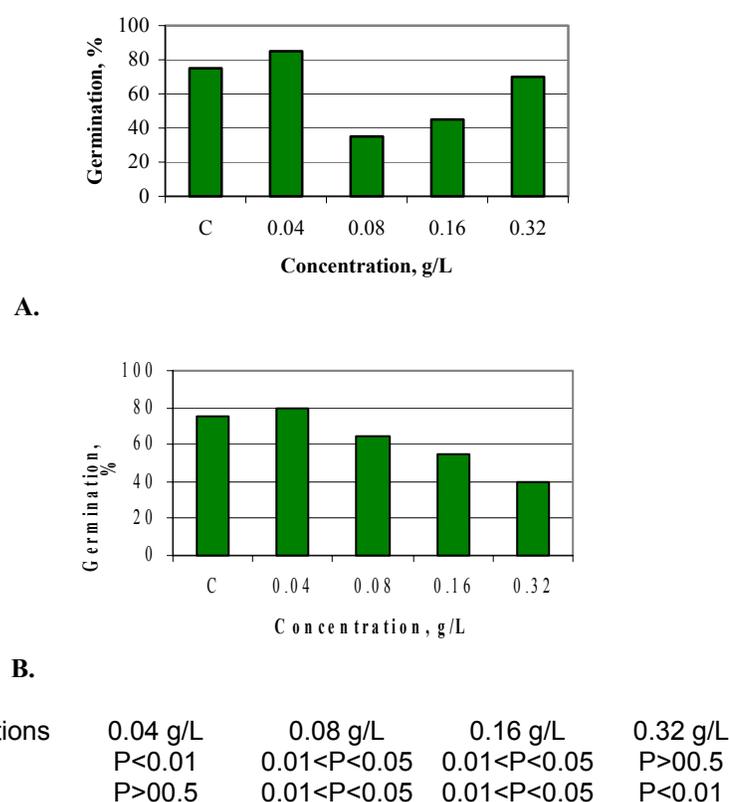
The leaf area was estimated using a mathematic model, without destruction of the plant. In the first experimental group, the leaf area was stimulated at a concentration of 0.08 g/L, while the other concentrations tested had slight effects of the studied feature (Fig. 3 A). The repeated treatment with solutions containing 0.04 g/L and 0.32 g/L spruce extract had stimulatory effects on leaf area (Fig. 3 B). The concentration of 0.16 g/L in bioactive compounds induced a strong inhibitory effect.

The single treatment with phenolic extract had a positive influence on the established plantlets of *L. esculentum*, while the repeated use of polyphenols had better influences on plantlets' height and leaf area.

Due to the complex composition of the spruce bark extract, the growth processes can be influenced by many factors, including the synergism of polyphenols or the presence in small amounts of compounds with strong bioactivity. However, the influence of every compound from spruce bark has not been established, making it difficult at this moment to elucidate the mechanism in which the growing processes in plants are modified.

Our previous data (Simionescu et al. 1991) showed that polyphenols extracted from spruce bark have some effects similar to endogenous plant hormones, depending on the dosage used in the experiments of cells tissue culture. At present we have obtained information about the composition of polyphenols existing in crude spruce bark extracts (Bălaş A. unpublished data). At the same time, some experiments are developed to establish a correlation between the structure of polyphenols and their capacity to influence plant development and different enzymatic system biosynthesized by plants.

Therefore, it is possible for polyphenols to interact or interfere with hormones produced by plants, influencing thus growth process. One cannot exclude the possibility that polyphenols may be transformed or destroyed at the soil level during plant development.

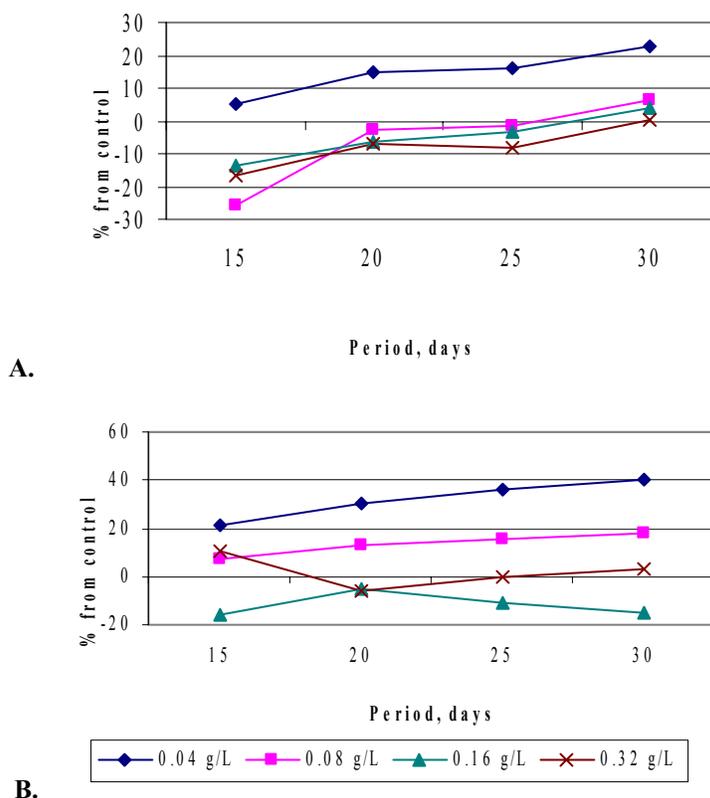


**Figure 1.** The influence of spruce extract on established plantlets at different treatment: single (A) and repeated (B). F values for each concentrations used and treatments.

### The Influence of Lignin on Plant Growth

The presence of lignin at dosages of 100 kg/ha and 200 kg/ha had stimulatory effects on the number of established plants (Fig. 4). The growth speed of *L. esculentum* plantlets was accelerated in the first 20 days of the experiments, but reduced in the next 10 days (Fig. 5). After 30 days, the plantlets' height had increased by 10 % at dosage 150 kg/ha and with 5 % for concentrations 100 kg/ha and 200 kg/ha. The addition of 250 kg/ha of lignin had a slight inhibitory effect on the plantlets' height. The most significant influences on leaf area were observed at concentrations 100 kg/ha and 200 kg/ha. These

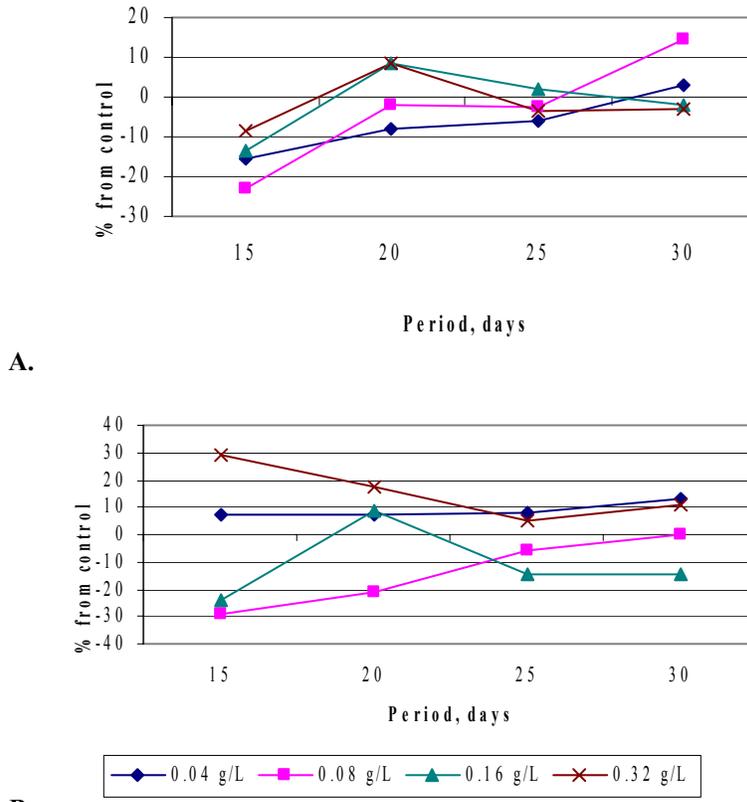
results are in concordance with those previously obtained in the case on bean cultivation (Dumitru et al. 2005). In this case, some observations have been made concerning the accumulation and developing of some microorganisms in sandy soil treated with lignin.



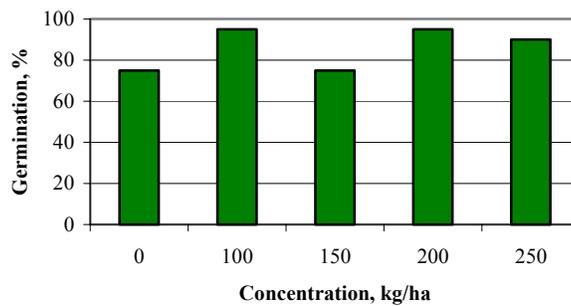
**Figure 2.** Variation of plantlets' height compared to control for single (A) and repeated (B) treated with spruce extract. Values are presented as percentage differences from control.  $P < 0.01$  (A) and  $0.01 < P < 0.05$ .

At the same time cultivation of the plants and following the whole vegetation cycle allowed us to obtain information regarding the productivity. Evidence of an interesting phenomenon, from genetic point of view, was observed, concerning the regulation of cell division.

The influence of lignin on plants' development was reduced in comparison to the polyphenolic extract, and these effects can be correlated with its low accessibility in medium. The modifications of the studied parameters can be explained by the structural lignin modification under the influence of soil microorganisms, which can lead to the formation of degradation products with stimulatory effects on plant growth. Previous data showed that the presence of lignin has a beneficial influence on plantlets development even after a short period of time, (Dumitru et al. 2003; Popa et al. 2005). Also, the plantlets' growth may be influenced by the hemicelluloses content of the lignin extract, which can be degraded by soil microorganism, thus contributing to the fertilization.

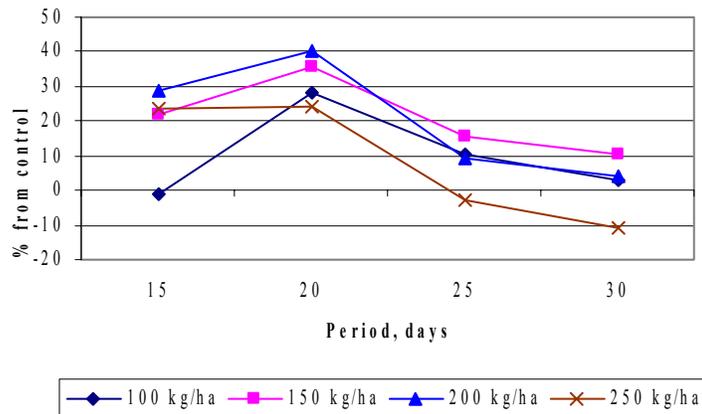


**Figure 3.** Variation of leaf area compared to control for single (A) and repeated (B) treatment with spruce extract. Values are presented as percentage differences from control. P<0.01

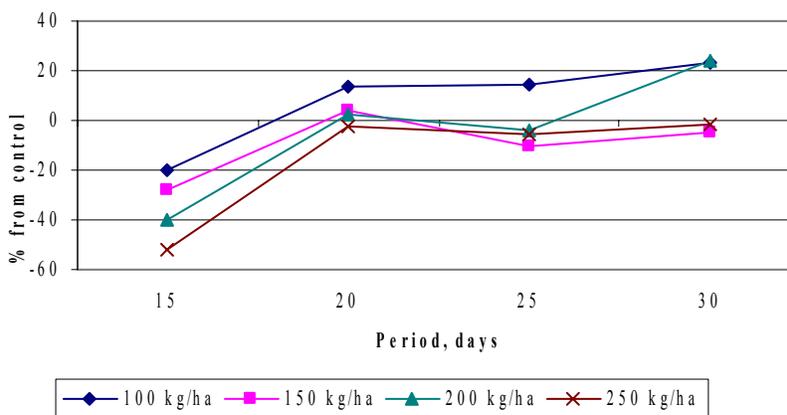


Concentrations	150 kg/ha	150 kg/ha	200 kg/ha	250 kg/ha
F values	P<0.01	P>0.05	P<0.01	P<0.01

**Figure 4.** The influence of lignin on established plants of *L. esculentum*. F values for each concentrations used and treatments.



**Figure 5.** Variation of plantlets' height under the influence of lignin. Values are presented as percentage differences from control.  $P < 0.05$ .



**Figure 6.** Variation of leaf area under the influence of lignin. Values are presented as percentage differences from control.  $P < 0.05$ .

## CONCLUSIONS

1. The single treatment with spruce bark extract had positive effects on the number of established *L. esculentum* plantlets.
2. Repeated treatment with polyphenols had a beneficial influence on the plantlets' height and leaf area.
3. In both cases, at concentrations of 0.04 g/L the bioactive compounds induced better development of *L. esculentum* plantlets compared to the control.
4. Lignin had a positive influence on the plantlets growth at 100 kg/ha and 200 kg/ha, but lower compared to spruce extract.
5. The obtained results will be further developed with the aim of establishing the role of polyphenols in biosynthesis processes and their influence on genetic characteristics. A correlation of these parameters with structure of polyphenols will be investigated.

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## THE EFFECT OF CHEMICALLY COATED NANOFIBER REINFORCEMENT ON BIOPOLYMER BASED NANOCOMPOSITES

Bei Wang, and Mohini Sain\*

The aim of this work was to explore how various surface treatments would change the dispersion component of surface energy and acid-base character of hemp nanofibers, using inverse gas chromatography (IGC), and to investigate the effect of the incorporation of these modified nanofibers into a biopolymer matrix on the properties of their nanocomposites. Bio-nanocomposite materials were prepared from poly(lactic acid) (PLA) and polyhydroxybutyrate (PHB) as the matrix, and the cellulose nanofibers extracted from hemp fiber by chemo-mechanical treatments. Cellulose fibrils have a high density of –OH groups on the surface, which have a tendency to form hydrogen bonds with adjacent fibrils, reducing interaction with the surrounding matrix. It is necessary to reduce the entanglement of the fibrils and improve their dispersion in the matrix by surface modification of fibers without deteriorating their reinforcing capability. The IGC results indicated that styrene maleic anhydride coated and ethylene-acrylic acid coated fibers improved their potential to interact with both acidic and basic resins. From transmission electron microscopy (TEM), it was shown that the nanofibers were partially dispersed in the polymer matrix. The mechanical properties of the nanocomposites were lower than those predicted by theoretical calculations for both nanofiber-reinforced biopolymers.

*Keywords:* Cellulose nanofibers, Nanostructure, Microfibrils, Biopolymers, Inverse gas chromatography

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### INTRODUCTION

Minutization is a continuing trend in the development of technology. The prefix “nano” has become applied to new classes of materials intended for manufacturing, e.g. nano-materials and nanocomposites. Unfortunately, not many of the most recent developments of this nature are able to satisfy the core concept of sustainability. One way to address issues related to sustainability is to incorporate renewable materials as miniaturized elements of construction materials (Sain and Oksman 2006). The backbone of a plant or tree is a polymeric carbohydrate with an abundance of tiny structural entities known as “cellulose fibrils”. These fibrils are comprised of different hierarchical microstructures commonly known as nano-sized microfibrils, having high structural strength and stiffness (Wang and Sain 2007). Biopolymers from renewable resources have attracted much attention lately. Renewable sources of polymeric materials offer an

answer to maintaining sustainable development of economically and ecologically attractive technology. In recent years, scientists and engineers have been working together to use the inherent strength and performance of these nano-fibrils, combined with natural green polymers, to produce a new class nano-materials.

Poly(lactic acid) (PLA) is a class of crystalline polymers with relatively high melting point (Mohanty et al. 2000). Recently PLA has been highlighted because of its availability from renewable resources such as corn and sugar beets. PLA is synthesized by the condensation polymerization of D- or L-lactic acid or ring-opening polymerization of the lactide (Lunt 1998). Advanced industrial technologies of polymerization have been developed to obtain high molecular weight pure PLA, which leads to a potential for structural materials with enough lifetime to maintain mechanical properties without rapid hydrolysis. Poly( $\beta$ -hydroxybutyrate) (PHB) is a biotechnologically produced polyester that constitutes a carbon reserve in a wide variety of bacteria and has attracted much attention as a biodegradable thermoplastic polyester (Holmes 1988). However, it suffers from some disadvantages compared with conventional plastics, for example, brittleness and a narrow processability window.

Many studies have been done on extracting cellulose microfibrils from various natural sources and on using them as reinforcement in composite manufacturing (Bhatnagar and Sain 2005; Chakraborty et al. 2006; Nakagaito and Yano 2005; Sain and Bhatnagar 2003). The use of cellulose nanofibers as nanoreinforcement is a new field in nanotechnology, and as a result there are still some disadvantages. Firstly, the separation of nanoreinforcement components from natural materials and the associated processing techniques have been limited to the laboratory scale (Oksman et al. 2006). Secondly, the fiber isolation process consumes a large amount of energy, water, and chemicals. The production is time-consuming and is still associated with low yields. Thirdly, due to their strong hydrogen bonding between cellulose chains, it is necessary to reduce the entanglement of the fibrils and improve their dispersion in the solid phase polymer matrix by surface modification of nanofibers without deteriorating their reinforcing capability. It has been reported that the surface modifications of cellulose nanofibers to make them compatible with non-polar solvents or non-polar polymers. Such an approach has been attempted for polyolefins and other commodity polymers (Goussé et al. 2004). The treatment of the fibers may be by bleaching, grafting of monomers, acetylation, and so on. In this way, high performance composite materials can be processed with a good level of dispersion. Interaction of cellulose with surfactants has been another way to stabilize cellulose suspensions into non-polar systems (Heux et al. 2000).

Poor interfacial adhesion between nanofibers and the polymer matrix leads to a decline in mechanical properties of nanocomposites. In recent years a deeper understanding has been achieved related to surface phenomena. This has led to an introduction of more sophisticated approaches, which allow for a study of thermo-dynamic and kinetic information. One technique, which has been shown to be very valuable, is inverse gas chromatography (IGC). In IGC, a solid material under investigation is used as the stationary phase. An empty column is filled with the (porous) material (adsorbent) and the adsorbate molecules in the mobile phase probe the surface of the adsorbent (Thielmann 2004). The surface energy of a material can be described by the sum of a dispersion component and a specific interaction component (Gulati and Sain 2006). The

dispersion component refers to London dispersion forces, and the specific component refers to the polar, ionic, electrical, magnetic, metallic, and acid-base interactions. Fowkes and Mostafa (1978) proposed that dispersion forces and acid-base interactions are the primary forces operating across the interface. IGC is an alternative method for measuring the changes in the thermodynamic properties of a nanofiber surface after treatment and for estimating the London dispersion component of the surface free energy of nanofibers (before and after treatment). Gulati and Sain (2006) reported that alkalization and acetylation make the hemp fibers amphoteric, thereby improving their potential to interact with both acidic and basic resins.

The goal of this work was to explore how various surface treatments would change the dispersion component of surface energy and the acid-base character of hemp nanofibers, using IGC. The cellulose nanofibers were extracted from hemp by chemo-mechanical treatments. PLA- and PHB-based nanocomposites using cellulose nanofibers were prepared by injection molding and hot compression. The cellulose nanofibers used in this study were treated by five different chemicals. Uncoated cellulose was used as a reference. Transmission and scanning electron microscopy were used to investigate the nano-structure of the nanocomposites and the dispersion of fibers within the matrix. The potential use of chemically coated nanofibers as reinforcing agents in biocomposites was also explored. The mechanical properties of the nanocomposites were studied by means of tensile testing.

## EXPERIMENTAL

### Materials

#### *Matrix*

Poly (lactic acid) (PLA), Nature Works™ 4031D, was supplied by Cargill Dow LLC, Minneapolis, USA. The material has a density of 1.25 g/cm<sup>3</sup>, a glass transition temperature ( $T_g$ ) of 58 °C, and a melting point of 160 °C. Polyhydroxybutyrate (PHB), Biomer-P226 biodegradable polymer, was supplied by Biomer, Krailling, Germany. The material has a density of 1.17 g/cm<sup>3</sup> and melting point of 173 °C.

#### *Reinforcement*

The raw material used in this study was hemp fibers (*Cannabis sativa L.*) from southwestern Ontario, Canada (Hempline Inc., ON). These fibers have diameters of approximately 22-25 μm and lengths of 15-25 mm. The cellulose nanofibers were extracted from hemp fiber by chemo-mechanical treatments. Isolated nanofibers were shown to have diameters between 50-100 nm and lengths in the micrometer scale, which results in a very high aspect ratio (87.5).

#### *Chemicals*

Reagent grade chemicals were used for fiber isolation and bleaching, namely, sodium hydroxide, hydrochloric acid, sodium chlorite, chlorine dioxide, peroxide, and sulfuric acid. Michem® Prime EAA (ethylene acrylic acid) copolymer dispersions-4983R (Michelman, Inc., Cincinnati, OH) was the dispersant, which exhibits excellent adhesion

to cellulosic substrates. Styrene Maleic Anhydride resins (SMA<sup>®</sup>) from Sartomer Company (Exton, PA) are low molecular weight styrene/maleic anhydride copolymers. Hydrophobic SMA resins are used as surface sizing compounds for paper and cross-linking agents for powder coatings. Kelcoloid HVF and LVF are stabilizers used for fiber coating. Kelcoloids (International Specialty Products, Wayne, NJ) are made of propylene glycol alginates (PGA), copolymers of mannuronic and guluronic acids. The key function of PGAs is to help stabilize an emulsion or high-solids suspension. Guanidine hydrochloride, 50940 BioChemika (Fluka Chemie AG, Buchs, Switzerland) was used for the fiber coating. It was originally designed for refolding of proteins.

## Methods

### *Nanofiber isolation*

The isolation of hemp nanofibers is a multi-step process. Chemical and mechanical treatments were applied to the fiber to make nanofibers. The chemical treatment included pre-treatment, acid hydrolysis, and alkaline treatment. The mechanical treatment was comprised of two parts: cryocrushing and high pressure defibrillation. Details of the nanofiber isolation process are outlined in the author's previous publication (Wang et al. 2007).

### *Nanofiber chemical coating*

Cellulose nanofibers were stored in water suspension after the chemo-mechanical isolation. Different types of chemicals were added to the suspension containing nanofibers in the proportion 1:2 (w/w), using an estimated weight of the cellulose nanofibers. In order to improve the dispersion of the coated nanofibers, the suspensions were prepared with continuous stirring by magnetic stirrer for 24 h at a room temperature. The suspensions containing nanofibers were freeze-dried in a Multi-Drier freeze-drying machine (Frozen in Time, Ltd.).

### *Processing of nanocomposites*

This project was focused on synthesizing nano-biocomposites, using PLA and PHB in the solid phase, by injection molding or hot compression. A solid-phase compounding method was used to mix the freeze-dried nanofibers with PHB in a high-intensity kinetic mixer (Werner and Pfleiderer Gelimat) at 3200 rpm with tip speed of 23 m/s. Product was discharged at a pre-set temperature of 150 °C. Test samples were compression-molded with a WABASH Hot Press into sheet form. The mold temperature was 180 °C, and the pressure was 50 MPa. PLA composites containing 5 wt.% SMA-coated nanofibers were prepared by melt blending the polymer with the fiber, using a Brabender mixer (C.W. Brabender Instruments Inc., NJ). The compounding temperature was 170 °C, and the rotating screw speed was 60 rpm for 5 min. Then the compound was granulated, using a C.W. Brabender Granulator (C.W. Brabender Instruments Inc., NJ). The granulates were then pre-heated to 100 °C for 1 h and injection molded using an Engel Injection molder (Model ES-28, ON, Canada) equipped with a standard ASTM mold for tensile, flexural, and impact test specimens. The typical injection molding conditions were: injection temperature 180 °C, injection time: 8 s, cooling time 25 s, and

mold opening time 2 s. All composites contained 5 wt.% loading of nanofibers with respect to total weight of the composite.

#### *Column preparation and IGC procedure*

IGC measurements were done with a Perkin-Elmer Autosystem XL Gas Chromatograph (GC) fitted with a flame ionization detector. To ensure flash vaporization, the injection port was kept at 423 K. All stationary phases, including 2-4g uncoated hemp nanofibers (HPN) or coated-HPN, were dried in an oven at 70 °C for 24h and packed under vacuum with a vibrator into a copper column (length 33 cm and internal diameter of 4 mm) of which the end was plugged with glass wool. The columns were maintained overnight at 105 °C in a nitrogen stream to remove moisture and other volatiles from the cellulose fibers before each experiment. The columns were first cleaned with acetone before use to get rid of greases used in copper processing.

The IGC probes used in the present study were chromatography grade solvents (Sigma-Aldrich). The probes were used without further treatment. Their physicochemical properties are listed in Table 1. Helium was used as the carrier gas. The corrected flow rate of helium was 10 mL/min. Small quantities of probes were injected into the column using Hamilton syringes. Peaks were found to be symmetrical and the area under each peak was directly related the amount adsorbed/desorbed. In the present study, the temperature dependence was determined within the temperature range 40 to 100 °C. Averages of three measurements were taken to calculate retention volumes, with air as the marker.

**Table 1.** Physicochemical Properties of the IGC Probes used In the Present Study (Schultz et al. 1987; Guttman 1983)

Probe	Area (A <sup>o</sup> 2)	$\gamma_1^d$ (mJ/m <sup>2</sup> )	DN	AN	Character
Hexane	51.5	18.4	0	0	Neutral
Heptane	57	20.3	0	0	Neutral
Octane	62.8	21.3	0	0	Neutral
Nonane	68.9	22.7	0	0	Neutral
Chloroform	44	25.9	0	23.1	Acidic
Ethyl Acetate	48	16.5	17.1	9.3	Amphoteric
Ethyl Ether	47	15	19.2	3.9	basic
Tetrahydrofuran (THF)	45	22.5	20.1	8	basic
Acetone	42.5	16.5	17	12.5	Amphoteric

#### *Microscopy characterization*

The nanostructure of the composites was examined in a transmission electron microscope (TEM), Hitachi H-7000 TEM at an acceleration voltage of 100 kV. To examine the nanocomposites, the samples were cut and polished to rectangular sheets, embedded in epoxy, and allowed to cure overnight. The final ultra-microtoming was performed with a diamond knife at room temperature, generating foils approximately 90 nm in thickness. These foils were gathered onto Cu grids.

A scanning electron microscope (JEOL JSM-840, Tokyo, Japan) (SEM) was used as a routine for microstructural analysis of the nanofibers with and without surface

coatings. All images were taken at an accelerating voltage of 15 kV. The sample surfaces were coated with a thin layer of gold on the surface, using an Edwards S150B sputter coater (BOC Edwards, Wilmington, MA) to provide electrical conductivity.

### *Tensile testing*

The mechanical behavior of nanofiber-blend-PHB film or nanofiber-blend-PLA nanocomposite was tested by an Instron 5860 (Grove City, PA) in tensile mode with a load cell of 2 kN or 30 kN in accordance with ASTM D 638. The specimens were cut in a dumbbell shape with a die ASTM D 638 (type V). Tensile tests were performed at a crosshead speed of 2.5 mm/min. The values reported in this work result from the average of at least 5 measurements.

## BACKGROUND

### Determination of the Acid-Base Characteristics of Lignocellulosic Surfaces by IGC

The surface energy of a material can be described by the sum of the London dispersion component and specific interactions. Thus, the work of adhesion can be written as,

$$W_a = W_a^d + W_a^{AB} \quad (1)$$

where  $W_a$ ,  $W_a^d$ , and  $W_a^{AB}$  are the total work of adhesion, the work of adhesion due to dispersion forces and acid-base interactions, respectively. Acid-base interactions are useful for surface modification (Dwight et al. 1990). Hence, in order to design new modification methods for improving fiber-matrix adhesion and meaningful interpretation of the existing methods, quantitative determination of surface acid-base characteristics of natural fibers is important. Data generated in this study explored surface modification for lignocellulosic fibers and their compatibilization with biopolymers.

### Background of IGC

IGC has become a widely used technique to characterize the surface properties of organic and inorganic materials. Acid-base probes are used to measure the acid-base characteristics of the solid surface, and saturated n-alkane probes are used to measure the dispersion component of the surface energy of interaction. In the present study, retention times of saturated n-alkane and acid-base probes injected at infinite dilution were used to calculate the dispersion component ( $\gamma_s^d$ ) of the surface energy, the free energy of adsorption ( $\Delta G^{AB}$ ), and the enthalpy of adsorption ( $\Delta H^{AB}$ ) corresponding to acid-base surface interactions. Papirer's approach, as described by Schultz et al. (1987; 1991), was used to estimate the acceptor ( $K_A$ ) and donor ( $K_D$ ) parameters of the test substrates.

The fundamental parameter in the IGC measurements is the specific retention volume,  $V_n$ , defined as the volume of carrier gas required to elute a probe from a column.  $V_n$  is related to experimental variables by the following equation,

$$V_n = F^*(T_r - T_o) \quad (2)$$

where  $T_r$  and  $T_o$  are the retention times of the probe and the air marker, respectively;  $F^*$  is the corrected flow rate of the carrier gas, defined as,

$$F^* = FJ \quad (3)$$

where  $F$  is the corrected gas flow rate in mL/min;  $J$  is the correction factor for the gas compressibility,

$$J = 1.5 [(P_i/P_o)^2 - 1]/[(P_i - P_o)^3 - 1] \quad (4)$$

where  $P_o$  is the carrier gas pressure at the column outlet, and  $P_i$  is the carrier gas pressure at the column inlet.

The interaction of neutral probes, such as saturated n-alkanes, with the substrate material is dominated by the van der Waals dispersion forces of interaction. Molar free energy of adsorption is related to net retention volume by the following relation,

$$\Delta G = RT \ln(V_n) + C \quad (5)$$

where  $R$  is the gas constant,  $T$  is the column absolute temperature, and the value of  $C$  depends on the reference state. The free energy of adsorption is related to work of adhesion by the following relation (Mukhopadhyay and Schreiber 1995),

$$\Delta G = NaW_a = 2Na(\gamma_s^d)^{1/2}(\gamma_l^d)^{1/2} + C \quad (6)$$

where  $N$  is Avogadro's number;  $a$  is the surface area of a single probe;  $W_a$  is the work of adhesion;  $\gamma_l^d$  is the dispersion component of the surface energy of the probe; and  $\gamma_s^d$  is the dispersion component of the total surface energy of the interacting solid. Combining equation (5) and (6), we get:

$$RT \ln(V_n) = 2Na(\gamma_s^d)^{1/2}(\gamma_l^d)^{1/2} \quad (7)$$

A plot of  $RT \ln(V_n)$  versus  $2Na(\gamma_l^d)^{1/2}$  should give a straight line with slope  $(\gamma_s^d)^{1/2}$  in the case of probes interacting only due to dispersion component of surface energy. From the slope of the straight line  $\gamma_s^d$  can be calculated.

The free energy of adsorption ( $\Delta G^{AB}$ ) corresponding to the specific acid-base interactions is related to the enthalpy of adsorption ( $\Delta H^{AB}$ ) by,

$$\Delta G^{AB} = \Delta H^{AB} - T\Delta S^{AB} \quad (8)$$

where  $\Delta S^{AB}$  is the entropy of adsorption corresponding to the specific acid-base interactions. A plot of  $\Delta G^{AB}$  versus  $T$  (temperature) should yield a straight line with intercept equal to  $\Delta H^{AB}$ . The enthalpy of adsorption corresponding to the specific acid-

base interaction is related to the acceptor and donor parameters,  $K_A$  and  $K_D$  of the fibers. According to Saint-Flour and Papirer (1982),

$$\Delta H^{AB} = K_A DN + K_D AN \quad (9)$$

where DN and AN are the donor and acceptor numbers, respectively, of the acid-base probe as defined by Guttmann (1983). A plot of  $\Delta H^{AB}/AN$  versus  $DN/AN$  should yield a straight line with slope  $K_A$  and intercept  $K_D$ . According to Schultz (1987) the specific interaction parameter,  $I$ , for acid-base interactions can be defined as,

$$I = K_A^f K_D^m + K_A^m K_D^f \quad (10)$$

where the superscripts  $f$  and  $m$  refer to fiber and matrix, respectively.

## RESULTS AND DISCUSSION

### Dispersion Component of The Surface Energy

Preliminary experiments were performed on the coated and uncoated cellulose powders to determine the optimum chromatographic conditions for reproducible measurements of the retention times of the probes. The chromatographic peak shape of each probe had to be as symmetrical as possible. The dispersion component of uncoated and chemically coated hemp fibers was calculated from a plot of  $RT \ln(V_n)$  versus  $2Na(\gamma_1^d)^{1/2}$ . The values for the dispersion component,  $\gamma_s^d$ , of the surface energy at different temperatures are summarized in Table 2.

**Table 2.** Dispersion Component,  $\gamma_s^d$ , of the Surface Energy of Lignocellulosic Particles at Different Temperatures

Material	$\gamma_s^d$ (mJ/m <sup>2</sup> )			
	313K	333K	353K	373K
Cellulose (Dorris and Gray 1979)	48	44	40	36
Uncoated HPN	42	40	34	28
SMA-Coated HPN	44	41	39	36
HVF-Coated HPN	46	44	43	38
LVF-Coated HPN	44	42	40	33
EAA-Coated HPN	46	43	41	37
Guanidium Hydrochloride Coated HPN	50	47	44	41
PLA	32	29	28	27
PHB	51	47	43	40

HPN: hemp nanofibers

The dispersion component of resin was also calculated similarly. The linear relationship vs.  $n$ -alkane chain length illustrates that this technique works well in case of natural fibers. Chemical treatments had the effect of increasing their respective  $\gamma_s^d$  values toward that of the cellulose powder. This is likely due to the dissolution of low energy surface impurities and surface exposure of relatively higher energy cellulose. In the

present study, the temperature dependence was determined in the temperature range 40 to 100 °C. Chemically coated fibers showed a negative temperature coefficient over this entire range due to chemical rearrangements. The London dispersion component was affected by the type of polymers and the treatment of fibers.

### Acid-base Interactions of the Surface Energy

Values of the free energy of adsorption,  $\Delta G^{AB}$ , corresponding to surface acid-base interactions are summarized in Table 3. The corresponding values of the enthalpy of adsorption,  $\Delta H^{AB}$ , determined from the plots of  $\Delta G^{AB}$  as a function of temperature (T) for all the probes in all cases, are given in Table 4. Some probes showed a negative acid-base free energy and enthalpy of adsorption on the cellulose. For example, the acid-base interaction between HVF-coated HPN (hemp nanofibers) and the ethyl ether probe was not favorable for adsorption. Considering that ethyl ether is basic (DN = 19.2), and the HVF-coated HPN used in this study was found to have a basic characteristic ( $K_D = 0.22$ ), this result is not surprising. A comparison of the enthalpy of adsorption between uncoated and coated fibers indicated that the interactions between the probes and SMA- and EAA-coated HPN were greater than those observed between the probes and uncoated HPN. The uncoated HPN had relatively low donor ( $K_D = 0.31$ ) and acceptor ( $K_A = 0.19$ ) parameters compared to the donor ( $K_D = 0.77$ ) and acceptor ( $K_A = 0.34$ ) parameters of SMA-coated HPN. The significant increase in the acceptor parameter  $K_A$  suggests that coated fiber may interact more strongly with a matrix (Marcovich et al. 1996).

The  $K_A$  and  $K_D$  values for the respective fibers were estimated from the slope and intercept of the respective linear regression lines of  $\Delta H^{AB}/AN$  as a function of DN/AN. These values are summarized in Table 5. Qualitatively, SMA-coated HPN showed relatively higher acid-base characteristics than uncoated HPN. A similar trend was observed in the case of EAA-coated HPN compared with uncoated HPN. The uncoated, SMA-coated, HVF-coated, and LVF-coated HPN showed a basic surface characteristic.  $K_A$  and  $K_D$  values appear to be consistent with the molecular structure of cellulose, where the hydrogen atoms in the hydroxyl groups act as electron acceptors and the oxygen atoms in the glycosidic linkages and hydroxyl groups act as electron donors. The EAA-coated HPN showed an amphoteric surface characteristic, and guanidium hydrochloride coated HPN showed a predominantly acidic characteristic. The relatively high  $K_A$  value indicates a surface that is rich in hydroxyl groups.

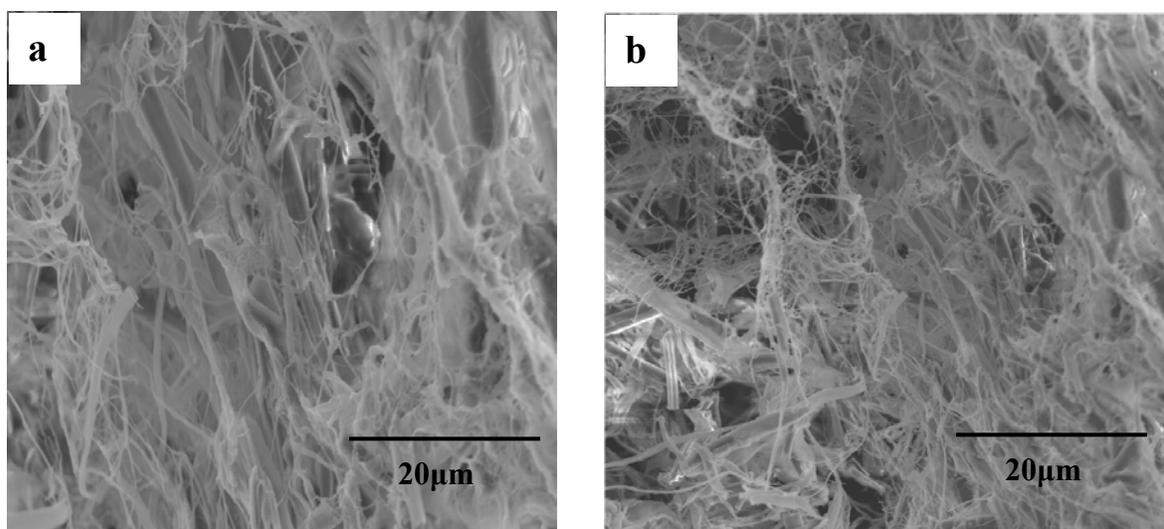
The surfaces of uncoated and chemically coated fibers were enriched by different classes of chemicals and extractives. Hemp fibers were found to be basic, which is probably due to presence of triglycerides, which exhibit a pronounced basic character (Tshabalala 1997). The removal of extractives and hemicellulose by chemical treatments had the effect of increasing the dispersion component of the surface energy of the HPN. The polymer matrix PLA used in this study was found to have an acidic character. By contrast, PHB showed a predominantly basic character, according to the  $K_A$  and  $K_D$  values.

Values of the specific interaction parameter, as defined by Schultz et al. (1987), were calculated for each type of fiber and resin combination. These values are shown in Table 6. Acid-base interactions with PLA increased by SMA- and EAA-coated HPN, and a very similar trend was observed for PHB matrix. EAA copolymers were used as a

dispersant in this study, bringing together in one product the benefits of both ethylene and acrylic acid. The crystalline structure of ethylene provides the barrier properties, flexibility, and resistance to water and chemicals. The acrylic acid comonomer imparts improved adhesion, hot-tack strength, and optical clarity. The nanofiber suspension containing EAA dispersant remains homogeneous indefinitely. It has excellent adhesion to cellulose and other polar substrates due to the high content of acrylic acid in the base copolymer. The EAA dispersant also exhibits outstanding adhesion to polyethylene and other plastics. Styrene maleic anhydride (SMA) resins are low molecular weight styrene/maleic anhydride copolymers. Altering the styrene to maleic anhydride ratio changes the hydrophilic/hydrophobic balance of the polymer. At their most hydrophilic, SMA resins form high solids solutions and can be used to produce fiber dispersions. These results are of special practical importance because surface acid-base interactions may be implicated in the adhesion of coatings and finishes to polymer and other lignocellulosic fibers. Adsorption occurred only when there was an exothermic interracial acid-base interaction. The present paper is only focused on the material structure and mechanical properties of SMA-coated HPN nanocomposites. The properties of EAA-coated HPN nanocomposites were discussed in the author's previous publication (Wang and Sain 2007).

### Material Structure

Figure 1 presents typical pictures of freeze-dried HPN. Figure 1(a) is a SEM image of uncoated HPN. Each particle of HPN is an aggregation of cellulose fibers due to the strong hydrogen bonds of adjacent molecules. The size of the fiber bundle is at the  $\mu\text{m}$  level. Figure 1(b) shows a picture of SMA-coated HPN with a well-organized web-like structure. The morphology of coated HPN appears distinguishable compared to uncoated HPN. The SMA-coated fibers formed loose networks during freeze drying. It is proved that SMA could reduce the entanglement of the nanofibers.



**Fig. 1.** Scanning electron micrographs of freeze-dried HPN samples: (a) uncoated and (b) SMA coated.

**Table 3.** Free Energy of Adsorption,  $\Delta G^{AB}$ , of the Acid-Base Probes at Different Temperatures

Substrate/Probe	$\Delta G^{AB}$ (KJ.mol <sup>-1</sup> )			
	313K	333K	353K	373K
Uncoated HPN				
Chloroform	3.76	1.50	-0.86	-2.37
Ethyl Acetate	5.36	4.61	3.25	1.66
Ethyl Ether	3.15	-0.20	-0.52	-3.06
Tetrahydrofuran (THF)	4.40	2.88	1.52	-1.32
Acetone	4.61	2.23	0.07	-2.37
SMA-Coated HPN				
Chloroform	12.86	12.68	8.89	5.55
Ethyl Acetate	12.10	10.79	8.76	6.58
Ethyl Ether	8.20	7.45	6.39	5.42
Tetrahydrofuran (THF)	13.07	12.55	12.21	11.43
Acetone	12.86	11.91	11.28	9.21
HVF-Coated HPN				
Chloroform	2.30	1.10	-1.63	-4.38
Ethyl Acetate	2.07	0.90	-0.80	-1.64
Ethyl Ether	-1.05	-3.43	-7.34	-9.93
Tetrahydrofuran (THF)	2.31	0.41	-0.05	-0.97
Acetone	1.64	-0.14	-1.63	-4.63
LVF-Coated HPN				
Chloroform	3.59	2.64	-0.17	-1.49
Ethyl Acetate	1.79	0.86	0.36	-0.73
Ethyl Ether	-0.07	-1.20	-3.40	-4.53
Tetrahydrofuran (THF)	1.86	0.95	0.33	-0.26
Acetone	2.73	1.76	1.35	0.27
EAA-Coated HPN				
Chloroform	12.70	11.33	10.66	9.83
Ethyl Acetate	11.71	10.99	9.99	9.08
Ethyl Ether	10.29	8.13	7.00	6.37
Tetrahydrofuran (THF)	13.56	12.47	11.54	10.78
Acetone	9.96	9.35	8.79	8.71
Guanidium Hydrochloride Coated HPN				
Chloroform	-1.87	-3.41	-4.81	-5.09
Ethyl Acetate	-3.05	-3.57	-3.89	-4.04
Ethyl Ether	-4.26	-4.54	-4.81	-5.09
Tetrahydrofuran (THF)	-3.47	-3.48	-3.49	-3.50
Acetone	2.96	2.87	2.71	2.62

**Table 4.** Enthalpy of Adsorption,  $\Delta H^{AB}$ 

Probe	$\Delta H^{AB}$ (KJ.mol <sup>-1</sup> )					
	Uncoated HPN	SMA-Coated HPN	HVF-Coated HPN	LVF-Coated HPN	EAA-Coated HPN	Guanidium Hydrochloride Coated HPN
Chloroform	5.70	16.43	5.04	5.65	13.45	1.04
Ethyl Acetate	6.83	14.20	3.34	2.58	12.67	2.81
Ethyl Ether	4.58	9.21	2.20	1.59	11.17	3.98
Tetrahydrofuran (THF)	6.50	13.63	3.00	2.47	14.41	3.46
Acetone	6.91	14.21	3.88	3.47	10.28	3.09

**Table 5.** Surface Acid-Base Characteristics,  $K_A$  and  $K_D$ 

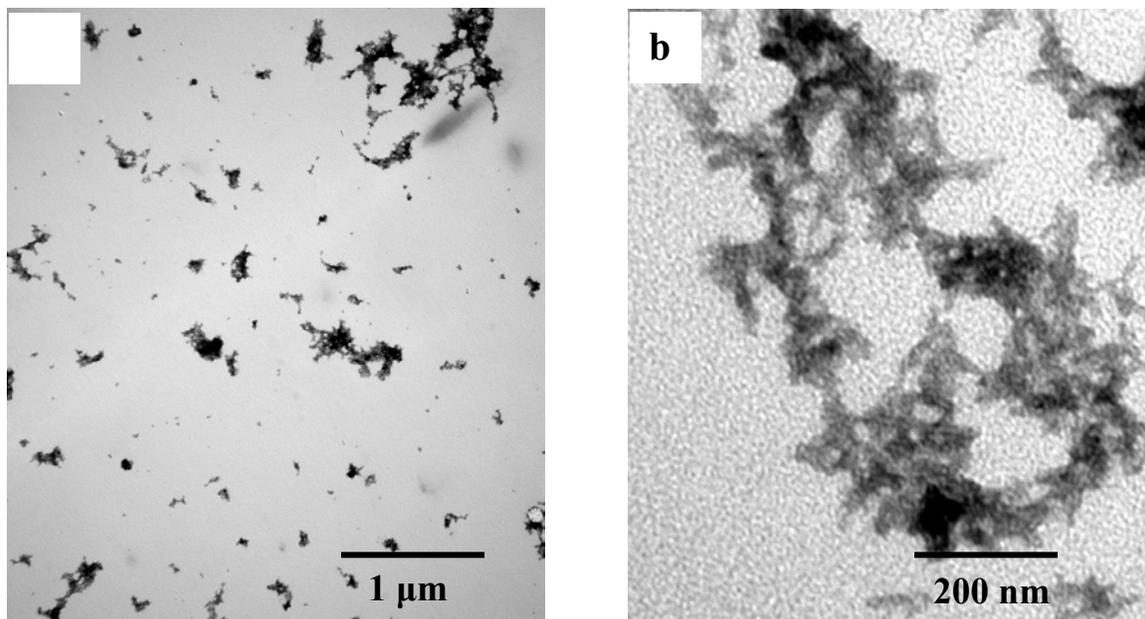
Material	$K_A$ (a.u.)	$K_D$ (a.u.)
Uncoated HPN	0.19	0.31
SMA-Coated HPN	0.34	0.77
HVF-Coated HPN	0.07	0.22
LVF-Coated HPN	0.04	0.23
EAA-Coated HPN	0.49	0.45
Guanidium Hydrochloride Coated HPN	0.20	0.02
PLA	0.18	0.12
PHB	0.22	0.69

**Table 6.** Values of Specific Interaction Parameter

$I = K_A^f K_D^m + K_A^m K_D^f$	PLA	PHB
Uncoated HPN	0.08	0.20
SMA-Coated HPN	0.18	0.41
HVF-Coated HPN	0.05	0.10
LVF-Coated HPN	0.05	0.08
EAA-Coated HPN	0.14	0.44
Guanidium Hydrochloride Coated HPN	0.03	0.14

The processing of cellulose nanocomposites renders several challenges. The major difficulty is to achieve uniformly dispersed nanofibers in the polymer matrix. The nanofibers have a very large surface-to-volume ratio and have a tendency to aggregate when dried. The injected composites were examined using a transmission electron microscope (TEM) to study the composite morphology at nanoscale. Figure 2(a) shows an overview picture of the PLA/SMA-coated HPN composite. It was difficult to see any cellulose nanofibers in this sample. There are some dark spots, indicating that the nanofibers were not uniformly dispersed in the PLA matrix, and it is possible that the cellulose was degraded during processing. In Fig. 2(b) a more detailed view of the composite with PLA is shown. It can be seen that the nanofibers were partly dispersed in PLA. Agglomerates were present in the PLA/SMA-coated HPN nanocomposite. The structure can therefore not be described as fully networked. The dispersion and distribution of nanofibers can be affected and improved by optimizing the chemical surface treatments and the compounding process. Figure 2(b) shows the presence of a

non-homogeneous structure of nanofibers in the PLA based nanocomposites. This fact will be reflected in the mechanical properties, since there is a strong link between the morphology of nanocomposites and the improvement in properties of the polymer matrix.



**Fig. 2.** Transmission electron micrograph of the PLA/SMA-coated HPN composites: (a) an overview and (b) detailed view.

### Mechanical Properties of Nanocomposites

The chemical surface modifications of cellulose fibers were studied with the aim of improving their interfacial compatibility with PLA and PHB, that is, to enhance the mechanical properties of the ensuing composite. The mechanical properties of the prepared nanocomposites are presented in Table 7. There were some improvements in the properties of the nanocomposite materials, compared to pure PLA and PHB. Table 7 also shows that the improvements were similar for both nanoreinforcements. The PHB/SMA-coated HPN nanocomposite showed a 17% increase in the yield strength and a 24.5% increase in modulus in comparison to PHB/uncoated HPN nanocomposite. There was a 35% increase in the yield strength and a 37% increase in modulus relative to pure PHB. The PLA/SMA-coated HPN nanocomposite showed only a 3% increase in tensile strength and a 7% increase in modulus compared to PLA/uncoated HPN. There was a 8.6% increase in tensile strength and a 10% increase in modulus, compared to pure PLA. These results were lower than expected. Theoretical calculations were therefore performed in order to better understand the results and to see the potential effect of both nanoreinforcements.

**Table 7.** Tensile Properties of the Nanocomposites.

Materials	Max. Stress (MPa)	S.D.	E-Modulus (GPa)	S.D.
PHB	15.32	1.00	1.41	0.16
PHB/Uncoated HPN	17.68	1.68	1.55	0.11
PHB/SMA-coated HPN	20.68	6.66	1.93	1.25
PLA	65.49	0.21	2.72	0.09
PLA/Uncoated HPN	68.97	0.40	2.80	0.06
PLA/SMA-coated HPN	71.14	0.64	2.99	0.01

The Halpin-Tsai equation was used to calculate the theoretical tensile modulus for the two nanocomposite materials, see Eq. (11) – Eq. (14) (Agarwal and Broutman 1990),

$$E = E_m(1 + \zeta\eta\Phi)/(1-\eta\Phi) \quad (11)$$

where  $E_m$  is the Young's modulus of the matrix,  $E_f$  represents Young's modulus of the filler,  $\zeta$  is a shape parameter dependent upon filler geometry, orientation, and loading direction, and  $\eta$  is given by,

$$\eta = (E_f/E_m - 1) / (E_f/E_m + 1) \quad (12)$$

$$\zeta = 2 \times \text{Length/Diameter} \quad (13)$$

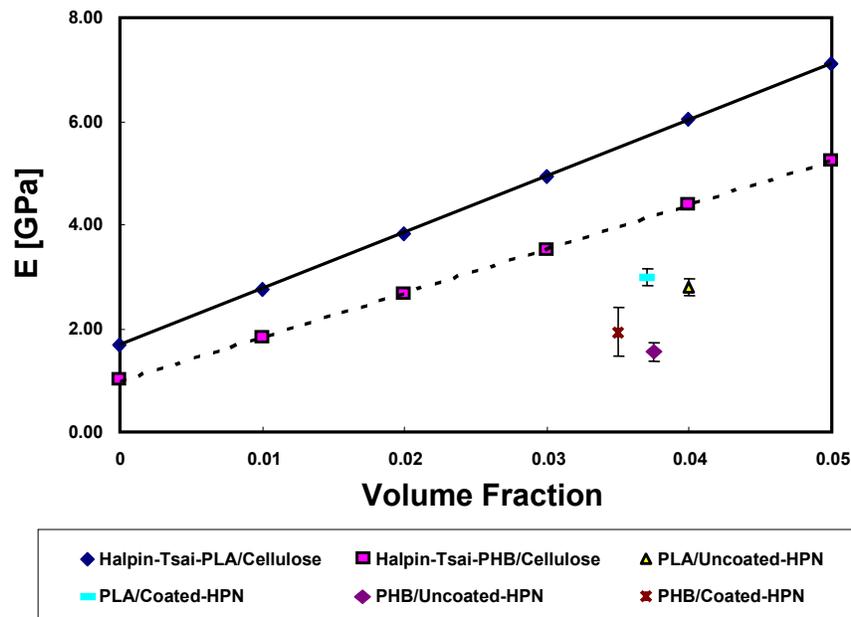
$$\Phi = \text{volume fraction} \quad (14)$$

The Halpin-Tsai equation is normally used to predict the modulus for aligned fiber composites, but it has been used before to predict the modulus of nanocomposites (Wu et al. 2004; Fornes and Paul 2003). It was chosen because it demanded the least amount of assumptions to be made about the materials. The Halpin-Tsai equation can only be applied to predict the modulus of fiber/matrix nanocomposites in the range of low fiber volume fractions. At high filler concentration, the predicted value is lower than the experimental data. It is assumed that the filler apparent volume is related to the dispersion of filler, and that the larger apparent volume may originate in better dispersion, which results in a higher modulus of the composite. When the predicted values at filler volume concentrations of less than 6%, it is well fitted to the experimental data (Wu et al. 2004). By comparing model predictions with the two-dimensional finite element calculations for discontinuous oriented square fiber-reinforced composites, Ashton et al. (1969) determined that  $\zeta = 2$  (length/diameter of the fiber) =  $2 \times$  aspect ratio provided good agreement for longitudinal modulus.

The volume fraction of each nanoreinforcement was calculated using Eq. (15) (Luo and Daniel 2003),

$$\Phi_f = (w_f/\rho_f)/((w_f/\rho_f) + (1 - w_f)/\rho_m) \quad (15)$$

where,  $w_f = 5\%$ ,  $\rho_{\text{cellulose}} = 1.58 \text{ g/cm}^3$  (Ganster et al. 1999),  $\rho_{\text{SMA-coated HPN}} = 1.70 \text{ g/cm}^3$ ,  $\rho_{\text{PLA}} = 1.25 \text{ g/cm}^3$ , and  $\rho_{\text{PHB}} = 1.17 \text{ g/cm}^3$ . The volume fractions for the PLA/uncoated HPN and the PLA/SMA-coated HPN were determined to be 4% and 3.7%, respectively. The volume fraction for the PHB/uncoated HPN and the PHB/SMA-coated HPN were 3.75% and 3.5%, respectively. The following data were used in the calculation:  $E_{\text{PLA}} = 1.7 \text{ GPa}$ ,  $E_{\text{PHB}} = 1.0 \text{ GPa}$ ,  $E_{\text{cellulose}} = 167.5 \text{ GPa}$  (Petersson and Oksman 2006), aspect ratio of uncoated HPN is 88, and aspect ratio of SMA-coated HPN is 82 (Wang et al. 2007). A comparison between the theoretical and experimental results can be seen in Fig. 3. When comparing the results, one has to keep in mind that theoretical calculations are based on PLA/cellulose and PHB/cellulose systems, where the nanoreinforcement is aligned in the longitudinal direction and has perfect interfacial adhesion to the matrix. From Fig. 3, we can draw the conclusion that both systems have large potentials for strength development, which this experiment was unable to reach. One can also see that the PLA system has the largest potential and that the PLA/nanofiber system, due to its agglomerated structure, was farthest away from its theoretical value. When it comes to the PLA/uncoated HPN and the PLA/SMA-coated HPN system, the uncoated nanofiber PLA system should have higher theoretical tensile strength value, compared to the SMA coated nanofiber PLA system, due to its lower volume fraction. In contrast, the chemical treatments on the fiber surface increased the interfacial adhesion between fiber and matrix; the experimental results showed the PLA/SMA-coated HPN system as having higher tensile properties.



**Fig. 3.** Experimentally measured tensile modulus data compared to theoretical predictions by Halpin-Tsai.

## CONCLUSIONS

1. Inverse gas chromatography (IGC) at infinite dilution has proven to be a convenient tool for measurement of surface energy and acid-base characteristics of natural fibers and polymer matrix. Changes in final properties of the composites due to the effect of various chemical treatments on the fiber surface can also be explained using this technique. Acid-base interactions with PLA were increased by SMA- and EAA-coated HPN, and the same trend was observed for the PHB matrix.
2. SEM pictures showed SMA-coated HPN having a well-organized web-like structure and proved that the size of nanofibers is indeed in the nano-level. Current TEM pictures showed the presence of a non-homogeneous structure of nanofibers in the PLA based nanocomposites. The properties shown here will most probably be improved if it is possible to disperse the nanofibers more evenly within the polymer matrix. A uniform nanofiber dispersion in a matrix, coupled with a high aspect ratio of the nanofibers will indicate a strong potential for the use of these biocomposite films.
3. In both PLA and PHB systems, the SMA-coated HPN as the reinforcement enhanced the mechanical properties over the systems containing uncoated HPN or pure polymer. The theoretical calculations made in this article showed that the PLA has the largest potential to improve the mechanical properties, compared to the PHB system. This experiment was a step in the direction of creating fully renewable biopolymer based nanocomposites.

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## USE OF SURFACTANTS IN ENZYMATIC HYDROLYSIS OF RICE STRAW AND LACTIC ACID PRODUCTION FROM RICE STRAW BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

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Rice straw (RS) is one of the most abundant lignocellulosic waste by-products worldwide and provides an alternative substrate to produce useful chemicals such as bioethanol and lactic acid. However, higher enzyme loadings are needed to obtain a higher product yield, which makes the large-scale utilization economically difficult. The presence of non-ionic surfactants and poly(ethylene glycol) (PEG) during the enzymatic hydrolysis of lignocellulosics has been found to increase the conversion of cellulose into fermentable sugars. We have found that adding 0.2g g<sup>-1</sup> substrate of polyoxyethylene(20) sorbitan monooleate (PSM) or high-mass PEG increased the sugar yield by 22% and 12%, respectively, when enzyme loading was at 10FPU g<sup>-1</sup> for 24h. PSM behaved better than PEG when different substrate concentrations, temperatures, and enzyme loadings were investigated. PSM provides an opportunity to reduce enzyme dosage while still keeping the same extent of hydrolysis. We also investigated the effect of PSM on the simultaneous saccharification and fermentation of pretreated RS to lactic acid. Results showed that addition of 0.7g L<sup>-1</sup> PSM improved the lactic acid production by 24% compared to the reference without PSM addition at 72h.

*Keywords:* Rice straw, Cellulose, Surfactant, Lactic acid, Simultaneous saccharification and fermentation

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### INTRODUCTION

Lactic acid and its derivatives have been widely used in the food, pharmaceutical, cosmetic and industrial applications. Recently, lactic acid has gained increasing interest as a raw material for manufacture of poly(lactic acid), a bio-compatible and biodegradable plastic (Hujanen and Linko 1996). However, the economics of ultimate lactic acid fermentation is still a problem, and medium compositions contribute most to the overall cost of lactic acid production. In order to reduce the production cost of lactic acid, many studies have been published using the hydrolysate of cellulosic materials as carbon sources to produce lactic acid (Hofvendahl and Hahn-Hagerdal 2000; Wee et al. 2006). The research along this line has yielded two efficient bioprocess schemes, which are termed “simultaneous

saccharification and fermentation” (SSF) and “separate hydrolysis and fermentation” (SHF), of which the SSF of lignocellulosic materials for production of lactic acid has been extensively investigated (Bustos et al. 2005; John et al. 2006; Tanaka et al. 2006).

In the bioconversion of lignocellulosic biomasses to value-added products, hydrolysis of pretreated lignocellulosic materials to fermentable sugars is of great importance. It is the rate-limiting step in both SSF and SHF (Lyer and Lee 1999; Alkasrawi et al. 2003). From previous studies by different authors (Wyman et al. 1992; Gregg and Saddler 1996), it is apparent that a high degree of bioconversion of lignocellulose to reducing sugars requires low substrate concentration, prolonged hydrolysis time, and high enzyme loading. However, high enzyme concentration contributes significantly to the overall process cost due to the high enzyme cost. For instance, enzyme loading can amount to as much as 60% of the process cost of biomass conversion (Wilke et al. 1981; Nguyen and Saddler 1991; Helle et al. 1993). Therefore, it is urgent to find methods to enhance the saccharification yield of cellulose while reducing cellulase dosage. Towards this aim, it has been reported that applying both non-ionic surfactants and bovine serum albumin (BSA) treatment can effectively increase the hydrolysis rate of lignocellulose (Eriksson et al. 2002; Yang and Wyman 2006).

In China, rice straw (RS) is the most abundant agricultural residue, and the annual production is estimated to be approximately 200 million tons (Zhu et al. 2006). In this study, the effect of poly(ethylene glycol) (PEG), an ethylene oxide (EO) containing polymer, and polyoxyethylene(20) sorbitan monooleate (PSM) (Tween® 80) on the enzymatic cellulose hydrolysis was investigated using pretreated RS as substrate. Further, the effect of PSM on SSF of pretreated RS to lactic acid by *Lactobacillus casei* YQ-06 was also studied. To the best of our knowledge, there have been few studies on lactic acid production by SSF with addition of surfactant.

## EXPERIMENTAL

### Microorganism

*Trichoderma viride* YQ-02, isolated by our laboratory, was used for cellulase production. The stock culture was stored on potato-glucose-agar (PDA) and subcultured every two months.

*Lactobacillus casei* YQ-06 was acclimatized from *Lactobacillus casei* GIM 1.159 (originally from ATCC 334). The strain can grow well at 45 °C and was maintained on deMan, Rogosa and Sharpe (MRS) slant (DeMan et al. 1960).

### Lignocellulosic Materials and Pretreatment

Raw RS was obtained from local farmers in Hefei, Anhui province, China. Prior to pretreatment, it was cut to 1-2 cm length and washed thoroughly with tap water until the washings were clean and colorless and then dried in an oven at 65 °C to constant weight. Oven-dried RS was then ground to 80-120 mesh with an electric grinder and was used in the experiments. The smashed RS was stored at room temperature until the time of use. The initial composition of the RS was determined to be 33.3% cellulose, 23.32% hemicellulose, and 17.5% lignin.

A two-step chemical pretreatment: dilute acid pretreatment and alkaline peroxide pretreatment was carried out as described by Curreli et al. (2002). The cellulose, hemicellulose, and lignin contents of pretreated RS were 68.8%, 9.4%, and 8.9%, respectively.

### Cellulase Production

Cellulase was produced by solid state fermentation. A two-stage technique was employed. In the first stage, the fungus was grown on PDA slants for 5 days at 30 °C. In the second, 10 mL of deionized sterile water was added to a PDA slant, and a spore suspension containing  $10^7$  spores  $\text{mL}^{-1}$  was used to inoculate Erlenmeyer flasks (250 mL) containing 5 g of RS and wheat bran (dry weight ratio is 3:2) moistened with the following mineral medium ( $\text{g L}^{-1}$ ):  $(\text{NH}_4)_2\text{SO}_4$  10;  $\text{KH}_2\text{PO}_4$  4;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5, and  $\text{CaCl}_2$  0.5. (Chen et al. 2004). The initial pH value of the medium was adjusted to 5.0 after sterilization at 121 °C for 30 min, and the water content of the substrate was 75%. The flasks were incubated at 30 °C for 96h under static conditions.

### Enzyme Extraction and Concentration

The enzyme was extracted by a simple contact method (Krishna and Chandrasekaran 1996). The fermented samples were shaken (150rpm) with 50mL of 0.1M citrate buffer of pH=4.8 for one hour and filtered through filter paper. The filtrates were centrifuged at 10,000 rpm (4°C) for 15 min to remove spores of the organism, and supernatants were further concentrated by ammonium sulfate precipitation. The enzyme salt solutions were dialyzed with 0.1M citrate buffer of pH4.8 for 24h with continuous stirring and the buffer was changed from time to time (3 to 5 times) for removing ammonium salt. The enzyme was concentrated with PEG20,000 to the required concentration. Concentrated cellulase solutions with a filter paper activity (FPU) of 5.028 IU  $\text{mL}^{-1}$  and a cellobiase activity (CBU) of 3.017 IU  $\text{mL}^{-1}$  were used in the hydrolysis and SSF experiments.

### Enzymatic Hydrolysis

Hydrolysis experiments were carried out in 250 mL flasks with a working volume of 100mL in the presence of different concentrations of polyoxyethylene(20) sorbitan monooleate (PSM) or high molecular mass polymer of ethylene oxide (PEG). The PSM was Tween® 80, provided by Shanghai Shenyu Pharmaceutical & Chemical Co. Ltd., China. The PEG was PEG 6000, provided by Shantou Xilong Chemical Industry Factory Co., Ltd., China.

Pretreated RS was saccharified with different volumes of concentrated cellulase solutions in 0.1M citrate buffer (pH 4.8) at a substrate concentration of 2% (w/v). Sodium azide ( $30 \mu\text{g mL}^{-1}$ ) was added to the reaction mixture to prevent microbial and fungal contamination. The flasks were incubated at 50 °C on an orbital shaker agitated at 150rpm. Sample aliquots of 2mL were taken at different times, centrifuged, and the supernatants were analyzed for reducing sugars. Sugar yield was expressed as mg reducing sugars produced by enzymatic action per g dry biomass.

### Simultaneous Saccharification and Fermentation (SSF)

*L. casei* inoculum was prepared by transferring a loop of stock culture to

10mL sterile MRS medium in 50mL screw cap tubes and maintained at 45°C for 24h. This culture (1mL) was then transferred to 10mL MRS medium in a 100mL screw cap conical flask and incubated at 45°C for 24h.

SSF experiments were carried out in 250mL conical flasks (working volume 100mL) placed in orbital shakers (150rpm) under anaerobic conditions. SSF reaction mixtures contained 6g of pretreated RS (previously autoclaved for 15min at 121°C); 12mL of crude cellulase extract (10FPU g<sup>-1</sup> substrate); 5mL *L.casei* inoculum; different concentrations of PSM, ranging from 0 to 5.6 g L<sup>-1</sup>; and basal medium were added to make up the volume to 100mL. The medium pH was adjusted to 5.0 with 0.1M citrate buffer (Lyer and Lee 1999). The basal medium contained (g L<sup>-1</sup>): peptone 10; yeast extract 5; NaCH<sub>3</sub>CO<sub>2</sub> 0.5; MnSO<sub>4</sub>·H<sub>2</sub>O 0.05; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.2; FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01; NaCl 0.01; and ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.2.

### Analytical Methods

Filter paper activity and cellobiase activity were assayed according to a standard cellulase activities analytical procedure recommended by the International Union of Pure and Applied Chemistry (IUPAC) (Ghose 1987). One unit of filter paper activity (FPU) was defined as the amount of enzyme required to liberate 1 μmol of reducing sugars as glucose per minute per milliliter of crude enzyme extract under assay conditions. Cellulose, hemicellulose, and lignin were determined according to the procedures described elsewhere (Goering and VanSoest 1970). The amount of reducing sugars was determined by the DNS method (Miller 1959). L-lactic acid was estimated by a colorimetric method (Kimberley and Taylor 1996).

Three parallel samples were used in all analytic determination, and data are presented as the mean of three replicates. Relative standard deviations in the assays of reducing sugars and lactic acid were below 5%.

## RESULTS AND DISCUSSION

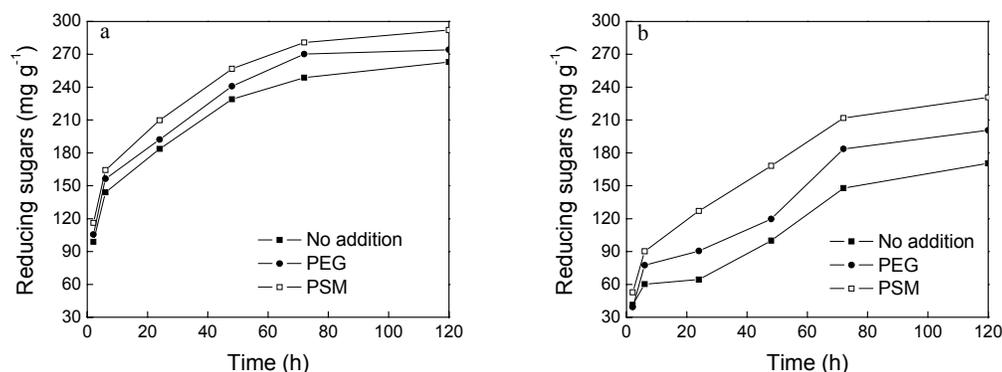
### Evaluation of Saccharification Conditions with Surfactant Addition

#### *Hydrolysis at lower temperature with addition of surfactants*

The temperature 50 °C has been used as the optimum for enzymatic hydrolysis of different lignocellulosics (Vlasenko et al. 1997; Krishna et al. 1999; Ortega et al. 2001; Adsul et al. 2005; Chen et al. 2006; Xu et al. 2006). This is also true of for the cellulase from *T. viride*.

Hydrolysis of pretreated RS with addition of 0.2g g<sup>-1</sup> surfactants was investigated at 40 °C and 50 °C (Fig. 1). As expected, samples with surfactant additions had higher conversion, with PSM performing better than PEG at both temperatures. At 40 °C, sugar yields were 184 and 212 mg g<sup>-1</sup> dry biomass with PEG and PSM addition, respectively, while for the sample without surfactant addition, the sugar yield was only 148mg g<sup>-1</sup> dry biomass at 72h. At a temperature of 50 °C, the conversion increased by 13% and 8% when adding PSM and PEG in the reaction mixture, compared to the control without surfactant addition, respectively. It has been reported that surfactants could increase the stability of cellulase (Kaar and Holtzapple 1998). Surfactant as an enzyme stabilizer possibly contributes to better performance

of cellulase at lower temperature.



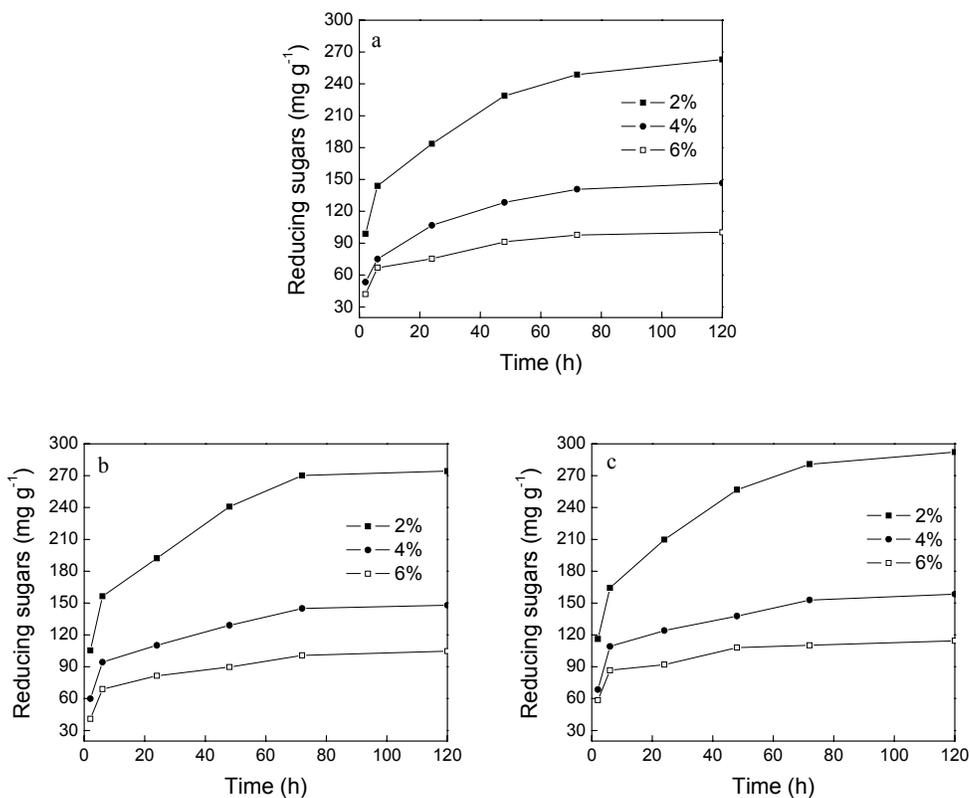
**Fig. 1.** Time course of RS hydrolysis with surfactant addition at different temperatures. Enzyme loading 10FPU g<sup>-1</sup> substrate, substrate concentration 2% (w/v), pH 4.8. Surfactant concentration 0.2g g<sup>-1</sup> substrate. (a) 50 °C; (b) 40 °C.

#### *Effect of substrate concentration*

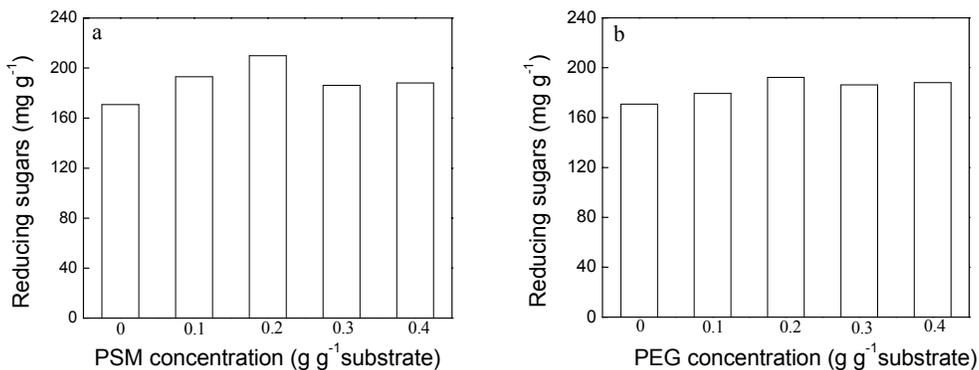
The effect of substrate concentration on enzymatic hydrolysis was investigated with addition of surfactant at a fixed ratio of cellulase dosage to substrate (10FPU g<sup>-1</sup> substrate and 6CBU g<sup>-1</sup> substrate). As shown in Fig. 2, the rate of conversion decreased when higher substrate concentration than 2% (w/v) was used. Further increase in substrate concentration limits the saccharification yield because of stirring difficulties, reduction of the aqueous movable phase, and end-product inhibition (Lee and Fan 1982; Szczodrak 1987). Substrate concentrations of 2% have also been found optimum to release reducing sugars from different lignocellulosic biomass (Krishna. et al. 1998; Krishna et al. 1999). Accumulation of glucose and cellobiose as end-products, especially cellobiose, strongly inhibits the cellulase in the reaction mixture. In order to reduce the inhibition of end-product, several methods have been developed, including supplementation with excess  $\beta$ -glucosidase during hydrolysis, and the use of a high enzyme loading and removal of sugars during hydrolysis by SSF (Stenberg et al. 2000; Chen et al. 2006).

#### *Effect of surfactant concentration*

A portion of the data from surfactant loading experiments is plotted in Fig. 3. Addition of surfactants to the reaction mixture enhanced the enzymatic hydrolysis of pretreated RS in comparison to the surfactant-free samples. Addition of 0.2 g g<sup>-1</sup> of PSM and PEG increased the sugar yield to 210 and 192mg g<sup>-1</sup> dry biomass from 172mg g<sup>-1</sup> dry biomass at 24h, respectively. Higher surfactant concentration than 0.2g g<sup>-1</sup> substrate resulted in decreased conversion. A likely explanation for this may be that the hydrophobic part of the lignin in the lignocellulosic materials needs a certain concentrations of non-surfactants to be occupied through hydrophobic interactions to prevent unproductive binding of cellulases to lignin (Eriksson et al. 2002). Thus, more free cellulose is available for hydrolysis of cellulosic substrate. When higher surfactant concentration is used, micelles of non-surfactant may be formed (Jonstromer and Strey 1992; Schomacker and Strey 1994). Formation of micelles may prevent cellulase from contacting the cellulose substrate, thus reducing the conversion rate.



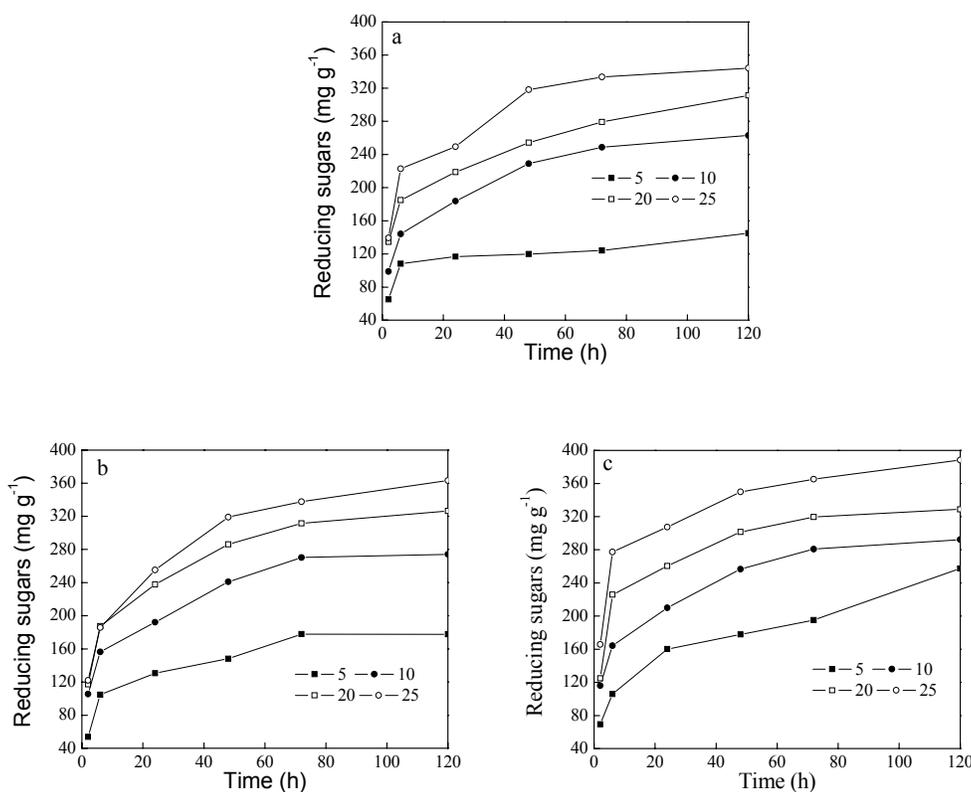
**Fig. 2.** Effect of substrate concentration (% (w/v)) on enzyme hydrolysis with addition of surfactants. Enzyme loading 10FPU g<sup>-1</sup> substrate, pH 4.8, surfactant concentration 0.2g g<sup>-1</sup> substrate, temperature 50 °C. (a) no surfactant addition; (b) PEG addition; (c) PSM addition



**Fig. 3.** Effect of surfactant concentration on enzyme hydrolysis. Enzyme loading 10FPU g<sup>-1</sup> substrate, substrate concentration 2% (w/v), temperature 50 °C, pH 4.8, incubation time 24h. (a) PSM addition; (b) PEG addition.

### Effect of enzyme loading

The effect of enzyme loading (5-25FPU  $\text{g}^{-1}$  substrate) on the enzymatic hydrolysis of pretreated RS has been studied and the results are presented in Fig. 4. Higher enzyme loading resulted in higher sugar yield. The sugar yield increased sharply when increasing enzyme dosage from 5FPU  $\text{g}^{-1}$  to 10FPU  $\text{g}^{-1}$  substrate for all of the samples. Thereafter the increase observed was less. Most samples leveled off after 72h, but the sample of enzyme concentration at 5FPU  $\text{g}^{-1}$  substrate without surfactant addition leveled off after 6h.



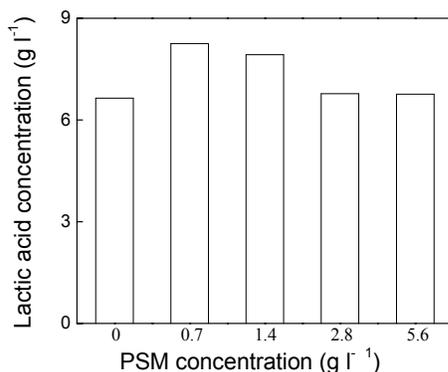
**Fig. 4.** Effect of enzyme loading (FPU  $\text{g}^{-1}$  substrate) on enzyme hydrolysis with addition of surfactants. substrate concentration 2% (w/v), pH 4.8, surfactant concentration 0.2g  $\text{g}^{-1}$  substrate, temperature 50 °C. (a) no surfactant addition; (b) PEG addition; (c) PSM addition.

### SSF of RS to Lactic Acid with PSM Addition

As mentioned above, PSM performed better in the enhancement of enzymatic hydrolysis of pretreated RS. So PSM was chosen to investigate the effect of surfactant on SSF of pretreated lignocellulosics to lactic acid.

The effect of PSM on the SSF of cellulosic materials to lactic acid is plotted in Fig. 5. Results indicated that a small amount of PSM (0.7g  $\text{l}^{-1}$ ) could increase the production of Lactic acid by *L. casei*, while higher PSM concentration had a negative effect on lactic acid production. The positive effect of PSM in the SSF experiments was likely due to the increase in conversion rate of lignocellulosic materials by

preventing the unproductive adsorption of cellulase to the lignin and allowing more enzyme to be available for hydrolysis of cellulose. But when higher PSM concentration is used, the PSM may dissolve the lipid in the cell membrane and cause the death of the cell. PSM provides an opportunity to reduce the enzyme loading while still maintaining the same extent of lactic acid production.



**Fig. 5.** Effect of PSM concentration on SSF of pretreated RS to Lactic acid. Enzyme loading 10FPU g<sup>-1</sup> substrate, substrate concentration 6% (w/v), temperature 45 °C, pH 5.0, incubation time 72h.

Further research on the effects of surfactant in SSF of lignocellulosics to lactic acid and economic analysis of the production process of lactic acid from cellulosic substrate with addition of surfactants are being carried out in our laboratory.

## CONCLUSIONS

Addition of a non-ionic surfactant polyoxyethylene(20) sorbitan monooleate (PSM, Tween® 80) or a high-mass polymer of ethylene oxide (PEG) can effectively improve the enzymatic hydrolysis of pretreated rice straw (RS). But PSM performed better than PEG when different substrate concentrations, temperatures, and enzyme loading were investigated. The simultaneous saccharification and fermentation (SSF) of pretreated RS to lactic acid was improved by the addition of PSM. Lactic acid production increased by 24% when adding 0.7g L<sup>-1</sup> PSM, compared to the reference without PSM addition at 72h.

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## KEY CONSIDERATIONS IN THE DETERMINATION OF POLYELECTROLYTE CONCENTRATION BY THE COLLOIDAL TITRATION METHOD

Paulina Mocchiutti and [Miguel A. Zanuttini](#)\*

Medium or high charge density cationic polyelectrolytes are frequently used for water treatment. In the papermaking wet-end they are used as retention agents or as flocculating aids. Negative polyelectrolytes that enter the papermaking system increase the demand for cationic polyelectrolytes. Polyelectrolyte concentration can be determined by the colloidal titration method, using either of two options for detecting the endpoint: i) visual observation or spectrophotometric determination of the colour change of an indicator, or ii) streaming current measurement. This work discusses the best conditions for the application of the titration using spectrophotometric measurement for the end point detection. Polydiallyldimethylammonium chloride was used as the cationic polyelectrolyte, potassium polyvinyl sulphate as the negative polyelectrolyte, and o-toluidine blue as the positive indicator dye. The polyelectrolyte concentration range, interference from the metal ions affecting the indicator color change, the optimal indicator concentration to avoid precipitation problems, and the effect of adding a surfactant to the indicator solution were also considered. Titration curves were analyzed and optimized. Under these conditions the technique provided results with acceptable precision.

*Keywords:* Polyelectrolytes, Colloidal titration, Cationic demand, O-toluidine blue indicator.

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### INTRODUCTION

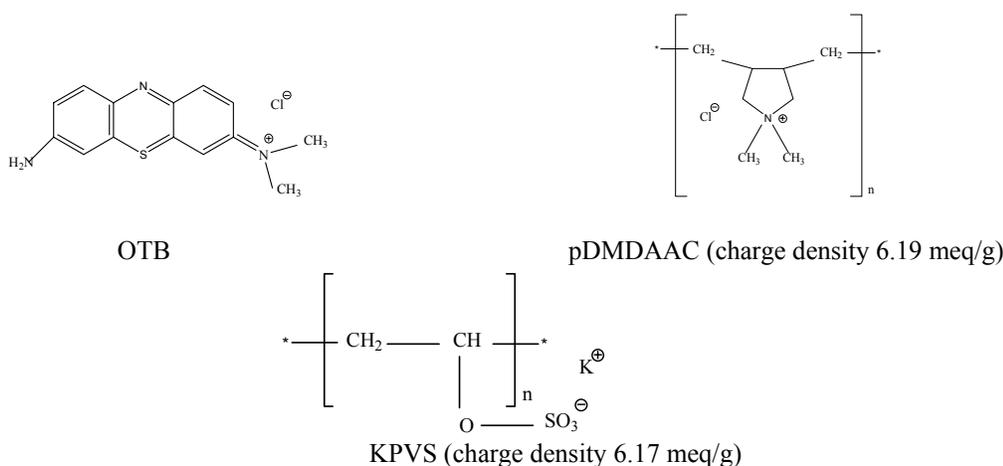
In the paper industry, polyelectrolytes are added as retention agents (e.g. polyamines or polyacrylamides) in order to control the dissolved and colloidal substances present in the machine white water (fines, particles, fillers, pigments). Polyelectrolytes are also used to improve drainage during paper formation or as aids to increase the dry strength of paper (e.g. cationic starch).

In order to quantify or characterize these polyelectrolytes, or to determine the “cationic demand” of a pulp suspension, the colloidal titration first proposed by Terayama (1952) is one of the most frequently employed methods. Direct colloidal titration consists of determining the concentration of a cationic polyelectrolyte in the presence of a positive indicator, using a polyelectrolyte of opposite charge as titrant. When there is an excess of the titrant, the indicator adsorbs onto it and changes its color. The technique is based on the fact that the complex formation between two polyelectrolytes of high and opposite charge density is generally, though not always,

stoichiometric or 1:1 (Terayama 1952; Eklund and Lindström 1991). On the other hand, indirect titration consists of determining the concentration of anionic polyelectrolytes after adding an excess of a known quantity of the positive polyelectrolyte.

Terayama (1952), Winter et al. (1986), Wågberg et al. (1989), and Fors (2000) used linear polyelectrolytes of high molar mass and high charge density to apply this method. They used poly-diallyldimethylammonium chloride (pDMDAAC) as the cationic polyelectrolyte, potassium polyvinyl sulphate (KPVS) as the anionic polyelectrolyte, and o-toluidine blue as the cationic indicator (OTB) (Fig. 1). In the final point of the titration, the indicator changes from blue to pink-violet, due to the presence of an excess of KPVS. Nevertheless, the visual detection of this color change is difficult and requires considerable experience.

Furthermore, Kam and Gregory (1999) stated that the mutual neutralization of polyelectrolyte charges can result in precipitation. Under these conditions, it is even more difficult to detect the indicator color change.



**Fig. 1.** Chemical structures of OTB indicator dye (o-toluidine blue), pDMDAAC (poly-diallyldimethylammonium chloride), and KPVS (potassium polyvinyl sulphate).

This work discusses the proper conditions for the application of the colloidal titration technique and the determination of the equivalence point when the OTB indicator is used. Different titrations were carried out, varying the pDMDAAC concentration but keeping the indicator and NaCl concentrations constant. The variation coefficient of the technique was determined.

## EXPERIMENTAL

### Materials

The pDMDAAC preparation started from a Mw: 400,000-500,000 pDMDAAC aqueous solution, at an approximate concentration of 20 % (g/L) (Aldrich). A standard solution of approximately 100 meq/L was prepared and its concentration was determined, using the methods proposed by Rice and Roeraade (2003): the solid material content was

determined from the average weight of six samples of the solution after drying at 60°C during 12 hours. The dry material was 19.8 % (g/L). Then, a solution of pDMDAAC 0.0015N in NaCl 0.01N was prepared, which was used to determine the KPVS solution concentrations.

In the literature, other methods for determining the concentration of KPVS have been proposed. They are based on the colloidal titration method, and they make use of cationic surfactants as standard solutions: cetyltrimethylammonium bromide (Kam and Gregory 1999) or zephiramide (Masadome 2003). Nevertheless, to use these standard solutions it is necessary to know the corresponding optimal conditions of the colloidal titration method.

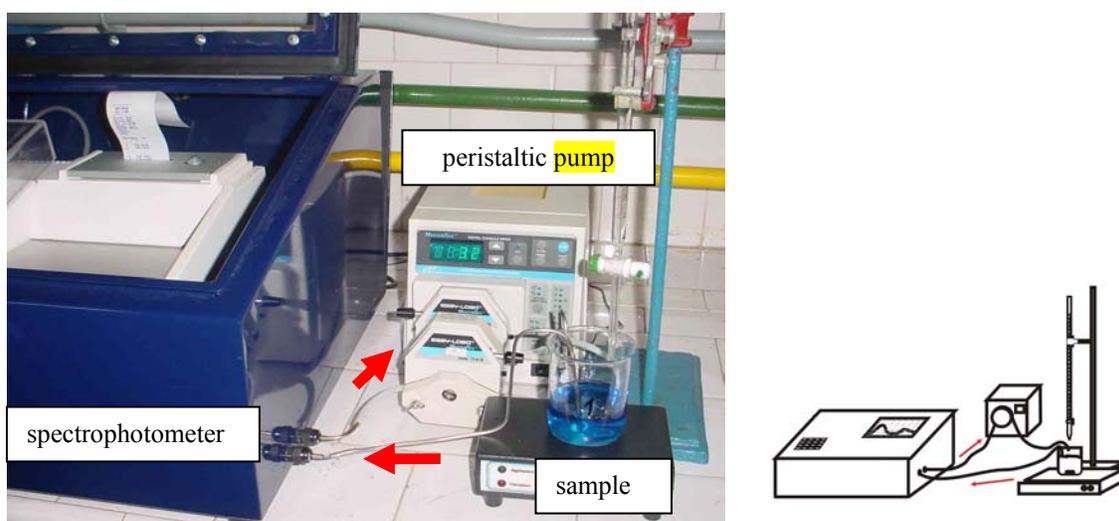
KPVS was provided by Nalco (Argentina) as a 0.0025 N solution. KPVS solutions of 200  $\mu$ N and 500  $\mu$ N were prepared. These solutions are stable only for a few months.

A 980  $\mu$ N solution of o-toluidine blue (ICN Biomedical) in 0.1%(w/v) Tween 20 (Anedra) solution was prepared as the indicator original solution.

## Methods

The colloidal titration was carried out using the arrangement shown in Fig. 2. Volumes of 0.3 to 3.5 mL of the cationic polyelectrolyte (pDMDAAC) solution and a known amount of OTB were diluted to 100 mL using 0.01N NaCl. Every 30 seconds, under stirring, 0.2 mL of the anionic polyelectrolyte (KPVS) were added from the microburette. Using a peristaltic pump in a closed circuit, the sample was forced to pass through the quartz flow cell (path length: 10 mm) of the spectrophotometer (CECIL 3055) where the absorbance at 628 nm was being registered. On the basis of these data, a titration curve was drawn and the equivalence point was determined.

The spectrophotometric measures facilitated the detection of the indicator color change; a quartz cell was used instead of a glass one, as suggested by Rice and Roeraade (2003), in order to minimize the adsorption of the polyelectrolytes.

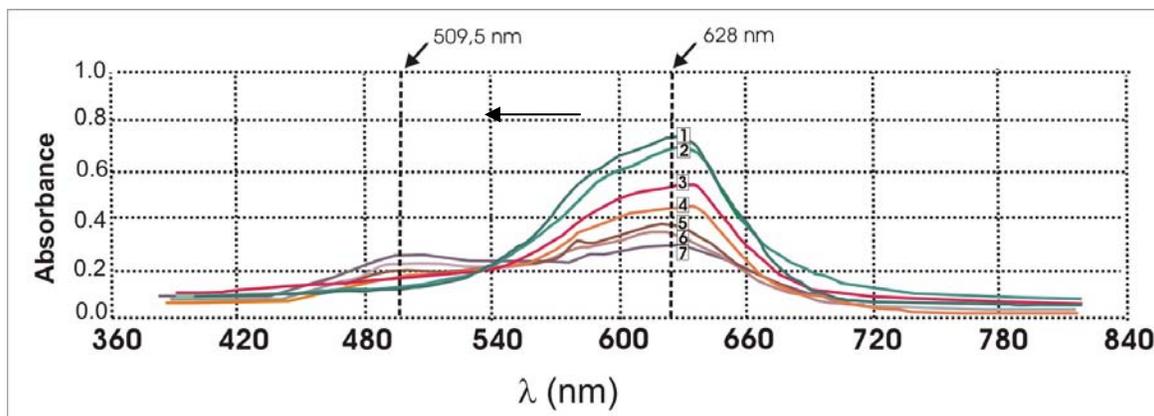


**Fig. 2.** Photograph and scheme of the arrangement used for polyelectrolyte titration. The liquid recirculates in the quartz flow cell (path length: 10 mm) of a spectrophotometer, where the solution absorbance is recorded as long as the KPVS is being added.

## RESULTS AND DISCUSSION

### Optimal Wavelength

Figure 3 shows the absorbance spectra obtained (range: 400-800 nm) during the titration of a 100 mL solution containing 2.6  $\mu\text{eq}$  of pDMDAAC in 0.01N NaCl and 2.6  $\mu\text{eq}$  of OTB. Increasing quantities of KPVS 200  $\mu\text{N}$  (from 0 to 6.4  $\mu\text{eq}$ ) were added (Fig. 3). It should be observed that in this case the initial concentration of pDMDAAC was similar to the indicator concentration.



**Fig. 3.** Absorbance spectra of a 100 mL sample containing 2.6  $\mu\text{eq}$  pDMDAAC in 0.01N NaCl and 2.6  $\mu\text{eq}$  OTB after addition of increasing quantities of KPVS 200  $\mu\text{N}$  (the curves from 1 to 7 correspond to quantities of KPVS added from 0 to 6.4  $\mu\text{eq}$ ).

Figure 3 shows that initially (curve 1) the maximum absorbance wavelength was at 628 nm. From curve 3 it can be seen that there was a hypsochromic shift of the band from 628 nm to 509.5 nm that can be ascribed to the adsorption of indicator on the excess of KPVS. The decrease in absorbance value at 628 nm during titration was greater than the increase in absorbance value at 509.5 nm. For this reason, the 628 nm wavelength was chosen to follow the titration. Sjöding and Ödberg (1996) also used this wavelength (628 nm) for measuring the amount of dye adsorbed onto the KPVS.

### Optimal Indicator Concentration

There is a critical concentration of ions in a titration medium, above which no color change of the indicator can be observed (Eklund and Lindström 1991; Sjödin and Ödberg 1996). In the presence of NaCl, the critical concentration of ions is 0.02N, and in the presence of divalent cations it is even lower (Eklund and Lindström 1991). To avoid this problem, Sjödin and Ödberg (1996) proposed to increase the indicator concentration, because they considered the interference of the ions as an ion-exchange process with KPVS as ion-exchanger. However, these authors found that if the concentration of o-toluidine blue was greater than 20  $\mu\text{N}$ , the indicator tended to form a precipitate with the excess of KPVS.

Rice and Roeraade (2003) used a 25  $\mu\text{N}$  OTB solution, but they added Tween 20 0.1% (a non-ionic surfactant) to reduce the OTB/KPVS complex precipitation and/or adsorption in the quartz cell. In our work, a concentration of 30  $\mu\text{N}$  was adopted to observe the indicator color change using absorbance data. Under this condition, the initial

absorbance of the sample was around 0.800 at 628 nm and decreased to values of 0.300-0.350 during the titration. Despite the fact that the indicator initial concentration was high (30  $\mu$ N), the addition of Tween 20 reduced precipitation problems.

A possible alternative is to use a flow cell of greater optical path-length in the spectrophotometer, allowing work with lower indicator concentrations. Nevertheless, it is important to bear in mind that if the concentration of the indicator is too low, the ionic strength of the medium could reduce the change in absorbance.

### Ionic Strength

Kam and Gregory (1999) showed that, when a polymer of high charge density (2.5 to 4 meq/g) and high molecular mass ( $M_w$ :  $1.10^6$  to  $3.10^6$ ) is titrated with KPVS, using spectrophotometric determination of the colour change of the OTB indicator, the equivalence volume at break-point is independent of the ionic strength up to  $5.10^{-2}$  M NaCl. Nevertheless, using streaming current analysis, Chen et al. (2003) found that the presence of salt levels higher than  $9.10^{-4}$  M  $CaCl_2$  disturbs the 1:1 stoichiometry between pDMDAAC ( $M_w$ :  $4.10^5$ - $5.10^5$ ) and KPVS.

Considering that the colloidal titration method would be applied to the industrial suspensions or industrial waste water, a medium level of  $1.10^{-2}$  N NaCl was adopted in this work. In the lab, this salt concentration was used to build the adsorption isotherms of pDMDAAC onto cellulosic fibers in order to determine their surface charge (Winter et al. 1986, Wågberg et al. 1989, Laine et al. 1996).

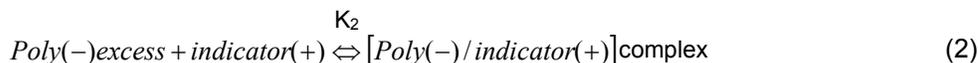
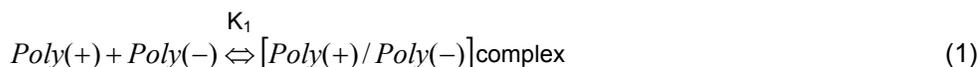
For all the experimental titration curves presented in this work, the initial NaCl concentration was  $1.10^{-2}$  N.

### Titration Curves

Figure 4 shows the absorbance data recorded during polyelectrolyte titration as a function of the volume of KPVS added.

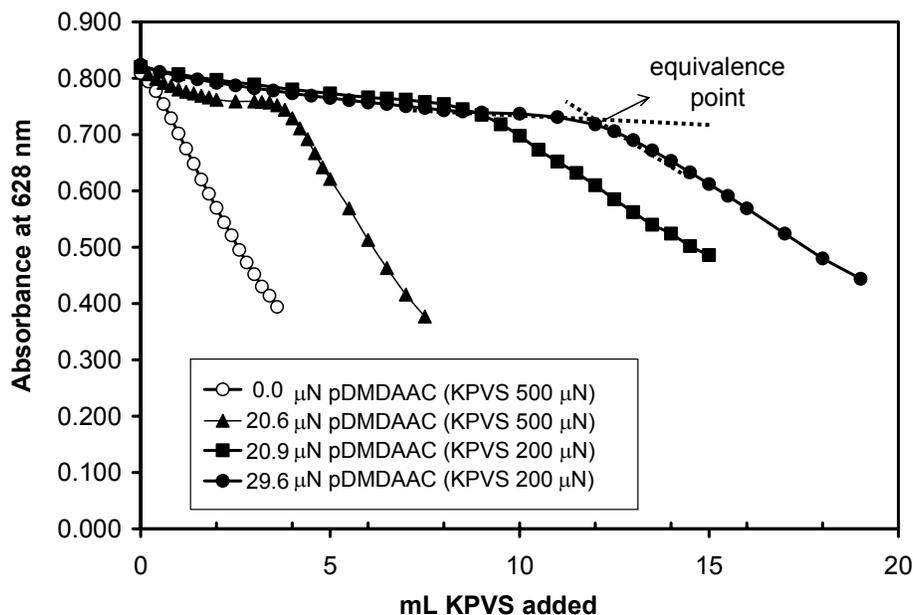
At the beginning of titration, the absorbance value of the solution at 628 nm decreased only because the titrant diluted the solution. KPVS formed a complex together with the pDMDAAC present in the solution, without reacting with OTB. Once all the pDMDAAC had reacted, an excess of KPVS formed a complex with OTB, the absorbance value at 628 nm decreased, and the absorbance value at 509.5 nm increased.

The equilibrium reactions which took place during the polyelectrolyte titration can be expressed as follows:



Where  $K_1 \gg \gg K_2$

where  $K_1$  and  $K_2$  are the equilibrium constants (Eklund and Lindström 1991).



**Fig. 4.** Titration curves corresponding to different initial pDMAAC concentrations. The OTB concentration in the solution to titrate was  $30\mu\text{N}$  (in the presence of Tween 20) and two different KPVS concentrations ( $200\mu\text{N}$  and  $500\mu\text{N}$ ).

Considering the same equilibrium reactions (1) and (2), Kam and Gregory (1999) stated that if  $K_1$  is 100 times as much as  $K_2$  or higher, reaction (2) only takes place when reaction (1) is completed, meaning when all the cationic polymer forms a complex with the anionic polymer. Then, the point where the absorbance of the sample solution just begins to fall can be taken as the equivalence point of the titration (Fig. 4).

However, Rice and Roeraade (2003), who used the same polyelectrolytes, found that it is difficult to identify the exact equivalence point of titration because a certain non-specified quantity of the OTB is adsorbed onto KPVS before its color changes. They proposed to add enough KPVS to adsorb all of the indicator, and then to calculate the equivalence point by subtracting the KPVS in excess consumed to neutralize the indicator.

In the present work, using indicator concentrations greater than  $20\mu\text{N}$ , it was observed that titrating further than the break-even point, which means adding greater quantities of KPVS, precipitations and/or depositions onto the surface of the cell or other parts of the circuit can occur. To avoid this problem, the intersection of the curves asymptotes (where the absorbance of the solution just begins to fall) was taken as the equivalence point of the titration (Fig. 4). Masadome (2003) used the same criterion to determine the equivalence point of a polyelectrolyte titration using the crystal-violet indicator.

### Accuracy of the Technique

Table 1 shows the variation coefficients obtained when solutions of increasing concentration of pDMAAC ( $20\text{--}48\mu\text{N}$ ) were titrated in duplicate in the presence of  $30\mu\text{N}$  OTB and  $0.01\text{N}$  NaCl, using spectrophotometric measurements. The coefficient was found to vary between 1.7 and 2.1%.

**Table 1.** Variation Coefficients in the Colloidal Titration Technique

pDMAAC concentration	mL of KPVS 200 $\mu$ N spent in titration*	$\bar{x}$ (mL of KPVS)	standard deviation	Variation coefficient $\left(\frac{s}{\bar{x}}\right) \cdot 100$
20 $\mu$ N	8.97 8.72	8.85	0.18	2.1
28 $\mu$ N	12.49 12.20	12.34	0.20	1.7
38 $\mu$ N	19.46 18.90	19.18	0.40	2.1
40 $\mu$ N	19.45 19.92	19.68	0.33	1.7
48 $\mu$ N	24.30 24.94	24.62	0.46	1.8

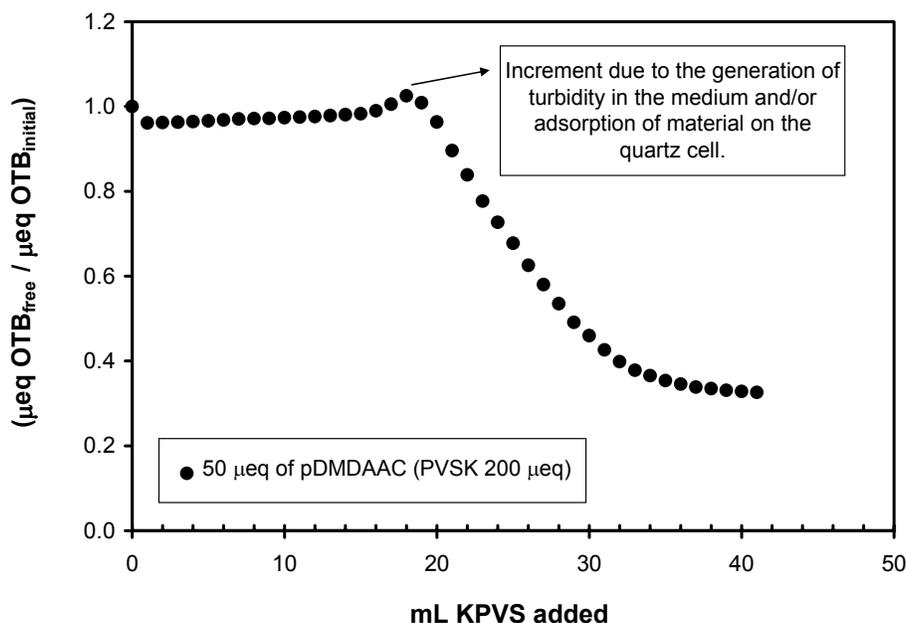
\* original and duplicate

### Concentration of the Polyelectrolyte to Titrate

Figure 5 shows the free to initial value of the indicator ratio,

$$\mu\text{eq}_{\text{free}}^{\text{OTB}} / \mu\text{eq}_{\text{initial}}^{\text{OTB}} \quad (\text{see equation (3) from the Appendix})$$

as a function of the mL of KPVS added to a solution initially containing 50  $\mu$ N of pDMAAC. This kind of figure makes it possible to see the break-even titration curve with greater accuracy than the absorbance plotted as a function of the volume of KPVS added (Fig. 4).



**Fig. 5.** Titration curve of a sample containing 50 $\mu$ N pDMAAC and 30  $\mu$ N OTB concentrations (in the presence of Tween 20), and 200  $\mu$ N KPVS titrant concentration.

Figure 5 also shows that for high pDMDAAC concentration in the solution to titrate (50  $\mu\text{N}$ ) there is an increase in the absorbance value at 628 nm near to the equivalence point of titration. This behavior can be ascribed to the generation of turbidity in the medium and/or the adsorption of material on the quartz cell. To avoid this problem, concentrated pDMDAAC solutions were diluted for titration, keeping constant both the ionic strength of the medium and the indicator concentration.

## CONCLUSIONS

1. The colloidal titration technique is possible only if the ionic strength of the titration medium, the indicator concentration and the concentration of the polyelectrolyte to titrate are taken into account.
2. There exist a minimum and a maximum level of the indicator concentration to clearly determine its color change. The minimum quantity is limited by the ionic strength of the titration medium and by the need of detectable absorbance values by spectrophotometer. The indicator maximum quantity is determined by the possibilities of the KPVS/OTB precipitation. The addition of a surfactant as Tween 20 to the indicator solution is a possibility to minimize the latter.
3. The titration of concentrated cationic polyelectrolyte solutions is possible if beforehand the solutions are diluted to a concentration lower than 50  $\mu\text{N}$ , provided that the indicator concentration is preserved.
4. High ionic strength solutions (greater than 0.02 N NaCl) should be diluted and/or the indicator concentration should be increased before titration.
5. The determination of the equivalence point using spectrophotometric measurements has acceptable precision results.

### *Appendix: Determination of the ( $\mu\text{eq}_{\text{free}}^{\text{OTB}} / \mu\text{eq}_{\text{initial}}^{\text{OTB}}$ ) ratio*

The following equation was used to draw Figure 5. It is a correction to account for the dilution effect caused by addition of the KPVS.

$$\frac{\mu\text{eq}_{\text{free}}^{\text{OTB}}}{\mu\text{eq}_{\text{initial}}^{\text{OTB}}} = \frac{A_{\text{diluted solution}} \cdot (V_{\text{initial}} + V_{\text{KPVS}})}{A_{\text{initial}} \cdot V_{\text{initial}}} \quad (3)$$

where:

$\mu\text{eq}_{\text{initial}}^{\text{OTB}}$  = initial OTB microequivalents in the solution to be titrated.

$\mu\text{eq}_{\text{free}}^{\text{OTB}}$  = OTB microequivalents not adsorbed onto the KPVS (uncomplex).

$A_{\text{initial}}$  = absorbance at 628 nm of the initial solution to be titrated.

$A_{\text{diluted solution}}$  = absorbance of the solution during titration.

$V_{\text{initial}}$  = initial volume of the solution to be titrated.

$(V_{\text{initial}} + V_{\text{KPVS}})$  = total volume of the solution.

## ACKNOWLEDGMENTS

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## STUDY OF SORPTION PROPERTIES OF LIGNIN-DERIVATIZED FIBROUS COMPOSITES FOR THE REMEDIATION OF OIL POLLUTED RECEIVING WATERS

S. Nenkova

The sorption properties of lignin-wool composites towards oil pollution at different concentrations of the contamination were studied. The release ability of oil pollutant was studied by a gravimetric method and by determining the chemical oxygen demand of cleaned water. It has been established that technical hydrolysis lignin-wool composites display a low release ability of oil-based pollutants and a slow rate for achieving release equilibrium.

*Keywords:* Technical hydrolysis lignin, Wool shoddy, Sorbent, Oil pollution of water, Release equilibrium

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### INTRODUCTION

Oil spills that occur from river-going vessels, in ports, and from manufacturing facilities adjacent to rivers are an exceptionally great ecological problem. Nowadays, the ecological protection against oil pollution is being carried out in different ways and means, depending on the kind, the composition, and the location of the pollution.

A number of papers (Masahiro 1994; Fletcher 1994; Davis 1994) are related to using biological means for cleaning waters, but these methods are not applicable for typical situations. In those cases the use of highly effective fibrous-polymer sorbents may be of great importance.

Papers and patents are available that deal with highly fibrillated fibrous materials for cleaning failing protein water (Chem 1998), micro-fibrous sorbents on the basis of thermoplastic polymers (Eur. Patent Appl. EP0608884 1994), and wool fibrous materials of high surface area for cleaning of oil spills in the Persian Gulf in 1990 (Ronald 1993). Testing of wood saw dusts for the cleaning of water surface from average oil spills (Hianov 1995), preparation of highly effective sorbents on the basis of fibrous waste from paper production (Kugin 1994), and the use of lignin-containing waste for cleaning water (Grigoriev 1996) have also been reported.

The possibilities for cleaning oil pollution by sorbents on the basis of fibers, polymer, and wood, however, have not been well investigated. Good remediation of oil-polluted water can be achieved with wood-fibrous sorbents, and in particular with fibrous and lignin-containing materials due to their high sorption capacity towards oil, oil products, and heavy metals. These fibrous and lignin-containing materials are cheap, as well as being waste products with high sorption ability. Of special interest in this respect are the wool shoddy (WS) and technical hydrolysis lignin (THL). These materials possess

highly developed surfaces, which together with different functional groups determine their high sorption ability. Technical hydrolysis lignin (THL), synthetic fabrics, or fibrous wastes from their production are used for collection of oil and lubricants from water and soil (Russian Patent RU2049543 1995).

The sorption of lignin under dynamic turbulent conditions has been investigated. The increasing of the degree of turbulence leads to a higher sorption rate and velocity. The rate and velocity of sorption depends on the ratio of oil product to lignin. Two technological schemes for the remediation of oil-polluted water with hydrolyzed lignin have been developed (Finland Patent FI912863 1992).

The criteria necessary to achieve selection of suitable sorbents for the remediation of polluted water are not only the initial and final sorption capacity but also the ability for retention of the oil pollutants, i.e., it must possess a low desorption rate.

Data on the ability of lignin and textile fibers to retain of oil pollutants once the sorption equilibrium has been reached has not been reported. Therefore, the release equilibria at different concentrations of oil pollutants have not been previously investigated. The determination of such a correlation would be of great practical importance for remediation oil spills that possess different thicknesses on a receiving water surface.

The purpose of the present investigation is to provide a study of the release characteristics of oil pollutants of wood - fibrous composites based on lignin and wool shoddy at different contamination concentrations.

## EXPERIMENTAL

### Materials

All work was conducted with the following materials:

- Technical hydrolysis lignin (THL), obtained by acid hydrolysis of wood in industry and situated in 2001 year near the town Razlog, Bulgaria. The size fraction with 0.125-0.315 mm was used in the present study.
- Fibrous waste materials (the so-called wool shoddy (WS) consisted of 50 weight percent wool, 25 weight percent viscose, and 25 weight percent polyamide)

The ratios of THL:WS were 50:50; 75:25 and 90:10.

A crude oil with 0.85 g/cm<sup>3</sup> specific weight and pH 7 was used for oil pollution at a ratio of oil to water of 10:0 (100 weight% oil), 6:4 (60 weight % oil), and 4:6 (40 weight % oil).

### Techniques

Two methods to study release characteristics have been developed:

#### *1. Weight method for the determination of oil flow*

Desorption properties were investigated at constant temperature (20°C). A glass tube with diameter  $d=25$  cm and height  $h=55$ cm was used. Wood-fibrous material (THL/WS) was put into the tube, and then the oil or mixture of oil/water was poured on.

The oil released quantity was measured during 270 min at regular intervals after a 15 min waiting time for obtaining the maximal sorption of oil pollution.

## 2. Chemical Oxygen Demand (COD) testing

The release of bound oil was determined by Chemical Oxygen Demand (COD) testing. The COD was ascertained by using potassium dichromate, a well known method for the assessment of the concentration of organic pollutants. 50 ml of water was poured into the THL with WS that was in the glass tube after completion of the desorption process as described above (weight method). After one minute, water was drawn out for the next 5 minutes. This process was repeated until release equilibrium (flowing of clear water out of the tube) was achieved. The COD of the oil-containing fractions was then determined.

## RESULTS AND DISCUSSION

The experimental data corresponding to the release of the oil pollutants from a lignin-fibrous mixture (THL:WS-50:50) at three ratios of oil to water, are shown in Fig.1. It can be seen from Fig. 1 that the release at 100 % (10:0) oil pollutant concentration was steady, and the release equilibrium was reached after 240 minutes.

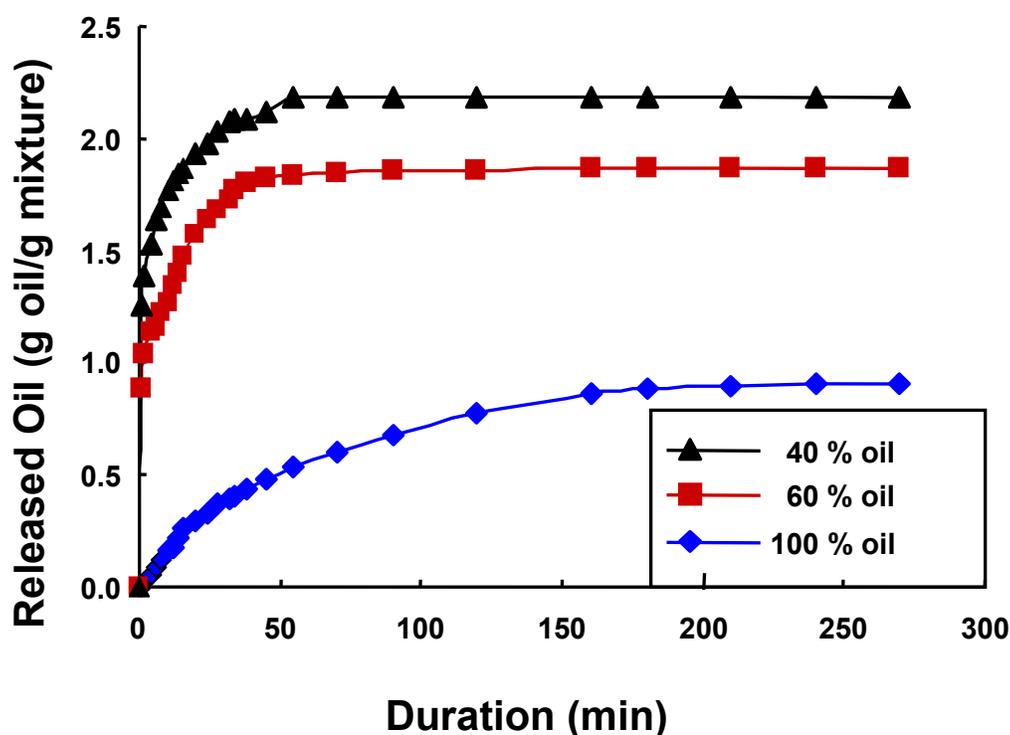


Fig.1. Released quantity of oil by THL:WS (50:50) at different ratios of oil:water

At the ratios 6:4 and 4:6 oil to water it was seen that initially only water release occurred from the mixture (7 min for 60 % oil and 10 min for 40 % oil). The release equilibrium was reached after 240 minutes at 100 % oil and after only 60 min at 40 % oil. 100 % retention of oil pollutants was observed at the ratio 4:6. Therefore, the composite mixture of THL/WS – 50:50 is expected to be very suitable for use as a sorbent in the case of oil spills having low oil concentration.

The dependence of oil released quantity on lignin-fibrous composite (THL/WS-75/25) at three ratios of oil:water over 260 min is given in the Figure 2. The data show that the process of release of oil at 100 % oil pollution was rapid up to 60 minutes, and afterwards was steady. The equilibrium at the other ratios was reached very quickly, i.e., after approximately 30 minutes.

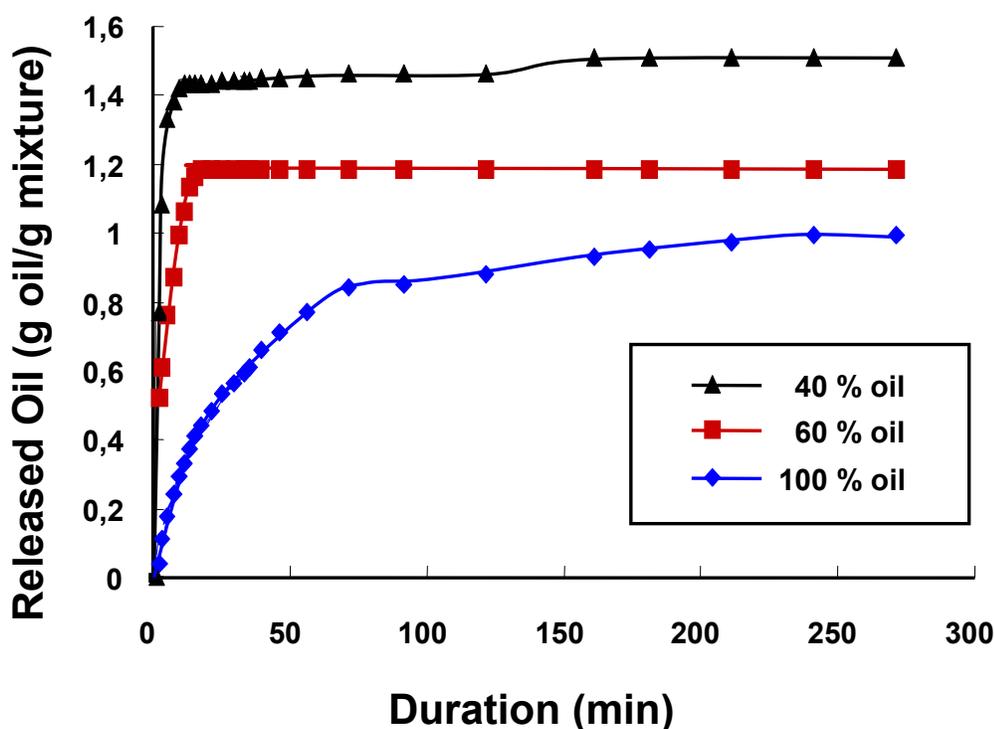


Fig. 2. Released oil quantity by THL:WS (75:25) at different oil percentage fractions

The data for the composite of 90 %THL and 10 % WS demonstrates the following dependencies:

- the release equilibrium was reached very quickly (38 minutes),
- initial water release was not observed, and
- released oil quantities at the three ratios of oil to water (10/0, 6/4, 4/6) were similar ( Fig. 3).

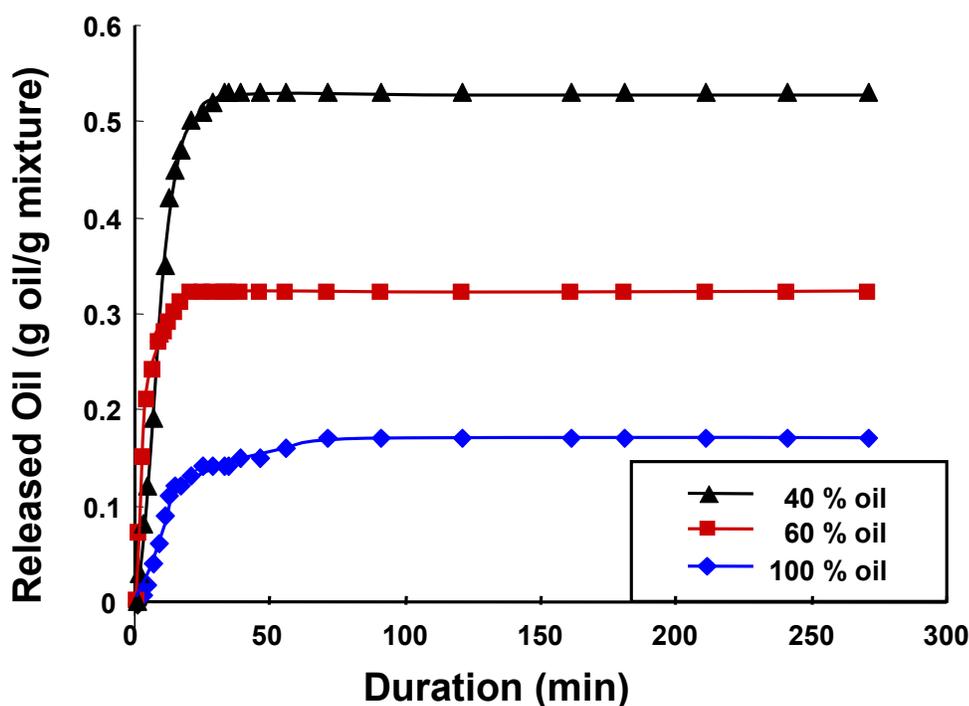


Fig. 3. Released oil quantity by THL:WS (90:10) at different oil percentages

Undoubtedly, these findings can be attributed to the favorable influence of the two components in the composites with the highest content of lignin. The WS gave higher maximal sorption, but THL displayed a higher capacity to retain the oil.

The established dependencies were confirmed from the results of the retained oil quantities at the three ratios of oil to water (Table 1).

A summary of the previous dependences is as follows:

- It is observed that there was a gradual decrease of the initial sorption capacity of composites when the level of WS ranged from 50 % to 10 %;
- The release process was similar at the three ratios of THL to WS;
- The release process of the three composites at the ratio of oil/water (6:4) was similar, but there were significant differences for the ratio of oil to water (4:6);

Therefore, it can be concluded that the THL/WS composites have significant advantages over the separate use of the two components in the process of release of oil

The data for COD, characterizing the sorption in different batches of materials, at the three ratios of oil/water - 10:0; 6:4; 4:6, are presented in Figs. 4, 5, and 6. The dependencies were similar to those for the physical desorption, as determined by the weight method.

The average rate of release was determined on the basis of the COD data. By washing water from the sorbent, the oil situated in the capillary system, i.e. connected by sorption process occurring during the pour and partially by physical adsorption, was separated. The contamination of this rinse water was determined by COD analysis.

**Table 1.** Oil, Retained Quantity (g oil/g mixture)

Duration (min)	THL:WS – 50:50			THL:WS – 75:25			THL:WS – 90:10		
	100 %	60 %	40 %	100 %	60 %	40 %	100 %	60 %	40 %
0.0	4.53	4.81	5.15	3.00	3.72	3.99	3.19	3.29	3.54
0.5	4.53	4.18	4.35	3.00	3.31	3.57	3.12	3.28	3.52
1.0	4.53	3.93	3.91	2.97	3.20	3.22	3.03	2.22	3.51
1.5	4.52	3.81	3.84	2.94	3.15	3.02	2.99	2.17	3.48
2.0	4.51	3.77	3.78	2.92	3.11	2.91	2.95	2.14	3.46
3.0	4.48	3.73	3.71	2.88	3.03	9.72	2.88	2.10	3.45
4.0	4.46	3.37	3.64	2.85	2.96	2.66	2.82	2.08	3.42
5.0	4.44	3.66	6.58	2.83	2.86	2.62	2.74	2.07	3.40
6.0	4.43	3.62	3.53	2.80	2.85	2.61	2.73	2.05	3.35
7.0	4.42	3.62	3.51	2.78	2.78	2.59	2.67	2.04	3.30
8.0	4.40	3.59	3.48	2.76	2.73	2.57	2.62	2.02	3.26
8.5	4.39	3.58	3.45	2.75	2.70	2.57	2.60	2.02	3.24
9.0	4.39	3.57	3.43	2.74	2.69	2.57	2.59	2.01	3.22
9.5	4.38	3.56	3.41	2.73	2.68	2.56	2.58	2.01	3.21
10.0	4.37	3.54	3.40	2.72	2.66	2.56	2.57	2.01	3.19
10.5	4.37	3.53	3.39	2.71	2.64	2.56	2.55	2.00	3.18
11.0	4.36	3.52	3.38	2.70	2.63	2.56	2.53	2.00	3.15
11.5	4.35	3.50	3.37	2.70	2.61	2.56	2.52	2.00	3.14
12.0	4.35	3.47	3.36	2.69	2.59	2.56	2.50	2.00	3.12
12.5	4.35	3.46	3.35	2.69	2.58	2.56	2.49	2.00	3.11
13.0	4.35	3.44	3.34	2.68	2.57	2.56	2.49	2.00	3.09
13.5	4.35	3.42	3.33	2.67	2.57	2.56	2.48	2.00	3.09
14.0	4.33	3.41	3.32	2.66	2.56	2.56	2.48	1.99	3.09
14.5	4.33	3.39	3.31	2.66	2.55	2.56	2.47	1.99	3.08
15.0	4.32	3.37	3.31	2.65	2.54	2.56	2.46	1.99	3.08
16.0	4.31	3.34	3.30	2.64	2.54	2.56	2.46	1.98	3.07
17.0	4.29	3.32	3.28	2.63	2.54	2.56	2.46	1.98	3.07
20.0	4.26	3.24	3.24	2.60	2.54	2.56	2.46	1.97	3.04
24.0	4.24	3.17	3.19	2.57	2.54	2.55	2.46	1.97	3.03
26.0	4.23	3.15	3.16	2.55	2.54	2.55	2.46	1.97	3.02
28.0	4.21	3.13	3.14	2.54	2.54	2.55	2.46	1.97	3.02
34.0	4.16	3.04	3.09	2.50	2.54	2.55	2.46	1.97	3.01
38.0	4.14	3.01	3.08	2.46	2.54	2.54	2.46	1.97	3.01
50.0	4.08	2.98	3.02	2.40	2.54	2.54	2.46	1.97	3.01
70.0	4.02	2.96	2.99	2.31	2.54	2.53	2.46	1.97	3.01
100.0	3.93	2.95	2.99	2.27	2.54	2.53	2.46	1.97	3.01
140.0	3.84	2.95	2.99	2.25	2.54	2.48	2.46	1.97	3.01
180.0	3.75	2.94	2.99	2.21	2.54	2.48	2.46	1.97	3.01
240.0	3.66	2.94	2.99	2.15	2.54	2.48	2.46	1.97	3.01
270.0	3.66	2.94	2.99	2.15	2.54	2.48	2.46	1.97	3.01

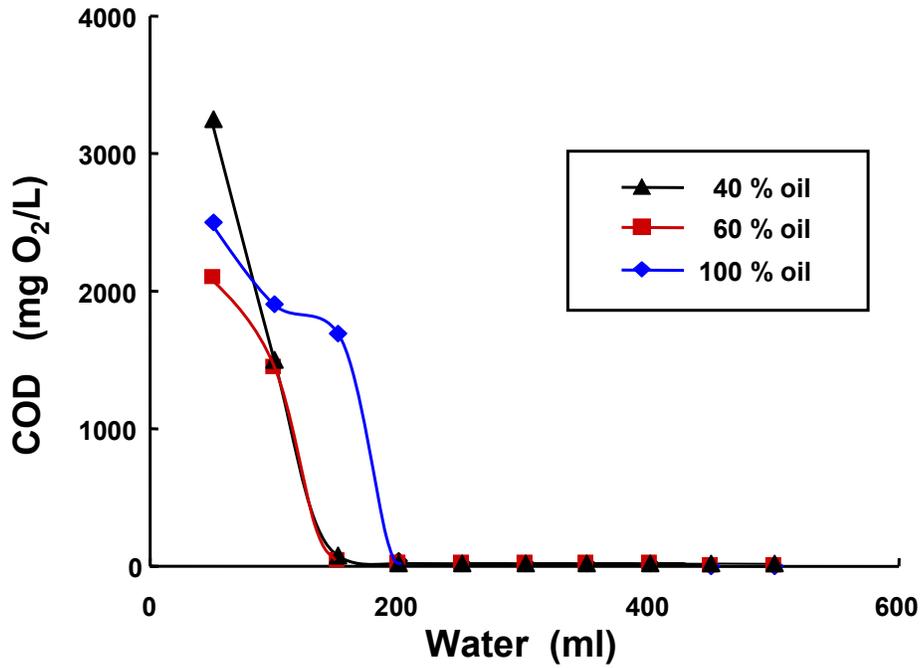


Fig. 4. Dependency of COD at different oil percentages by THL:WS (50:50) at different ratio oil:water

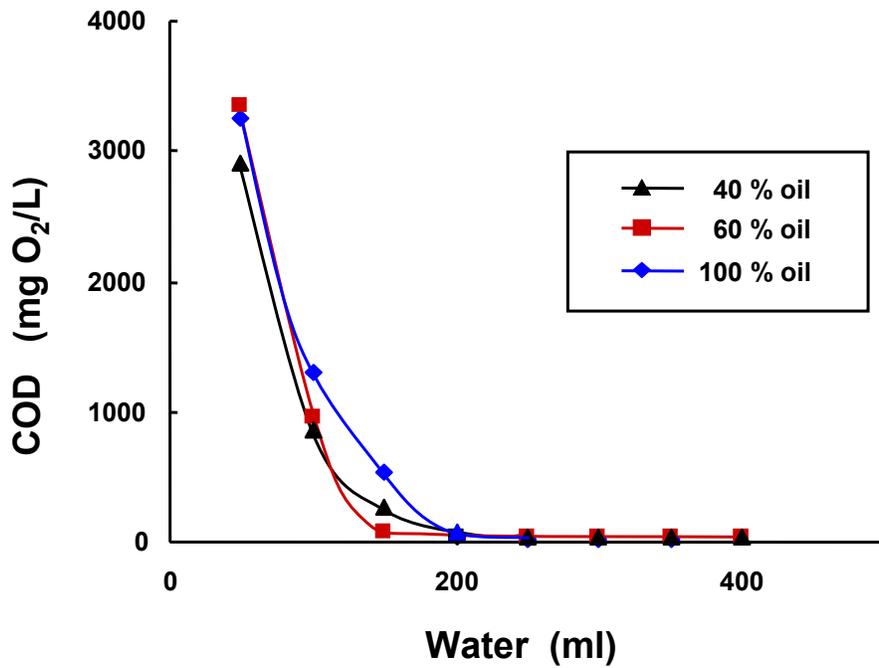
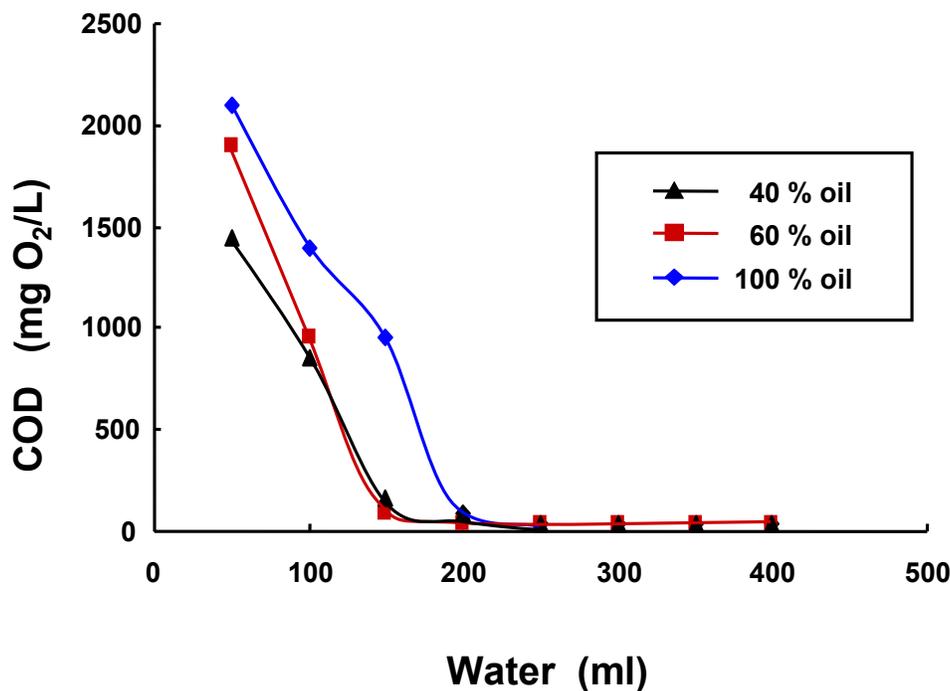


Fig. 5. Dependency of COD at different oil percentages by THL:WS (75:25) at different ratio oil:water



**Fig. 6.** Dependency of COD at different oil percentages by THL:WS (90:10) at different ratios of oil:water

The average rate of release of oil pollution from the sorbent was estimated by:

$$v_{av} = \frac{\Delta COD}{V \Delta \tau}, \quad (1)$$

$v_{av}$  - average rate, mg O<sub>2</sub>.L<sup>-1</sup>.min<sup>-1</sup>;

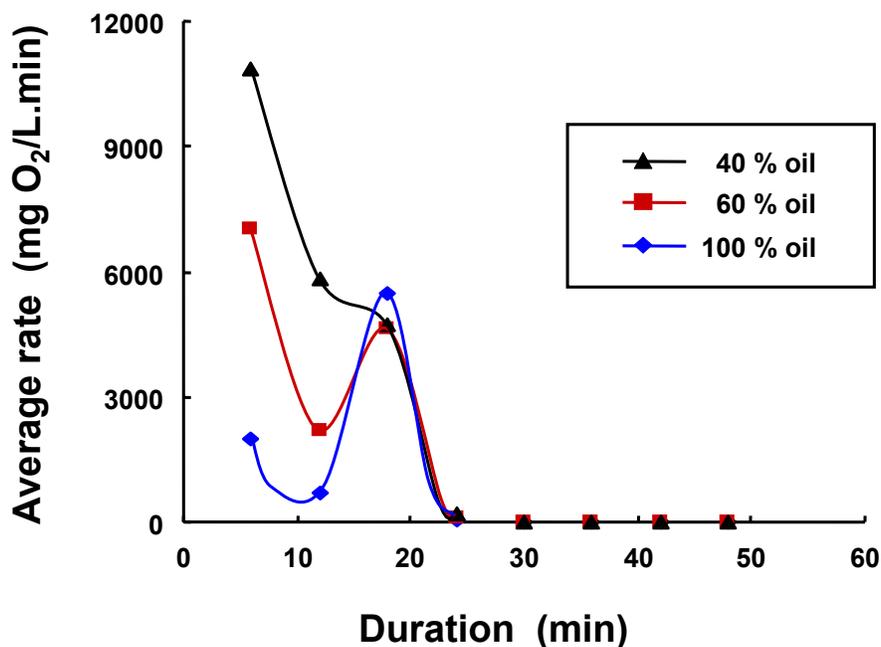
COD - Chemical Oxygen Demand, mg O<sub>2</sub>.L<sup>-1</sup>;

$\tau$  - time, min;

$V$  - volume of washing water, L.

This formula describes the COD change of polluted water for one minute. Data for the average rate of release for the three composites are shown in Figs. 7, 8 and 9.

It was established that the release of oil from THL:WS (50:50) occurred with a high rate, independently of the ratio of oil to water. However, the behavior of the curves was different. It was observed that the ratio of oil release was slowed up in the interval of 6-18 minutes at 100 % and 60 % oil. At the same time the average rate at 40 % oil was higher and the release continued uniformly. In spite of this, the composition of THL: WS (50/50) was suitable for use, because such a sorbent will retain great quantities of oil (it possesses a high sorption capacity but shows a lower release ability) (Fig.7).



**Fig. 7.** Dependency of average rate of release ability at different oil percentage by THL:WS (50:50) at different ratios of oil:water

The addition of 25 % WS to THL (mixture of THL:WS equals 75/25) led to a slight increase in the average release rate in the beginning, but the initial sorption capacity of mixture was sufficiently high such that it was optimum for the sorption process. Different dependencies for the individual ratios of oil to water were observed. The rate decreased at 100 % oil in the interval up to 18 minutes. The average rate was higher at 40 % and 60 % oil (Fig. 8).

The comparison of Figs. 7, 8 and 9 shows that the average rate of release was lowest at 10 % WS in the composite. This was attributed to the good ability of THL to retain the oil, most likely due to the THL pore structure; in fact it was found that minimal release was reached at a composition of THL: WS of (90/10).

Hence, the addition of minimal WS quantities led to a decrease in the average rate of release. This fact has practical importance, because such a composite sorbent derived from THL with 10 % WS can retain the oil pollutants for a sufficiently long time to allow the oil polluted sorbent to be recycled.

## CONCLUSIONS

1. The release properties of composites based on technical hydrolysis lignin (THL) with 50 %, 25 %, and 10 % wool shoddy (WS) towards oil pollution at different ratios of oil to water were studied.

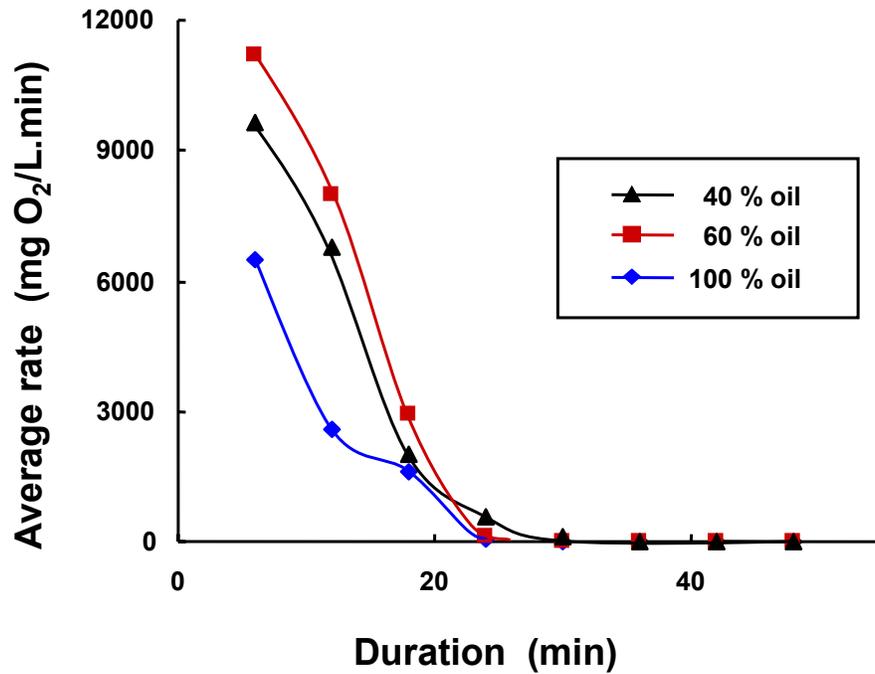


Fig. 8. Dependency of average rate of release ability at different oil percentages by THL:WS (75:25) at different ratios of oil:water

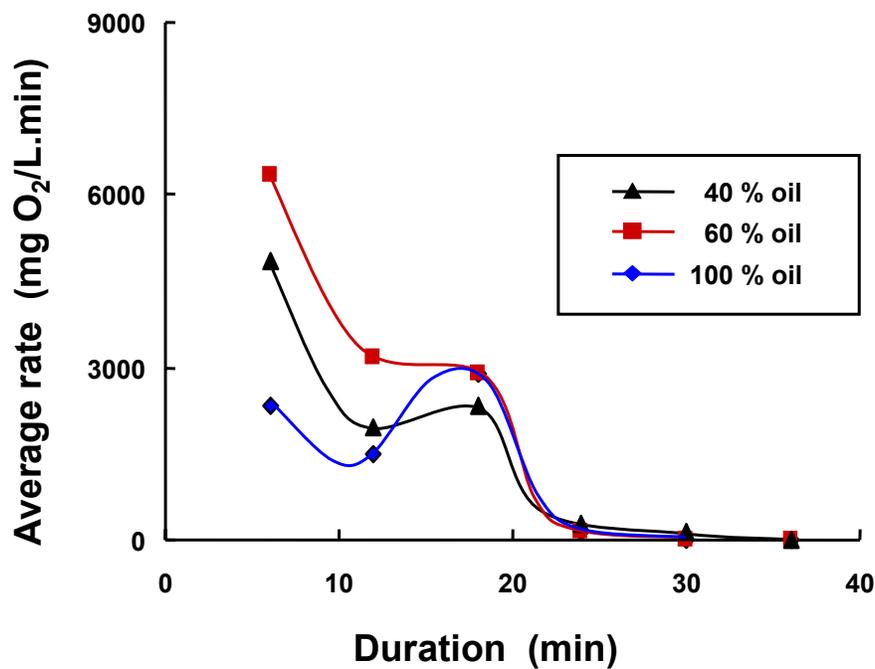


Fig. 9. Dependency of average rate of release ability at different oil percentages by THL:WS (90:10) at different ratios of oil:water

2. It was noted that:
  - the composites were suitable for use as oil sorbents;
  - the initial sorption capacity increased considerably with an increase in the WS addition; and
  - the WS addition increased the release of oil from the composite .
3. The optimal results towards sorption and release of oil pollution were reached at a ratio of THL to WS equal to 90:10.

## ACKNOWLEDGMENTS

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## L-LACTIC ACID PRODUCTION FROM LACTOBACILLUS CASEI BY SOLID STATE FERMENTATION USING RICE STRAW

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In order to make full use of rice straw (RS) produced at large quantity in China and to reduce the production cost of L-lactic acid, attempts were made to utilize the hydrolysate of RS as sole carbon source and the lignocellulose as inert support for producing L-lactic acid using solid state fermentation (SSF). The pretreated rice straw was enzymatically hydrolyzed by cellulase, and the hydrolyzate, containing reducing sugars supplemented with a minimum of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, MnSO<sub>4</sub>, and yeast extract, was used as moistening agent to impregnate 5g of RS, which was used as the inert support for SSF. Maximum L-lactic acid production of 3.467g per 5g of support was obtained at 37 °C, using *Lactobacillus casei* as inoculum, after 5 days of fermentation with optimized process parameters such as 72% moisture content, 4g per 5g support of reducing sugars, 2.5ml per 5g support of inoculum size, 3g per 5g support of CaCO<sub>3</sub>, and pH 6.5.

**Keywords:** L-lactic acid, Solid state fermentation, Rice straw, Hydrolysate, *Lactobacillus casei*

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### INTRODUCTION

Lactic acid is a versatile chemical, widely used in the food, pharmaceutical, textile, cosmetic, and chemical industries (Yun et al. 2004). In recent years there has been increasing interest in the manufacture of poly(lactic acid), a biocompatible and biodegradable plastic. Selective production of stereospecific L(+) lactic acid has been extensively studied by microbial fermentation because chemical synthesis results in racemic mixture of lactic acid (Garlotta 2001; Naveena et al. 2005a). However, only a few lactic acid bacteria, such as *Lactobacillus casei*, produce a predominately optically pure L(+) form of Lactic acid (Senthuran et al. 1997).

The economics of L(+)-lactic acid production is still a problem, and media composition contributes significantly to the overall cost of L(+)-lactic acid fermentative production. In order to bring down the production cost of L(+)-lactic acid, research efforts have been undertaken to replace the expensive carbon and nitrogen sources with cheap raw materials in the media (Hofvendahl and Hahn-Hagerdal 2000). Utilization of renewable, cheap, and abundant agro-industrial waste for the production of L(+)-lactic acid is an increasing trend in recent years. Two bioprocess schemes, termed simultaneous saccharification and fermentation, and separate hydrolysis and fermentation, have been developed for lactic acid production using lignocellulosics (Yun et al. 2004; Bustos et al. 2005; Shen and Xia 2006).

Solid state fermentation (SSF) processes involve the growth of microorganism on a solid material in the absence or near-absence of free-flowing water (Krishna 2005). It usually uses agro-industrial waste as support and/or carbon source for

production of various value-added products, such as single-cell protein, industrial enzymes, secondary metabolites, and fine chemicals. The subject has been widely studied (Robinson et al. 2001; Soccol and Vandenberghe 2003; Couto and Sanroman 2005; Couto and Sanroman 2006). Compared to submerged fermentation, the SSF process is a simple process with improved product characteristics, higher product yields, reduced energy requirements and initial capital cost, lower water output, and easier product recovery (Ooijkaas et al. 2000; Adinarayana et al. 2003).

There have been quite a few studies published in these years concerning lactic acid production from lignocellulosic materials under submerged liquid fermentation (John et al. 2007; Zhang et al. 2007). However, to the best of our knowledge, there have been no reports regarding the production of L(+)-lactic acid by *Lactobacillus casei* under SSF using rice straw (RS) as an inert support and hydrolysate of pretreated RS hydrolyzed by cellulase as sole carbon source.

## EXPERIMENTAL

### Microorganism and Inoculum Preparation

*Trichoderma viride* YQ-02, isolated by our laboratory, was used for cellulase production. The stock culture was stored on potato-glucose-agar (PDA) at 4°C and subcultured every two months. *Lactobacillus casei* GIM 1.159 (originally from ATCC 334), a homofermentative lactic acid producer, was used for L(+)-lactic acid production. The strain was maintained on deMan, Rogosa and Sharpe (MRS) slant (DeMan et al. 1960), and subcultured every month. A loop of culture was inoculated into 250ml conical flasks containing 45ml MRS medium and incubated at 37°C for 20 h. The 20h old bacterial culture was used as the inoculum for SSF.

### Lignocellulosic Materials and Pretreatment

Raw RS was obtained locally in Hefei, Anhui province, China. It was ground to 80-120mesh with an electric grinder and was used in the experiments. The smashed RS was stored at room temperature until its use. The initial composition of the RS was determined to be 33.3% cellulose, 23.32% hemicellulose and 17.5% lignin. A two-step chemical pretreatment: dilute acid pretreatment and alkaline peroxide pretreatment, was carried out as described by Curreli et al. (2002). The cellulose, hemicellulose, and lignin contents of pretreated RS were 68.8%, 9.4%, and 8.9%, respectively. The pretreated RS was used as substrate for enzymatic saccharification.

### Cellulase Production and Extraction

Cellulase was produced by SSF. A two-stage technique was employed. In the first stage, the fungus was grown on PDA slants for 5 days at 30°C. In the second, 10ml deionized sterile water was added to a PDA slant and a spore suspension containing 10<sup>7</sup> spores/ml was used to inoculate the solid state medium (2.5cm thick) in 500ml Erlenmeyer flasks containing RS and wheat bran (dry weight ratio is 3:2) moistened with the following mineral medium (g/l): (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 10; KH<sub>2</sub>PO<sub>4</sub> 3; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5, and CaCl<sub>2</sub> 0.5 (Jecu 2000). The initial pH value of the medium was adjusted to 5.0 after sterilization at 121°C for 30 min, and the water content of the substrate was 75%. The flasks were incubated at 30°C for 96 h under static conditions.

Cellulase was extracted by suspending the solid state medium in 10-fold 0.1 M

citrate buffer of pH 4.8 for one hour and centrifuged to remove spores of the organism. The supernatants obtained were further concentrated by ammonium sulfate precipitation. The enzyme salt solutions were dialyzed for removing ammonium salt and were further concentrated with poly(ethylene glycol) (PEG) 20,000. Concentrated cellulase solutions with a filter paper activity (FPU) of 5.028IU/ml and a cellobiase activity (CBU) of 3.017IU/ml were used in the hydrolysis experiments.

### Enzymatic Hydrolysis of Pretreated RS

Enzymatic saccharification was carried out at 50°C for 48 h with the pH adjusted to 4.8 by addition of 0.1M citrate buffer solution. The enzyme loading was 50 FPU/g substrate, and the substrate concentration was 50g/l. The hydrolysate were centrifuged, and clear supernatants containing reducing sugars were used as sole carbon source for SSF.

### Solid State Fermentation (SSF) for L-Lactic Acid Production

Experiments were conducted in 250 ml conical flasks containing 5g inert support (powered RS). The concentrated hydrolyzate, supplemented with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.5g/5 g support) and MnSO<sub>4</sub> • H<sub>2</sub>O (0.1g/5 g support) and a growth promoter, yeast extract (0.5g/5 g support), was used as moistening agent to impregnate the support. 2.5g/5 g support of CaCO<sub>3</sub> was also added into it as a buffering agent to neutralize the lactic acid produced by microbial fermentation. The flasks were autoclaved at 121°C for 15min. After cooling, the sterilized solid medium was inoculated with 2 ml cell suspension (20h old), mixed thoroughly, and incubated at 37°C for 5 days.

### Optimization of Process Parameters

Optimum physico-chemical and nutrient parameters required for maximum L(+)-lactic acid production by *Lactobacillus casei* under SSF were determined for initial moisture content of the support (45-90% w/w), initial reducing sugar concentration (2-4.5g/5g support), initial pH of the medium (pH 5 to 7), Tween 80 concentration (0-0.2g/5g support), incubation temperature (25-42°C), inoculum size (1.5-3.5ml), yeast extract concentration (0-2g/5g support), MnSO<sub>4</sub>·H<sub>2</sub>O concentration (0-0.15g/5g support), and CaCO<sub>3</sub> concentration (1.5-3.5g/5g support). The protocol used for optimization of various process parameters was to evaluate the effect of an individual parameter and to incorporate it at the optimized level into the experiment before optimizing the next parameter. After optimizing various parameters, a time course experiment was conducted, incorporating all the optimized parameters.

### Analytical Methods

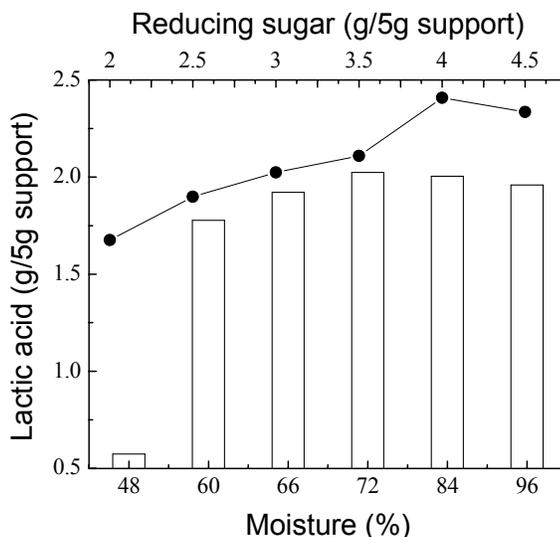
Filter paper activity (FPU) and cellobiase activity (CBU) were assayed according to a standard cellulase activities analytical procedure recommended by the International Union of Pure and Applied Chemistry (IUPAC) (Ghose 1987). One unit of enzyme activity was defined as the amount of enzyme required to liberate 1μmol of reducing sugars as glucose per minute per milliliter of crude enzyme extract under assay conditions. Cellulose, hemicellulose, and lignin were determined according to the procedures described elsewhere (Goering and VanSoest 1970). The total of reducing sugars was determined by the Dinitrosalicylic acid method (Miller 1959). L(+) lactic acid was extracted with 1M H<sub>2</sub>SO<sub>4</sub> and was estimated by the colorimetric method of Kimberley and Taylor (1996). The moisture content of the RS was estimated according

to the method described by Adinarayana et al. (2003).

Three parallel samples were used in the analytic determination, and data are presented as the mean of three replicates. Relative standard deviations in all the assays were below 5%.

## RESULTS AND DISCUSSION

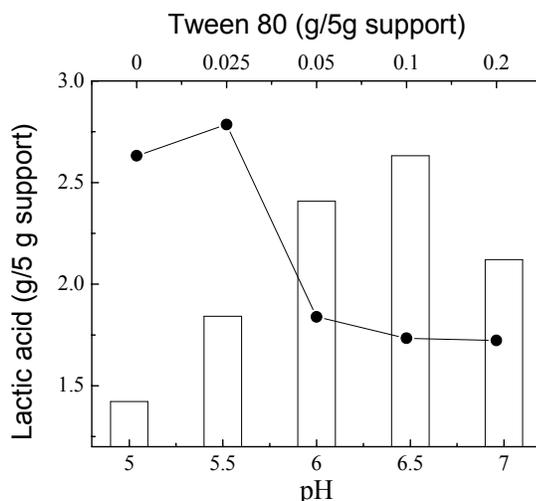
For SSF, a certain moisture content of the substrate is a critical factor, because this parameter has influence on the growth and biosynthesis and secretion of different metabolites (Krishna and Chandrasekaran 1996). In the case of some fungi a wide moisture level, ranging from 20 to 70%, supports superior growth and metabolic activity, but in the case of bacteria, only a higher moisture content of the support can yield superior performance (Sabu et al. 2006). The effect of moisture level of the support on lactic acid production was investigated by moistening the support with a minimum amount of concentrated rice straw hydrolyzate-based medium in such a way that the initial reducing sugars were kept constant at 3g/5g support. The results are presented in Fig. 1.



**Fig. 1.** Effects of moisture content of the support (□) and reducing sugar concentration (●) on lactic acid production using *Lactobacillus casei* under SSF.

As shown in Fig. 1, maximum lactic acid production was observed at an initial 72% moisture content of the support. A decrease in lactic acid production was observed when the moisture level was higher or lower than the optimum. This result was in good agreement with that of Rojan et al. (2005) and John et al. (2006), who also reported that an initial moisture content 72% was best for lactic acid production using *Lactobacillus casei* and *Lactobacillus delbrueckii*, respectively. Higher initial moisture content in SSF leads to suboptimal product formation due to reduced mass and oxygen transfer process and a decrease in porosity of the solid matrix. On the other hand, lower moisture content causes a reduction in solubility of nutrients of the substrate, a low

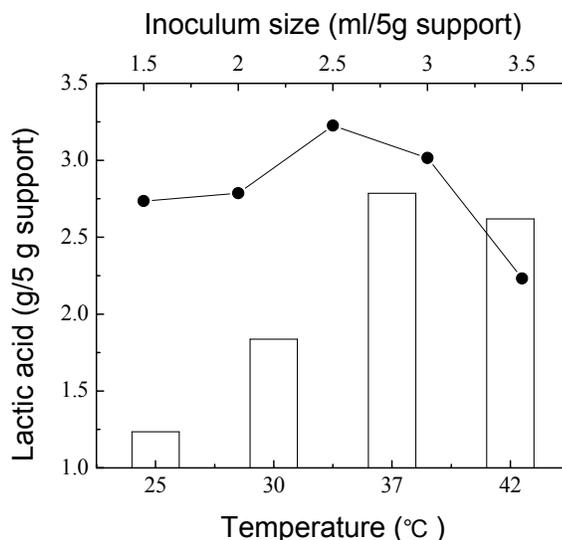
degree of swelling and a high water tension (Lonsane et al. 1985; Babitha et al. 2006). Initial reducing sugar is also a major factor that can significantly influence the lactic acid production. To optimize the concentrations of reducing sugars from the hydrolysate of pretreated RS, 5g of inert support was moistened with different volumes of concentrated hydrolyzate, varying in concentrations of reducing sugars; however, the moisture content of the support was maintained at 72%. As shown in Fig. 1, 4g initial reducing sugars per 5g of support was found to be optimum for conversion of sugars to lactic acid.



**Fig. 2.** Effects of pH of medium (□) and Tween 80 concentration (●) on lactic acid production using *Lactobacillus casei* under SSF.

The effect of initial pH of the medium on  $L(+)$  lactic acid production was tested using a pH range of 5 to 7 with a constant  $\text{CaCO}_3$  addition of 2.5g per 5g of support. As shown in Fig. 2, the lactic acid yield increased with an increase in initial pH of the medium up to pH 6.5 (2.63g/5g support), and thereafter followed a marked decrease. In SSF, an optimum pH value 6.5 for *Lactobacillus casei* obtained in this investigation did not agree with those of Rojan et al. (2005) who found pH 5.5 to be the best for producing lactic acid.  $L(+)$  lactic acid production increased to 2.79g/5g support with addition of 0.025g/5g support of Tween 80 (Fig. 2). Tween 80, a non-ionic surfactant, has been reported to enhance the microbial ability to produce some enzymes (Reese and Manguire 1969; Goes and Sheppard 1999; Feng et al. 2006; Zeng et al. 2006). Tween 80 has also been proved beneficial in the cultivation and fermentation process of some *lactobacillus* (Duggan et al. 1959), in the case of most *Lactobacillus delbrueckii* strain, unsaturated fatty acids such as Tween 80 are essential growth factors (Partanen et al. 2001). Oh et al. (1995) reported that the growth of *Lactobacillus casei* was strongly affected by Tween 80. In the production of  $L(+)$  lactic acid from wheat bran by *Lactobacillus amylophilus* in SSF, both Naveena et al. (2005b) and Nagarjun et al. (2005) pointed out that Tween 80 was found to influence the lactic acid productivity. When more than 0.025g/5g support of Tween 80 was used, Tween 80 had an adverse effect on lactic acid production (Fig. 2). An explanation to this may be that Tween 80 as a surfactant could dissolve the lipid in the cell membrane, destroy the membrane

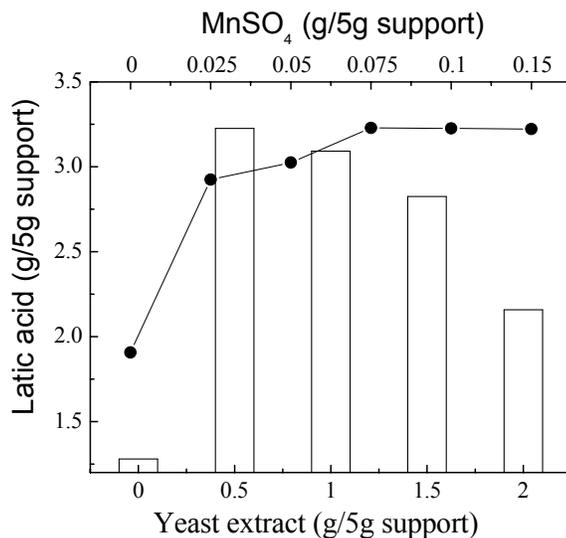
structure, and then cause the death of the cell.



**Fig. 3.** Effect of temperature (□) and inoculum size (●) on lactic acid production using *Lactobacillus casei* under SSF.

Temperature influences the bioactivity of proteins, including enzymatic activity. Thus it can be expected that temperature could promote or inhibit production of a particular metabolite. Figure 3 shows that the maximum production of L(+) lactic acid was obtained at 37°C. Higher or lower temperatures than the optimum resulted in reduced lactic acid production, although *Lactobacillus casei* could produce lactic acid within the temperature range of 30°C to 44°C (Linko and Javanainen 1996). The size of the inoculum plays a significant role in the production of metabolites under SSF. In the present study, bacterial cells were used as inoculum, and different inoculum sizes were tested in order to enhance the lactic acid production. As shown in Fig. 3, an inoculum size of 2.5ml was found to be best for production of lactic acid (3.23g/5g support). Lower inoculum sizes resulted in insufficient biomass for product production, which causes decreased lactic acid production, whereas higher inoculum size produced too much biomass and caused the depletion of nutrients necessary for lactic acid production.

Yeast extract, a costly source of nitrogen, has been commonly used in laboratory scale lactic acid fermentation, because no other nitrogen sources were competitive to yeast extract in the production of lactic acid (Wee et al. 2006). In an economic analysis for lactic acid production, the cost of yeast extract accounted for over 30% total production cost (Mulligan et al. 1991). Therefore, it is necessary to minimize the yeast extract addition while still maintaining a high lactic acid production. Figure 4 shows that addition of 0.5g/5g support of yeast extract gave the maximum lactic acid production.



**Fig. 4.** Effects of yeast extract (□) and Mn<sup>2+</sup> (●) on lactic acid production using *Lactobacillus casei* under SSF.

The effect of Mn<sup>2+</sup> on lactic acid production was studied by supplementing the SSF medium with different amounts of MnSO<sub>4</sub>. It was found that *Lactobacillus casei* produced the maximum amount of lactic acid with addition of 0.075g/5g support of Mn<sup>2+</sup>. Archibald and Fridovich (1981b) reported that some lactobacilli strains required high concentrations of manganese for growth. It has been known that lactobacilli strains were deficient in catalase activity, and the presence of manganese could act as a scavenger of toxic oxygen species such as superoxide anion (O<sub>2</sub><sup>-</sup>) or hydrogen peroxide in the microaerophilic condition (Archibald and Fridovich 1981a). In the case of *Lactobacillus casei*, it also had been reported that manganese could serve as a constituent of lactate dehydrogenase responsible for lactic acid production (Krischke et al. 1991).

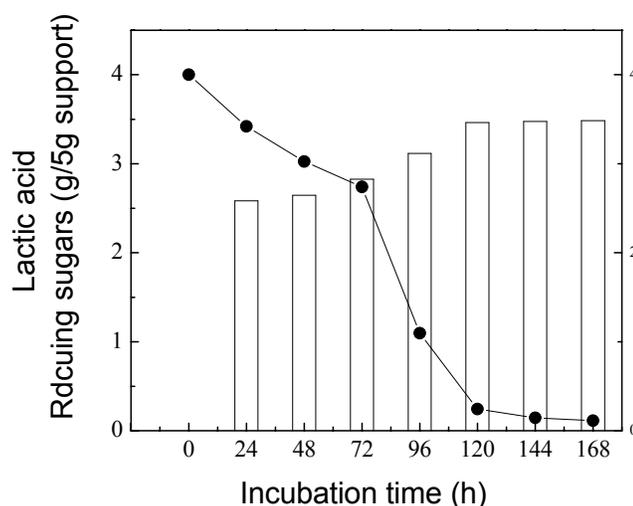
**Table 1.** Effect of CaCO<sub>3</sub> Concentration on Lactic Acid Production using *Lactobacillus casei* under SSF.

CaCO <sub>3</sub> (g/5 g support)	1.5	2	2.5	3	3.5
Lactic acid (g/5 g support)	2.495	2.880	3.225	3.468	2.901

CaCO<sub>3</sub> was used as a neutralizer of lactic acid produced by bacterial fermentation to prevent pH decrease of the medium. Table 1 reveals that CaCO<sub>3</sub> had a significant effect on the production of L(+)-lactic acid. Addition of 3g/5g support of CaCO<sub>3</sub> resulted in a 14% increase in L(+)-lactic acid production compared to addition of 2.5g/5g support of CaCO<sub>3</sub>. Altaf et al. (2006) also reported that lactic acid production was significantly effected by CaCO<sub>3</sub> in single step fermentation of starch to L(+)-lactic acid by *Lactobacillus amylophilus* GV6 under SSF.

Finally, after optimizing various process parameters, a time course study was conducted to see the cumulative effect of various parameters. The experiment was carried out, incorporating all the optimized parameters, and samples were taken every 24h. Figure 5 shows that it took just 120h to get maximum production of L(+)-lactic

acid of 3.467g/5g support, and thereafter, lactic acid production leveled off.



**Fig. 5.** Time course study of production of lactic acid (□) and consumption of reducing sugars (●).

## CONCLUSIONS

SSF for the production of  $L$ -lactic acid by *Lactobacillus casei* were conducted using RS as the inert support. It was found that *Lactobacillus casei* was able to grow on RS and effectively utilize the sugar prepared from enzymatic hydrolysis of pretreated RS. Optimization results showed that the strain produced 3.467g/5g support of  $L$ -lactic acid after 5 days of fermentation. This process provides a cost-effective and eco-friendly technology to produce  $L$ -lactic acid. Our continuing research will be focused on replacing yeast extract with an inexpensive nitrogen source to further reduce the production cost of  $L$ -lactic acid.

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## A STUDY ON OXYGEN DELIGNIFICATION OF MELOCANNA BACCIFERA (MULI BAMBOO) KRAFT PULP

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The response of kraft pulp of *Melocanna baccifera* (Muli bamboo) to different conditions of oxygen delignification and subsequent bleaching using CEHH sequence was studied. Oxygen delignification caused the kappa number of the pulp to drop between 40 and 75% over the range of temperature 70-100 °C, oxygen pressure 2-7 bar, alkali charge 2-4%, and reaction time 5-60 min. The oxygen-delignified pulp could be bleached to a brightness level of 86%. The conditions that favored greater kappa reduction also caused a greater reduction in the pulp viscosity, which called for an economic balance between the environmental benefits and the degradation of the pulp. Reaction temperature during oxygen delignification had an effect on the fiber curl and kink, while other variables such as oxygen pressure, alkali dose, and the reaction time had no significant effect on fiber deformation.

*Keywords:* *Melocanna baccifera*, *Muli bamboo*, *Oxygen delignification*, *Bleaching*, *Kappa number*, *Curl index*, *Kink index*

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### INTRODUCTION

For any bleaching method, environmental and/or economical considerations demand that the lignin content of the pulp should be reduced as much as possible before bleaching. Alkaline oxygen delignification (ODL) allows extended delignification of chemical pulps without a serious loss in pulp yield and with positive environmental impacts (Rodriguez et al. 2007; Gullichsen and Fogelholm 1999; Mukherjee and Bandyopadhyay 1993). ODL fits particularly well with kraft pulping, because oxidized white liquor can be used as an alkali source, and its spent liquor is recoverable with kraft liquor.

An oxygen stage before bleaching reduces the need for bleaching chemicals and the caustic soda required in the first extraction stage, roughly in proportion to the amount of delignification achieved in the oxygen stage. ODL decreases formation of chloroorganics (expressed as AOX) in bleach plant effluents when chlorine-based chemicals are used in subsequent bleaching of the pulp. Regardless of the bleaching chemicals used, ODL decreases BOD, COD, and color of the effluents. Interestingly, the decrease in color is more than expected on the basis of the lignin removed in the oxygen stage.

Although almost all the lignin in kraft pulp can be removed with an oxygen stage, oxygen is less specific at lignin removal than chlorine or other bleaching agents currently in use. Therefore, ODL is used to reduce the value of kappa number of the pulp by only

about 50%, because an attempt at greater reduction in kappa is expected to lead to unacceptable degradation of carbohydrates in the pulp and loss of pulp strength (Biermann 1996). However, oxygen delignification does not affect the pulp yield as negatively as other methods of extended cooking do.

Bamboo is widely used as a non-wood fibrous raw material for production of paper and paperboard in Asia (Atchison 1998). Because of their long fibers (often comparable with softwood), bamboo chemical pulps are used as reinforcing fibers for blending with hardwood and non-wood pulps in many paper products. Bamboo responds well to kraft pulping (Misra 1981). In India, bamboo is often cooked along with hardwoods.

ODL can be applied to bamboo kraft pulps for economical solutions to bleach plant environmental issues (Gomide et al. 1991; Kishore et al. 1995; Singh et al. 1995; Mittal and Maheshwari 1996). Vu et al. (2004) in a study on *bambusa procera acher* found that a pulp suitable for ECF or TCF bleaching could be produced by conventional kraft pulping followed by ODL. They observed that a high sulphidity (35-45%) with lower effective alkali (14-16%) resulted in both high yield and high pulp viscosity compared with low sulphidity (0-15%) with high EA (16-18%) at the same degree of delignification.

Muli bamboo is an important fiber source for the Indian paper industry. Muli bamboo is naturally distributed in Myanmar, Bangladesh, and northeastern states of India, where it represents between 60 and 95% of the region's bamboo resources. Besides northeastern states, Muli bamboo is also found in Orissa, and other lower altitude places of eastern India (Benton 2004). Muli bamboo is a medium-sized ever-green bamboo, 10–20 m tall, having culms of relatively thin walls, 5-12 mm, with 200-500 mm long internodes. The prices of muli bamboo in northeastern states of India are nearly 60% of the prices for other thick walled varieties available in the region. Muli bamboo is a good pulping raw material, except for some difficulties it poses in handling of chips in chip-silos, conveyers, and digester-feed systems because of its thin-walled structure.

In the present work, we have studied the response of kraft pulp of *Melocanna baccifera* (Muli bamboo) to different conditions of oxygen delignification and subsequent bleaching. A study on response of muli bamboo to ODL is of interest to the pulp mills whether they are using the conventional or a modern bleaching sequence. A CEHH (Chlorination-Extraction-Hypochlorite-Hypochlorite) sequence for bleaching of the ODL pulp was used in the present study, since elemental chlorine and hypochlorite are still widely used for bleaching of bamboo pulps in India and other Asian countries. It will take a few years before pulp bleaching will be completely ECF or TCF in this region. Many existing mills have, however, moved towards partial substitution of chlorine by chlorine dioxide in chlorination stage and using oxygen-based chemicals in extraction stages.

## EXPERIMENTAL METHODS

### Pulping and Bleaching

Samples of muli bamboo were obtained from an integrated pulp and paper mill located in Assam, India. The supply of muli bamboo to the mill was from the forests of North-Eastern States of India. One sample of bamboo freshly arrived from the forests and

another sample of bamboo stored for about six months in the yard of the mill were chipped separately. A 50:50 mixture of the two types of chips was used for further study.

The bamboo chips were cooked by kraft pulping process in a laboratory rotary digester consisting of six bombs rotating in an electrically heated polyethylene glycol bath. Each bomb was charged with 300 g chips and an appropriate amount of white liquor of 20% sulphidity. The schedule of digester heating consisted of 30 min for heating from ambient temperature to 100 °C, 90 min for heating from 100°C to 165°C, and 120 minutes at 165°C. The pulp obtained was washed with warm demineralized water and screened in a laboratory vibratory screen having 0.2-mm slots.

Oxygen delignification (ODL) of the pulp was carried out in a 3-L, Mark V, Quantum reactor at 10% consistency, using 250 g (o.d.) pulp for each run. The pulps after oxygen delignification were bleached using a CEHH sequence under the conditions given in Table 1.

**Table 1. Bleaching Conditions**

	C-stage	E-stage	H1-stage	H2-stage
Consistency, %	3	10	10	10
Retention time, min	30	60	120	120
Temperature, °C	25	65	45	40 - 45
Chlorine dose (as active chlorine), % of o.d. pulp	0.25 to 0.27 times kappa number		1.2	1.2
NaOH, % o.d. pulp		1.5 – 2.0%		
NaOH as buffer, % of calcium hypochlorite used			20	20

### Evaluation of the Pulp

Kappa number and brightness of the pulps were determined using TAPPI standard procedures T 236 and T 525 respectively. Pulp viscosity in CED (cupriethylene diamine) solution was determined using SCAN-C15:62 method and expressed as intrinsic viscosity (ml/g). Fiber dimensions and fiber deformations were determined using OpTest (OpTest 2000) laboratory fiber quality analyzer (FQA).

## RESULTS AND DISCUSSION

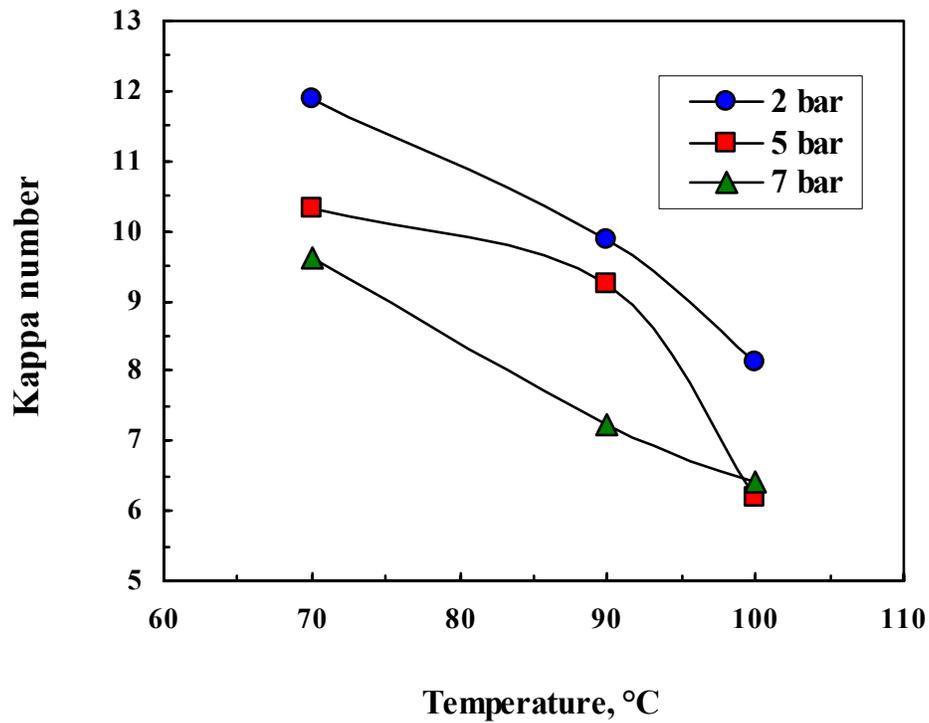
In the current industrial practice, the kappa number for bleachable grade bamboo kraft pulp was kept at some value between 16 and 18. During this study, several cooks were prepared in the laboratory by varying the active alkali charge and the maximum temperature of cooking. It was targeted to obtain a pulp of kappa number 20 so that after the oxygen delignification, a pulp of kappa number of about 10 could be obtained. Some conditions were screened out because the kappa number, pulp yield, and the residual active alkali content in black liquor were outside the acceptable ranges for this study. Four pulps, as shown in Table 2, were used to further study the response of these pulps to oxygen delignification and bleaching.

**Table 2.** Response to Pulping, ODL, and Bleaching

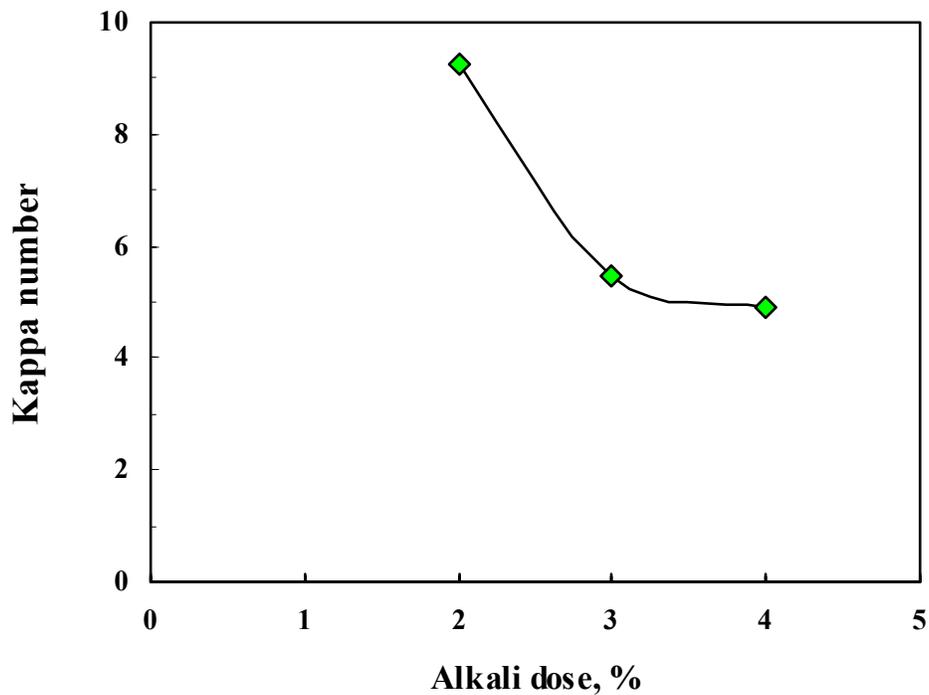
	Pulp 1	Pulp 2	Pulp 3	Pulp 4
<i>Kraft pulping, sulfidity 20%, raw material to liquor ratio 3:1, Maximum temperature 165 °C</i>				
Active alkali charge (as Na <sub>2</sub> O), %	13	14	15	16
Kappa number	32.3	22.7	20.2	16.4
Unscreened pulp yield	56.0	55.1	53.2	52.2
Screened pulp yield, %	51.3	52.9	52.4	47.7
pH of black liquor	11.0	11.2	11.9	12.1
Residual active alkali in black liquor (as Na <sub>2</sub> O), g/L	4.8	6.9	8.6	10.0
CED viscosity, ml/g			1066	
<i>Oxygen delignification (ODL): O<sub>2</sub> pressure 5 bar, Temperature 90 °C, Alkali charge 2%, Consistency 10%, Residence time 60 min</i>				
Kappa number	15.1	10.5	9.3	7.7
ODL pulp yield, %	48.9	51.9	51.7	46.2
CED viscosity, ml/g	917	988	910	837
<i>Bleaching sequence CEHH, Chlorine charge 0.25*Kappa number at C-stage, 1.2 % at H1-stage and 1.2% at H2-stage, 1.5% NaOH at E-stage</i>				
Bleached pulp yield, %	45.1	49.4	49.5	43.7
Brightness (ISO), %	86.0	86.9	86.2	86.4

The pulp produced using 15% active alkali charge was chosen for oxygen delignification as it gave the desired kappa number and adequate residual active alkali content of the black liquor. The CED viscosity of this pulp was found to be 1066 ml/g. This pulp was used for oxygen delignification under different conditions of oxygen pressure, temperature, alkali charge, and residence time in the oxygen reactor.

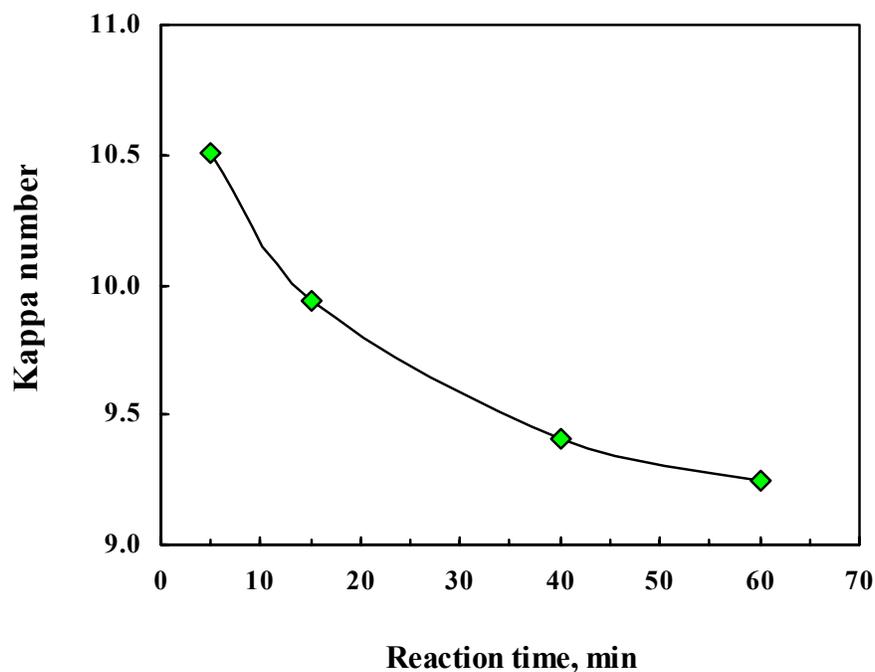
The ODL pulps obtained under different conditions were subsequently bleached using CEHH sequence. Figures 1 to 3 show the effect of temperature, oxygen pressure, alkali charge, and the residence time on the kappa number of the ODL pulp. The ODL caused the kappa number of the pulp to drop between 40 and 75% over the conditions used in these experiments. The kappa number reduction during ODL was greater at higher reaction temperature, higher oxygen pressure, higher alkali dose, and longer residence time. The oxygen reaction was very fast in the beginning and only five minutes were sufficient to cause about 50% drop in kappa number.



**Fig. 1.** Effect of oxygen pressure and temperature during ODL on kappa number. (Kappa number before ODL 20.2, Alkali charge 2%, Residence time 60 min)

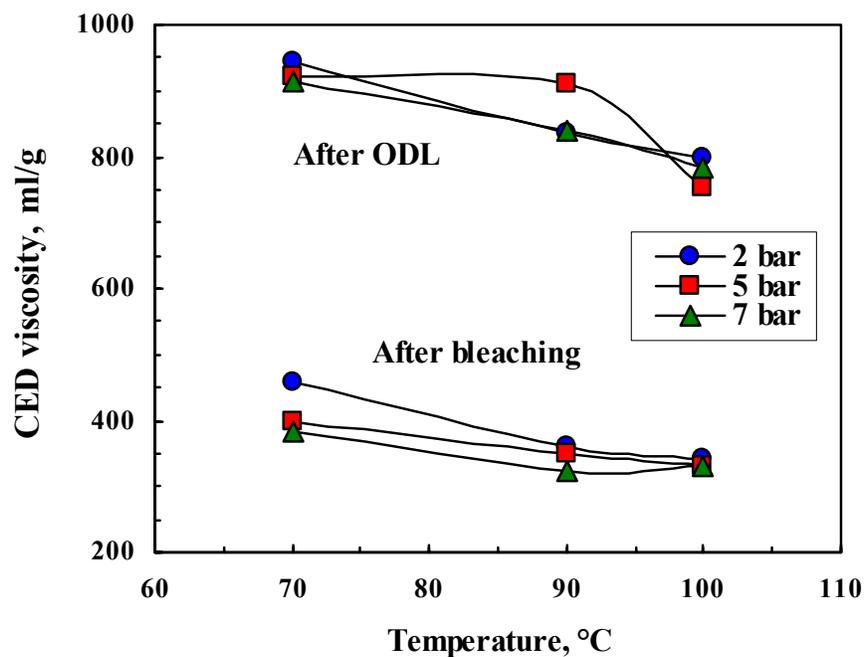


**Fig. 2.** Effect of alkali charge on kappa number of ODL pulp. (Kappa number before ODL 20.2, Oxygen pressure 5 bar, Temperature 90 °C, Residence time 60 min)

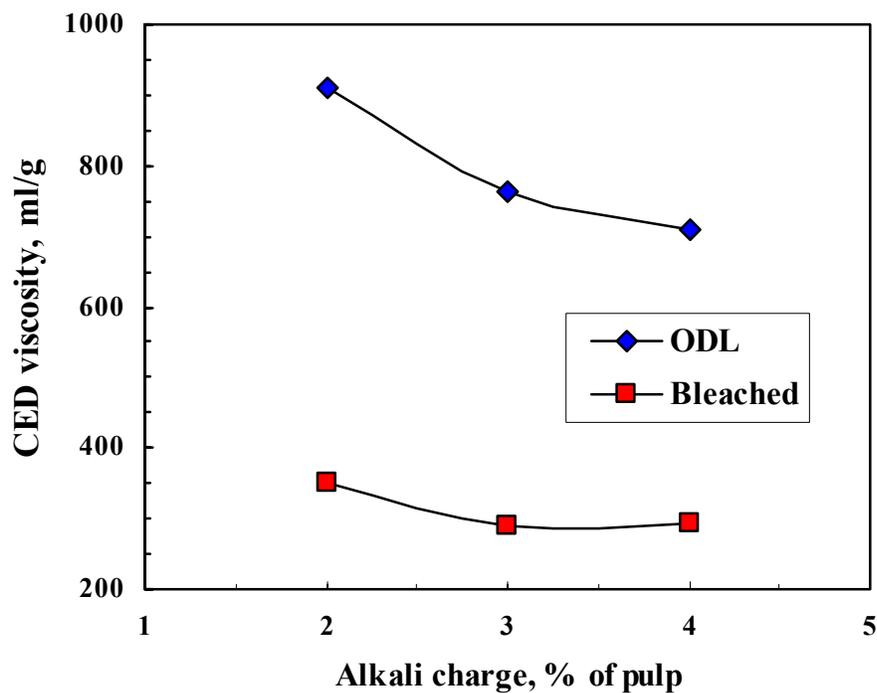


**Fig. 3.** Effect of ODL reaction time on kappa number of ODL pulp. (Kappa number before ODL 20.2, Oxygen pressure 5 bar, Temperature 90 °C, Alkali charge 2%)

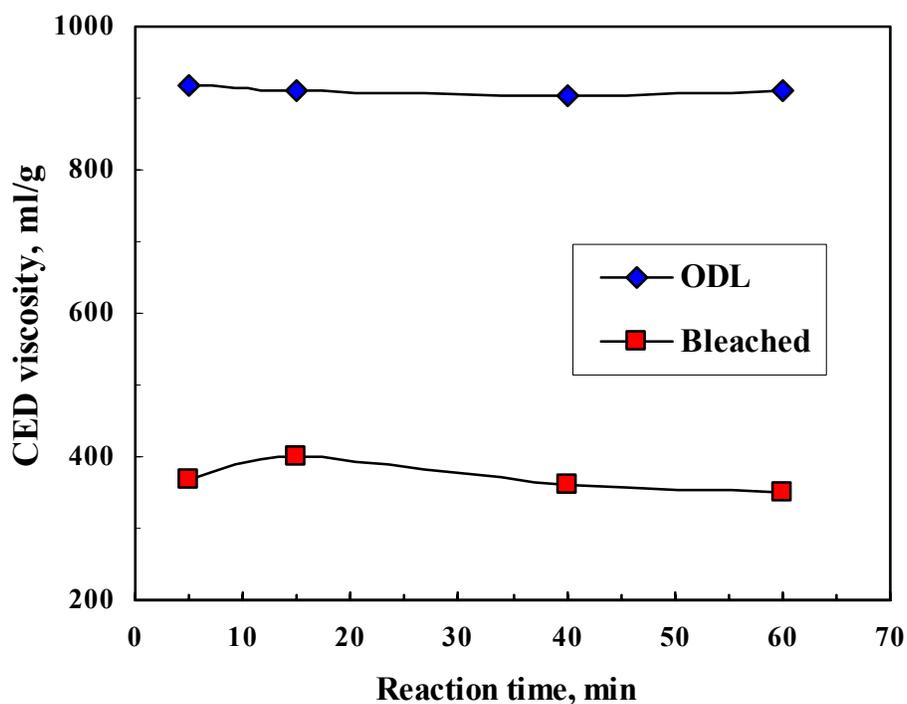
Figures 4 to 6 show the effect of ODL conditions on the pulp viscosity after the ODL and after subsequent bleaching.



**Fig. 4.** Effect of oxygen pressure and temperature during ODL on pulp viscosity after ODL and after final bleaching. (Viscosity before ODL 1066 ml/g, Alkali charge 2%, Residence time 60 min)



**Fig. 5.** Effect of alkali charge on viscosity of ODL pulp. (Viscosity before ODL 1066 ml/g, Oxygen pressure 5 bar, Temperature 90 °C, Residence time 60 min)

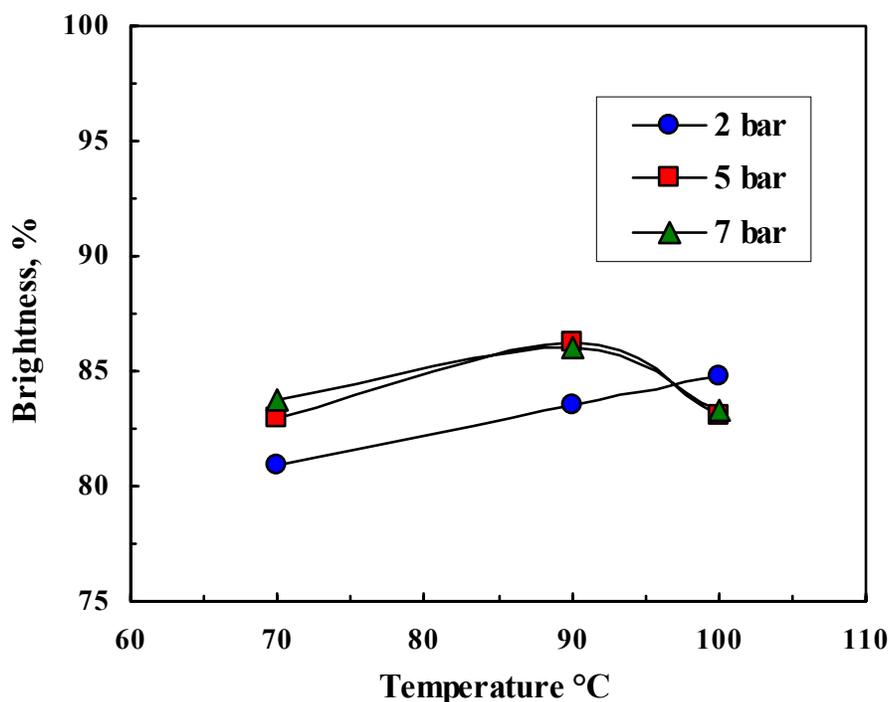


**Fig. 6.** Effect of ODL reaction time on viscosity of ODL pulp. (Viscosity before ODL 1066 ml/g, Oxygen pressure 5 bar, Temperature 90 °C, Alkali charge 2%)

As shown, the conditions that favored greater kappa reduction also caused a greater reduction in the pulp viscosity. Thus, the ODL conditions need to be chosen to provide the economic balance between the environmental benefits and the degradation of the pulp. Oxygen pressure had a relatively minor effect on the pulp viscosity.

Figure 7 shows the effect of ODL condition on the brightness of the finally bleached pulp. The maximum brightness attainable was 86.2% when the temperature during ODL was kept at 90 °C and the oxygen pressure was maintained at 5 bar.

The OCEHH bleaching has distinct advantages over the conventional CEHH bleaching sequence in terms of reduced chlorine consumption, increased pulp yield, and improved pulp brightness. For conventionally bleached pulp of muli bamboo, the data available in our laboratory suggest that the optimized conditions would be to obtain an unbleached kraft pulp with kappa number 16-17 and to bleach it to brightness 82-84%. These conditions correspond to 16% active alkali charge in pulping, 6-7% total active chlorine charge in bleaching, and the viscosity of the resulting pulp 450-500 ml/g.



**Fig. 7.** Brightness of bleached pulp as function of oxygen pressure and temperature during ODL. (Alkali charge 2%, Residence time 60 min)

### Fiber Quality Analysis of the ODL Pulp

The bleached pulp was analyzed for such fiber characteristics as fiber length, curl index, and kink index using OpTest laboratory fiber quality analyzer. The FQA fiber length measurements give similar results as given by the Kajaani FS-200 fiber length analyzer (Robertson et al. 1999). The mean fiber length and width were measured for different conditions of ODL. It was observed that the fiber dimensions were not affected

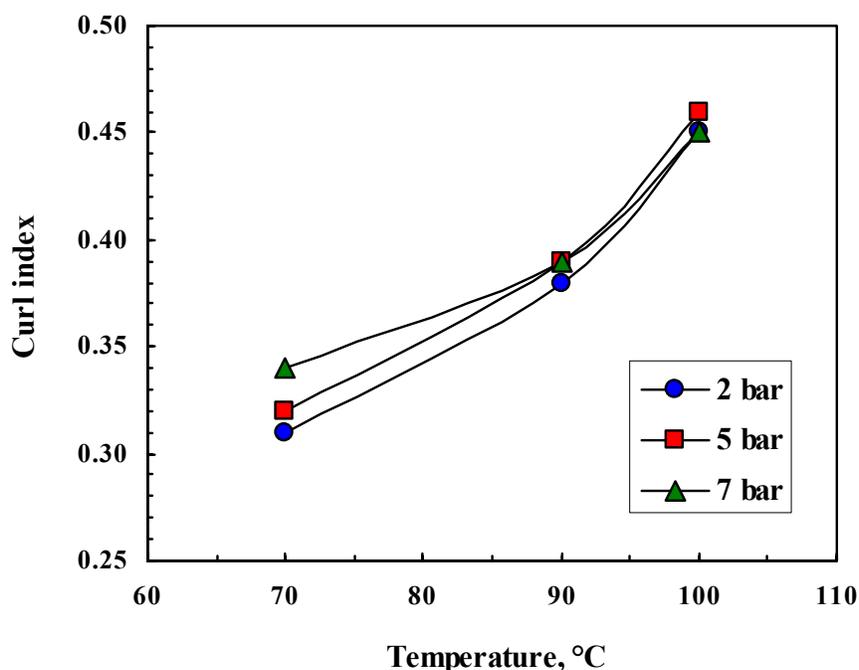
by the oxygen delignification process. The mean values and the standard deviations of the fiber dimensions of the pulps obtained under different conditions of ODL are given in Table 3.

**Table 3.** Mean Dimensions of the Fibers

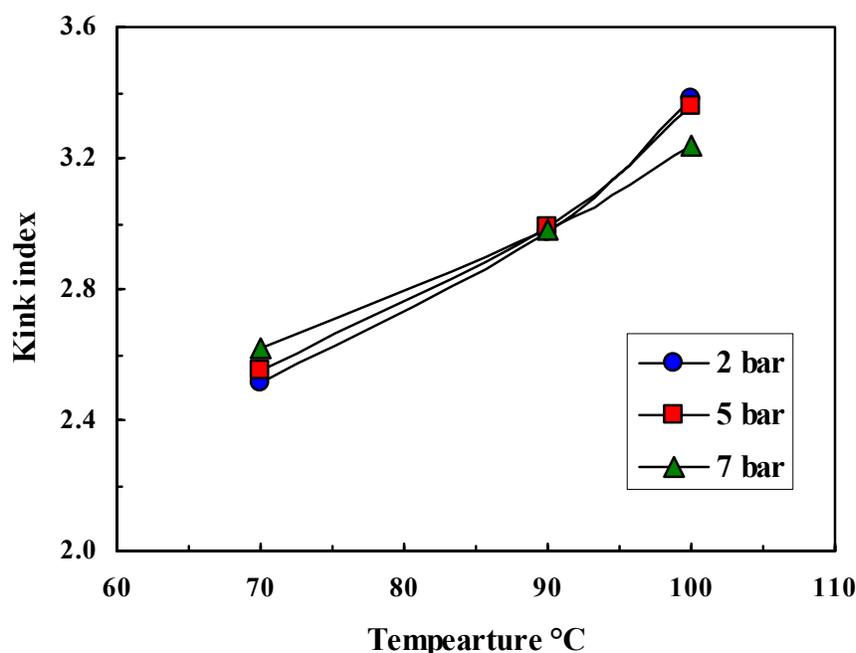
Weight weighted mean fiber length, mm	2.04 (Standard deviation = 0.043)
Length weighted mean fiber length, mm	1.56 (Standard deviation = 0.034)
Arithmetic mean fiber width, $\mu\text{m}$	17.43 (Standard deviation = 0.166)

In industrial applications, fiber deformation is very important fiber characteristic. Fibers in the plant stem are straight, but they become curly during pulping, mixing, and refining operations because of being subjected to bending and axial compressive stresses, particularly at medium and high consistencies (Robertson et al. 1999). The inclusion of fiber deformation with other fiber characteristics in pulp evaluation could greatly improve the understanding of the relationship among fiber properties, pulping and papermaking processes, and paper quality (Mohlin et al. 1996). In general, an increase in fiber deformation results in a decrease in tensile index and an increase in stretch-to-break of the paper.

The FQA device allows measurement of fiber deformation in terms of curl index, number of kinks per fiber, kink angles, and kink index. It was observed that these measurements of fiber deformation correlated highly with each other for the ODL pulps obtained under different conditions. Values of curl index and kink index only have been included in this discussion.



**Fig. 8.** Curl index of ODL pulp as function of oxygen pressure and temperature during ODL. (Alkali charge 2%, Residence time 60 min)



**Fig. 9.** Kink index of ODL pulp as function of oxygen pressure and temperature during ODL. (Alkali charge 2%, Residence time 60 min)

Fiber curl describes the deviation from straightness of the fiber axis. In FQA, the curl index is defined as the relative increase in the length of a fiber when it is straightened but not stretched (Page et al. 1985). Kink refers to an abrupt change in fiber curvature. The FQA uses a modified Kibblewhite equation for calculating kink index (OpTest 2000). As evident from Figures 8 and 9, the fiber curl and kink increased with increase in temperature. The oxygen pressure, alkali dose, and the retention time in oxygen reactor did not significantly affect the curl or kink index.

## CONCLUSIONS

1. For muli bamboo, a kraft pulp of kappa number of 20 could be produced using an active alkali charge of 15% (as  $\text{Na}_2\text{O}$ ) at the usual cooking conditions (sulphidity 20%, maximum temperature 165 °C). These conditions produced a pulp of high yield, high bleached brightness, low pulp degradation, and adequate residual active alkali content of the black liquor.
2. The ODL caused the kappa number of the pulp to drop between 60 and 30% over the range of temperature 70-100 °C, oxygen pressure 2-7 bar, alkali charge 2-4%, and reaction time 5-60 min. The kappa number reduction during ODL was greater at higher reaction temperature, higher oxygen pressure, higher alkali dose, and longer residence time. The oxygen reaction was very fast in the beginning and only five minutes were sufficient to cause about 50% drop in kappa number. This should be

economically advantageous, as a shorter retention time in an ODL reactor could be required.

3. The conditions that favor greater kappa reduction also caused a greater reduction in the pulp viscosity. However, oxygen pressure had a relatively less effect on the pulp viscosity. The ODL conditions should be chosen to provide the economic balance between the environmental benefits and the degradation of the pulp.
4. The maximum brightness attainable after bleaching was 86.2% when the temperature during ODL was kept at 90 °C and the oxygen pressure was maintained at 5 bar.
5. Fiber curl and kink increased with increase in temperature during ODL. The oxygen pressure, alkali dose, and the retention time in oxygen reactor did not significantly affect the curl index or kink index of the fibers. The increase in fiber curl and kink should help in increasing tensile energy absorption of the paper.

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## INTERFIBER BONDING AND FIBER SEGMENT ACTIVATION IN PAPER

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Bonding and activation in paper were studied with the help of laboratory test sheets and common paper strength tests. Different papermaking furnishes and raw material treatments were used to examine the effects they have on bonding and activation. Furthermore, various boundary conditions during drying were included to single out the influence of bonding and activation on paper properties. It was found that bonding is clearly increased by beating of kraft pulp, starch addition, and thermomechanical pulp fines, whereas activation benefited most from beating and addition of reinforcement fibers to mechanical pulp based furnishes. Subjecting test sheets to increasing amounts of drying stress affected activation positively, and bonding negatively. The increase in activation did not seem to be dependent on the beating degree of chemical pulp fibers. Bonding, on the other hand, deteriorated more significantly in sheets made of extensively beaten kraft fibers, i.e. in sheets where the initial bonding potential was higher. Commonly used paper strength measurements provide dependable and accurate tools for assessing the effect of different variables on both bonding and activation. A short literature survey of bonding and activation is also provided.

*Keywords: Bonding, Activation, Paper strength properties, Fines, Beating, Drying, Drying stress*

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### INTRODUCTION

Traditionally, the mechanical properties of paper, i.e. the strength of fiber networks, has been thought to arise from interfiber bonding – the strength of individual bonds and all the bonds within the network – and from the (axial) strength of individual papermaking fibers (Robinson 1980; Stratton 1991). More recently, the behavior of fibers in the paper network during the papermaking process has been added to this relationship (Niskanen 1993; Kettunen 2000; Tanaka *et al.* 2001a; Hiltunen 2003). The area of bonds and the strength of the bonds are important factors influencing the mechanical properties for most of the paper grades (Retulainen 1997). Bonding affects the structure of paper – both the development of structure and the final structure of the paper web.

Fiber segment activation is not a new idea; it was first put forward by Giertz (1964), and the idea of an increment in load-bearing capacity of fibers taking place in the network, of something happening to the unbonded lengths of fibers, during processing can be found in several sources (van den Akker *et al.* 1966; Lobben 1975 and 1976; Giertz and Rødland 1979; Niskanen 1993; Retulainen 1997; Wahlström 1999; Hiltunen 2003; Wathen 2006).

### Interfiber Bonding

Interfiber bonding is essential to sheet strength, which, according to Stratton (1991), is a function of two factors, the strength of an individual fiber and the strength of the

interfiber bonds. An interfiber bond can be defined as the zone where two fibers are so close to each others that chemical bonding, van der Waals' interaction, or molecular entanglement can occur (Retulainen *et al.* 1998). Bonds hold fibers together and therefore contribute to the internal cohesion of paper. In addition to mechanical properties, fiber bonds affect optical properties, electrical properties, and dimensional properties of paper (Retulainen 1997).

Formation of interfiber bonds begins as solid content increases during the papermaking process. At first, bonding happens through surface tension forces pulling fibers together when water is removed. This mechanism of fiber bonding was first explained in detail by Campbell (1959), and it is known as the *Campbell Effect*. The Campbell effect changes gradually to other types of bonding, so the solids content at which actual interfiber bonding starts to happen is not known exactly. An estimate for the start of bonding can be derived from the elastic modulus of paper during drying: the change in the modulus must happen because of bond formation since wet fibers have higher rigidity than wet bonds (otherwise the fibers would disintegrate into the solution). In kraft pulp, the elastic modulus starts to increase significantly at 50 % solids content (Retulainen *et al.* 1998).

During drying, pulp fibers shrink laterally, which causes shear stresses in the bond area, because of the discrepancy between the tendency for lateral and longitudinal shrinkage of fibers. The amount of shrinkage depends on the swelling degree of the wet fiber wall, which in turn is affected by internal fibrillation and chemical composition of the fiber wall. Shrinkage forces are largest at the peripheral area of the bond, and when the system is loaded, bond edges will take the load first. Shrinkage stresses generate axial compressive forces on the crossing fibers and may cause deformations in bonded fiber segments (microcompressions) in freely dried sheets. Stresses at bond area and fiber walls, as well as the microcompressions, modify the mechanical properties of the bonded segments, so that the properties of bonded fibers will differ from those of freely dried fibers. Mechanical properties of fibers in a network are therefore related to interfiber bonds (Retulainen 1997; Retulainen *et al.* 1998).

The structure of interfiber bonds is influenced by beating, pressing and drying. Other important factors affecting bond structure include fiber morphology, pulping procedure, beating equipment and the mode of drying (free versus restrained). The final structure of a fiber-fiber bond of two chemical pulp fibers is a combination of S1-S1, S1-S2 or S2-S2 layers. Flexible fibers can form wrap-around type of bonds and fibrils, and fines form bridges between fibers. On a microscopic scale, fibrils also form entanglements (Uesaka 1984). The structure of fiber bonds between chemical pulp fibers was studied by Nanko and Ohsawa (1989), who presented the following structural features in their work: an amorphous bonding layer is formed between the S1 layer of two beaten fibers by external fibrils, and probably polymer chains as well. The more beaten the fibers are, the thicker the bonding layer and the better the contact between two fibers will be, partly because of the increased amount of fines. According to Nanko and Ohsawa (1989), the skirt is an elongated part of the S1 layer extending from bond edges, and the covering layer consists of external fibrils and fines covering smooth edges.

### **Fiber Segment Activation**

Activation is one of the relevant properties of fibers within a network. It means that originally kinky, curly or otherwise deformed fiber segments, unable to carry load in the

network, can be modified into active components of the network (Fig. 1). Activation of the fiber network occurs during drying, when lateral shrinkage of fibers is transmitted to axial shrinkage of the neighboring fibers at bonded areas. If this shrinkage is restrained, the free fiber segments dry under stress and are therefore removed of their slackness (Giertz and Rødland 1979; Lobben 1975). Once the segments are activated the axial elastic modulus of fibers increases, which leads to further increment of the drying stress and both the segments and bonded areas are capable of bearing load. Interfiber bonding and shrinkage of fibers are prerequisites for activation. The amount of drying stress needed to activate free segments depends on the morphology of the fibers (Retulainen 1997).

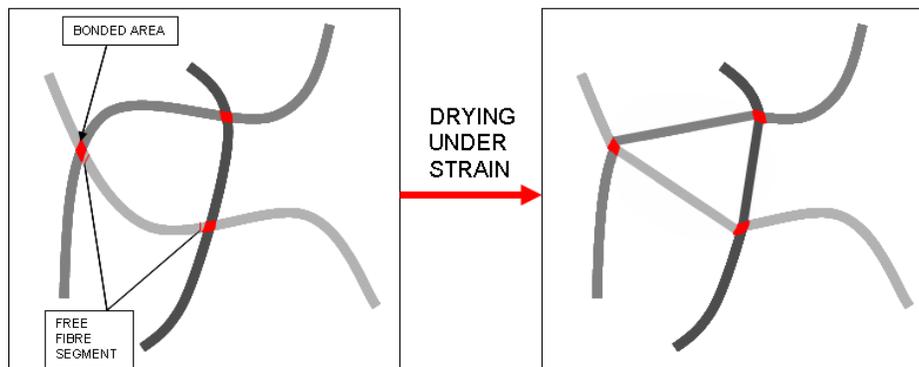


Fig. 1. A schematic illustration of activation.

During restrained drying of handsheets, the lateral shrinkage tendency of fibers is converted to axial compression of bonded fibers. Activation in bonded segments evens out larger stress concentrations at the fiber interfaces and inside the fiber wall by rearranging the lamellae and fibrils. The whole fiber is capable of bearing load more evenly. Activation of the free segments not only makes the segment straighter and fibrils more capable of bearing load, but it also may increase the order of cellulose and hemicelluloses inside the fibrils and decrease the fibril angle (Retulainen 1997).

Activation takes place during drying, and as drying shrinkage is restricted, and the fiber network is unable to contract and shrink, the level of activation becomes higher (Retulainen 1997; Tanaka *et al.* 2001). Straightening of initially slack free fiber segments leads to an increase in the elastic modulus of paper (Htun 1980). The tensile properties of paper are generally improved by increasing drying stress; especially the elastic modulus is favorably affected (Htun and de Ruvo 1978; Wahlström *et al.* 2000; Zhang *et al.* 2001). Tensile stiffness values five times the original have been reached with increasing strains (Wahlström 1999).

Activation can be improved by maximizing the bonded area and total fiber length. Pulp with low coarseness and large fiber width are favorable for both chemical and mechanical pulp. Strength of the blend can be improved by beating chemical pulp, which increases fiber flexibility and therefore bonded area can be increased. Beating increases also the number of bonds and the number of free segments and decreases the length of the free segments (Lobben 1976). The amount of fines in mechanical pulp should be high enough to tolerate reduction of fines-fiber ratio without reaching the limiting state (a function of sheet density and amount of chemical pulp fibers). The activity of chemical

pulp fibers can be improved by using pulp that has smaller fracture elongation and fewer micro-compressions and kinks in fibers.

### Factors Affecting Bonding and Activation

Unbeaten fibers cannot form strong paper, at least if not mixed with other pulps. The concept of fiber bonding, ever since the beginning of papermaking history, is related to development of strength properties (Page 1989). Beating clearly promotes interfiber bonding, but has also other effects on the fiber network, for example, the effect of beating on fiber flexibility affects subsequent fiber segment activation during drying. Contact between fibers increases, and the surface of fibers changes physically. In addition to increasing RBA, refining increases bond strength (Retulainen 1997). According to Robinson (1980), the most significant effect of beating is the increment of flexibility through delamination, swelling, and dislocation within individual fibers. This permits adjacent wall segments and fibers to mould and conform to one another during pressing and drying (Robinson 1980; Retulainen 1997). Beating has been found to increase activation (Giertz and Lobben 1967; Page 1989; Hiltunen *et al.* 1998; Hiltunen 2003). Fiber swelling and increase in elastic modulus of fibers increases paper strength through the Jentzen effect, i.e., the primary strength-enhancing effect of beating comes from enhanced bonding in the wet web, which is then put to effect by drying stresses (Niskanen 1998).

Commonly, the fines fraction is defined as the fraction of pulp that passes the 200 Bauer-McNett mesh (Hiltunen 1999). The properties of fines differ considerably from the properties of the fiber fraction. The particle size is small and surface area large, which means that fines are able to bind more water and hence swell more than fibers (chemical pulp fines swell more than mechanical, since mechanical pulp fines contain more extractives and hydrophobic lignin). In mechanical pulps, the fines content has a strong influence on the structure and properties of the fiber network (Retulainen 1997; Retulainen *et al.* 1998). The primary effect of fines on the paper network is an increase in density, which results in an increased number of bonds and in that way, improves tensile strength properties of the sheet. During the wet state of sheet formation, Campbell forces are increased by fines filling free spaces and extending the volume of bound water between fibrils of neighboring fibers. Fines can also behave as loosely bonded filler material, creating new light scattering surfaces and open structures (Luukko 1999). This effect seems to be valid for mechanical pulp fines, which contain both fibrillar and flake-like material, but not for chemical pulp fines. They contain mostly fibrillar material, which have such a high bonding ability that light-scattering coefficient is not improved (Luukko and Paulapuro 1999). Fines act as a bridge between fibers, thus contributing to coherent paper network formation. This way the local stress concentrations, evolved in the network during straining, are reduced or evened out, which leads to more uniform stress distributions and improved strength properties (Luukko 1998).

Additives that affect interfiber bonding positively are high molecular weight, hydrophilic colloids, either natural or synthetic, such as starch, proteins, vegetable gums and water-soluble resins (particularly polymers of acrylamide) (Robinson 1980; Ketola and Andersson 1999). They act by adsorption onto fiber surfaces, where fiber-to-binder-to-fiber bonds can evolve and contribute to bond and sheet strength. Addition of dry strength chemicals (e.g. starch, carboxymethyl cellulose,) is an ideal way of improving interfiber bonding and tensile strength of paper: they cause no increment in

sheet density or deterioration of light scattering coefficient. The strengthening ability of starch is related to the degree of substitution in starch, and it is not very effective if fibers are stiff and the bonded area small to begin with (Retulainen 1997). The increment in tensile strength with starch addition is largely due to improved specific bond strength and increased stiffness of bonded areas. The rupture process of interfiber bonds is also affected by starch addition: it does not seem to change the starting point of the rupture (stress or strain at which rupture starts is not increased), but the rate of bond ruptures is decreased considerably. For example, Lindström *et al.* (2005) and Hubbe (2006) have written extensive reviews on the relationship between dry strength chemicals and bonding.

The mechanical properties of bonds and bonded fiber segments are closely coupled by the drying stresses that act across every interfiber bond (Retulainen *et al.* 1998). During drying, the shrinkage of component fibers causes contraction of the whole fiber network and creates internal stresses in the paper. Fiber shrinkage influences various properties of paper, such as sheet structure, mechanical properties, and hygroscopic behavior (Nanko *et al.* 1991). Drying of previously undried virgin pulp fibers under axial tension increases considerably the tensile strength and decreases the breaking strain. Laboratory experiments have even shown that an 8 % increase in strain from -4 % (shrinkage) to +4 % (stretch) can yield a tensile stiffness five times the original (Wahlström 1999).

Drying and wet straining during drying affect fiber strength. Jentzen (1964) studied the effect of free drying and drying under load on the properties of individual fibers. Drying under axial tension can considerably increase the tensile strength and decrease breaking strain of single fibers. According to Jentzen (1964), the changes in mechanical properties are brought about by two factors: an increase in crystallite orientation and, secondly, by a more even distribution of stress within the fiber (among fibrils). An increase in crystalline orientation, taking place due to drying under load, leads to increasing tensile strength and Young's modulus (also, the ultimate fiber elongation decreases). Drying under load also induces a redistribution of stress within the fiber.

## Objectives

The objective of this work was to examine interfiber bonding and fiber segment activation as basic phenomena with the help of laboratory scale analyses: how different papermaking processes affect bonding and activation, and in turn, how bonding and activation relate to the end properties of paper, especially to different mechanical properties. Investigating the effect of drying and drying stress and their usefulness in examining bonding and activation was our special interest. At first, only two different drying modes were used (preliminary test series): free drying, where the test sheets were allowed to shrink freely during drying, and restricted drying where drying shrinkage was completely inhibited. Later on, a special drying frame construction (the Paper Drying Rheometer, PDR) enabling biaxial straining of test sheets was used to introduce different levels of drying stresses to the sheets at the beginning of drying.

Furthermore, the objective was to use common, widely used paper strength tests and analyses, and to study how well these can be used in examining bonding and activation. The goal was to establish testing procedures that would be comprehensive enough to yield feasible information on the phenomena, but at the same time, keep the testing so simple that it could be performed without very laborious or difficult analyses, with the equipment available for most paper mills and research facilities.

## EXPERIMENTAL

### Materials

ECF bleached TMP [Finnish commercial thermomechanical pulp made from Norway spruce (*Picea abies*)] was used in the experiments. The pulp was collected from the mill post-bleaching wash press before latency removal, and stored in a freezer. Before sheet forming, the TMP was hot-disintegrated according to SCAN-M 10:7. Freeness of the pulp, 44 ml CSF, was measured according to SCAN-C 21:65. For examining the effect of fines on TMP based paper properties, the mechanical pulp was fractionated, and the long fiber and middle fractions separated with a Bauer-McNett apparatus so that the R30 and R50 fractions were collected, and the R100, R200 and P200 fraction discarded. The fractionation was done according to SCAN-M 6:69 with some modifications: no filter papers were used but the fractions to be used were collected into buckets.

The chemical pulp used in these experiments was bleached kraft pulp [Finnish commercial kraft pulp made from Scotch pine (*Pinus sylvestris*)]. The pulp was disintegrated and beaten in a Valley beater according to SCAN-C 25:76. In this work, four different beating levels were used: SR° 14, 17, 22 and 34, as well as an unbeaten pulp (SR° 12).

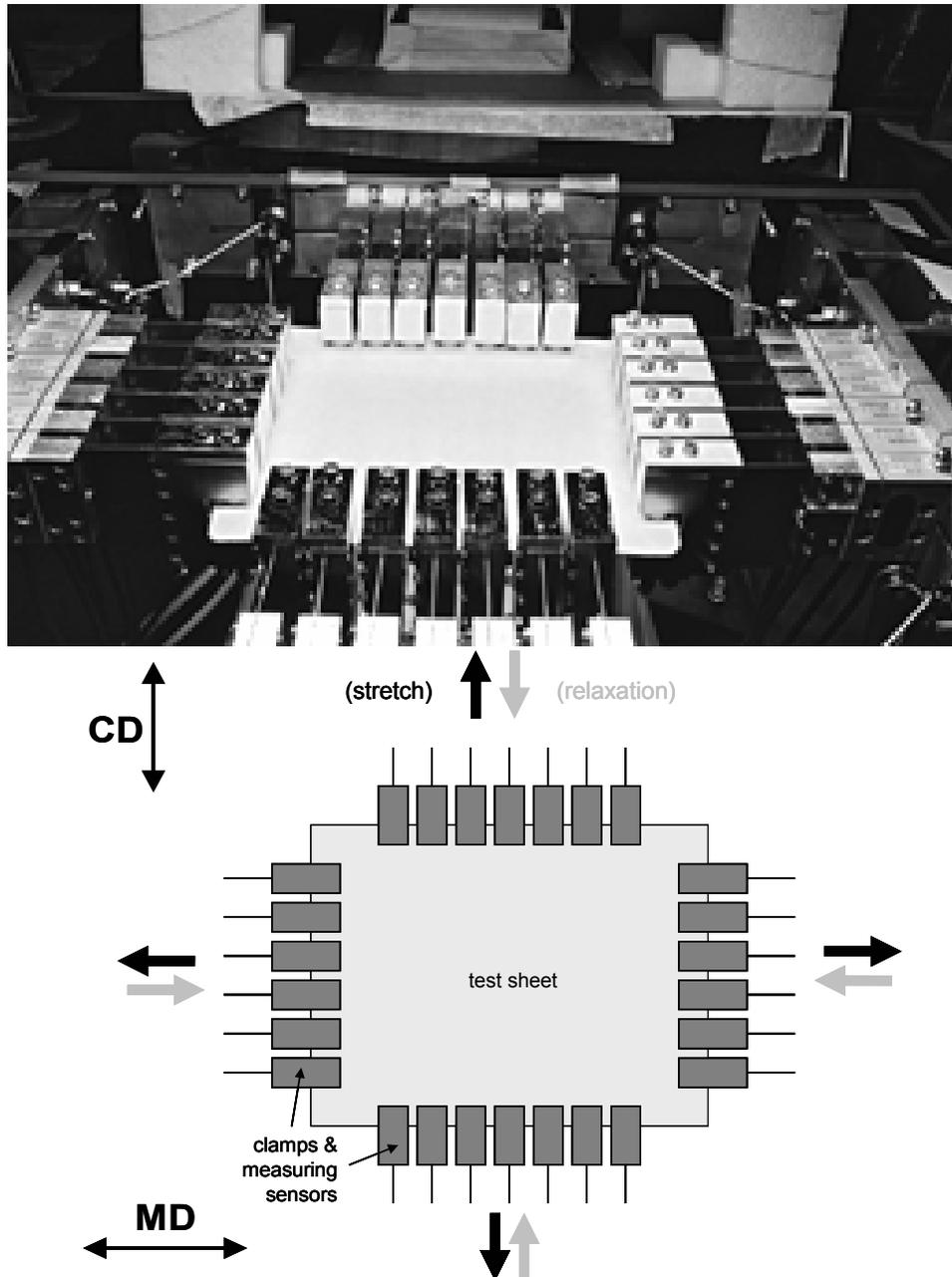
The cationic starch used in the experiments was commercial potato starch from Kemira Oyj, and it was added to the furnish prior to sheet forming. Four different addition levels were used for both 100% kraft pulp and 100% TMP pulp sheets: 0, 2, 5 and 10%. The starch was adsorbed onto fibers that had been first washed into sodium form (Swerin and Wågberg 1994). The consistency in the adsorption experiments was 5 g/L, ionic strength 0.5 mM NaHCO<sub>3</sub> and reaction time under magnetic stirring 30 min.

### Methods

The laboratory sheets were formed with a semi-automatic sheet mould, producing 165 mm x 165 mm handsheets, according to the standard SCAN-C 26. White water filtrate was circulated in order to balance the amount of fines, when sheets containing TMP were made. For those test series in which the sheets were dried with the PDR device, a different sheet mould, producing 240 mm x 290 mm laboratory sheets, was used. Apart from the size of the sheets produced, the mould and sheet forming complied with SCAN-C 26.

The laboratory sheets were wet pressed right after sheet forming and couching, according to the standard SCAN-C 26:27. Ordinary 165 mm x 165 mm laboratory sheets formed with the normal sheet mould were then dried; it was necessary to obtain different levels of drying stress with the help of a simple method. Gloss plates (squares the size of laboratory handsheets, cut from thin, laminated board) were used to restrict the shrinkage of test sheets during drying. This would correspond roughly to 0% strain. Propylene films (Millipore polypropylene prefilter, type 2.5 µm AN25) were used to allow the sheets to shrink completely freely during drying, corresponding up to a point with the negative strains of the PDR device. These sheets were dried in a conditioned room (23°C, 50 % RH) for at least 24 hours. Apart for using the propylene films in free drying, these methods of drying comply with the standard SCAN-C 26:27. The larger sheets, bound to PDR drying, were stored in a cold storage room after wet pressing prior to drying. These sheets were wrapped in double plastic bags, stacked and turned regularly to ensure even moisture distribution within the stacks.

PDR is a construction with 6-7 separate clamps for each side of the paper sample (Fig. 2). Each clamp is connected to high-precision sensor that measure position and force throughout the experiment. The drying procedure is controlled with a computer, and the measurements from the sensors are recorded on-line with special computer software. The PDR device utilizes both hydraulic and pneumatic drives for moving beams onto which the clamp-sensor constructions are attached. By controlling the beam movement, the paper sample can be for example subjected to different controlled strains during drying. Drying is achieved by six 500 W halogen lamps.



**Fig. 2. Above:** Paper Drying Rheometer (PDR) with an attached test sheet (right), and **Below:** A schematic illustration of the PDR test setup.

In the test series of this work, the idea was to achieve different levels of drying stress by straining the sheets before drying. The sheets were strained immediately after wet pressing at approximately 30% solids. First, the sheets were attached to the drying device and a preliminary straining was done to obtain a 'zero' stress level. Drying was then commenced straight after this. Strain levels between -2 (negative strain, i.e. the sheets were let to relax) and +2% on both of the in-plane directions (MD and CD) of the test sheet, were used. The principle of PDR drying and straining of test sheets can be seen in Fig. 2. Unfortunately, the actual final (maximum) drying stresses could not be examined due to unexpected data losses on the PDR measuring system. Based on the results of other similar drying experiments performed with the PDR, it can be said with confidence that the different strains will produce statistically significantly different drying stress levels.

The dried sheets were conditioned (23°C, 50 % RH) and tested for tensile properties, bond strength (Scott bond strength and elastic breaking strain) and in-plane tear strength, which was measured with MTS 400 tensile tester according to the procedure described by Kettunen and Niskanen (2000a). Also damage analysis was carried out. It produces two parameters, damage width and pull-out width: damage width measures the extent of damage or fiber de-bonding from the actual crack line, and pull-out width describes the extending of fiber ends from the crack line (Kettunen and Niskanen 2000b). Certain assumptions were made when assessing the results of this study. Tensile stiffness or elastic modulus is an indicator of the level of activation in the sheets. Scott bond strength represents the z-directional bond strength, whereas elastic breaking strain, calculated from tensile index divided by tensile stiffness index, gives an indication of bonding in the in-plane direction. A calculated variable (in-plane tear index divided by damage width) is assumed to combine these two aspects of bonding at least to a certain extent. Damage width indicates how far a fracture will progress perpendicular to the fracture line in a paper network, and its extent depends of bond strength and fiber strength in the fiber network.

## RESULTS AND DISCUSSION

### Using Paper Strength Properties to Examine Bonding and Activation

During this work, cause-effect relationships between the different paper properties and bonding/activation were closely investigated. Tables 1 and 2 were designed for examining the relationships between different strength properties and bonding and activation. These tables can be utilized in clarifying the effects of for example drying, beating or different fiber surface treatments on bonding and activation. Different strength property pairs and combinations, such as in-plane tear strength and damage width or tensile strength and density, can be then used to examine the effect of different variables on bonding and activation. The tables, when correctly modified, are applicable to all possible variations of processes (beating, wet pressing, drying) and raw materials (pulp type, amount of fines, additives, fiber surface treatments). In Table 1, activation is divided into two different categories: in one, activation is increased through increasing fiber swelling and in the other, by increased straining during drying. In Table 2, interfiber bonding is divided into two categories, in which bonding is thought to be increased either by dry strength additives or by fines. Activation is thought to be affected mainly by drying stress.

**Table 1.** Chemical Pulp Paper Properties and Bonding/Activation

<i>Property</i>	<i>Interfiber bonding</i> conventional dry strength additives	<i>Fiber segment activation</i>	
		fiber swelling (e.g. low-intensity beating)	drying stress
Density	<b>0</b>	<b>++</b>	<b>0</b>
Tensile strength	<b>++</b>	<b>++</b>	<b>++</b>
Elastic modulus	<b>0 or +</b>	<b>++(+)</b>	<b>+++</b>
Elastic breaking strain	<b>++</b>	<b>+</b>	<b>--</b>
Z-directional strength	<b>+++</b>	<b>++</b>	<b>--</b>
In-plane tear strength	<b>+ or -</b>	<b>+ or -</b>	<b>--</b>
Damage width	<b>--</b>	<b>--</b>	<b>--</b>

**Table 2.** Mechanical pulp paper properties and Bonding/Activation

<i>Property</i>	<i>Interfiber bonding</i>		<i>Fiber segment activation</i>
	conventional dry strength additives	TMP fines	drying stress
Density	<b>0</b>	<b>++</b>	<b>0</b>
Tensile strength	<b>+</b>	<b>++</b>	<b>+(+)</b>
Elastic modulus	<b>0</b>	<b>++</b>	<b>++</b>
Elastic breaking strain	<b>0</b>	<b>++</b>	<b>-(-)</b>
Z-directional strength	<b>++</b>	<b>+++</b>	<b>--</b>
In-plane tear strength	<b>-</b>	<b>++</b>	<b>--</b>
Damage width	<b>-</b>	<b>-</b>	<b>+</b>

**Key for both tables:** + positive correlation, - negative and 0 no significant effect, the amount of signs indicates the strength of the correlation).

### Results from Preliminary Test Series

Adding starch to the TMP or kraft furnish prior to sheet forming did not affect tensile stiffness, as could be expected, since tensile stiffness or elastic modulus largely depends on the axial strength of fibers (or fiber segments in the network). These are governed by beating, wet pressing and the properties of the pulp mixture components. Drying stresses influence the elastic modulus as well (the elastic modulus increases if drying shrinkage is limited) (Niskanen and Kärenlampi 1998). In other words, activation of fiber segments influences the elastic modulus. This can be seen in Fig. 3: tensile stiffness of the restrictedly dried sheets were significantly higher than that of the freely dried sheets. Restricting the drying shrinkage resulted in significantly higher tensile stiffness values than what was observed in the freely dried sheets.

The effect of beating on activation in 100 % kraft sheets can be seen in Fig. 3. Beating increased tensile stiffness significantly, although the most significant changes seemed to take place already with quite small beating intensity increment. Beating clearly promoted interfiber bonding (Fig. 4, right), but had also other effects on the fiber network, for example, the effect of beating on fiber flexibility affected subsequent fiber segment activation during drying. As can be seen in Fig. 3, increasing the drying stress by restricted drying led to higher tensile stiffness – and activation in the kraft test sheets. Again, starch addition had no effect on tensile stiffness/activation.

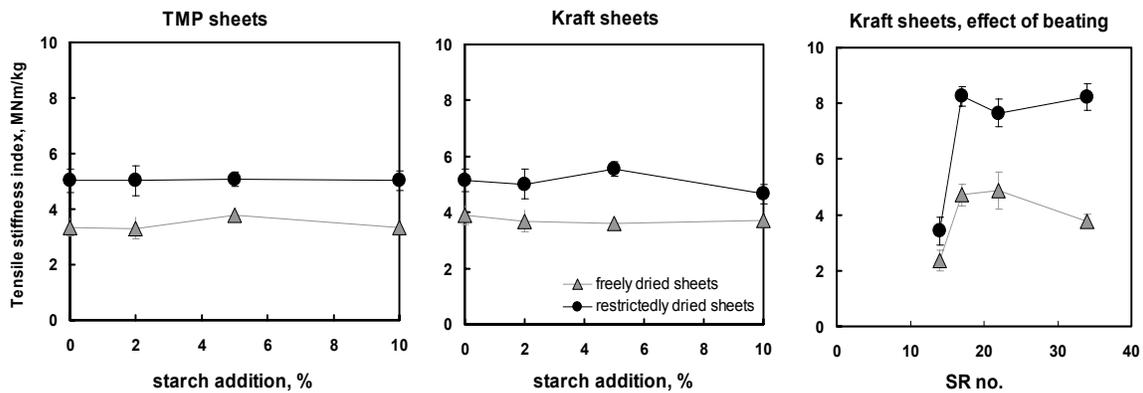


Fig. 3. Effect of starch addition and kraft pulp beating on activation in TMP and kraft sheets

Starch addition and beating increased the bond strength of kraft hand sheets (Fig. 4), although here as well, the increment seems to have been most significant already at a relatively low increase in beating intensity (from SR°14 to SR°17), and with the lowest starch addition (2%). There were no differences between the two drying modes in the differently beaten kraft handsheets, but in the starch-treated handsheets there were statistically significant differences between freely and restrictedly dried sheets, suggesting that the mechanism in which these two treatments affect bonding and bond strength is different. According to Retulainen (1997), the increment in tensile strength with starch addition is largely due to improved specific bond strength and increased stiffness of bonded areas. Starch affects bond strength directly by increasing the actual strength of bonds, whereas beating increases both bond strength and the bonded area in the fiber network. Drying stress decreases bonding in starch-treated sheets. Beating, on the other hand, affects fiber swelling, flexibility, conformability and bonding potential of chemical pulp fibers by creating external fibrillation and secondary fines (Robinson 1980; Page 1989; Niskanen 1998; Hiltunen 2003). External fibrillation increases the strength of interfiber bonds and enhances sheet consolidation (Retulainen *et al.* 1998). It provides a mechanism by which cellulosic surfaces can attain the close contact, without which the formation of hydrogen bonds would not take place. Beating not only increases the specific bond strength, but also the area of bonds.

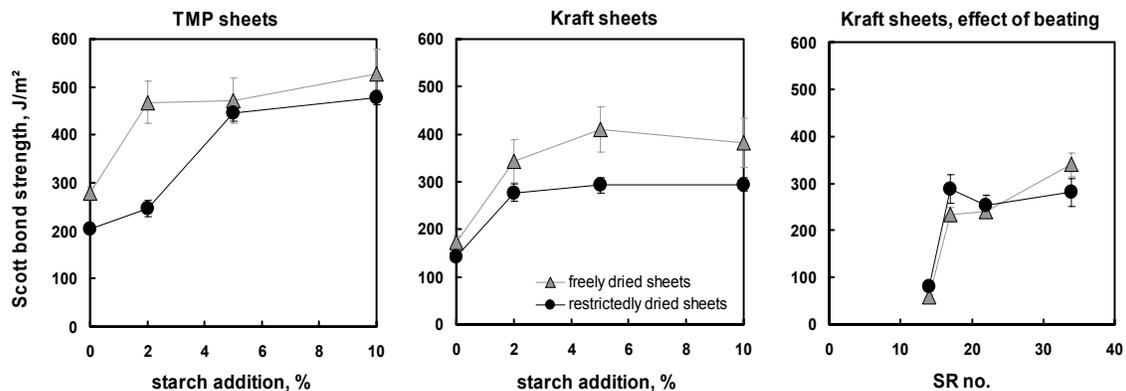
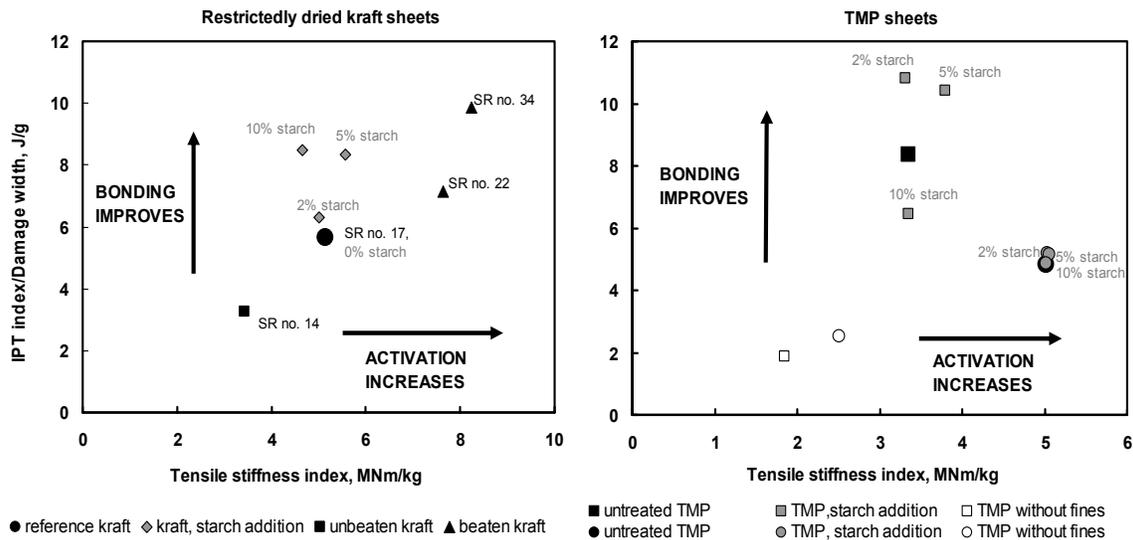


Fig. 4. Effect of starch addition and beating of kraft pulp on bonding in TMP and kraft sheets.

Figure 5 depicts the relationship between tensile stiffness index and a calculated variable in-plane tear index/damage width, and also the relationship of bonding and activation. In the kraft sheets (Fig. 5, left), beating seems to have affected both bonding and activation, whereas starch addition influenced only bonding. The effect of starch addition on bonding and activation in TMP sheets was either non-existent (restrictedly dried sheets) or ambiguous (freely dried sheets) (Fig. 5, right). On the other hand, removal of fines decreased both bonding and activation. The role of fines in bonding is widely recognized. But contrary to previous beliefs, their role in activation seems to be quite important as well. The role of fines in the development of activation in mechanical pulp sheets appears quite vital. Originally, the TMP fines were thought to affect only bonding. TMP fines and their influence on activation have been discussed in more detail by Vainio *et al.* (2007).



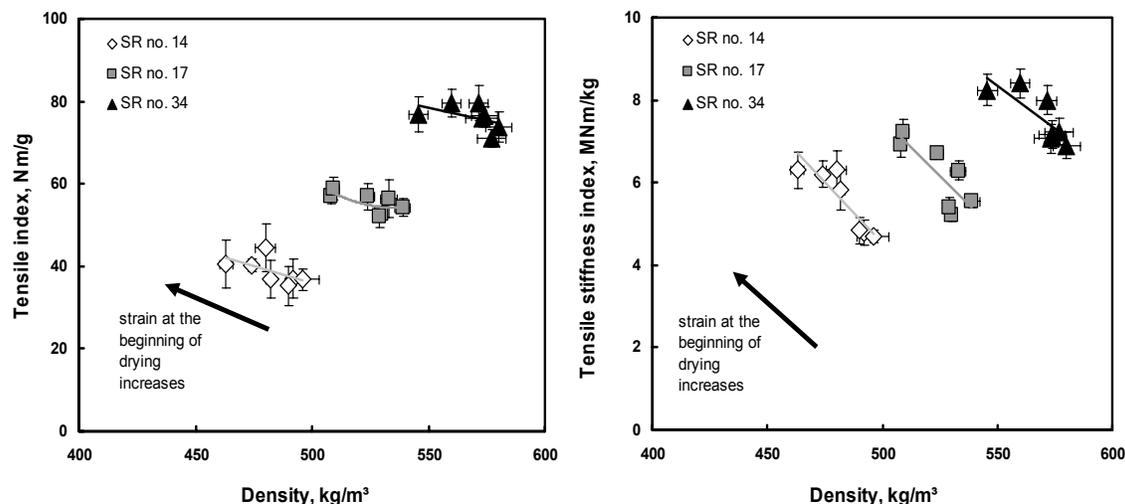
**Fig. 5.** Bonding and activation in restrictedly dried kraft sheets and TMP sheets. For TMP sheets, square markers represent freely dried sheets and round markers restrictedly dried sheets; open markers represent TMP without fines and black markers normal, unfractionated, untreated TMP; gray markers are for TMP sheets treated with starch.

## Results from PDR Test Series

Fiber segment activation (or elastic modulus of dry paper) is affected by fiber properties, but the strategy used for drying the paper network plays a significant role for the development of activation (Htun 1980, Zhang *et al.* 2001). In most sheets, activation increases linearly with increasing drying stress. During wet straining of paper, fiber segments become permanently elongated, and if the straining of paper is relatively small, i.e., not extensive enough to cause network failure, initially unloaded, non-bonded fiber segments become activated as the segment lengths stretch and bonded areas around them rupture (Niskanen 1993).

The tensile properties of sheets made of 100% kraft were generally improved with increasing drying stress (Fig. 6), although tensile strength increased only slightly. Tensile stiffness was affected very positively. All three beating levels seemed to behave quite similarly; the increase in tensile strength or tensile stiffness was almost the same in all to the three different beating levels. The initial level of tensile strength/stiffness was naturally

higher the higher the beating degree of the kraft pulp was. Beating did not seem to have much influence on the improvement in activation (Fig. 6, right), although the fibers should have different swelling properties due to their different beating levels. The results confirm deductions made on the effect of drying on paper properties: tensile stiffness index, understood here to reflect activation, increases with drying strain. When initially slack, free fiber segments straighten during drying under strain, and the elastic modulus of paper web increases (Htun 1980, Wahlström 1999). Tensile strength is thought to reflect both activation and bonding, and therefore the overall effect of drying stress on tensile strength is not very extensive.



**Fig. 6.** The effect of drying stress on the in-plane strength properties of handsheets.

Z-directional bond strength decreased with increasing drying stress (Fig. 7, left). The decrease was most drastic in sheets made from highly beaten pulp, probably because the initial ‘capacity’ for bonding was higher than in the sheets made from less beaten kraft pulp. Beating creates fines material and external fibrillation and increases fiber flexibility, which all promote bond strength and increase the bonded area (Page 1989; Robinson 1980). A similar negative effect can be in elastic breaking strain values of the test sheets: also the in-plane directional bond strength decreased with increasing drying stress (Fig. 7, right). Here, the degree of beating did not seem to influence the extent of deterioration. Increased drying stress reduces the bonded area and can also break up bonds in the network (reflected as decreasing sheet density). Similar results have been published by Wahlström *et al.* (2000). When paper is strained or elongated, as was done in this test series prior to drying with the PDR device, microscopic failures and even smaller scale ruptures take place in the network. These failures affect not only the internal structure of paper but also fiber-to-fiber bond structures and hydrogen bonds, which deteriorate either gradually or are destroyed altogether (Niskanen 1993).

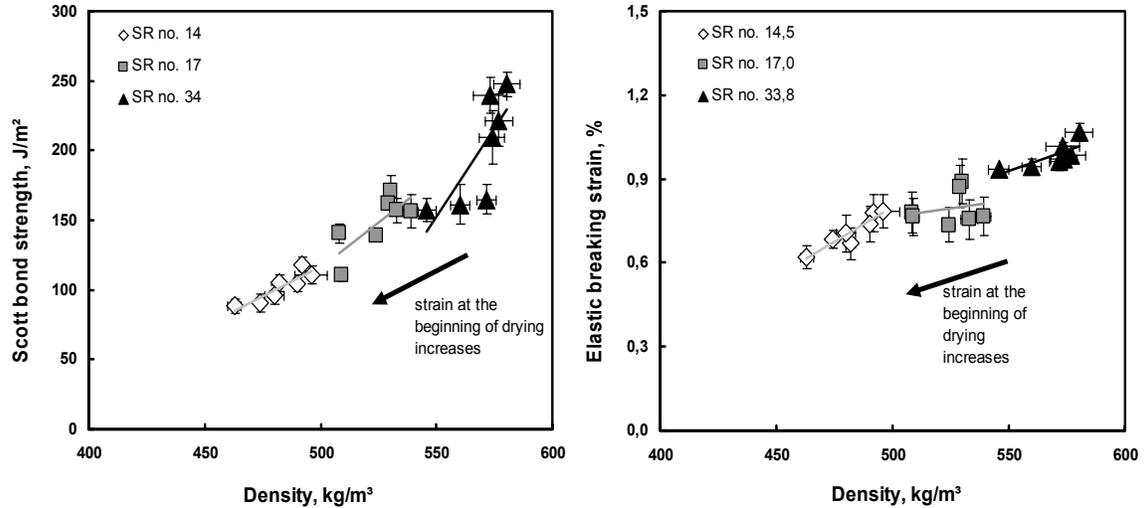


Fig. 7. The effect of drying stress on the bonding properties of handsheets.

Examining the relationship between bonding and activation in the test sheets (Fig. 8), it can be seen quite clearly that the overall bonding was affected negatively by increasing drying stress, although the extent of the decrease in bonding depended quite strongly on the degree of treatment that the kraft has been subjected to in beating. In the sheets made of very gently beaten kraft pulp, bonding almost stayed at a level independent of the drying stress, whereas in the sheets made of more beaten pulp, the effect was much more drastic. Activation, and its development with increasing drying stress, did not seem to depend on the beating level.

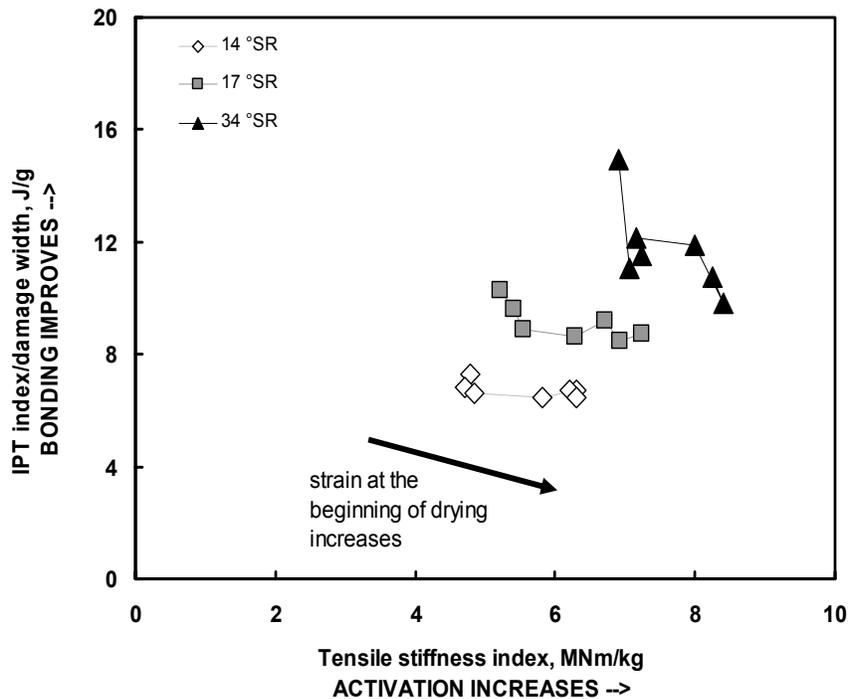


Fig. 8. Bonding and activation in handsheets made of differently beaten kraft pulp.

## CONCLUSIONS

1. Bonding and activation in paper can be well described with different strength properties and their combinations (Tables 1 and 2). The relationships between bonding and activation and different paper properties in kraft sheets are quite well understood; for example, beating and dry strength chemicals improve bonding. In TMP sheets, the cause-effect relationships seem to behave somewhat differently than in kraft sheets, and require further investigation to be fully understood.
2. Straining of test sheets before or during drying has a clear effect on both in-plane and z-directional strength properties: in general, activation of network increases by straining and bonding deteriorates with increasing straining.
3. Bonding is strongly affected by variables such as beating, furnish components and strength additives. For bonding, there are clear differences between different beating levels and sheet behavior in drying. Bonding potential develops during beating and refining, and those processes also have a significant influence on the structure and strength of bonds, which in turn seem to affect how bonds and bonding in general develops during drying.
4. Activation is affected by pulp properties (chemical vs. mechanical fibers; beating of chemical fibers) to some extent, but the role of drying strategy is significant. Activation is increased by beating, independent of the actual beating intensity, which could suggest that drying and drying stresses are more significant than fiber properties in the development of activation.
5. Furthermore, TMP fines seem to have some sort of role in activation, not only in bonding. The mechanism in which fines affect activation cannot be clarified with the results of this work.

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## ESTIMATION OF HARDWOOD LIGNIN CONCENTRATIONS BY UV SPECTROSCOPY AND CHLORINE DEMETHYLATION

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Major projects are underway in our laboratory focusing on mildly acidic (pH>3) and alkaline (pH<10) pretreatments of hardwood chips prior to incineration for electric power or prior to pulping for paper manufacture. Production of lignocellulosic ethanol from the hemicelluloses in the hydrolyzates will be attempted. It is of great interest to quantify the concentrations of lignin in these hydrolyzates, since lignin fragments are suspected as fermentation inhibitors. UV spectroscopy is normally used to estimate the concentration of aqueous soluble lignin. However, the 203 nm absorbance gave unreliable results for these hydrolyzates, and on some occasions the 278 nm absorbance was unduly influenced by high absorbance in the 260-265 nm range. A credible method that uses chlorination to generate methanol from the methoxyl groups in lignin will be described. Model compound experiments showed that syringyl lignin units, with two methoxyl groups, gave a methanol yield of ~1.0 mmole/mmole of aromatic rings.

*Keywords: Chlorination, Lignin, Guaiacyl units, Syringyl units, Methanol, Methoxyl groups,*

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### INTRODUCTION

In the first of two projects presently underway in our laboratory, mildly acidic solutions (acidolysis or A-stage) are been used to extract some of the hemicelluloses from hardwood chips prior to combustion or pulping. Approximately 70% of the hemicelluloses dissolve in the cooking liquor during kraft or soda/anthraquinone pulping and consume a significant fraction of the alkali in the process of being degraded to low molecular weight compounds. It would be technically difficult to remove these low molecular weight compounds from alkaline pulping effluents and purify their product streams to a degree adequate enough for use as commodity chemicals. Native hemicelluloses in wood or their degradation products in alkaline pulping effluents have high oxygen to carbon ratio and relatively low calorific values during combustion. A superior route would be to pre-extract the hemicelluloses and further hydrolyze them to monomers. Fermentation can then be used to convert the monomers to ethanol. Since lignin sub-structures are known fermentation inhibitors (Parajo et al. 1996; Jonsson et al. 1998; Cantarella et al. 2004), it would be helpful if the A-stage could be optimized to minimize the amount of lignin in the hydrolyzate (A-stage effluent).

The second project involves a pre-cooking stage with Na<sub>2</sub>CO<sub>3</sub> before kraft (NaOH and Na<sub>2</sub>S) or soda/antraquinone (SAQ) pulping. Hooper (1932) reported on the ability of this pretreatment (C-stage) to subsequently decrease the alkali demand in straight soda (NaOH only) and kraft pulping. After a C-stage we are observing improvements in the SAQ process that are much more significant than for the kraft process. The C-stage significantly improves selectivity (pulp yield at a given residual lignin content) for the SAQ but not the kraft process. It is of great interest to discover if lignin or carbohydrate reactions in the C-stage are primarily responsible for these improvements. The ability to estimate the quantity of lignin solubilized in the C-stage is obviously critical. It should also be noted that mildly alkaline solutions can be used to extract hemicelluloses (Carpita 1984; Matulewicz and Cerezo 1987) instead of the acidic process above.

The quantification of acid insoluble or Klason lignin is a well-established and widely used procedure in the wood chemistry community (TAPPI Method T222 om-88, 1988). However, quantification of soluble lignin has been much more unreliable and controversial. Lignin determination methods have been reviewed fairly recently (Hatfield and Fukushima 2005; Dence 1992), with much of the discussion focusing on the weakness or inconvenience associated with almost all of them. The more common techniques can be divided into four categories; 1) UV spectroscopy, 2) chemical modifications combined with UV/visible spectroscopy, 3) NMR spectroscopy, and 4) chemical consumption during exhaustive oxidation.

In the present investigation unreliable results were being obtained from the most widely used of the UV spectroscopic methods, i.e. lignin dissolved in 3 wt. % sulfuric acid (Tappi Method UM 250, 1985). A wide range of the other UV methods (some including solvent/water mixtures), NMR spectroscopy and oxidant consumption methods were preliminarily investigated and deemed to be unreliable or overly complicated.

This report considers the use of chlorination to estimate the lignin content of hydrolyzates by quantifying the amount of methanol generated from the methoxyl groups by the proposed mechanism in Fig. 1 (Gierer and Sundholm 1971). Time and resources were invested in this technique because it gave credible results for softwood lignin (Ni et al. 1990) and it was known that the lignin in A-stage effluents (pH>3.0) from softwoods (Casebier et al. 1969) and hardwoods (Casebier et al. 1973) were rich in methoxyl groups.

## MATERIALS AND METHODS

### Lignin Model Compounds (LMCs)

The chemical structures of the LMCs used are shown in Figure 2. Compounds **I**, **II**, **IV**, and **VI** were obtained from Aldrich Chemical Company while **III** and **V** were obtained from Fluka Chemical Corp.

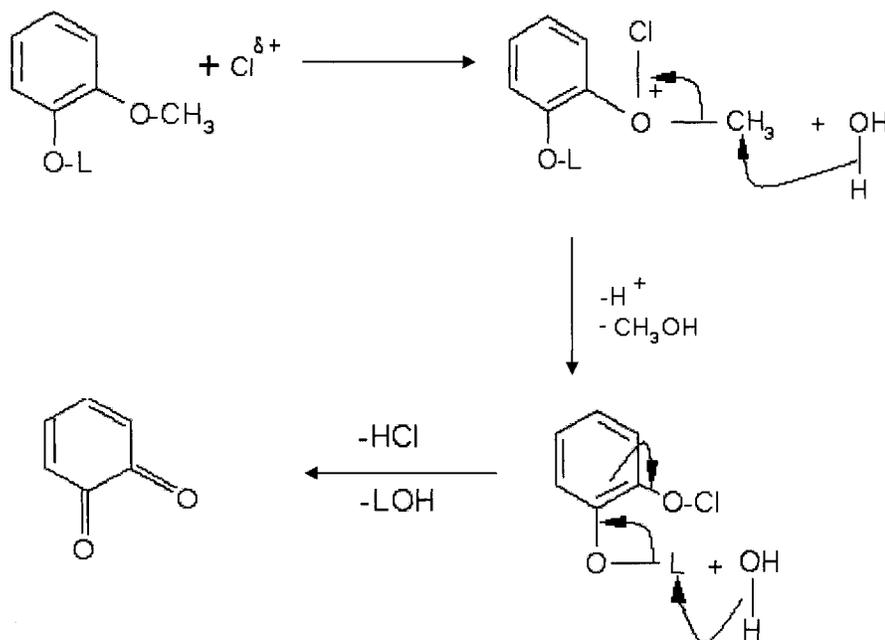


Fig. 1. Proposed mechanism for methanol generation during lignin chlorination.

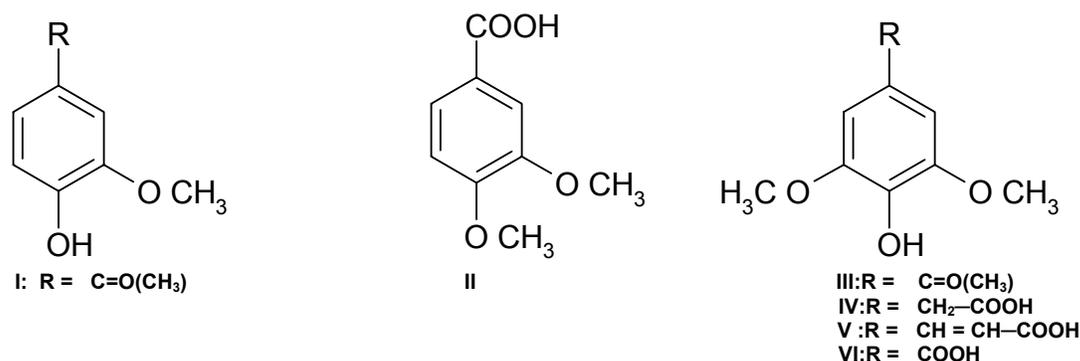


Fig. 2. Lignin model compounds (LMCs) used in this investigation

### Chlorination of Lignin Model Compounds

One mmole of each LMC was dissolved in 2ml of dioxane then diluted to 25ml with warm water. One-tenth of this solution was added to 4ml of 4M  $\text{H}_2\text{SO}_4$  in a polyethylene bottle, and then 5 ml of household bleach (6%  $\text{NaOCl}$ ) was added. The bottle was sealed, mixed, and placed in a  $60^\circ\text{C}$  water-bath for at least 2h. At the end of the treatment, 1ml of a solution containing 150 g/l of both  $\text{NaHCO}_3$  and  $\text{Na}_2\text{SO}_3$  was added, as well as 1ml of a solution containing the internal standard (1.6 g/l ethanol in  $\text{H}_2\text{O}$ ).

### Chlorination of Lignin-Containing Solutions

Hydrolyzates were adjusted to pH~9 and filtered through a 1.2µm Versapor porous disk (Pall Life Sciences) with the aid of a slight pressure difference. A volume of the filtrate (2.0 – 5.0 ml) was then taken and added to 4ml of 4M H<sub>2</sub>SO<sub>4</sub>. The chlorination treatment was identical to that used for the LMCs. This procedure is a slight modification to that of Ni et al. (1990) who used an aqueous solution of chlorine directly.

### Acid and Carbonate Pre-extraction Stages

Sugar maple (*Acer saccharum*) chips were treated at a 4:1 liquor to wood (L:W) ratio with water or dilute acetic acid solutions at 120°C, 150°C, and 165°C. The time to temperature was 30 minutes for 120°C and 150°C, and 60 minutes for 165°C. The acetic acid dose was 3.0%, 1.5%, and 0% respectively for the 3 temperatures. The pH of all of the A-stage hydrolyzates that were collected was in the range of 3 to 4. A-stages were also applied to 20-mesh woodmeal from 13 poplars after they were extracted with ethanol-toluene in accordance with TAPPI method T204 om-88 (1988). Woodmeals were placed in 100ml autoclaves at a L:W≥20. Several autoclaves were placed simultaneously in an M&K digester with recirculating hot water. The first condition was 1h to 175°C and 45 min at that temperature. The extracting solution was comprised of 20% ethanol (v/v) and 0.01M H<sub>2</sub>SO<sub>4</sub>. The second condition was 30 minutes to 150°C and 1h at that temperature with only water as the extracting fluid.

A chip mixture comprised of ~60% of an eastern cottonwood (*Populus deltoides*) clone, ~20% white birch (*Betula papyrifera*), and ~20% sugar maple was used for the carbonate pretreatment. The Na<sub>2</sub>CO<sub>3</sub> dose was 7.7% on chips, the L:W ratio was 4:1, and 60 minutes to 165°C was used. Once 165°C was attained hydrolyzates were collected after 15, 30, and 60 minutes.

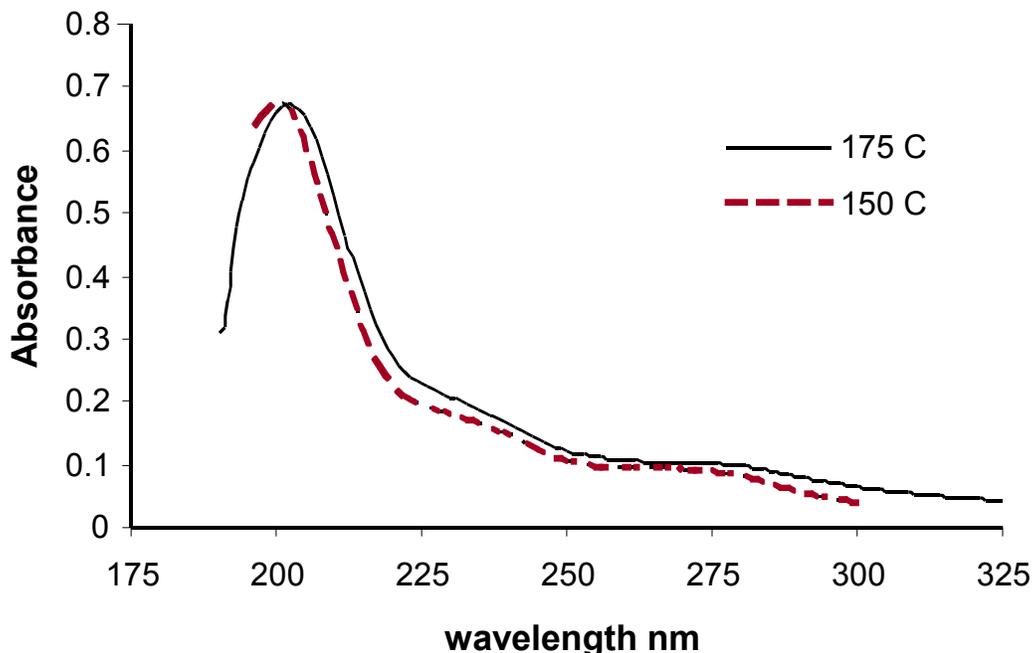
### UV and Gas Chromatographic Analyses

The methanol in the reaction products was analyzed on a HP5890 gas chromatograph with flame ionization detection. A 1.83m long Tenax TA 80/100 packed column was used with a column head pressure of ~120kPa. A Perkin Elmer Lambda 650 UV/Vis spectrometer was used to obtain UV spectra of lignin-containing solutions. The filtered solutions above (pH~9) were diluted by a factor of at least 100 and adjusted to 3 wt. % H<sub>2</sub>SO<sub>4</sub> before analysis.

## RESULTS AND DISCUSSION

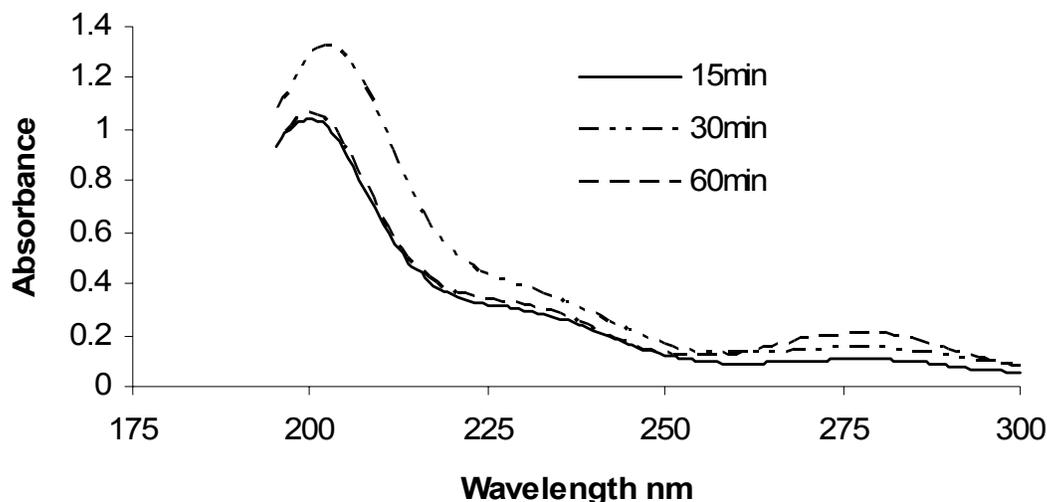
Thirteen poplars were acidolyzed at 150°C and 175°C to see if there was any correlation between syringyl to guaiacyl (S:G) ratio and acid soluble lignin. The S:G ratios of the 13 poplar woodmeals were known beforehand. The ratio was determined by a previously described method that involved both nitrobenzene and potassium permanganate oxidations (Francis et al. 2005). UV spectra with well defined peaks at 203 nm and 278 nm were obtained for 10 of the 13 A-stage effluents. However, three of the effluents did not show a peak at 278nm due to higher than normal absorbance in the 260-265 nm range. A possible explanation is that those samples had higher than normal

generation rates of furfural and/or hydroxymethylfurfural (Dence 1992). However, there may be other explanations. The spectra for 150°C and 175°C effluents (different dilution factors) for one of the three poplars are shown in Figure 3. If the 260-265 nm absorbance were primarily due to furfurals and the 278 nm to lignin, then it would be highly coincidental that the 260 nm to 278 nm absorbance ratio was nearly identical for treatments at 150°C (without ethanol) and 175°C (with ethanol).



**Fig. 3.** UV spectra without a well defined 278 nm peak for acid hydrolyzates (150°C and 175°C) from a specific hybrid poplar.

The 203 nm absorbance readings were very erratic for these effluents, particularly when the acidolysis temperature was 175°C. Several diluted effluent samples were further diluted 1:1 with 3% H<sub>2</sub>SO<sub>4</sub>. Typically the 278 nm absorbance decreased by 50 ± 3%. However, the 203 nm absorbance would decrease anywhere from 10-80%. An excellent example of this is shown in Fig. 4 for acidolysis of sugar maple chips at 165°C. Effluents were collected at 15, 30 and 60 min and diluted in exactly the same manner to a final dilution factor of 800. The 278 nm absorbance increased in a logical manner from 0.106 to 0.160 to 0.217. However, the corresponding 203 nm absorbance were 1.04, 1.34, and 1.07.

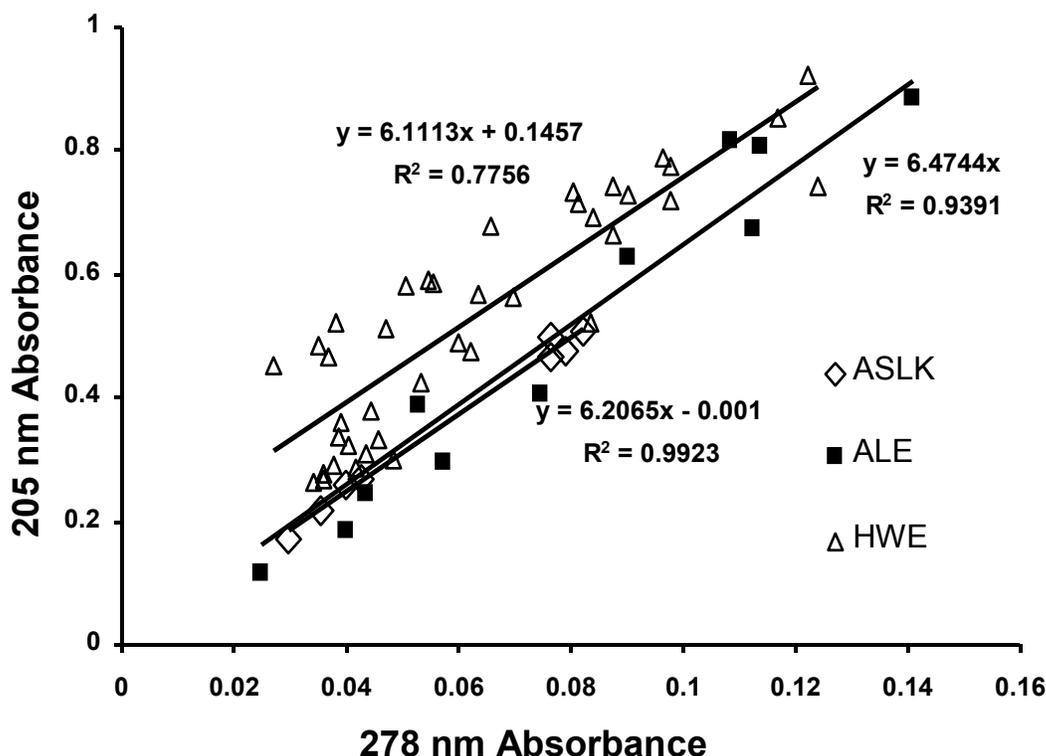


**Fig. 4.** UV spectra of acid hydrolyzates from sugar maple collected after 15, 30 and 60 minutes at 165°C. The dilution factor was 800 for all three samples.

It appears as if acidolysis temperature is a significant variable in the erratic nature of the 203 nm absorbance. In a simultaneous investigation, the leaching of sugar maple lignin into 0.1M NaOH at room temperature has been investigated. Close to classical UV lignin spectra were obtained despite the alkaline conditions. It can be seen in Figure 5 that the ratio of 203 nm to 278 nm absorbance was fairly constant at ~6.5. Similarly, acid soluble lignin from the Klason method (TAPPI Method T 222 om-88, 1988; TAPPI Method UM 250, 1985) was analyzed for 4 of the poplars. The 203 nm absorbance is also plotted against the 278 nm absorbance in Fig. 5 after dilution by factors of 15 and 30.

It can be seen that the ratio was very similar to that of the alkali-leached lignin; a slope of 6.2 was obtained with an  $R^2$  value of 0.992. In the Klason test the slurry is refluxed in 3%  $H_2SO_4$  at a low temperature of ~100°C. The data for most of the 150°C poplar effluents are also shown in Fig. 5 at various levels of dilution. It can be seen that the ratio of 203 nm to 278 nm absorbance was approaching that of the earlier two cases. However, for a 278 nm absorbance of 0.048, the 203 nm absorbance varied from 0.30 to ~0.55. Dilution experiments with selected samples indicated that most of the error was in the 203 nm absorbance.

UV spectroscopy has some clear drawbacks when used to estimate the concentration of some aqueous soluble lignin and other analytical techniques were investigated.



**Fig. 5.** Ratio of 203 nm to 278 nm absorbance for acid-soluble lignin from Klason test (ASLK), alkaline leachate at room temperature (ALE), and hot water extraction of poplar woodmeals at 150°C (HWE).

### Preliminary Investigation of Other Analytical Techniques

Some of the more credible methods discussed by Dence (1992) were attempted. The first alternative UV method to be investigated was a solvent comprised of phosphoric acid and water (8:2 v/v) (Bethge et al. 1952; Francis and Reeve 1987). This solvent appeared to give higher absorbance in the 260-265 nm region of the spectrum. In another approach, A-stage effluents were further acidified then extracted with dichloromethane. The dried extracts were dissolved in 2-methoxyethanol/water (8:2 v/v) and 95% ethanol and analyzed by UV spectroscopy (Lin 1992). The two major problems observed with 3% H<sub>2</sub>SO<sub>4</sub> were still apparent, i.e. absence of a sharp 278nm peak in some samples and erratic readings for the 203 nm peak.

Proton NMR is used in our laboratory for quantification of carbohydrates in hydrolyzates (Kiemle et al. 2004; Francis et al. 2006). Signature peaks for lignin were observed in both A- and C-stage effluents. However, the clearly identifiable lignin peaks were different for A- and C-stage effluents. Also, under both acid and alkaline conditions the quality of the spectra (peak resolution and baseline) varied significantly with treatment time. The methoxyl protons (~3.8 ppm) were observed in all spectra. This method maybe effective if the lignin is extracted from the hydrolyzates and acetylated

prior to NMR analysis. Sample preparation for such an approach can be found in the literature (Lundquist 1992).

The kappa number was the first and last of the oxidant consumption methods to be investigated. The only change to the method (Tappi T 236 cm-85, 1985) was replacing fibers with effluent. In one of early trials 25ml of a particular effluent consumed 25.1ml of 0.1N KMnO<sub>4</sub>, but when the effluent volume was decreased to 20ml it consumed 24.3 ml of the permanganate.

At this stage it was decided that a method with an established and specific reaction would be the next one investigated, and chlorine demethylation was selected.

### Methanol Yields from Syringyl Units

An excellent review on dealkylation of lignin by chlorine is in the literature (Dence, 1996). That publication referred to the high methanol yields from guaiacyl (G) LMCs (Gierer and Sundholm 1971) and the residual lignin in softwood kraft pulps (Ni et al. 1990). However, the Dence review made no mention of quantitative data for substrates such as hardwood lignin that contain significant amounts of syringyl (S) units, and we have been unable to find any such data published since 1996.

Methanol yield was quantified for the LMCs in Fig. 2, and the results are documented in Table 1. It can be seen that the methanol yield for the 4 syringyl monomers averaged ~1.0 mmole/mmole of aromatic rings. Repeat experiments were performed for three of the LMCs at a later date and those results are in parentheses in Table 1. The method appears to be decently reproducible. From the present data for two guaiacyl monomers, coupled with data from the literature (Gierer and Sundholm 1971; Ni et al. 1990), we estimated a methanol yield of ~0.8 mmole/mmole of phenylpropane (C<sub>9</sub>) units in the guaiacyl fraction of lignin. The present yield of 0.7 for **II** is almost equal to the 0.67 obtained Gierer and Sundholm (1971) for 1, 2-dimethoxybenzene (veratrole). A decent estimate of methanol yield from chlorination of hardwoods would be 0.9-1.0 mmole/mmole of aromatic rings, since most hardwoods appear to contain more S than G units. Ten of the thirteen poplars involved in this investigation had S:G ratios ≥ 1.2.

**Table 1.** Methanol Yield from LMCs

Compound	Methanol Yield <sup>1</sup>
I	0.8
II	0.7
III	0.9 (0.8) <sup>2</sup>
IV	1.1
V	1.2 (1.1)
VI	0.8 (1.0)

<sup>1</sup> mmoles/mmole of aromatic rings    <sup>2</sup> Experiments performed at a later date

The effect of substituents on methanol yield is beyond the scope of the present investigation. Our hope was that the methanol yield for S units would be close to that obtained for G units (~1.0 mmole/mmole) and this was indeed observed. If that were not the case, then the S:G ratio would have to be involved in the calculations correlating methanol yield to lignin content.

An average methanol yield of 1.0 mmole/mmole of aromatic rings along with an average molecular weight (MW) of 210 for the C<sub>9</sub> units in hardwood lignin will be used

in our calculations. A wide range of literature data was examined, and it appears as if the structural formula for various native hardwood lignins is fairly constant, except for the number of methoxyl groups. The average formula is close to  $C_9H_{8.7}O_{2.9}(OCH_3)_x$  with  $x$  varying from 1.30 to 1.57 (Gellerstedt et al. 1988, Fengel and Wegener 1989; Dence and Lin 1992). If  $x = 1.50$  is assumed, then a MW of 210 is obtained. It should also be noted that varying  $x$  from 1.30 to 1.57 only changes the  $C_9$  MW by 8.4 units.

### Lignin Content of Hydrolyzates

The number of mmoles of methanol in a given volume of hydrolyzate was obtained by GC. This value was multiplied by 210 to give mg of lignin. All of the A- and C-stages conducted with chips used 4 liters of liquor/kg of wood (oven dried or OD basis). Typically 5ml of hydrolyzate was chlorinated and that corresponded to 1,250mg of chips at the beginning of the process.

A 1-cm quartz cuvette was used for UV analysis on all occasions. Therefore, lignin concentration in g/l can be obtained by simply dividing the absorbance by the absorptivity in accordance with equation [1] (Dence 1992). The value obtained is then multiplied by the dilution factor to arrive at the lignin concentration in the hydrolyzate. One liter of solution would have treated 250g of chips.

$$A = \epsilon \ell C \quad [1]$$

where  $A$  = absorbance;  $\epsilon$  = absorptivity in  $lg^{-1}cm^{-1}$ ;  $\ell$  = light path in cm;  $C$  = lignin concentration in g/l

Many prior investigations have concluded that the absorptivity of native softwood and hardwood lignin is  $\sim 20 lg^{-1}cm^{-1}$  at 278 nm (Johnson et al. 1961; Sjöström and Enström 1966; Marton 1967; Hardell et al. 1980; Iiyana and Wallis 1988). This value was used in our calculations, and an assumed value of  $124 lg^{-1}cm^{-1}$  ( $20 \times 6.2$ , Fig. 5) was used for 203 nm. The chlorination method is compared to the UV method (203 nm & 278 nm) for several hydrolyzates in Table 2.

It can be seen that the chlorination method compared favorably with the 278 nm absorbance results. Therefore, for samples where the 278 nm absorbance appears to be unduly influenced by chromophores absorbing in the 260-265 nm or 300-310 nm range, chlorination can be used. The 203 nm absorbance should be used with great care on all occasions. Once again decent reproducibility was obtained.

**Table 2.** Comparison of Lignin Content of Hydrolyzates by Chlorination and UV Spectroscopy

Hydrolyzate	Chlorination	278nm	205nm
A-stage (Maple chips)	1.4 <sup>1</sup> 2.3 (2.6) <sup>2</sup> 3.1	1.7 2.6 3.5	2.7 3.5 2.8
A-stage (Poplar, 150°C)	1.4 (1.2)	1.4	1.7
C-stage (MH chips <sup>3</sup> )	0.4 (0.4) 2.2	0.4 1.8	0.4 1.5
<sup>1</sup> % on chips or wood meal <sup>2</sup> Repeat experiment at a later date			
<sup>3</sup> MH = mixed hardwoods			

Wood chips were subjected to A- and C-stage treatments, and the chlorination method was used to estimate lignin content of the hydrolyzates (Fig. 6). The value was 0.3% lignin on chips after 60 minutes at 120°C; 0.7% on chips after 20 minutes at 150°C; and 1.4% on chips after 15 minutes at 165°C. These results appear to be internally consistent, based on the temperature effect normally observed in wood depolymerization. The results for the C-stage hydrolyzate were somewhat unusual. After a one-hour heat up time and 30 minutes at 165°C, the lignin content in the hydrolyzate was only 0.4%. This chlorination analysis was repeated, and a nearly identical result was obtained. Also, the UV estimate (278 nm) was in the range of 0.4% on chips for several dilutions. However, the lignin content then increased to 2.2% on chips with 30 minutes of additional retention. A credible explanation is that atmospheric O<sub>2</sub> in the digester at the start of the treatment oxidized and degraded lignin during the early stages. On no occasion did we purge the digester at the start of a C-stage. The pH of the hydrolyzate was 9.6 when 165°C was attained (0 minutes at temperature) and 8.4 after 30 minutes. In that pH range one would expect oxidation by O<sub>2</sub>, catalyzed by the carbonate radical (HCO<sub>3</sub><sup>•</sup>), as reported by Mih and Thompson (1983). The explanation for the significant increase in lignin content after 30 minutes would be the complete consumption of the O<sub>2</sub> in lignin and other oxidation reactions.

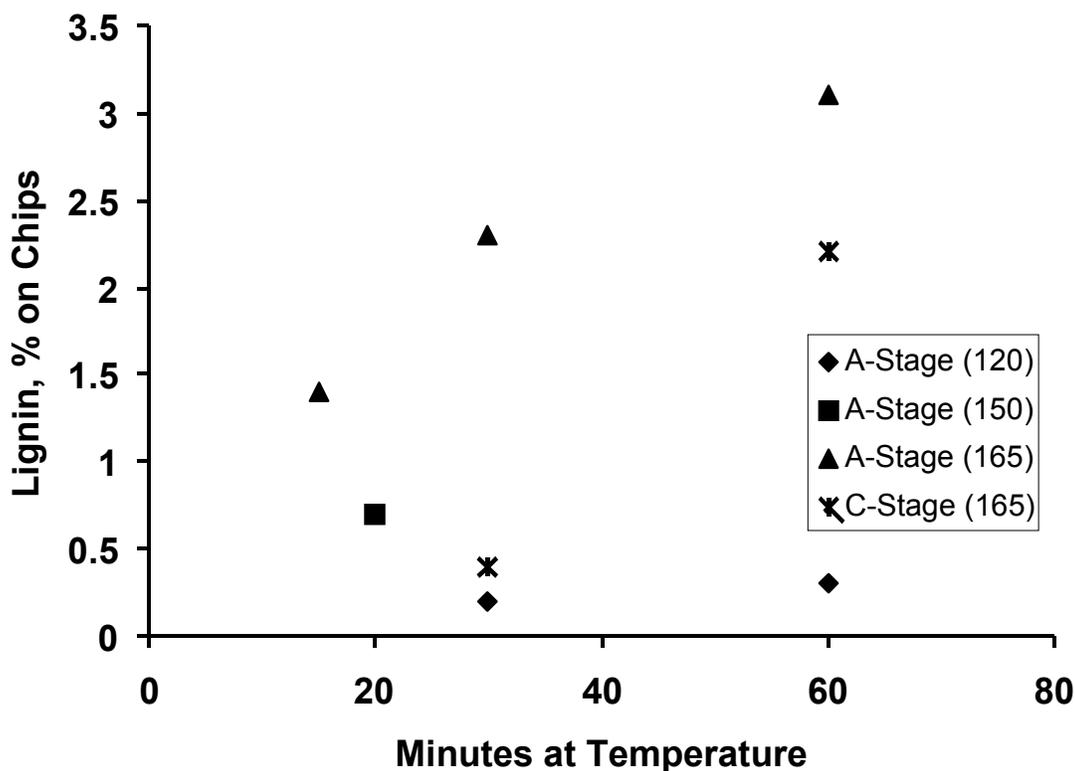


Fig. 6. Lignin content of A-stage and C-stage hydrolyzates determined by chlorination

## CONCLUSIONS

In the process of investigating mild acid and alkaline pretreatments of hardwoods we found the UV method of quantifying lignin in the hydrolyzates to be unreliable. In some cases a well-defined 278 nm peak was not obtained, and in many cases the 203nm peak did not afford a credible result. Preliminary investigations were conducted with different solvents for UV spectroscopy, NMR spectroscopy, and quantification of oxidant consumption per ml of effluent (kappa number). These methods were either inconvenient or unreliable. A method based on methanol formation from methoxyl groups during lignin chlorination was investigated for hardwoods. Syringyl model compounds were investigated and they averaged ~1.0 mmole of methanol/mmole of aromatic rings. When the chlorination method was used to analyze both acid and alkaline hydrolyzates, the results obtained all fell in the expected ranges and were always close to the estimate based on the 278 nm UV absorbance, i.e. when the 278nm peak was not unduly influenced by chromophores absorbing in either the 260-265nm or 300-310nm range. Based on several repeat experiments, an error of plus or minus 10% (maximum) is estimated for the chlorination method. However, enough data were not collected for a detailed statistical evaluation. It should be noted that a 10% error is quite reasonable for these samples. Even larger errors were reported by Hatfield and Fukushima (2005) for determinations using some of the more conventional methods and involving larger masses of lignin.

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## ACID-BASED HYDROLYSIS PROCESSES FOR ETHANOL FROM LIGNOCELLULOSIC MATERIALS: A REVIEW

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Bioethanol is nowadays one of the main actors in the fuel market. It is currently produced from sugars and starchy materials, but lignocelluloses can be expected to be major feedstocks for ethanol production in the future. Two processes are being developed in parallel for conversion of lignocelluloses to ethanol, "acid-based" and "enzyme-based" processes. The current article is dedicated to review of progress in the "acid-based-hydrolysis" process. This process was used industrially in the 1940s, during wartime, but was not economically competitive afterward. However, intensive research and development on its technology during the last three decades, in addition to the expanding ethanol market, may revive the process in large scale once again. In this paper the ethanol market, the composition of lignocellulosic materials, concentrated- and dilute-acid pretreatment and hydrolysis, plug-flow, percolation, counter-current and shrinking-bed hydrolysis reactors, fermentation of hexoses and pentoses, effects of fermentation inhibitors, downstream processing, wastewater treatment, analytical methods used, and the current commercial status of the acid-based ethanol processes are reviewed.

*Keywords:* Lignocellulosic materials, Dilute-acid hydrolysis, Ethanol, Fermentation

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### INTRODUCTION

Ethanol is nowadays an important product in the fuel market. Its market grew from less than a billion liters in 1975 to more than 39 billion liters in 2006 and is expected to reach 100 billion liters in 2015 (Licht 2006). Less than 4% of the ethanol is produced synthetically from oil, while the rest is produced by fermentation from bioresources. Ethanol is now produced from two major groups of bioresources: sugar substances and starchy materials. There is a competition between these two feedstocks for fuel ethanol production. While sugar substances were the feedstock for more than 60% of fuel ethanol production at the beginning of the 2000s, its share decreased to 47% by 2006, when grains accounted for 53% of the production (Licht 2006).

Ethanol has a potential market as big as the oil market. It can potentially replace the entire fuel market for gasoline. Furthermore, plastics such as polyethylene can be produced from ethanol through ethylene. However, the amounts of sugar substances and grains are limited in the world. They are relatively expensive feedstocks for ethanol production, and ethanol competes with human food for these raw materials. This competition may lead the price of grains and sugar to higher levels in the future.

Furthermore, the economy of the ethanol production process from grains depends on the market for its by-product, i.e. distillers' dried grains with solubles (DDGS) as animal food, which may not expand like the ethanol market in the future. In addition to the price, there is concern about damaging forests by increasing farming area as a result of more ethanol production from, for instance, sugar cane in Brazil.

Lignocellulosic materials are renewable, largely unused, and abundantly available sources of raw materials for the production of fuel ethanol. Lignocellulosic materials can be obtained at low cost from a variety of resources, e.g. forest residues, municipal solid waste, waste paper, and crop residue resources (Wyman 1996). These materials contain sugars polymerized in form of cellulose and hemicellulose, which can be liberated by hydrolysis and subsequently fermented to ethanol by microorganisms (Millati et al. 2002; Palmqvist and Hahn-Hägerdal 2000).

## COMPOSITION OF LIGNOCELLULOSIC MATERIALS AND THEIR HYDROLYZATES

Lignocellulosic materials predominantly contain a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives, and ashes. The term "holocellulose" is often used to describe the total carbohydrate contained in a plant or microbial cell. Holocellulose is therefore comprised of cellulose and hemicellulose in lignocellulosic materials.

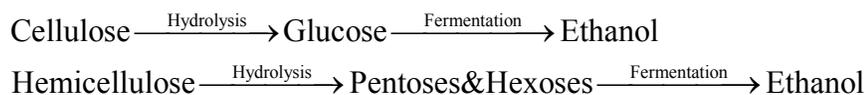
Cellulose is an unbranched linear polymer. The length of a cellulose molecule (polymer) is determined by the number of glucan units in the polymer, referred to as the degree of polymerization. The degree of polymerization of cellulose depends on the type of plants and typically is estimated to be from 2000 to 27000 glucan units. Hemicelluloses belong to a group of heterogeneous polysaccharides. The amount of hemicellulose is usually between 11% and 37% of the lignocellulosic dry weight. Hemicelluloses are relatively easily hydrolyzed by acids to their monomer components consisting of xylose, mannose, glucose, galactose, arabinose, and small amounts of rhamnose, glucuronic acid, methyl glucuronic acid, and galacturonic acid (Morohoshi 1991; Sjöström 1993).

Lignin is a very complex molecule constructed of phenylpropane units linked in a three-dimensional structure. Generally, softwoods contain more lignin than hardwoods. Although the principal structural elements in lignin have been largely clarified, many aspects of its chemistry remain unclear. Chemical bonds have been reported between lignin and hemicellulose and even cellulose. Lignins are extremely resistant to chemical and enzymatic degradation (Palmqvist and Hahn-Hägerdal 2000; Taherzadeh 1999).

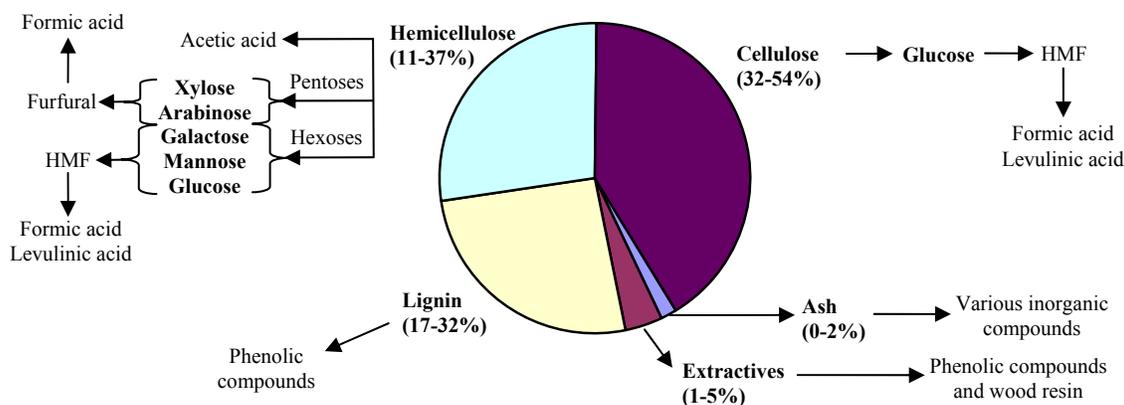
Extractives are wood compounds that are soluble in neutral organic solvents or water. The extractives usually represent a minor fraction (between 1-5%) of lignocellulosic materials. They contain a large number of both lipophilic and hydrophilic constituents. The extractives can be classified in four groups: (a) terpenoids and steroids, (b) fats and waxes, (c) phenolic constituents, and (d) inorganic components (Sjöström 1993; Taherzadeh 1999).

The amounts of the carbohydrate polymers and lignin depend on the type of material. Garrote et al. (1999) and Wyman (1996) have compiled the compositions of lignocelluloses from different hardwoods, softwoods, and agricultural residues reported in publications. The hardwoods such as white birch, aspen, red maple, *Eucalyptus*, *Populus*, and oak contain 39-54% cellulose, 14-37% hemicellulose, and 17-30% lignin. The corresponding values for softwoods, e.g. pines and firs, are 41-50% cellulose, 11-27% hemicellulose, and 20-30% lignin. The composition of different agricultural residues varies widely. For instance, rice straw consists of 32-47% cellulose, 19-27% hemicellulose, and 5-24% lignin.

The carbohydrate polymers in the lignocellulosic materials need to be converted to simple sugars before fermentation, through a process called hydrolysis. However, several products can result from hydrolysis (Fig. 1). There are several possible methods to hydrolyze lignocelluloses. The most commonly applied methods can be classified in two groups: chemical hydrolysis and enzymatic hydrolysis. Cellulose and hemicellulose can be converted to ethanol, while lignin remains as a by-product:



In addition, there are some other hydrolysis methods in which no chemicals or enzymes are applied. For instance, lignocelluloses may be hydrolyzed by gamma-ray or electron-beam irradiation, or microwave irradiation. However, these processes are far from being commercially applied (Taherzadeh 1999).



**Fig. 1.** Composition of lignocellulosic materials and their potential hydrolysis products

## OVERALL PROCESS OF ETHANOL PRODUCTION FROM LIGNOCELLULOSIC MATERIALS BY CHEMICAL HYDROLYSIS

A generally simplified representation of the process for ethanol production from lignocellulosic materials by chemical hydrolysis is shown in Fig. 2. The lignocellulosic raw materials are milled initially to sizes of a few millimeters, and then they are hydrolyzed to obtain fermentable sugars. However, several by-products may be formed or released in this step. If highly toxic hydrolyzates are formed, a detoxification stage is necessary prior to fermentation. The hydrolyzates are then fermented to ethanol in bioreactors. The ethanol is distilled to 90-95% purity by distillation. If fuel ethanol is desired, it should be further dehydrated to >99% by e.g. molecular sieves, to enable its blending with gasoline.

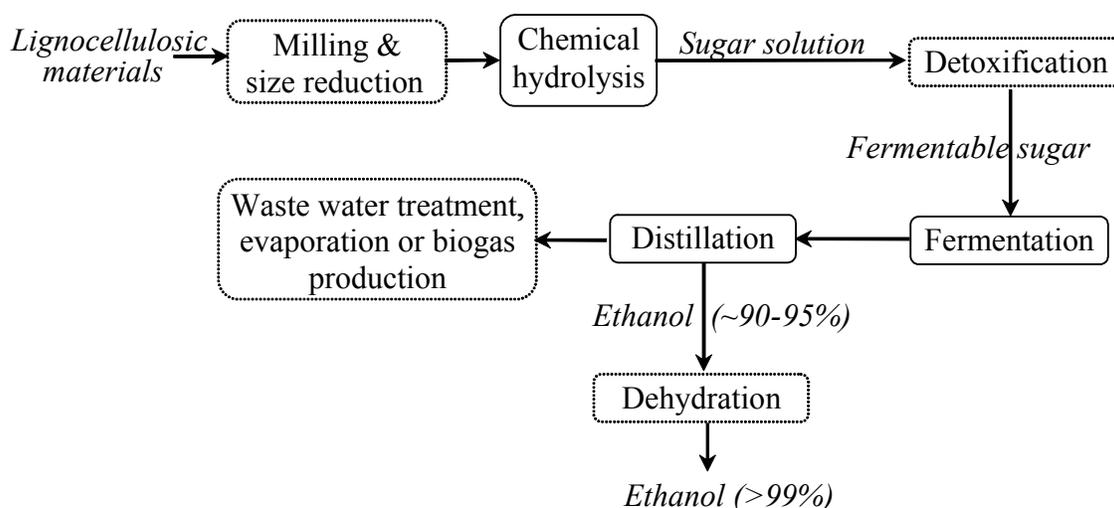


Fig. 2. Overall process scheme for ethanol production from lignocellulosic materials

## CHEMICAL HYDROLYSIS

Hydrolysis involves cleaving the polymers of cellulose and hemicellulose into their monomers. Complete hydrolysis of cellulose results in glucose, whereas the hemicellulose gives rise to several pentoses and hexoses. While softwood hemicellulose is mainly composed of mannose, the dominant sugar in hemicellulose derived from hardwood and crop residues is usually xylose (Karimi et al. 2006b; Taherzadeh et al. 1997a). The hydrolysis can be carried out chemically or enzymatically.

Chemical hydrolysis involves exposure of lignocellulosic materials to a chemical for a period of time at a specific temperature, and results in sugar monomers from cellulose and hemicellulose polymers. Acids are predominantly applied in chemical hydrolyses. Sulfuric acid is the most investigated acid (Harris et al. 1945), although other acids such as HCl (Hashem and Rashad 1993) have also been used. Acid hydrolyses can be divided into two groups: (a) concentrated-acid hydrolysis and (b) dilute-acid

hydrolysis. A comparison between concentrated- and dilute-acid hydrolysis methods is presented in Table 1.

**Table 1.** Comparison between Concentrated- and Dilute-Acid Hydrolysis Methods

Hydrolysis method	Advantages	Disadvantages
Concentrated-acid process	<ul style="list-style-type: none"> <li>- Operated at low temperature</li> <li>- High sugar yield</li> </ul>	<ul style="list-style-type: none"> <li>- High acid consumption</li> <li>- Equipment corrosion</li> <li>- High energy consumption for acid recovery</li> <li>- longer reaction time (e.g. 2-6 h)</li> </ul>
Dilute-acid process	<ul style="list-style-type: none"> <li>- Low acid consumption</li> <li>- Short residence time</li> </ul>	<ul style="list-style-type: none"> <li>- Operated at high temperature</li> <li>- Low sugar yield</li> <li>- Equipment corrosion</li> <li>- Formation of undesirable by-products</li> </ul>

### Concentrated-Acid Hydrolysis

Hydrolysis of lignocelluloses by concentrated sulfuric or hydrochloric acids is a relatively old process. Braconnot in 1819 first discovered that cellulose can be converted to fermentable sugar by concentrated acids (Sherrard and Kressman 1945). Concentrated single-stage hydrolysis with sulfuric acid, and concentrated hydrolysis by liquid or vapor phase with hydrochloric acid were used.

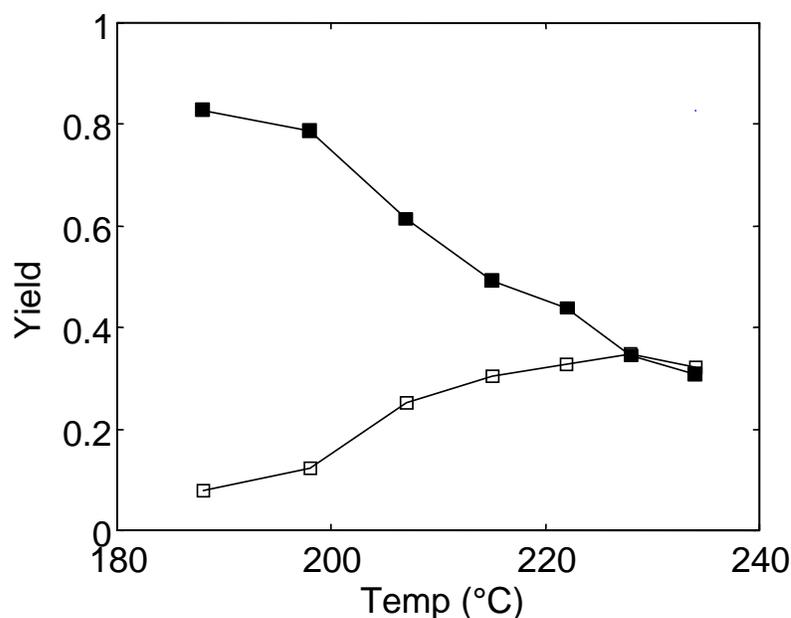
Concentrated-acid processes are generally reported to give higher sugar yield (e.g. 90% of theoretical glucose yield) and consequently higher ethanol yield, compared to dilute-acid processes. Furthermore, the concentrated-acid processes can operate at low temperature (e.g. 40°C), which is a clear advantage compared to dilute-acid processes. However, the concentration of acid is very high in this method (e.g. 30-70%), and dilution and heating of the concentrated acid during the hydrolysis process make it extremely corrosive. Therefore, the process requires either expensive alloys or specialized non-metallic constructions, such as ceramic or carbon-brick lining. The acid recovery is an energy-demanding process. In addition, when sulfuric acid is used, the neutralization process produces large amounts of gypsum. Furthermore, the environmental impact strongly limits the application of hydrochloric acid. The high investment and maintenance costs have greatly reduced the potential commercial interest of this process (Jones and Semrau 1984; Katzen et al. 1995; Wyman 1996).

Despite the disadvantages, the concentrated-acid process is still of interest. It has recently been exploited by a Dutch research group in a process called “Biosulfurol.” In this process, the biomass is impregnated with 70% sulfuric acid and subsequently hydrolyzed by adding water. The acid is then recovered partly by anion membranes, and partly in the form of H<sub>2</sub>S from anaerobic wastewater treatment. The process was claimed to have a low overall cost for the ethanol produced (van Groenestijn et al. 2006). Furthermore, two full-scale cellulose-to-ethanol projects are under development in North America by Arkenol Inc. ([www.arkenol.com](http://www.arkenol.com)) and Masada Resource Group ([www.masada.com](http://www.masada.com)) based on concentrated-acid hydrolysis.

### Dilute-Acid Hydrolysis

Among the chemical hydrolysis methods, dilute-acid hydrolysis is probably the most commonly applied. It is a method that can be used either as a pretreatment preceding enzymatic hydrolysis, or as the actual method of hydrolyzing lignocellulose to the sugars (Qureshi and Manderson 1995). The first established dilute-acid hydrolysis process was probably the Scholler process (Faith 1945). This was a batch process, in which the wood material was kept in 0.5% sulfuric acid at 11-12 bar for approximately 45 minutes. Nowadays, most of dilute-acid hydrolysis processes are performed in a batch mode with a retention time of a few minutes (e.g. cf. Karimi et al. 2006b).

Batch reactors have been the most widely used reactors for kinetic study of hydrolysis and for laboratory and pilot study of ethanol production from lignocellulosic materials (e.g. Brandberg et al. 2005; Harris et al. 1984; Millati 2005; Purwadi et al. 2004; Saeman 1945; Sanchez et al. 2004; Sues et al. 2005; Taherzadeh et al. 1997a; Taherzadeh et al. 2000c). An example of a kinetic study for one-stage dilute-acid hydrolysis is presented in Fig. 3, where 0.5% sulfuric acid was used at temperatures of 188-234°C and a retention time of 7 minutes (Taherzadeh et al. 1997a). A major part of the hemicellulose (more than 80%) could be hydrolyzed by dilute-acid hydrolysis at temperatures less than 200°C, but the maximum overall glucose yield occurred at a hydrolysis temperature higher than 220°C. This is due to the larger recalcitrance of cellulose to hydrolysis.



**Fig. 3.** Glucose yield from glucan (□) and mannose yield from mannan (■) in a one-step dilute-acid hydrolysis of (25% dry weight) spruce as a function of hydrolysis temperature (adapted from Taherzadeh 1999).

A main drawback of dilute-acid hydrolysis processes, particularly in one stage, is degradation of the sugars in hydrolysis reactions and formation of undesirable by-products. This not only lowers the yield of sugars, but also several of the by-products severely inhibit the formation of ethanol during the fermentation process. The possible

inhibitors that can be formed during the dilute-acid hydrolysis, and their original components, are illustrated in Fig. 1. Potential inhibitors are furfural, 5-hydroxymethylfurfural (HMF), levulinic acid, acetic acid, formic acid, uronic acid, 4-hydroxybenzoic acid, vanillic acid, vanillin, phenol, cinnamaldehyde, formaldehyde, etc. (Larsson et al. 2000; Taherzadeh 1999). Some inhibitors, such as terpene compounds, are initially present in the wood, but apparently most of the inhibitors are formed during the hydrolysis process.

In order to avoid degradation of monosaccharides at high temperatures and formation of the inhibitors, dilute-acid hydrolysis is carried out in two (or more) stages. In the first stage, which should be carried out under relatively mild conditions, hemicellulose is converted to sugar monomers. It is considered as equivalent to a dilute-acid pretreatment step. In the second stage, the residual solid is hydrolyzed under more severe conditions, allowing cellulose to be hydrolyzed (Harris et al. 1984). In a one-stage pretreatment, a temperature between 140 and 170°C can be used, but two treatments at about 120°C for a longer time may also be applied (Torget and Hsu 1994).

The “two-stage” dilute-acid process is usually preferred to one-stage dilute-acid hydrolysis because:

- a) The separate stages for hydrolysis of the hemicellulose and cellulose should result in higher sugar yield. Furthermore, a product with high hexose sugar content can be obtained in the second-stage hydrolysis, which can easily be fermented to ethanol. Mixtures of pentose and hexose are usually problematic for fermentation, because of the difficulty in fermentation of pentoses.
- b) The energy consumption should be minimized, since liquid is removed before the second-stage hydrolysis.
- c) The resulting sugar solution should be more concentrated.
- d) Less sugar degradation from the hydrolyzed materials in the first stage leads to a higher overall yield of sugars.
- e) Fewer fermentation-inhibiting components are formed during the two-stage hydrolysis.

Generally, the maximum yield of pentoses and hexoses recovered from hemicelluloses in the first stage of the hydrolysis is high (i.e. 80-95% of the total sugars available), while the yield of cellulose hydrolysis to glucose is usually low (e.g. 40-60%). However, a low yield of glucose may not be considered as a serious problem because of the low price of lignocellulosic materials, as well as the possibility of drying and burning the residual cellulose and lignin in an energy complex to produce electricity and heat.

### **Dilute-Acid Hydrolysis Reactors and Processes**

Batch reactors are traditionally used for hydrolysis of lignocellulosic materials in lab and pilot plants. However, research also has been carried out concerning development of other reactor types for this purpose. In this section, we review plug flow, percolation, countercurrent, and shrinking-bed reactors for hydrolysis of lignocellulosic materials by the dilute-acid processes.

### *Plug flow reactor*

The CSTR (continuous stirred-tank reactor) and plug flow reactor are generally used in continuous processes. The CSTR has not been of significant interest for the hydrolysis of lignocellulosic materials, possibly due to problems associated with mixing of very high solid content material, the sealing of the stirrer in a high-pressure reactor, and the high energy demand for mixing. However, there have been some studies concerning continuous hydrolysis in plug flow reactors (Church and Wooldridge 1981).

Thompson and Grethlein (1979) developed an isothermal plug flow reactor to study the kinetics of acid hydrolysis of cellulosic substrates. The kinetic parameters in a model that gives glucose formation from purified cellulose (Solka-Floc) were obtained at temperatures from 180 to 240°C, sulfuric acid concentrations from 0.5 to 2.0%, and slurry concentrations from 5.0 to 13.5%. It was determined that the glucose formation from newsprint can be predicted from the kinetic model developed for Solka-Floc. It was found that at least 50% of the potential glucose can be obtained at 240°C, 1% acid, and 0.22 minutes of residence time. This was a significant improvement in yield of conversion of cellulose to glucose, compared to the batch reactor, where the maximum yield was only 40%. Moreover, the retention time is significantly shorter in plug flow reactors. Further improvement of plug flow reactors has been reported by McParland et al. (1982), who obtained 55-58% glucose yield at 240°C and only 6 seconds residence time.

The plug flow reactor, having less than 30 seconds residence time and higher yield of glucose (e.g. 50-60%) compared to batch reactors, might be a process of commercial interest. However, no more improvement may be forthcoming for this system, since it has two inherent problems: difficulty in controlling the retention time in the range of a few seconds, and heat transfer limitation within the biomass particles (Lee et al. 1999).

### *Percolation reactor*

The percolation reactor is a packed-bed flow-through reactor. There are certain advantages in this reactor in comparison to batch and plug flow reactors for hydrolysis of lignocellulosic materials. Lower sugar decomposition can result, since the sugar product is removed immediately after formation in this reactor. A high concentration of sugar can be produced by using a packed-bed reactor, since a high solid/liquid ratio can be used. Furthermore, unlike the batch and plug flow reactors, this process does not need a solid/liquid separation (Lee et al. 1999).

In a study by the National Renewable Energy Laboratory (NREL) in the USA, a continuously flowing, two-stage percolation reactor resulted in more than 95% of the theoretical yield of solubilized xylose sugars (monomeric and oligomeric) from yellow poplar sawdust (Torget and Hsu 1994). They studied the two-temperature (140/170°C) dilute-acid prehydrolysis of hybrid poplar using a percolation process, and found soluble xylose to be 92% of the theoretical value and 2% of the xylan to be degraded to furfural. Acid-impregnated steam explosion (0.4% sulfuric acid, 200-230°C, 1-5 min) was reported to solubilize 90-95% of the hemicellulose from softwood, and 90% of the remaining cellulose can be hydrolyzed by cellulase enzymes (Nguyen et al. 1998).

Percolation reactors were used in most of the old wood sugar processes such as the Scholler process, the Madison wood sugar process, and some of the Soviet processes

(Lee et al. 1999). Two methods of modification have been applied in percolation reactors: two-stage step-change and two-stage reverse-flow. These processes involved a first treatment stage at a low temperature followed by a second stage at a high temperature. In two-stage step-change percolation reactors, the fresh biomass is first treated at a low temperature in the first reactor, followed by treatment in the second reactor at a higher temperature. In two-stage reverse-flow percolation reactors, the treated biomass at low temperature is treated at a high temperature, then the liquid is passed through the second reactor filled with fresh biomass at the low temperature (Kim et al. 1993; Lee et al. 1999; Torget and Hsu 1994).

#### *Countercurrent reactor*

A countercurrent reactor is a moving-bed reactor in which the directions of solid and liquid are reversed. The major part of the sugar is produced near the liquid outlet point of the reactor. Countercurrent flow of liquids and solids minimizes sugar degradation and product dilution by removing the sugars from the reaction zone before substantial degradation can occur, and consequently raising the yield and concentration of sugar with minimal inhibitory formation.

The progressing-batch reactor is one of the attempts that is based on a countercurrent scheme. A progressing-batch reactor uses several percolation reactors in series to simulate countercurrent flows, while retaining the simplicity of the percolation reactor. Wright et al. (1987) made the first attempt at design of such systems. They used seven percolating reactors: one reactor for filling, another for emptying, and five in operation.

#### *Shrinking-bed reactor*

Bed shrinking is a phenomenon that can improve the reactor performance. Bed shrinking occurs in a packed-bed reactor due to partial solubilization of the solid biomass during the reaction. This reactor has a fixed and a movable end; the movable end is supported by a compressed spring. Incorporation of a spring-loaded plunger into the packed-bed reactor is intended to continuously reduce the bed depth as the reaction progresses. It reduces the liquid throughput, which results in raising the sugar concentration. This method has resulted in sugar yields higher than 95% from hemicellulose and 85% from cellulose in a multiple percolation reactor system simulating countercurrent operation (Lee et al. 2000).

Kim et al. (2001) studied the kinetics of cellulose hydrolysis using batch and shrinking-bed reactors. The maximum yield of glucose obtained from batch reactor experiments was about 60% for pure cellulose, whereas the maximum glucose yields from yellow poplar feedstock were subsequently lower, falling in the range of 26-50%. In experiments using the shrinking-bed hydrolysis reactor, glucose yields of 87.5, 90.3, and 90.8% of the theoretical yield were obtained for yellow poplar feedstocks at 205, 220, and 235°C, respectively. Furthermore, the hydrolysis times for glucan were about three times lower with the shrinking-bed reactor than with the batch reactor. What causes this difference is not yet fully understood, and further research is necessary to investigate the details of the reaction mechanism of these heterogeneous catalytic reactions.

## EFFECTIVE PARAMETERS IN HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS

Lignocellulosic materials and hydrolysis processes are very complicated. Factors influencing the yields of the lignocellulose to the monomeric sugars and the by-products are:

### (a) Properties of the Substrate

The properties of the substrate can affect the hydrolysis. These properties are: neutralizing capacity, proportion of easily hydrolyzable hemicellulose and cellulose, amount and rate of hydrolysis of the difficult-to-hydrolyze materials, the length of the macromolecules, degree of polymerization of cellulose, configuration of the cellulose chain, and association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicellulose, proteins, mineral elements, etc. Particle size is also one of the effective parameters (Kosaric et al. 1983; Taherzadeh et al. 1997a).

### (b) The Acidity of the System

Another parameter affecting the hydrolysis is the acidity of system. The acidity is dependent on the type and concentration of acid used, amount of acid solution, amount of acid (e.g. acetic acid) released from the biomass during hydrolysis, liquid to solid ratio, the neutralizing capacity of the lignocellulose, and movement of the solution during heating. When dilute-acid hydrolysis is applied in a continuous process such as a screw-fed co-current reactor, it requires a relatively short residence time. Therefore, the penetration of acid catalyst into the biomass, as well as dispersion in the reactor, can significantly affect the overall reaction, and consequently the reactor performance. Diffusivity of sulfuric acid is dependent on the nature of the lignocellulosic materials. It has been shown that the diffusivity of sulfuric acid is significantly higher in agricultural residues than in hardwood (Kim and Lee 2002).

### (c) Rate of Decomposition of Hydrolysis Products during Hydrolysis

The rate of decomposition of the products during the hydrolysis process depends on temperature, acidity, reaction time, and the concentration of sugars. Under hydrolysis conditions that produce a solution containing in excess of 10 percent glucose, reversion phenomena are suggested to be very important. The reversion phenomena result in much of the glucose being present not as free glucose but as dimers, oligomers, and anhydrosugars which are unavailable to the microorganisms used in fermentation (Harris et al. 1984). It was recently reported that metals and/or metal ions can also catalyze glucose decomposition during the acid hydrolysis of lignocellulosic materials. Thus, the material used in the construction of the hydrolysis reactor should also be carefully selected (Xiang et al. 2004).

## FERMENTATION OF THE HYDROLYZATES

Fermentation of the lignocellulosic hydrolyzates is more difficult than the well-established processes of ethanol production from *e.g.* sugar-cane juice or grains. Hydrolyzates contain a broader range of inhibitory compounds, whose composition and concentration depend on the type of lignocellulosic materials and on the chemistry and nature of the pretreatment and hydrolysis processes. Secondly, the hydrolyzates of hemicelluloses contain not only hexoses but also pentoses, where xylose is the dominant sugar in the hydrolyzates from hardwood hemicelluloses (Nigam 2001; Pessoa et al. 1997; Vanzyl et al. 1988; Wyman 1996). Therefore, the fermenting microorganism should be able to produce ethanol from the hydrolyzates with a high yield and productivity, withstand potential inhibitors, and produce ethanol from pentoses, as well as being safe for humans. Baker's yeast (*Saccharomyces cerevisiae*) is the most commercially used microorganism for ethanol production, but it cannot ferment xylose (Björling and Lindman 1989; Hahn-Hägerdal et al. 1988; Ho et al. 1998; Jeffries 2006; Jeppsson et al. 2002; Sreenath and Jeffries 2000). A large number of yeasts, bacteria, and filamentous fungi are reported to produce ethanol as the main fermentation product, and have been reviewed in literature (Ingram et al. 1998; Jeffries 2006; Lin and Tanaka 2006; Olsson and Hahn-Hägerdal 1996).

The effects of the inhibitory compounds, different fermentation techniques, and the xylose-fermenting microorganisms are discussed briefly here.

### (a) The Effects of Inhibitory Compounds on Fermentation

The by-products mentioned earlier inhibit fermentation by different mechanisms. As a function of conditions and method of hydrolysis, different inhibitors may dominate in terms of concentrations. However, a combination of the action of several substances is the reason for observed inhibition (Clark and Mackie 1984). Furthermore, it is not only the quantitatively dominant inhibitors that determine the fermentability of a hydrolyzate. The toxicity of a hydrolyzate is found to differ from that of a synthetic medium with the same amount of the major hydrolyzate inhibitors added, indicating the importance of other inhibitors present in trace amounts (Taherzadeh et al. 1999).

Acetic acid, formic acid, and levulinic acid are the most common carboxylic acids found in the hydrolyzates. Acetic acid is not only a by-product of hydrolysis (Schneider 1996), but is also a well-known by-product of fermentation (Oura 1977). Acetic acid is mainly formed from acetylated sugars in the hemicellulose, which are cleaved off already under mild hydrolysis conditions. Therefore, the acetic acid yield in the hydrolysis does not significantly depend on the severity of the hydrolysis process, and acetic acid can be formed at concentrations even higher than 10 g/l (Taherzadeh et al. 1997b). It is generally accepted that the effect of the undissociated part of the acid is larger than the effect of the dissociated part (Thomas et al. 2002). The undissociated carboxylic acids can diffuse through the cell membrane, dissociate there, and decrease the internal pH (Gottschalk 1987; Verduyn et al. 1992). It is reported that the baker's yeasts can tolerate up to about 5 g/l undissociated concentration of acetic acid (Taherzadeh et al. 1997b).

The rate of sugar decomposition under dilute-acid hydrolysis conditions (*e.g.* 0.8% acid and 180°C) is ordered as follows (Xiang et al. 2004):

## Xylose&gt; Arabinose&gt; Mannose&gt; Galactose&gt; Glucose

Therefore, the xylose is more sensitive to the acidity and high temperature conditions, and is decomposed to furfural. Glucose is more resistant to harsh conditions.

Furfural and HMF are the only furans usually found in hydrolyzates in significant amounts (Taherzadeh et al. 1997a). Furfural has been found to inhibit the *in vitro* activity of several important enzymes in the primary carbon catabolism, such as hexokinase, aldolase, phosphofructokinase, triosephosphate dehydrogenase, and alcohol dehydrogenase. Among these enzymes, the latter two appear to be the most sensitive (Banerjee et al. 1981). However, the inhibition of certain non-glycolytic enzymes, such as pyruvate dehydrogenase and aldehyde dehydrogenase, is even more severe (Modig et al. 2002). Consequently, cell growth is more sensitive to the presence of furfural than is the ethanol production from glucose. Furfural can be converted by the yeast to less inhibitory compounds, furfuryl alcohol and furoic acid (Taherzadeh et al. 2000c).

HMF is not as severely toxic to *S. cerevisiae* as furfural (Taherzadeh et al. 2000b). This is in line with the observation that the *in vitro* inhibition of the enzymes pyruvate dehydrogenase and aldehyde dehydrogenase is lower by HMF than by furfural. On the other hand, the conversion rate of furfural is about 4 times faster than that of HMF. Hence, HMF remains much longer than furfural in the medium, and consequently, the effects of HMF last longer than those of furfural. It should be mentioned that early in the hydrolysis, HMF is the major degradation product of hexoses, but subsequently levulinic and formic acids predominate (Harris et al. 1984).

A large number of phenolic/aromatic compounds have been detected in dilute-acid hydrolyzates (Larsson 2000; Palmqvist and Hahn-Hägerdal 2000). These are believed to be degradation products of lignin during the hydrolysis. However, the aromatic compounds may also form as a result of sugar degradation and are present in lignocelluloses as extractives. Larsson (2000) studied the influence of lignocellulose-derived aromatic compounds on oxygen-limited growth and ethanol production by *S. cerevisiae*. The influence of hydroxymethoxybenzaldehydes, diphenols/quinones, and phenylpropane derivatives on *S. cerevisiae* cell growth and ethanol formation was assayed using a defined medium and oxygen-limited conditions. The inhibitory effect of the hydroxymethoxybenzaldehydes was highly dependent on the positions of the substituents. A major difference in inhibition by the oxidized and reduced form of a diphenol/quinone was reported, with the oxidized form being more inhibitory. Transformations of aromatic compounds occur during the fermentation, including aldehyde reduction, quinone reduction, and double bond saturation. Aromatic alcohols were detected as products of reductions of the corresponding aldehydes, namely hydroxymethoxybenzaldehydes and coniferyl aldehyde. High molecular mass compounds and the corresponding diphenol have been detected as products of quinone reduction. Together with coniferyl alcohol, dihydroconiferyl alcohol was identified as a major transformation product of coniferyl aldehyde (Larsson 2000). Among the phenolic compounds, vanillin and syringaldehyde are the important inhibitors. However, they can be assimilated by *S. cerevisiae* in the fermentation process (Delgenes et al. 1996), and

conversion of vanillin to vanillyl alcohol by *Klebsiella pneumoniae* has also been reported (Nishikawa et al. 1988).

It should be noticed that reported concentrations of the phenolic/aromatic compounds are normally a few milligrams per liter (Larsson et al. 1999). This could be due to the low water solubility of many of the phenolic compounds, or to a limited degradation of lignin during the hydrolysis process. Among the phenolic compounds, less heavily substituted phenolics are probably the most inhibitory materials in the hydrolyzates (Clark and Mackie 1984; Nishikawa et al. 1988).

### **(b) Fermentation Techniques**

Several of the inhibitory compounds found in hydrolyzates can be biotransformed, or, in a few cases, even be fully metabolized by yeast. Conversion occurs for several of the carboxylic acids, furans, and phenolic compounds. This suggests that continuous *in situ* detoxification of the hydrolyzate during the fermentation might be possible. However, it requires a suitable mode of operation, and the bioconversion of inhibitors must be taken into account in the design of the process.

In batch processes of ethanol production, the microorganism works in a high substrate concentration initially and a high product concentration finally. Generally, batch fermentations are characterized by low productivity, and they are labor-intensive. Batch cultivation is not a suitable method for cultivation of lignocellulosic hydrolyzates, since a high concentration of the inhibitors at the beginning of fermentation deactivates the yeasts and stops the process. The “fed-batch” technique is a promising method for the fermentation of dilute-acid hydrolyzates, and its application for this purpose has recently been studied (Nilsson et al. 2001; 2002; Taherzadeh et al. 2000a; Taherzadeh et al. 1999). The basic concept behind the success of this technique is the capability of *in situ* detoxification by the cells. Since the yeast has a limited capacity for the conversion of the inhibitors, the achievement of a successful fermentation strongly depends on the feed rate of the hydrolyzate. At too high a feed rate, using an inhibiting hydrolyzate, both ethanol production and cell growth can be expected to stop, whereas at a very low feed rate the hydrolyzate may still be converted but at a very low productivity, which was experimentally confirmed (Taherzadeh et al. 1999). Consequently, there should exist an optimum feed rate (or dilution rate) specific to a particular hydrolyzate. If the hydrolyzate is only slightly inhibitory, then a high feed rate can be applied. On the other hand, for a severely inhibiting hydrolyzate, a low feed rate is necessary to prevent build-up of the concentration of inhibitors in the bioreactor to levels that completely stop the cellular metabolism. Therefore, an optimum feed rate should be provided to the bioreactor by e.g. an adaptive control of the process (Nilsson et al. 2001; 2002; Taherzadeh 1999; Taherzadeh et al. 2000c).

“Continuous cultivation” is the third mode of operation for fermentation of hydrolyzates. The major drawback of continuous fermentation is that inhibitors present in the medium will limit the specific growth rate of the cells. This will result in wash-out of the bioreactor, unless a very low dilution rate is applied, giving a very low productivity. Furthermore, at a very low dilution rate the conversion rate of the inhibitors can be expected to decrease, due to the decreased specific growth rate of the biomass. Thus, wash-out may occur even at very low dilution rate. Cell retention by “immobilization,”

“encapsulation,” “filtration,” and “cell recirculation” by using e.g. centrifuges or flocculating organisms are solutions to overcome the wash-out problem in continuous cultivation of dilute-acid hydrolyzates. Millati (2005) immobilized cells of *S. cerevisiae* entrapped in Ca-alginate to continuously ferment hydrolyzates. The method was then further developed by Purwadi (2006), and completed with cultivation with flocculating yeast. Cell recirculation was examined by Brandberg et al. (2005). Continuous fermentation of wheat-supplemented lignocellulose hydrolyzate with different types of cell retention was compared by Brandberg et al. (2007). Encapsulation of *S. cerevisiae* was reported to be a powerful means for continuous cultivation of toxic dilute-acid hydrolyzates (Talebnia and Taherzadeh, 2006). All of these methods indicate new possibilities of using fermentation with high-cell-density cultivation.

### (c) Pentose Fermentation

An important factor in production of ethanol from lignocellulosic materials is efficient fermentation of the sugars available in hydrolyzates to ethanol with a high yield and high productivity rate. Although a large number of yeasts, bacteria, and filamentous fungi can produce ethanol as the main fermentation product, none of these microorganisms naturally meets all the requirements for the lignocellulosic ethanol production. One of the major challenges in this field is fermentation of xylose. *S. cerevisiae*, which is the most widely microorganism in ethanol production, does not have genes encoded for xylose reductase (XR) and xylitol dehydrogenase (XDH) and cannot utilize xylose (Jeffries and Jin 2004; Karhumaa et al. 2006; Katahira et al. 2004; Kötter and Ciriacy 1993; Jeppsson et al. 2002; van Maris et al. 2006). There have therefore been intensive efforts to introduce other wild type organisms that can utilize xylose and produce ethanol or genetically modify the organism for this purpose. The result of these intensive efforts is to obtain microorganisms that are able to produce ethanol anaerobically from xylose with high yield (e.g. more than 0.40 g/g sugars) and high productivity of more than 1.0 g/g.h (van Maris et al. 2006). The challenge is therefore now to introduce these strains in large-scale ethanol production from lignocellulosic feedstocks that are rich in xylose.

#### *Organisms for producing ethanol from xylose*

There are several naturally occurring ethanol-producing bacteria, yeast and fungi that utilize xylose. The yeast species *Candida*, *Pichia*, *Schizosaccharomyces*, *Kluveromyces* and *Pachysolen*, fungi of species *Fusarium*, *Mucor*, *Rhizopus*, *Monilia*, and *Paecilomyces*, and bacteria of species *Clostridium*, *Bacillus*, *Bacteroides*, *Thermoanaerobacter*, and *Ervinia* are mentioned as ethanol producers from xylose (Abbi et al. 1996; Flores et al. 2000; Olsson and Hahn-Hägerdahl 1996; Jeffries and Kurtzman 1994; Millati et al. 2005; Sreenath and Jeffries 2000; Zaldivar et al. 2001). In research on ethanol production from pentoses and sugar-cane bagasse hemicellulose hydrolyzate, Ueng and Gong (1982) used *Mucor* and *Fusarium* species. Both of these are able to ferment various sugars and alditols, including glucose, pentoses and xylitol, to ethanol. *Mucor* can ferment sugar-cane bagasse hemicellulose hydrolyzate to ethanol, while *Fusarium* cannot ferment this hydrolyzate to ethanol. Zygomycetes fungi such as *Mucor indicus* and *Rhizopus oryzae* have recently been explored and showed good potential to

be used for ethanol production from xylose and other sugars in lignocellulosic hydrolyzates (Karimi et al. 2005; Karimi et al. 2006a; Millati 2005).

#### *Genetic modification of the strains*

Several attempts have been made to genetically modify *S. cerevisiae* and other microorganisms in order to produce ethanol from both hexoses and pentoses. There are some excellent reviews in this field (e.g. c.f. Jeffries 2006; van Maris et al. 2006). Several research groups tried to express either genes encoding for XR and XDH for assimilation of xylose through xylitol or xylose isomerase for direct conversion of xylose to xylulose in *S. cerevisiae*. Low ethanol yield and productivity from xylose, imbalance of NADH, and sensitivity to oxygen presence were among the big challenges in this field (van Maris et al. 2006). However, there are now engineered strains that are able to produce ethanol from xylose anaerobically with good yield of e.g. 0.41 g/g and productivity of e.g. 1.2 g/g.h (Kuyper et al. 2005).

Other strains than *S. cerevisiae* also have been engineered for ethanol production from xylose. The method principally involves addition and expression of all the genes that are not present in the genome of the microorganisms and are necessary to run the pathway from the sugars to ethanol. For example, the genes for xylose isomerase, xylulokinase, transketolase, and transaldolase were inserted into bacteria *Zymomonas mobilis*, which resulted in an organism producing ethanol from xylose (Zhang et al. 1995). Ingram and his group added the genes for pyruvate decarboxylase and alcohol dehydrogenase to *Escherichia coli* (Ohta et al. 1991). The resulting recombinant was able to produce 41 g/l ethanol from 80 g/l xylose with a productivity of 0.87 g/l.h. Furthermore, Ingram et al. (1999) made a similar recombination to *Klebsiella oxytoca*, which is natively able to ferment cellobiose and celotriose, in order to eliminate the need for  $\beta$ -glucosidase. The strain was further recombinated to contain endoglucanase. Recently, Japanese Research Institute of Innovative Technology for the Earth (RITE) developed a microorganism for ethanol production. RITE strain is claimed to be an engineered strain of *Corynebacterium glutamicum* that converts both pentose and hexose sugars into alcohol. They engineered the central metabolic pathway of *C. glutamicum* to produce ethanol. A recombinant strain which expressed the *Z. mobilis* gene encoding for pyruvate decarboxylase and alcohol dehydrogenase was constructed (Inui et al. 2004). Among the different fermentation parameters, xylose-utilizing cultures are known to be very sensitive to dissolved oxygen concentration. A low and well-controlled level of oxygenation is required for efficient ethanol production from xylose by most of the xylose-fermenting microorganisms. A certain level of oxygen is necessary to maintain cell viability, xylose transport, and high ethanol productivity. While many of the pentose-fermenting microorganisms rapidly lose viability without sufficient oxygen, excess oxygen completely stops ethanol production, and the cells respire the substrate to form biomass (Franzen 1997; Lee 1997; Toivari et al. 2001; Wyman 1996).

Production of ethanol from other carbon sources in lignocellulosic hydrolyzates (e.g. L-arabinose, galacturonic acid and L-rhamnose) may require extensive metabolic engineering. While xylose is the most abundant pentose sugar in the hemicellulosic fraction of biomass, L-arabinose is also present in appreciable amounts. L-Arabinose fermentation, based on the expression of a prokaryotic pathway in *S. cerevisiae*, has also

been established, but needs further optimization before it can be considered for industrial implementation. An emerging and major challenge is to achieve the rapid transition from proof-of-principle experiments under ‘academic’ conditions (synthetic media, single substrates or simple substrate mixtures, absence of inhibitory components) towards efficient conversion of complex industrial substrate mixtures that contain synergistically acting inhibitors (van Maris et al. 2006).

#### (d) Adaptation of Microorganisms

Increases in fermentation rate and ethanol yield by adaptation of microorganisms to the fermentation media have been reported in some studies (Amartey and Jeffries 1996; Oliinichuk et al. 1986). Adaptation of the microorganism to the lignocellulosic hydrolyzate was suggested as an alternative approach to detoxification.

Martin et al. (2007) reported that adaptation of a xylose-utilizing genetically engineered strain of *S. cerevisiae* to sugar-cane bagasse hydrolyzates can increase its tolerance against phenolic compounds, furaldehydes, and aliphatic acids, and lead to improved performance with respect to ethanol production. Amartey and Jeffries (1996) showed that adaptation of *P. stipitis* to corn cob acid-hydrolyzed hemicellulose resulted in a significantly higher fermentation rate.

Adaptation has been shown to increase the ability of a broad range of yeast strains to grow in lignocellulosic hydrolyzates. Adaptation of *P. stipitis* CBS 5776 by repeated recycling in an acid hydrolyzate from aspen resulted in increased productivity from 0.60 to 0.85 g/g.h and increased yield from 0.32 to 0.45 g/g. In another study, *P. stipitis* CBS 5776 was adapted to a detoxified hydrolyzate (red oak acid hydrolyzate) and was then able to ferment an undetoxified hydrolyzate with a larger inoculum, giving an ethanol yield of 0.30 g/g. The unadapted *P. stipitis* could not ferment the undetoxified hydrolyzate at all (Olsson and Hahn-Hägerdal 1996). At Nanjing Forestry University, extensive work on the adaptation of *C. shehatae* to Spent Sulfite Liquor (SSL) was carried out, resulting in strains that can tolerate high temperature (38°C), high acetic acid concentration (15 g/l), and a high pentose fraction (70%) (Olsson and Hahn-Hägerdal 1996). However, the ability to adapt *S. cerevisiae* to lignocellulosic hydrolyzates was shown to be strain-dependent. For instance, Keating et al. (2006) did not observe any improvement in adaptation of *S. cerevisiae* to SSL.

The adapted strains are seldom deposited in culture collections; they have only been used in individual laboratories, and verification of the performance of these strains is difficult. In addition, the stability of the adapted strains may present a problem. Isolation of strains from natural or industrial habitats has been reported to be a useful technique for finding strains with suitable properties for cultivating lignocellulosic hydrolyzates. A strain of *S. cerevisiae* isolated from a SSL fermentation plant was shown to be able to utilize glucose and galactose simultaneously in the presence of acetic acid, in contrast to the behavior of baker’s yeast. As an alternative to adaptation and isolation from harsh environments, genetic engineering might improve the microorganism to better withstand a specific inhibitor; however, this can only be attempted if the inhibiting mechanism is known (Olsson and Hahn-Hägerdal 1996).

## DOWNSTREAM PROCESSING

### Product Recovery

The fermentation broth (mash or beer) is a mixture of ethanol, water, cell mass, fusel oil, and other components available in the fermentation media such as residual sugars, nonfermentable sugars, and hydrolysis by-products. The concentration of ethanol produced from dilute-acid hydrolyzate is typically low (e.g. 10-35 g/l), since the total concentration of sugar is usually low (e.g. 20-80 g/l). Furthermore, it is difficult to work with highly concentrated hydrolyzates, since the concentration of inhibitors will increase by concentrating the hydrolyzates. On the other hand, if one could get rid of this problem, then the ethanol concentration in mash would be generally up to 10%. The cells can tolerate this level of ethanol concentration at 30°C, but their tolerance decreases with increasing temperature (Hamelinck et al. 2005).

Ethanol can be purified by distillation to a concentration just below its azeotropic point, i.e. 95%, which will be called “hydrated ethanol” (Hamelinck et al. 2005). However, the solid particles and fermenting microorganisms should be separated before distillation, by e.g. centrifuges or decanters. On the other hand, if flocculant yeast is used, then the natural capacity of these microorganisms for aggregation and flocculation can eliminate the need for separation of biomass and reduce the investment costs. Hydrated ethanol can be employed in high-ethanol-content fuel (e.g. E95). However, for mixing of the ethanol with gasoline, the ethanol should contain no more than 1% of water (anhydrous ethanol). Molecular-sieve technology is the common method for production of anhydrous ethanol.

### Residual Solids and Wastewater Treatment

The main solid residual from the process is lignin. Its amount and quality depend on the feedstock used and the process applied. The lignin and remaining solid materials can be burned to produce steam for the process (hydrolysis, distillation, and evaporation), electricity, and perhaps central heat (Hamelinck et al. 2005). This is possible due to the high energy value of the lignin that is released during its combustion (Cardona and Sanchez 2007). However, it can also be processed through e.g. gasification and Fischer-Tropsch process to produce synthesis gas and hydrocarbon fuel additives. Lignin can replace phenol in the widely used phenol formaldehyde resins, although the production costs and market value of these products are problematic.

The stillage, or wastewater remaining after distillation, exhibits a considerable pollution potential (Callander et al. 1986). Most of the components in the stillage of ethanol production from lignocellulosic materials originate from the soils on which these materials are grown, and therefore should be returned to soil. However, application of untreated stillage to standing pasture can result in phytotoxicity (Wilkie et al. 2000). The residual water contains significant amounts of organic compounds such as acetic acid, furfural, HMF, residual sugars, and other components, and needs processing before its disposal into the environment. Wilkie et al. (2000) reviewed the characterization of ethanol stillage from several lignocellulosic materials and compared them with stillage from conventional feedstocks. Generally, the characteristics of stillage from cellulosic materials appear to be comparable to those of conventional feedstocks (e.g. sugar cane

and corn), and, therefore, methods of stillage treatment and utilization applied to conventional feedstocks might also be applicable to cellulosic feedstocks. Two possible exceptions to the similarity of cellulosic and conventional stillage characteristics which deserve attention are the potential for higher levels of heavy metals from the acid hydrolysis processes and the presence of unusual inhibitors, such as hardwood extractives, associated with phenolic compounds present in the feedstock. However, the limited number of studies on stillage from different cellulosic feedstocks and hydrolysis methods means that predictions of treatment performance are prone to error.

A solution may be to recirculate the wastewater as much as possible and then concentrate the rest by multistage evaporation and incinerate the final concentrated wastewater. However, the evaporation requires a significant amount of energy that can have a negative impact on the energy balance of ethanol production (Faust et al. 1983; Wilkie et al. 2000). Running the process at higher solids consistency or with recirculated process streams, to maintain a high concentration of ethanol and dissolved solids, can reduce the energy requirements in the distillation and evaporation units. However, the effect of this stream circulation on the fermentation and stream handling should be examined. Finally, the evaporator condensate can undergo aerobic or anaerobic biological treatment or burning. The stillage can be used for production of single-cell protein or other viable biological products such as enzymes, chitosan, astaxanthin, plant hormones, and the biopolymers alternan and pullulan.

Aerobic and anaerobic digestion can serve as an effective means for removing COD from stillage and converting it to biogas, which is another biofuel. However, the presence of inhibitors in the stillage can cause some difficulties in biological digestion of stillage.

Reducing the color of stillage, in addition to COD reduction and nutrient removal, might be required in order to discharge treated stillage into surface waters without degrading water quality (Wilkie et al. 2000). The presence of phenolic compounds that are released during the degradation of lignin, melanoidins from Maillard reaction of sugars with proteins, caramels from overheated sugars, and furfural and HMF from acid hydrolysis, can make the cellulosic stillage's color more severe than that of other stillages.

### **Analysis of Lignocellulosic Materials and Acid Hydrolyzates**

The accurate compositional analysis of biomass is crucial in order to commercialize the technology for biomass to ethanol conversion. However, the analysis of lignocellulosic materials depends on the method of analysis and also on the sample preparation. A number of approaches involve milling a dried sample, and regrinding or discarding fines can cause major errors, since the fines can differ in composition and reactivity from the larger particles (Wyman 1996). It should be kept in mind that different parts of wood and agricultural residue have different composition.

The analysis of hydrolyzate and fermentation broths based on lignocellulosic hydrolyzates is not straightforward, since the solution contains a complex array of sugars, phenolic compounds, organic acids, furans, and other degradation products. Furthermore, the diversity of constituents in the hydrolyzates may interfere with the analysis. In addition, the ethanol produced during the process increases the solubility of hydrophobic

substances in the broth, so that the composition of the matrix continuously changes during fermentation (Olsson and Hahn-Hägerdal 1996; Taherzadeh 1999; Wyman 1996). Total reducing sugar (monosaccharide) concentration may be analyzed colorimetrically using a dinitrosalicylic acid (DNS) reagent (Miller 1959). The reducing concentrations can also be analyzed by Fehling titration (Xu et al. 2005). It is also possible to analyze total monosaccharides plus disaccharides concentration by the DNS or Fehling method, but the samples should be completely hydrolyzed by HCl before analysis. However, the presence of complex media can cause large errors in these methods. Chromatography seems to be the most reliable method for analyses of different sugars and furans in the hydrolyzate.

In research work, sugar utilization and solvent production in several fermentations of lignocellulosic hydrolyzates have been determined in two different ways: the sample components were determined using single-column liquid chromatography (CLC) analysis and with a combination of gas chromatographic analysis and calorimetric methods. The concentrations determined by the two methods were compared; the results for 15 out of 19 samples agreed within 80% similarity, but only 5 samples agreed within 95% similarity. Substantial differences in analysis results (a relative standard deviation of 12% in total sugar content) were also found when different laboratories altogether made 18 analyses of the same corncob hydrolyzates. One reason for the difference in analysis results is the lack of selectivity in the detection method employed (Olsson and Hahn-Hägerdal 1996).

During the development of analytical methods, careful evaluation of the selectivity is necessary. The use of two different detection principles may indicate impurities in the individual chromatographic peaks. In line with discussions of suitable fermentation alternatives, awareness of the difficulties and uncertainties in the analysis methods used for the determination of the fermentation characteristics is necessary. High-performance liquid chromatography (HPLC) seems to be the best and most reliable method for analysis of sugars, furfural, HMF, acetic acid, and fermentation products. However, usually the interactions between the different sugars' peaks in HPLC reduce the accuracy and reproducibility of this method for analyses of different sugars. Furthermore, separations of different peaks from the chromatograms are usually person-dependent and much care is needed for reliable and reproducible analyses of the sugars. Decomposition of some sugars such as sucrose is a typical problem in HPLC. When one analyzes the sucrose, a peak for glucose and another for fructose appears in some cases due to internal hydrolysis of the sugar. Other problems are the evaporation of ethanol during the fermentation, taking the sample, freezing and defrosting, and even during preparation of the standard samples used for calibration.

## COMMERCIAL PROCESSES BASED ON THE ACID PROCESS

Pilot plant and commercial-scale facilities for converting lignocellulosic biomass to ethanol by chemical hydrolysis have existed since the early 1900s. One of the earliest commercial hydrolysis processes was a dilute-sulfuric-acid process developed by EWEN and TOMLINSON during World War I. Two plants in the United States producing sugars

from wood were operative. For economic reasons, both plants were closed at the end of World War I. Dilute sulfuric acid hydrolysis of wood was re-examined by the Forest Products Laboratory, U.S. Department of Agriculture, at the request of the War Production Board in 1943.

In total, more than twenty alcohol plants utilizing lignocelluloses were operated in Europe, Russia, China, Korea, and the US prior to or during World War II. These plants used either the Scholler dilute (0.2-1%) sulfuric acid process or the Bergius concentrated (40-45%) hydrochloric acid process for wood hydrolysis. Yields for both processes were similar, with up to 45% fermentable sugar (w/w) from coniferous sawdust or bark-free chips. The Scholler process was less capital intensive and was therefore preferred compared to the Bergius process. In this process, dilute-acid of 0.5-0.6% concentration was percolated down through the packed bed with continuous removal of 5-6% sugar solution. After hydrolysis, the lignin residue was filter pressed to 50% moisture and burned to satisfy the steam requirement of the plant (Maiorella 1983). Many of the plants were closed for economic reasons after World War II (Kosaric et al. 1983; Maiorella 1983). Today, only a few of these old plants are still operating, with virtually all of them in Russia (Badger 2002; Nguyen et al. 1996).

Tavda Hydrolysis Plant located in Russia is one of the industrial plants operating nowadays. The process is somewhat similar to the Scholler process, and it was established in 1943. In 1970, the plant was reconstructed, to give a design production capacity of 13.5 million liters per year, and it currently produces approximately 12.4 million liters per year. About 70% of the ethanol production is derived from wood-cellulose hydrolyzate, together with 20% derived from wheat-starch hydrolyzate and 10% from beet molasses. All of the three feedstocks are combined in a single fermentation process. A mixture of about 80% woodchips and 20% sawdust are processed in a dilute-acid hydrolysis process. The process is a multistage dilute-acid process under different pressures (6, 7, 9, 12, and 12.5 atmospheres) for a period of 100 minutes. The total fermentation time is 6-8 hours. The yeast used in the fermentation is a strain of the fission yeast *Schizosaccharomyces*. Further information can be found at [www.distill.com](http://www.distill.com).

Inventa AG (Switzerland) developed a dilute-acid hydrolysis process. The technology is based on the dilute-acid hydrolysis of wood, which produced annually 10 million liters of fuel grade ethanol, using locally available softwoods. The hydrolysis is performed on wood chips at 140-180°C with dilute acid (0.6 wt.%). Solid residue leaving the reactor is used as a fuel. The vaporized furfural is collected in a recovery section. The cooled hydrolyzate is then neutralized with limestone, and the gypsum is separated. The fermentation of hydrolyzate takes place in a series of fermenters. A yeast adapted to wood hydrolyzates is used. The mash, having an ethanol concentration of about 2%, is separated, and a concentrated yeast suspension is recycled to the fermenter. Fuel-grade ethanol produced is concentrated by distillation. The yield of alcohol is 240 liter per tone of wood dry matter. The plant operated until 1956 in Domat/Ems (Kosaric et al. 1983).

BC International Corporation (BCI) in the United States, and SEKAB in Sweden, are commercializing the new technologies based on the latest research and developments on two-stage dilute-acid hydrolysis. In the first stage, hemicellulose is hydrolyzed under milder conditions (e.g. 170-190°C), and cellulose is hydrolyzed under more severe conditions (200-230°C).

## CONCLUDING REMARKS

Lignocellulosic materials are expected to be major feedstocks for ethanol production in the near future. However, the process of conversion of these materials is not as easy as conversion of sugar substances and starchy materials. Dilute-acid hydrolysis is one of the suitable methods for conversion of lignocellulosic materials to fermentable sugars. However, the process of hydrolysis is very complicated, depending on several parameters such as properties of the substrate, acidity, and rate of decomposition of the products during hydrolysis. Conversion of the hemicellulose present in lignocellulosic materials to simple sugars by dilute-acid hydrolysis can be easily carried out. However, obtaining a high yield of glucose from the cellulose part of lignocelluloses by dilute-acid hydrolysis is a challenge. A suitable reactor design can be applied in order to improve the yield of cellulose conversion by the dilute-acid process. Fermentation of a mixture of hexose and pentose and several major and minor inhibitors can introduce further problems. A suitable fermentation strategy, such as adaptive continuous or fed-batch processes, and employing a suitable natural or engineered microorganism that can convert both pentose and hexoses is necessary for the fermentation. The environmental impacts of an ethanol process should also be considered carefully. The residual solid materials and the wastewater should be further processed to produce several other products in an ethanol plant; otherwise they might create environmental problems.

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## REVIEW OF FACTORS AFFECTING THE RELEASE OF WATER FROM CELLULOSIC FIBERS DURING PAPER MANUFACTURE

Martin A. Hubbe and John A. Heitmann

The ease with which water is released from cellulosic fiber material during the manufacturing of paper can affect both the production rate and the consumption of energy during the manufacturing process. Important theoretical contributions to dewatering phenomena have been based on flow through packed beds of uniformly distributed fibers. Such descriptions are able to explain why resistance to dewatering increases as a function of the hydrodynamic surface area of fibers. More recent studies have demonstrated a critical role of finely divided matter. If the fines are unattached to fibers, then they tend to move freely through the fiber mat and plug channels in the paper web during the dewatering process. Dewatering also is affected by the deformability of cellulosic fibers and by whether the fibers easily slide past each other, thereby forming a dense mat. By emphasizing the role of fine matter, colloidal forces, and conformability of cellulosic materials, one can gain a more realistic understanding of strategies that papermakers use to enhance initial drainage and vacuum-induced dewatering.

*Keywords:* Dewatering, Drainage, Freeness, Fines, Water retention value, Kozeny-Carman, Choke-point mechanism, Mobility of fines, Sheet sealing, Sediment volume

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### INTRODUCTION

The removal of water from cellulosic fibers and other materials in the wet web constitutes the most energy-demanding part of the paper manufacturing process (McGregor and Knight 1996). This review considers various ways in which investigators have sought to explain the dewatering process and to understand factors that can either increase the rate of production or reduce the consumption of energy.

To put things into perspective, one can divide paper dewatering operations into phases. The first phase involves impingement of a low-solids fibrous suspension onto one or between a pair of highly permeable fabrics, which are manufactured as continuous belts. During operation of a modern paper machine, such fabrics can travel at surface speeds up to 1900 m/min. Because the suspension impinging onto a forming fabric typically has a solids content between 0.3 and 1%, some of the water will require only gravity and inertia to flow out of the cellulosic mixture.

The next phase of the dewatering process often involves a subtle disturbance of the developing web of paper, using devices such as hydrofoils. A hydrofoil is placed on the side of a fabric opposite from where the paper is being formed, and it is designed to apply a very short-term vacuum impulse. In addition to doctoring some water from the

back of the fabric, hydrofoils tend to jostle the wet web, freeing up drainage channels, and also tending to make the paper somewhat more uniform within the plane of the sheet.

A third phase of the dewatering process involves systematic application of vacuum, usually by means of vacuum flat-boxes and a perforated roll (the “couch” roll), over which the fabric travels. After passing over the couch roll, the paper web solids content is usually in the range 18-25%. Then it passes through a series of press nips, where water is forced from the sheet into the void spaces of continuous felts. After pressing, the paper web solids content usually is in the range of about 40-55%. The final operation in removing water from paper usually occurs as the sheet travels in serpentine fashion over a series of steam-heated rolls. The finished paper should have a moisture content of about 4-8%, roughly corresponding to the equilibrium moisture content of the paper under the humidity conditions at which it will be used.

According to McGregor and Knight (1996) the cost to remove one unit of moisture in the forming, pressing, and drying sections of a paper machine is related by the ratios 1:5:220. It is often possible to save energy by slowing down a paper machine, thus increasing the effectiveness of dewatering in the forming and pressing operations (Mansfield 1986). Because of the high capital costs of papermaking equipment, as well as the energy costs associated with papermaking, papermakers are motivated to find ways to produce more tons of product at a constant input of time and energy.

As noted in recent reviews, a variety of test methods have been developed to predict how rapidly water will be released during the production of paper (Kerekes and Harvey 1980; Roschy et al. 2002; Hubbe 2003). Briefly stated, these methods involve various standard conditions of filtration (Kerekes and Harvey 1980; Pires et al. 1989; Anon. 1994a), sometimes with automatic recording of the filtrate mass versus time (Sampson 1997; Bley and Falkenberg 2001), sometimes with application of vacuum (Gess 1984; Pires et al. 1989, Wang and Hubbe 2001; Roschy et al. 2002), or with pressure pulsations intended to simulate the environment of a modern paper machine (Persson and Österberg 1969; Britt et al. 1986; Lin and Schuster 1992; Räisänen et al. 1995; Sutman 2000). Some recent progress has employed computerized addition of papermaking chemicals, increasing the precision with which it is possible to evaluate different chemical strategies to promote more rapid release of water (Bley and Falkenberg 2001; Roschy et al. 2002). Even the traditional Canadian Standard Freeness test (Anon. 1994a) recently has been automated and improved in order to extract additional information that might be correlated to on-machine dewatering performance (Corcadden 2005).

Further progress has been achieved by measuring the water-retaining ability and particle size of cellulosic fines in a suspension; this approach was found to give a high correlation with the dewatering characteristics of combined furnish from which the fines were obtained (Kang and Paulapuro 2006). Because the fines fraction of papermaking furnish appears to play such a predominant role relative to dewatering rates, it should be emphasized that a wide variety of very small solid materials are apt to be present in papermaking stock. Cellulosic fines may consist of ray parenchyma cells (primary fines) or of fibril fragments removed from cell walls during refining (secondary fines). Mineral particles used as “fillers” in paper products are also counted as “fines” by many authors. Colloidal matter, including emulsion droplets, polyelectrolytes, and polyelectrolyte

complexes also can be considered as fines, depending on the scope of an investigation. When considering the mechanisms to be described in the following sections, it is quite likely that different types of fines behave differently.

## PACKED BED CONCEPTS

### Specific Surface Area

Kozeny (1927) showed that the resistance to flow through packed beds of granular materials could be explained in terms of the size and number of pores. His ideas were extended by Carman (1938,1939), who verified the equation and introduced such concepts as hydrodynamic radius, specific surface area, and the effects of tortuosity. A commonly cited form of the Kozeny-Carman equation is given in Eq. (1),

$$k = (\gamma/\mu) (2/C_{K-C}) (1/S_o^2) [e^3/(1+e)] \quad (1)$$

where  $k$  is the permeability (length/time),  $\gamma$  is the unit mass of the fluid,  $\mu$  is the fluid's dynamic viscosity,  $C_{K-C}$  is the Kozeny-Carman coefficient (usually taken to be about 5),  $S_o$  is the specific surface area per unit displacement volume of particulate material, and  $e$  is the fractional void volume (Carrier 2002; Capuis and Aubertin 2003). The permeability coefficient is defined in reference to d'Arcy's law,

$$dV/dt = k A \Delta P_f / (\mu L) \quad (2)$$

where  $V$  is the filtrate volume at time  $t$ ,  $A$  is the cross-sectional area available for flow (disregarding the presence of a solid phase),  $\Delta P_f$  is the pressure drop across the permeable material,  $\mu$  is the viscosity, and  $L$  is the linear length of the column through which the fluid passed through the permeable material. In cases where all of the other parameters can be determined, equations (1) and (2) sometimes are employed to estimate the specific surface area of material in a packed bed (Sullivan and Hertel 1942).

Ingmanson (1952, 1953) showed that the same concepts could be applied to compressible materials, such as cellulose. The situation considered was constant pressure dewatering through a fiber pad of uniform composition. The following equation was proposed to represent the average specific resistance, defined on a mass basis,

$$R_w = k S_w^2 \Delta P_f / \left\{ \int_0^p [(1-ac)^3 / c] dp \right\} \quad (3)$$

where  $S_w$  is the specific surface area of the fibers,  $a$  is the effective specific volume of the fibers,  $c$  is the mass of fibers in a uniform bed, and  $p$  is the compacting pressure.

Over the years, various researchers have built upon the work of Kozeny, Carman, and Ingmanson, verifying and fine-tuning the theory (Whitney et al. 1955; Tiller and Cooper 1960; Meyer 1962; Nelson 1964; Tiller and Shirato 1964; Han 1969; Kyan et al. 1970; Binotto and Nicholls 1979; Jackson and James 1986; Jonsson and Jonsson 1992a,b; Nordén and Kauppinen 1994; Mantar et al. 1995; Kumar et al. 1996; Ramarao and Kumar 1996). Whitney et al. (1955) and Jackson and James (1986) compared particulate

materials having a wide range of shapes; they found that all of the results tended to fall on the same line of flow resistance as a function of the square of the characteristic radius and the volumetric content of solid matter. Such results provide strong support for the general concept. Chan et al. (1996) showed that the Kozeny-Carman equations agreed well with experimental results involving mixtures of differently shaped particles, all having roughly the same specific surface area.

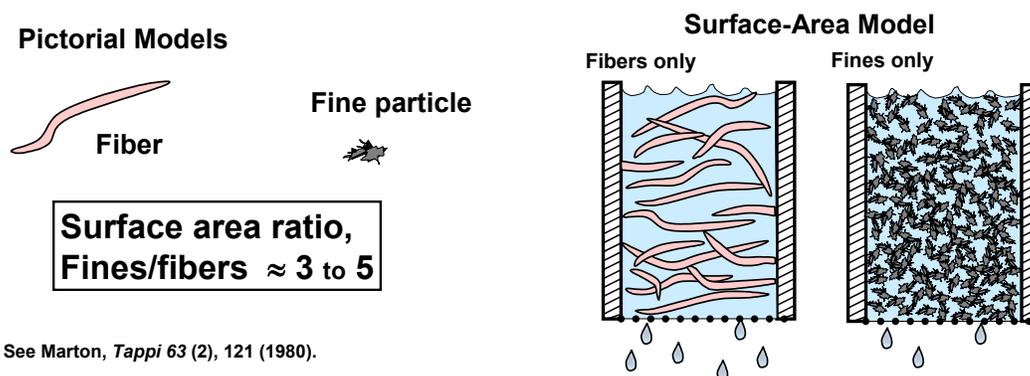
The Kozeny-Carman concept also can be adapted to specific situations. For instance, Han (1969) introduced concepts of viscoelastic creep, resulting in progressive densification of fiber mats exposed to constant pressure. Binotto and Nicholls (1979) showed that Kozeny-Carman concepts can be applied with good agreement to different fractions of classified pulp suspensions differing in fiber length and wall thickness. Ramarao and coworkers (Kumar et al. 1996; Ramarao and Kumar 1996) provided an analysis of gravity-assisted dewatering, making it possible to obtain specific filtration resistance data from conventional freeness test equipment. Their model predicts that the density of the fiber pad, especially the part near to the filter screen, will go through a maximum when a fiber suspension is dewatered by gravity. Zhu et al. (1995) showed that related concepts originally developed to predict flow through textile materials also can be applied with good accuracy in the case of flow through papermaking fiber mats.

### Effects of Fine, High-Surface-Area Suspended Matter

One of the hoped-for benefits of using calculations based on the Kozeny-Carman approach has been to account for effects of fine suspended matter having a relatively high surface area per unit mass, or “specific surface area.” Consistent with theory, it has been found that fine matter having the smallest size and highest specific surface area tends to have the greatest adverse effect on dewatering (Przybysz and Szwarcztajn 1973; Patel et al 1994; Liu et al. 2001). Fibrillar material, mainly composed of delaminated cell wall material, tends to cause greater reductions in dewatering rates, compared to fines having rounded or brick-like shapes, as in the case of parenchyma cells from the wood (Brecht and Klemm 1953; Steenberg et al. 1960; Waterhouse and Omori 1993; Krogerus et al. 2002; Hubbe 2002). Because fine fibrils tend to have a higher surface area per unit mass than blocky or rounded particles, such findings are consistent with the idea that specific surface area has a dominant effect on permeability.

The main effects predicted in the Kozeny-Carman equation are illustrated pictorially in Fig. 1. Consistent with the work of Marton (1980), it will be assumed that the fine matter has a much higher surface area per unit mass, compared to typical fibers in the suspension. As illustrated at right in the figure, a greater frictional resistance is expected when fluid flows through the bed of fines. Although Fig. 1 appears to imply a uniform packing of solid matter, we already have seen that not all users of the Kozeny-Carman equation have made such an assumption (e.g. Ingmanson 1952, 1953).

To apply the Kozeny-Carman equation to suspensions containing odd-shaped finely-divided matter, information about surface area is required. However, surface area within fiber lumens, within the cell wall, and within adsorbed macromolecular material at solid surfaces is not expected to affect the release of water during a conventional gravity-based or pressure-based dewatering experiment. Hence, researchers have sought various ways to assess the “hydrodynamic specific surface area” of suspended matter.



**Figure 1.** Left: Cartoon representations of typical fiber and typical fiber fine particle in a papermaking pulp suspension. Right: Illustration showing more rapid dewatering through a bed of coarse fibers, in comparison with fine matter, assuming uniform packing density.

Mason (1950) described how the effective hydrodynamic surface area can be estimated by either (a) determining the amount of silver needed to coat the accessible surface of suspended matter, (b) determining the efficiency of light scattering, which often is approximately related to surface area, or (c) by determining the specific resistance to filtration. In fact, Robertson and Mason (1949) were among the first to apply the concepts of Kozeny and Carman to papermaking applications. Marton and Robie (1969), as well as Wood et al. (1991) showed that related information can be obtained very conveniently by evaluating the rate at which fine particles settle out of an unstirred aqueous suspension. Wood and Karnis (1996) extended one of Mason's ideas, showing that turbidity test results, which are related to light scattering, can be used to estimate the hydrodynamic specific surface area of fiber fines. Kang and Paulapuro (2006) described use of a dynamic, centrifugal method to rapidly evaluate the rate of fine-particle sedimentation, and they also demonstrated how one can measure the viscosity of fractionated suspensions of fine matter as a means of estimating the degree to which such matter has become swollen with water.

### Effects of Enzymes on Surface Area

An elegant way to demonstrate the effect of specific surface area on dewatering resistance of cellulosic material is to use enzymes. Cellulase treatments can be optimized to systematically clean up or “polish” the surfaces of fibers and fiber fines, removing fibrillar material that may be projecting outwards from such surfaces. Such a mechanism can explain why cellulase treatment after refining of kraft fibers can provide a substantial increase in drainage rates (Jackson et al. 1996; Eriksson et al. 1997a,b; Gruber and Gelbrich 1997; Seo et al. 2000; Gong et al. 2003; Gong and Bi 2005).

### Effects of Wet Fiber Stiffness

Because the stiffness of fibers, when wet, can affect packing density, one would expect this parameter to affect rates of dewatering. Kayan et al. (1970) incorporated fiber bending into their model to predict filtration resistance. Lindsay and Brady (1993b) found that fibers that had been dried tended to promote more rapid dewatering, consistent with the expected somewhat irreversible effects of drying (Stone and Scallan 1966;

Klungness and Caulfield 1982; Lindström and Carlsson 1982; Nazhad and Paszner 1994; Weise et al. 1996; Maloney et al. 1998; Zhang et al. 2004). Britt (1981) also found that rapid drainage is favored by the presence of relatively stiff fibers. Paavilainen (1993) was able to quantify such concepts, using a new technique for measurements of wet-fiber flexibility.

### **Mixtures of Particles of Different Size**

Higher packing densities can be expected in the case of suspensions that have wider distributions of particle size (Dodds 1980; Ethier 1991; Andrade et al. 1992). In effect, smaller particles can fill in spaces that would necessarily occur within suspensions consisting only of larger particles. Consistent with this effect, resistance to dewatering has been found to be larger, in the case of mixtures, than could be explained in terms of a linear combination of results from dewatering tests with uniform suspensions (Abe et al. 1979; MacDonald et al. 1991).

### **Deviations from Kozeny-Carman Predictions**

Studies showing significant deviations from predictions based on the Kozeny-Carman concept provide evidence that other mechanisms may play significant roles in controlling rates of water release from cellulosic material. For example, Hawes and Doshi (1986) found that the origin of fiber fines, including whether or not they had been recycled, played a large role relative to dewatering rates, to a much greater extent than could be explained by differences in surface area. They proposed that the observed differences were due to differences in flexibility and conformability among the different kinds of fiber fines. As noted by Ingmanson and Andrews (1959) the classical concepts of Kozeny and Carman, even when modified to account for compressibility effects, cannot be expected to adequately deal with effects of “debris” that can contaminate an otherwise uniform porous mat.

One reasonable approach, to account for deviations from classical Kozeny-Carman concepts, would involve known differences in composition of fiber mats, as a function of distance in the direction of flow. Heath and Hofreiter (1978) provided an excellent demonstration of how simple filtration of a fiber suspension, using conventional handsheet forming equipment, can give rise to Z-directional differences in the proportions of fine materials. Under conditions of very slow, one-directional dewatering, as in the case of a Fourdrinier paper machine making a very heavy-weight product, it is possible to detect effects of more rapid gravity sedimentation of the larger fibers, leaving a higher proportion of fiber fines in the upper part of the sheet (Unbehend et al. 1989). Ramarao et al. (1994) showed that the proportion of fine matter in different layers of a mat formed by filtration can be predicted by the relative ages of different layers, during the forming process. Results were consistent with concepts proposed earlier by Parker (1972), noting that layers of fibers closer to a forming fabric have a higher probability of capturing mobilized fine matter. However, much more uniform composition in the Z direction can be achieved if the suspension is treated with a retention aid, which apparently binds fine matter to the surfaces of fibers (Tanaka et al. 1982; Ramarao et al. 1994). Mantar et al. (1995) found that dewatering results can be strongly affected by the initial solids content of fibrous suspensions. They proposed that the effects were due to

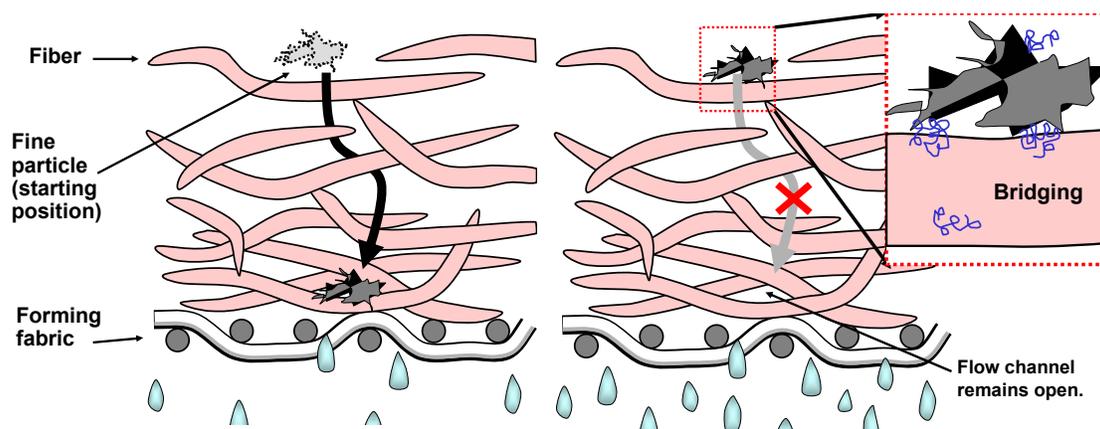
(a) increasing association between fine matter and fibers with increasing consistency, and (b) increasing tendency for fibers to entangle, forming flocs, with increasing consistency beyond a certain point (see Hubbe 2007). The distribution of fine matter in machine-made paper also is affected by the washing action of dewatering devices, such as hydrofoils and dewatering blades (Parker 1972; Zeilinger and Klein 1995).

## THE CHOKE-POINT HYPOTHESIS

### Fines and Dewatering

To understand the mechanisms grouped under the heading “Choke Point Hypothesis,” one needs to consider the behavior of unattached fine matter in a fiber suspension, and what can happen to such fines as water is being removed. Excellent articles have been written regarding the characteristics of cellulosic materials in fiber suspensions that are small enough, individually, to pass through a conventional forming fabric of a paper machine (Brecht and Klemm 1953; Steenberg et al. 1960; Kibblewhite 1975; Htun and de Ruvo 1978; Marton 1980; Lindholm 1983; Allen 1985; Scott 1986; Gruber et al. 1997; Moss and Retulainen 1998; Luukko and Paulapuro 1999; Blechschmidt et al. 2000; Pruden 2005). Some commonly noted features of the fiber fines fraction, in addition to their small size, include a relatively high ratio of surface area to mass and a tendency to increase the resistance to dewatering. The fines fraction of a papermaking furnish usually is determined by fractionation with a standard screen (Allen 1985; Anon. 1994, 1995; Luukko and Paulapuro 1999; Pruden 2005). In terms of size, one can make an argument that small, entrained air bubbles, which likewise can increase drainage resistance (Brecht and Kirchner 1959; Gertjeansen and Hossfeld 1967; Karras and Springer 1989; Rauch and Sangl 2000; Helle and Paulapuro 2004; Martorana and Kleemann 2006), ought to be considered as part of the fines component of a papermaking furnish.

The choke-point hypothesis is illustrated in Fig. 2. Several writers have proposed that unattached fiber fines, which can move freely through the paper web during the



**Fig. 2.** Left: Schematic illustration of “choke-point” mechanism in which unattached fine particles move through drainage channels to vulnerable points at which they block the flow; Right: Proposed effect of bridging polyelectrolytes on defeating choke-point mechanism by keeping fiber fines attached to fiber surfaces.

process of dewatering, have a high likelihood of blocking channels through which the water is able to flow (Britt et al. 1986; Patel and Trivedi 1994; Kumar et al. 1996; Räsänen 1996; Wildfong et al. 2000,2003; Paradis et al. 2002; Hubbe 2002). This can be thought of as a manifestation of Murphy's law, since the movement of water through the wet web is expected to transport the fine particles until they get stuck at "choke points," *i.e.* locations in the mat that happen to be particularly unfortunate with respect to dewatering efficiency. A version of the choke-point hypothesis was enunciated as early as 1969, when Han (1969) proposed that particulate matter in the water could accumulate in a fiber mat, slowing the dewatering rate. A detailed microscopic study by de Silveira et al. (1996) revealed that fines can play a wide variety of roles in a paper sheet, some of which appear to be consistent with the mechanisms just described.

### Support for the Choke-Point Mechanism: Fines Level and Basis Weight

If one begins by assuming that dewatering rates are predominantly controlled by the movement of fines to points where they tend to occlude dewatering channels, then it can be argued that the effect of fines on drainage ought to be nonlinear in character. For sake of discussion, let's envision a very simple mat of fibers in which there happen to be 100 identifiable passages for the flow of water. The first fine particle is drawn by the flow of departing filtrate into a position where it almost completely seals off flow through one of these channels, changing the overall filtration resistance by about 1%. The 50<sup>th</sup> of the fines blocks the 50<sup>th</sup> passage, changing the filtration resistance by about 2%. And the 99<sup>th</sup> fine particle seals off the next-to-last channel, changing the overall filtration resistance by a factor of two! Although this simple arithmetic is not meant to represent a realistic description of pores in a wet-web of paper, nor the efficiency with which a fiber fine would be likely to close off a passage through a wet web, the mechanism implies that resistance to dewatering ought to increase out of proportion to the content of fines, especially when the level of unattached fine begins to approach some critical level. Data generally agreeing with the expectations just cited have been reported in several studies (Molina et al. 1984; Springer and Pires 1988; Hubbe 2002).

A related qualitative analysis can be applied to the subject of basis weight. As noted earlier in this article, fine particles have the highest probability of ending up in a layer of the paper that becomes relatively dense early in the dewatering process. At the limit of a pure filtration mechanism of dewatering (see later discussion), that layer is expected to be near to the filter screen. If the basis weight of the sheet were to be doubled, then the flow of fines-containing water that passes through that layer also will be approximately doubled. As discussed already in the preceding paragraph, each successive fine particle approaching a given layer within the mat of fibers is expected to have a progressively greater adverse impact on dewatering, so the net result is that one expects dewatering resistance to increase out of proportion with increases in basis weight. Again, experimental data from simple filtration analyses tends to support the choke point mechanism in this regard (Gess 1991; Paradis et al. 2002; Wildfong et al. 2000a,b,2003). Though the choke-point model may not be the only way to explain an increase in dewatering resistance out of proportion to basis weight (see, for instance, the "sealing" mechanism, as described later), it is worth noting that Gess (1991) observed a large,

nonlinear increase in dewatering resistance with basis weight only in a case where the level of fiber fines had been artificially increased, compared to a default condition.

### Mobility of Fines

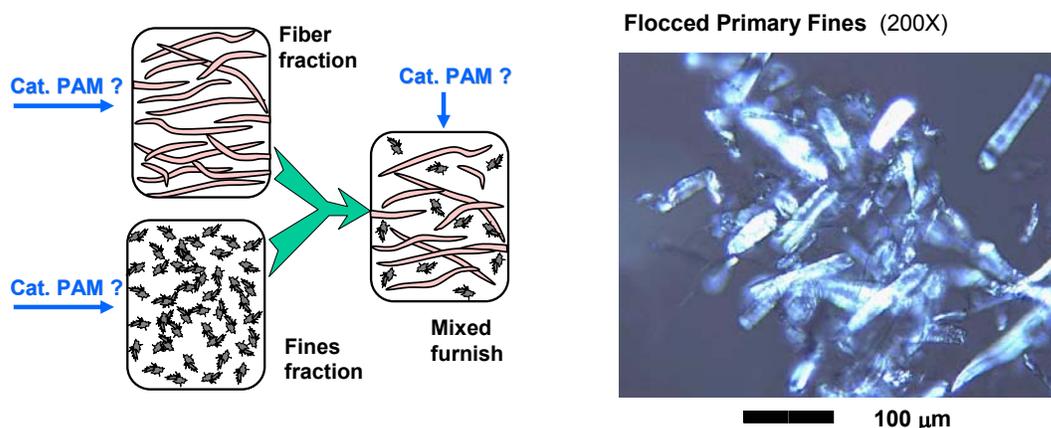
Further evidence of the action of a choke-point mechanism can be obtained by controlling whether or not fine matter is free to move, relative to its initial position vis-à-vis fibers. Issues related to whether or not fiber fines and other solids within a sheet of paper are free to move, relative to a surrounding network of fibers, have been described by Parker (1972). Britt (1981) concluded, based on experiments with controlled agitation of fiber suspensions, that a majority of fiber fines would remain unattached to fibers under typical papermaking conditions. Van de Ven (1984) concluded, however, that it is relatively unlikely for very small particles to become deposited onto fibers during sheet formation, due to hydrodynamic effects. In layman's terms, the water surrounding a fiber tends to act like a lubricant, preventing close approach of small particles as they are carried past the fiber in streamlines of flow. It follows, logically, that most of the collisions, resulting in sticking of fine matter to fiber surfaces must occur earlier in the process. Another view is that electrostatic attraction forces may be able to overcome hydrodynamic forces and bring about "sticking collisions" in such cases.

The concept that fines sometimes can move freely within a paper web during dewatering is supported by studies related to a "healing" mechanism, which appears to be responsible for moderate improvements in formation uniformity (Norman et al. 1995; Sampson 1997). The idea is based on the fact that the initial fibers or fiber flocs impinging onto a forming fabric will have a nonuniform distribution. However, flow toward the forming fabric will become suppressed in those areas already covered. In this way, later-arriving fibers and fines, initially in the upper portion of a jet of slurry landing on a Fourdrinier fabric, will tend to be steered away from high-basis-weight locations and towards voids or thinner parts of the wet web. Further evidence of the relative movement of fines in the thickness direction of paper, during its formation, is shown by non-uniform distributions of fine matter, especially in the case of paper made on Fourdrinier machines, where drainage occurs in one direction (Parker 1972; Tanaka et al. 1982).

At low to moderate levels of hydrodynamic shear the simplest way to prevent fine matter from migrating through the mat of fibers is to employ a very-high-mass acrylamide copolymer, an additive that papermakers refer to as a retention aid (Horn and Linhart 1991; Doiron 1998). Many studies have reported positive effects of retention aid addition on dewatering rates (Britt and Unbehend 1980; Lindholm 1980; Wegner 1987; Karras and Springer 1989). Such results might be explained by noting that the minimum shear stress needed to detach a particle from a fiber surface exposed to flow is a strong inverse function of particle size (Hubbe 1985). The shear stress level that is just sufficient to detach a pair of fibers from each other, overcoming any polymer bridging resulting from the retention aid use, will not ordinarily be sufficient to cause detachment of a smaller particle (Britt 1981; Hubbe 1984; Hubbe and Wang 2002; Huber et al. 2004; Rojas and Hubbe 2004). Thus it is to be expected that a significant proportion of the fines fraction, following treatment of the system with an effective retention aid, will be prevented from participating in a choke-point mechanism. Retention aids can be expected to be especially effective in binding the smallest categories of fines to fiber surfaces,

and such fines have been implicated in the most severe effects on dewatering (Liu et al. 2001). Small, unattached fines that are compact in character, e.g. filler particles, may have a high probability of passing through the rest of the fiber mat without becoming trapped by a filtration mechanism.

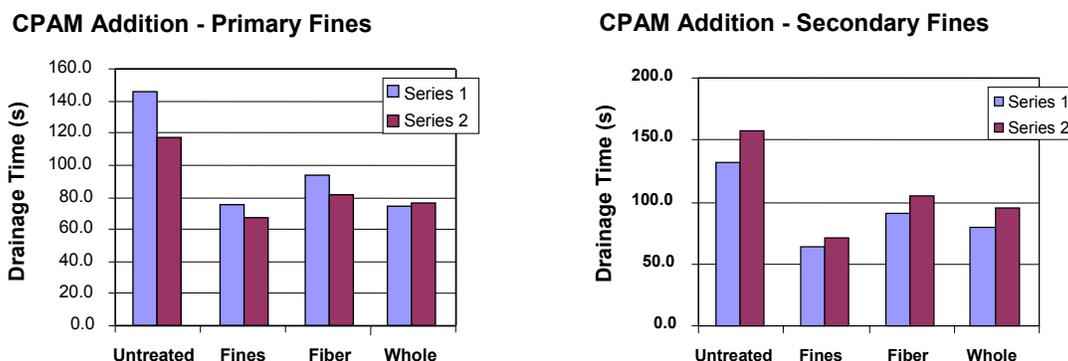
In an effort to evaluate various alternative interpretations, Hubbe (2002) carried out preliminary experiments in which a cationic acrylamide-type retention aid was added alternatively (a) just to the fines fraction, (b) just to the long-fiber fraction, or (c) to the combined furnish. These three situations are illustrated in Fig. 3, which also shows an example of how addition of the flocculant chemical to a suspension of primary hardwood fines caused the fines to become agglomerated. In each case, the fibers and fines were recombined before making a test sheet. Experiments were repeated for two kinds of cellulosic fines. The first set, primary fines, was obtained by fractionating unrefined hardwood pulp. The second set, secondary fines, was obtained by extensive refining of fines-free hardwood fibers. Parallel observations by light microscopy showed that treatments of type (a) caused agglomeration of fines, decreasing their hydrodynamic surface area. In addition, all of the treatments greatly increased the efficiency of retention during the sheet-forming process.



**Figure 3.** Summary of experimental procedure for treatment of fractionated hardwood kraft furnish either before or after recombining the fines and long fibers and noting the rate of dewatering during formation of paper. A: Treatment options. B: Agglomerated primary fines.

As shown in Fig. 4, each treatment scheme significantly increased the dewatering rates. Particularly large dewatering increases were obtained in cases (a) and (c), roughly corresponding to agglomeration of fines and attachment of fines onto long fibers. Both results are consistent with the choke-point mechanism. Related evidence can be found in studies relating the efficiency of retention to the surface charge and zeta potential of materials in fiber suspensions. Often such studies have shown maximum dewatering rates and maximum retention when aqueous conditions have been adjusted in such a way that the net electrical potential associated with the surface is near to zero (Horn and Melzer 1975; Bhardwaj et al. 2005; Hubbe et al. 2007a). Furthermore, it is well known that high-charge polyelectrolytes and multivalent ions having a charge opposite to that of

fiber suspensions tend to be effective dewatering aids (Britt and Unbehend 1985; Jaycock and Swales 1994; Maunier and Ramarao 1996; Gruber et al. 1997).



**Figure 4.** Experimental results for drainage time determinations with untreated bleached kraft furnish with three optional addition procedures of cationic flocculant, (a) addition to the fine fraction only; addition to the long fiber fraction only; and (c) addition to the recombined whole pulp. A: Primary hardwood fines. B: Hardwood fines resulting from refining action.

Liimatainen et al. (2006) found that scalenohedral precipitated calcium carbonate (PCC) of a type having a positive surface charge tended to promote dewatering, in contrast to other kinds of fillers that they tested. They attributed the anomalous results partly to colloidal attraction and efficient retention of the mineral onto the negatively charged surfaces of cellulosic materials. Curiously, this study was almost a mirror image of work reported by Solberg and Wågberg (2002). The latter researchers studied the retention efficiency when negatively charged ground calcium carbonate (GCC) particles were added to suspensions of positively charged fibers. In both cases attraction between surfaces of opposite charge promoted retention of filler particles on fiber surfaces.

### Cases in Which Polymeric Treatments Increase Resistance to Dewatering

In some cases the addition of polyelectrolytes, e.g. carboxymethylcellulose, has been found to increase the resistance to dewatering. Though such observations may at first appear contrary to the concepts mentioned in the previous subsection, on closer inspection some of the observations can provide further support of the choke-point mechanism. Dunham et al. (2002) observed cases in which the addition of a high-charge cationic polymer to a papermaking furnish having a high cationic demand caused a significant reduction in the rate of dewatering. It was observed that addition of the cationic polymer resulted in the formation of polyelectrolyte complexes, which remained suspended in the white water phase. This mechanism increased the particle size of colloidal material, i.e. “nano-fines,” in the white water phase from 1  $\mu\text{m}$  to about 20  $\mu\text{m}$ , which apparently allowed the material to behave similar to fines and choke drainage channels. Interpretation of such results needs to be done with care, however, since polyelectrolyte complexes may promote more rapid dewatering in other cases.

High-mass anionic retention aid polymers often have a negative effect on dewatering (Abson et al. 1980; Gess 1993; Miyanishi and Shigeru 1997; Lee and Lindström 1989), and it can be unclear whether or not such effects are related to a choke-point mechanism. Abson et al. (1980) reported related effects in the case of an anionic

retention aid, which was added to a system having aluminum sulfate present; one way to explain such results is to suppose that the alum formed a complex with the polyelectrolyte (Onabe et al. 1983). By becoming coagulated together with other such complexes, the colloidal material might become large enough to behave as small particles and block flow channels in the wet web of paper. Similar observations were reported by Polverari et al. (2001) and Pruszyński and Jakubowski (2002) in the case of high-charge cationic polymer addition to mechanical pulp furnishes, which tend to be rich in anionic colloidal material.

Related results have been reported in the case of a non-ionic retention system based on polyethyleneoxide (PEO) (Cadotte et al. 2005), except that it is not clear from the study whether or not the PEO was present in the form of complexes, or simply adsorbed onto the cellulosic surfaces. Similar effects sometimes can be observed with cationic acrylamide-type flocculants, but apparently only in cases where the system is overdosed with cationic material (Liu et al. 1986). When relatively large amounts of high-mass PEO or acrylamide copolymers are used to disperse long fibers, as in the production of wet-laid nonwoven fabrics, the same additives may be called “formation aids,” and the negative effects on dewatering rates can be very substantial (Lee and Lindström 1989).

### **Time Effects that Support the Choke-Point Mechanism**

The beneficial effect of drainage aids often has been found to pass through a maximum, several seconds after addition of the chemical to an agitated fiber suspension, and then to gradually decay with the further passage of time (Forsberg and Bengtsson 1990; Forsberg and Ström 1994; Hubbe and Wang 2002). Such observations can be explained in terms of an initial deposition of fine solids onto fiber surfaces, followed by gradual re-entrainment into the white water phase. It makes sense that the added cationic polyelectrolytes should initially form bridge-like or patch-like connections (La Mer and Healy 1963; Gregory 1976) between the fine matter and the fibers. However, the passage of time and the influence of hydrodynamic shear can shorten the molecular chains (Sikora and Stratton 1981; Tanaka et al. 1993; Forsberg and Ström 1994) and allow the polymeric additives to lie down flat on the fiber surfaces (Swerin and Ödberg 1997), or to become buried beneath a layer of fibrillation (Hubbe 2006).

Support for a mechanism involving conformational change and/or progressive migration of cationic polymers into pore spaces below fibrils at the fiber surface is provided by a study involving parallel measurements of dewatering and zeta potential (Ström and Kunnas 1991). Greater efficiency, in terms of dewatering, was observed in the case of higher-mass cationic polymers, which appeared to stay on the outer surfaces of suspended matter for a longer time. Based on such observations it makes sense to add dewatering aids relatively late in the approach system to a paper machine forming section, maximizing the degree to which fine particles are being held onto fiber surfaces during the forming process.

### **Water Retention Values (WRVs)**

Published evidence does not support that idea that high-charge polymeric additives have a large effect on the water that is held within the walls of cellulosic fibers.

Such a “de-swelling” action is among the possible mechanisms that might be used to explain the action of high-charge cationic dewatering aids (Auhorn 1982; Allen and Yaraskavitch 1991). Procedures involving centrifugation of damp plugs of fiber, allowing the filtrate to pass through a filter and into absorbent material, have been used for many years to estimate the amount of water that is contained within fiber cell walls (Thode et al. 1960; Jayme and Büttel 1968; Ahrens et al. 1999; Anon 1981, 2000). However, if drainage aids mainly functioned by penetrating within the cell walls of fibers, then one would expect their effectiveness to increase with decreasing molecular mass. In fact, the opposite is true (Ström and Kunnas 1991). Highly charged cationic polymers having relatively high mass were found to be much more effective at promoting dewatering, and they also had a much bigger effect in decreasing water retention values, as measured by the centrifugation. It was concluded that the WRV effects were mainly associated with water hold on the outsides of fibers, within layers of fibrillation. The most effective dewatering aids were those having capability to form large positive patches of charge, causing agglomeration of the fibrils. A related study showed that cationic polymers had relatively little effect on water retention values (Maunier and Ramarao 1996), but caused big increases in dewatering rates.

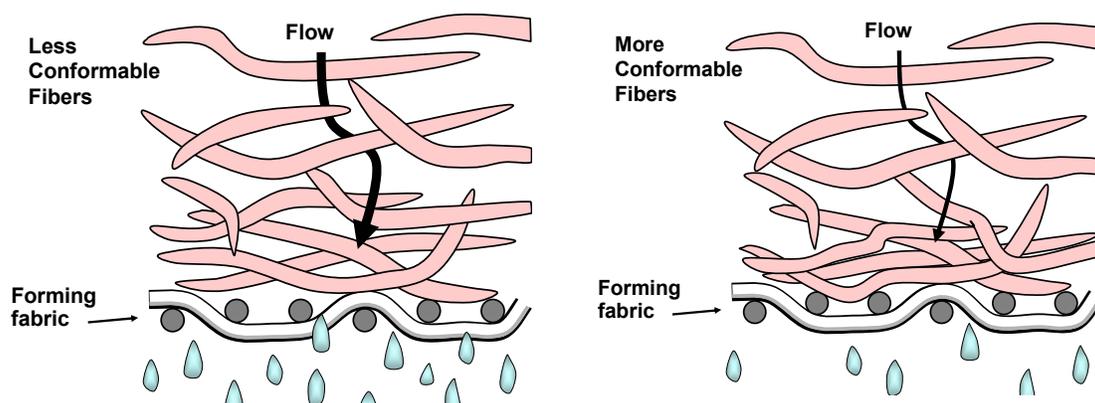
### **Pulse Dewatering as a Demonstration of the Choke-Point Mechanism**

A key piece of evidence can segue to the next topic. Dewatering tests involving controlled levels of flow or vacuum pulsations often show greatly accelerated rates of dewatering, and much less sensitivity to the presence of fines (Britt et al. 1986; Räsänen 1996; Räsänen et al. 1996; Mitchell and Johnston 2000; Rojas and Hubbe 2004). At least part of the dewatering enhancement effect probably can be attributed to the washing of fine material from the wire-side(s) of the wet web of paper (Egelhof and Bubik 1994; Zeilinger and Klein 1995; Räsänen et al. 1995; Räsänen 1996; Hubbe et al. 2006a), thereby defeating the choke-point mechanism.

### **SEALING AS A MECHANISM OF DEWATERING RESISTANCE**

Even in cases where fines do not move freely within a fiber suspension, there is another mechanism that can inhibit flow through the densest layers in the wet web of paper, as it is being formed. That mechanism is sometimes called “sealing.” The idea is that conformable cellulosic materials are forced together, as a result of applied vacuum or wet-pressing, such that they seal off passageways by which water might have more easily escaped from the wet web (Wildfong et al. 2000). The mechanism is illustrated schematically in Fig. 5.

As in the case of a rubber plug in an old-fashioned sink, the higher the pressure, the more effective becomes the sealing action. McDonald and Amini (1998) were able to apply this type of interpretation to explain dewatering resistance on a linerboard former. Wet sheets were pressed under different pressures in order to estimate the degree to which flow would be sealed off under different conditions of vacuum application during paper formation. Pires et al. (1989) observed cases in which resistance to flow increased out of proportion to the applied pressure, consistent with sealing.



**Figure 5.** Schematic illustration of “sealing” mechanism in which the fibers in a paper mat are sufficiently flexible that pressure causes them to squeeze together, sealing off flow

Evidence to support a sealing mechanism of resistance to dewatering comes, first of all, from measurements of fiber flexibility and conformability (Tam Doo and Kerekes 1982; Steadman and Luner 1985; Paavilainen 1993). More flexible fibers resulted in greater resistance to dewatering. Corroborating evidence comes from studies of fibers that have been recycled under laboratory conditions. An observed increase in freeness when never-dried fibers are formed into paper, dried, and then resuspended in water can be attributed to irreversible stiffening of the fibers (Paavilainen 1993; Dulemba et al. 1999; Zhang et al. 2004).

A sealing mechanism also can help explain the effectiveness of dewatering devices that produce short pulses of applied vacuum. If one assumes that sealing is the dominant mechanism limiting dewatering rates, then one would expect there to be diminishing rates of dewatering whenever vacuum is applied at a steady level (Mitchell and Johnston 2000). Indeed, the most effective dewatering, by means of vacuum flatboxes, usually requires optimization of the spacing and duration of vacuum pulses (Persson and Österberg 1969; Giles 1990; Räisänen 1996; Baldwin 1997). Lindberg (1970) observed that the application of vacuum in the form of pulses became increasingly important with increasing flexibility of the fibers, consistent with a sealing mechanism.

Many papermakers believe that sheet sealing effects mainly can be attributed to an interaction between fibers and the forming fabric. This subject has been reviewed in exquisite detail by Kufferath (1982). The idea is that, especially under conditions of rapid initial dewatering, fibers become pressed into the openings of a forming fabric, effectively rendering the fabric less porous (Giles 1990; Miller 1998). The importance of interactions between a fabric and the initial fibers impinging upon it has been demonstrated by turning a forming fabric upside-down and observing large differences in dewatering performance (Giles 1990). However, another careful study failed to find any special contribution that could be attributed to the first layer of fibers to land on the fabric (Herzig and Johnson 1999).

## INTER-FIBER FRICTION AND ITS INFLUENCE ON DEWATERING

In addition to the “sealing” mechanism, as just considered, another closely related mechanism appears to play a significant role with respect to the use of dewatering agents. That is, if fibers in a papermaking furnish are able to slide past each other when they come into contact, then one can expect that they will tend to form a relatively dense mat during the forming process. If, on the other hand, they tend to stick together and not slide past each other, then one can expect a more bulky, porous mat of fibers from which water can more easily flow. In other words, the degree to which the furnish components tend to become packed together is expected to play a major role in determining the permeability of the mat that is formed (Sampson and Kropholler 1995).

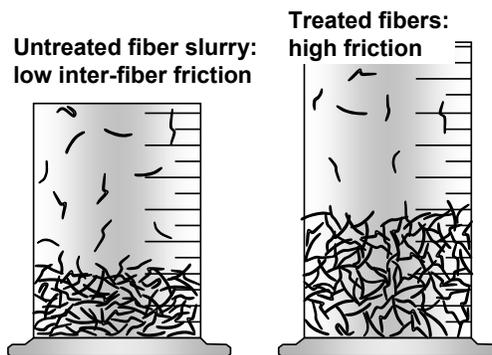
One way to find out whether fibers in suspension will tend to stick to one another on contact involves rheometric measurements. If the solids content of a fiber suspension is sufficiently high, then it can be feasible to measure the yield strength of a transient network that forms among the fibers upon secession of flow. Swerin et al. (1996) showed that such yield strength values could be greatly increased by the addition of flocculating polymers. At the other extreme, Zauscher (2000) measured frictional forces between submicroscopic surfaces, using atomic force microscopy (AFM). He found that the coefficient of friction generated between pairs of cellulosic surfaces in the presence of water could be greatly reduced by the addition of carboxymethyl cellulose (CMC).

Many of the effects that polyelectrolyte additives can have on inter-fiber friction in the wet state can be understood in terms of electrostatic forces of interaction. A particularly effective way to prevent cellulosic surfaces from sliding easily past one another is to treat the system so that the surfaces are partially covered with “patches” of high-charge cationic polymer. Indeed, polyelectrolytes having suitable molecular mass and high charge that is associated with a patch-type mechanism of agglomeration (Gregory 1976; Goossens and Luner 1976; Akari et al. 1996; Pfau et al. 1999), tend to be effective dewatering aids (Gruber et al. 1996, 1997). The general principle of patch-type agglomeration was demonstrated by Das and Lomas (1973), who treated half of a batch of cellulosic fines to make them strongly cationic. When such fines were recombined with untreated fines, having negatively charged surfaces, very strong agglomeration was apparent.

As observed by Noda et al. (2005), substantial decreases in dewatering resistance can be achieved by addition of cationic surface-active agents, which are often used as debonders to reduce the dry strength characteristics of the resulting paper. Because one can expect the positively charged groups on the surfactant to become associated with the negative surfaces of the fibers, it follows that the hydrophobic tails of the molecules will be free to self-associate, especially when a pair of fibers comes into contact. Such a mechanism would be expected to result in higher frictional forces between the fibers. The same mechanism also can explain why fiber mats formed in the presence of cationic surfactants also tend to be much more bulky and porous. Unfortunately, strategies based on the mechanism just described are not suitable for the majority of paper grades, where dry strength usually needs to be relatively high.

Inter-fiber friction also can be evaluated by allowing a treated fiber suspension to settle, and then evaluating the density of the sediment formed after a selected period of

time and under standardized conditions (Kline 1967; Alinec and Robertson 1974; Gruber et al. 1997; Hubbe et al. 2001). Relative to the other test methods, sediment volume tests can sense effects resulting from very small and transient forces of attraction or repulsion between fibers. Treatments that tend to increase values of sediment volume also tend to increase dewatering rates under controlled conditions. The effect is illustrated in Fig. 6.



**Figure 6.** Illustration of sediment volume test with papermaking fibers in the absence and presence of a coagulant

As proposed by Lindström (1989), the most effective polyelectrolyte-based dewatering aid programs tend to be those that can be classed as “reversible.” In other words, such systems will tend to form flocs again following application of sufficient hydrodynamic shear to completely redisperse the fibers from each other. The idea is that modern paper machine headboxes and other unit operations exert very strong forces of detachment on fiber systems, causing essentially all of the fiber-to-fiber polymer bridging contacts to be broken at least once before the sheet becomes established in the forming section. But in order for chemical systems to function most effectively as dewatering aids, they need to still have some residual agglomerating ability, even after being subjected to rather intense hydrodynamic shear. In addition to the cationic patch-type treatments already mentioned, microparticle-type drainage aid programs exhibit some reversibility in their flocculation behavior (Lindström 1989; Litchfield 1994; Swerin et al. 1997; Hedborg and Lindström 1996; Hubbe 2001, 2005).

## FIBER ALIGNMENT AND FLOW RESISTANCE

If one could align wet fibers in the manner that combed wet hair becomes aligned, then it is likely that one could achieve a significant decrease in permeability through a fiber mat. Such a mechanism even may be responsible for the “sheet sealing” effects described earlier. Evidence for this kind of mechanism has been obtained in studies where dewatering occurred in the presence of controlled hydrodynamic shear (Forsberg and Bengtsson 1990; Arslan et al. 1997; Paradis et al. 2003). For instance, controlled shear could be applied by using a rotor having the shape of a shallow cone positioned close to the dewatering screen (Paradis et al. 2003).

As a counter-example, it appears that significantly more permeable wet paper mats can be created under conditions where the fiber orientation is chaotic, including a high degree of out-of-plane alignment. It is well known that such chaotic alignment can be achieved by forming paper at relatively high solids content of the suspension. Paper formed under such conditions has been shown to have a reduced resistance to dewatering (Ingmanson and Whitney 1954; Ellis 1981).

## PAPER UNIFORMITY AND VACUUM RESPONSE

The remaining experimental evidence that will be considered has particular relevance to vacuum dewatering, in addition to dewatering within wet-press nips. In a classic piece of investigation Britt and Unbehend (1980) demonstrated positive effects of various dewatering aid treatments on the release of water during simple gravity-filtration of fiber suspensions. But parallel tests, carried out with application of vacuum, gave contradictory results. Rather than aiding in the dewatering, the cationic polymers, when used alone or in combination with an anionic acrylamide-type retention aid, resulted in substantially wetter fiber mats following a standardized application of vacuum.

The inconsistent results were attributed to the formation of persistent fiber flocs by the polyelectrolyte treatments. More rapid dewatering by gravity was achieved due to the ability of water to flow quickly within the large void spaces that surround fiber flocs. But once most of the water has been removed by application of vacuum, the same void spaces allow air to rush ineffectively through the wet web, failing to maintain a pressure differential across the thickness of the sheet. Confirmatory results were obtained by Scalfarotto and Tarvin (1984) and by Wegner (1987). The latter study also showed that the more highly flocculated sheets required longer application of heat in the drying operation in order to evaporate the remaining water.

Britt (1981) recommended at least moderate levels of refining of kraft fibers, in addition to vigorous agitation, in order to achieve uniform formation, as is required for an efficient response to vacuum application. Also, as observed by Britt and Unbehend (1985), the presence of a moderate level of fiber fines in the furnish can significantly improve vacuum dewatering, in comparison to a furnish from which the fines fraction has been removed. Not only do fiber fines tend to fill in void spaces within a wet web of paper, but also, as shown by Youn and Lee (2002), fines in the suspension can reduce the tendency of fibers to flocculate. All of these results are consistent with the formation of a tight, uniform wet-web that does not allow rapid leakage of air. Follow-up experiments on a pilot-scale paper machine showed that the most rapid dewatering could be obtained if, after addition of a highly effective flocculant system, including a highly charged cationic polymer, the furnish was agitated vigorously to fully disperse the fibers from each other (Britt and Unbehend 1980).

As one gets towards the upper limit of solids content that can be achieved by application of vacuum, it can be expected that a significant fraction of the water remaining within paper exists in thin films that occupy spaces between adjacent fibers and other solids (Maloney et al. 1999). Such films can help to explain why plugs of moist fiber that have been subjected to centrifugation tend to be wettest in the lower

layer, where the fibers were pressed together most tightly during dewatering (Abson and Gilbert 1980). As noted by Jones (1998) one can expect vacuum dewatering, at a given pressure differential, to reach a maximum, when all of the pores larger than a critical size have been emptied. Capillary pressures, which are inversely proportional to effective pore radius, can make it impossible to empty smaller pores.

## PUMPING AS A DEWATERING MECHANISM

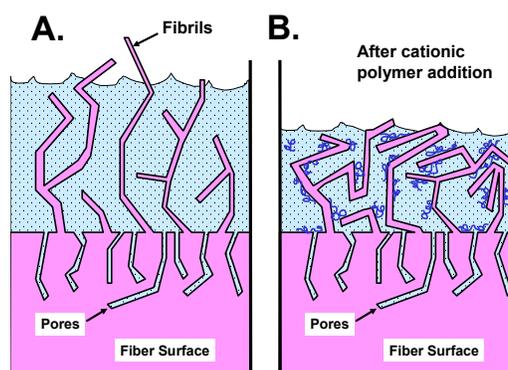
As already noted, the washing action of hydrofoils may tend to counter-act the choke-point mechanism of resistance to dewatering. However, there is a further effect of pulsating vacuum that may play a role once the wet web reaches the vacuum dewatering section of the forming zone. Räsänen et al. (1995) proposed that the part of the wet web nearest to the forming fabric can act as a sort of pump. The way in which this happens is by the layer becoming strongly compressed, when the wet web passes over a slot in a vacuum box. As the compressed layer recovers its equilibrium thickness, it may draw water from the rest of the wet web. The mechanism is supported by the relative futility of applying an individual vacuum pulse too long at a given pressure (Baldwin 1997; Jones 1998). Such a practice might be compared to application of further pressure to a spring after it has become almost fully compressed.

## WATER HELD WITHIN FIBERS

An even more difficult to remove category of water exists within the cell walls of fibers. As mentioned already, such water is commonly estimated by centrifugation of a damp plug of fibers, weighing the damp fibers, and then weighing them again after oven drying (Thode et al. 1960; Jame and Büttel 1968; Scallan and Carles 1972; Scallan and Tigerstrom 1992; Anon. 1981, 2000). The results of such tests are expressed as the water retention value (WRV), which is the ratio of water to fiber solids after centrifugation for a specific time at a specified level of acceleration (Anon. 1981, 2000). Cell-wall water content also can be evaluated by suspending a known mass of fibers in a known mass of water that contains a known concentration of high-mass dextran polymers (Scallan and Carles 1972; Scallan and Tigerstrom 1992; Maloney et al. 1999). One makes the assumption that the dextran molecules are too large to enter small pores within the cell walls of fibers and that they have no significant tendency to adsorb onto cellulose. By measuring the concentration of the sugar molecules in the bulk phase, one then back-calculates what must have been the net volume of pores that were too small to allow entrance of the polymers. Such a method even has been applied in the case of fiber fines, showing that recycled and rewetted fines tend to hold onto much less water, compared to their swollen state before their first cycle of drying (Laivins and Scallan 1996).

It appears doubtful that the swollen state of cellulosic materials can be affected to a significant extent by addition of dewatering aids, though more experimental evidence is needed. Swerin et al. (1990) reported significant decreases in water retention value following treatment of refined fiber suspensions with highly cationic polyelectrolytes of

low to moderate molecular mass. However, related results obtained by Ström and Kunnas (1993) provide evidence that most of the effect of such polyelectrolytes is restricted to a dewatering effect involving fibrillated layers. The mechanism is illustrated in Fig. 6, which depicts coagulation of a fibrillated layer by cationic polyelectrolyte molecules, though it is assumed that such molecules do not readily penetrate into the nanopores of the cell wall. As was noted earlier, the greater effectiveness of higher-mass polyelectrolytes in reducing the WRVs of treated suspensions probably can be attributed to enhanced effectiveness of the charged-patch mechanism of agglomeration, as well as a decreased tendency for the polyelectrolytes to become buried within smaller pores.



**Figure 7.** Illustration of how the coagulating effect of high-charge cationic additive (possibly acting by a charged patch mechanism) might decrease the amount of water held within layers of fibrils at fiber surfaces.

Some limited research has suggested that significant amounts of water can be associated with water-soluble polymers or polymer complexes held within a wet web of paper. For instance, Carlsson et al. (1977) measured significant increases in water retention with increasing levels of cationic acrylamide copolymer addition to mechanical pulp slurries. Hubbe et al. (2007b) observed increases in water retention when glass microfibers were treated with polyampholytes, which are polyelectrolytes having both negative and positive ionic groups. Further research is needed to determine whether such effects can be significant over a broad range of polyelectrolyte types and furnish conditions.

## COMPRESSION RESISTANCE AND WET-PRESS DEWATERING

It can be very challenging in the laboratory to estimate the maximum practical solids levels that can be achieved by wet-pressing of paper. Tests involving application of static pressure are not expected to give realistic predictions, due to the very short periods of time during which a wet web passes through a press nip. A number of researchers have attempted to evaluate wet-press dewatering by using devices that apply a hammer-like impulse (Zotterman and Wahren 1978; Davis et al. 1983; Carlsson 1984; Springer et al. 1989). Tests of wet-press dewatering generally have failed to show significant effects that could be attributed to prior treatment of the furnish with chemical

additives (Wegner 1987), though it is quite likely that the noise-to-signal ratio within the data would have obscured any such effects.

When a wet web of paper passes through a press nip, two types of forces are mainly responsible for preventing crushing of the sheet. One of these components of force results from hydrostatic pressure. Such pressure is a direct consequence of the factors that resist dewatering, i.e. frictional effects as water is squeezed through narrow passageways. But in addition to the hydrostatic component, the compressive forces also are resisted by the mechanical strength of solid components within the paper web (MacGregor 1983ab; Szikla and Paulapuro 1989). The interplay between these two classes of forces helps to explain, among other things, why paper sheets tend to become highly densified in the layers that lie nearest to a porous felt as the sheet passes through a press nip.

Very little research has been carried out to determine whether or not papermaking additives can contribute to the structural component of compression resistance in a wet web of paper. As an exception to this rule Fairchild (1992) showed that paper manufactured with a highly bulky, rosette-shaped form of precipitated calcium carbonate (PCC) filler tended to retain more water content after pressing, in comparison to paper that was made with PCC having a less bulky particle shape.

In the production of many paper grades, and in particular xerographic copy paper, file folder stock, and folding boxboard, it can be a great advantage to maintain a low apparent density in the final product. The challenge comes in trying to figure out how to press water effectively from a wet web of paper without irreversibly densifying it. One of the most promising strategies, in this regard, appears to involve the use of spring-like fibers, having the ability to recover some of their initial three-dimensional character after being squashed flat in a press nip. In this regard, mechanical pulp fibers usually can be described as being “tougher” in comparison to kraft fibers. For instance, it has been proposed to use chemithermomechanical pulp (CTMP) fibers in order to compensate for increased density when paper is prepared with a very high mineral content (Moberg 1985). Another approach involves directing the jet of fiber suspension at a relatively steep angle of impingement onto the forming fabric, i.e. pressure forming. By such means it is possible to achieve a higher proportion of fibers having orientations other than in the plane of the sheet. In principle, one expects there to be a relationship between out-of-plane fiber orientation and the ease of dewatering from paper. Issues of this nature deserve greater study in the future.

## **PRACTICAL STRATEGIES FOR DEWATERING OF PAPER**

Having discussed many different contributing mechanisms to explain the resistance to water release from paper, as it is being formed, this final section will be devoted to a summary of the main strategies that have been used to accelerate such dewatering. The goal here is to translate some of the chemical-related concepts outlined in this review to practical measures that can be implemented in a paper machine system. Items in the following list are arranged roughly in the same order as in the foregoing discussion:

**Table 1.** Common Strategies for Promoting Faster Dewatering

Dewatering Enhancement Strategy	Principle of Action
Minimize refining	Surface area minimization; keeping fibers stiff
Limited furnish treatment with cellulase	Reducing hydrodynamic surface area
Heat up the wet web (steam box)	Reducing viscosity of aqueous solution
Add high-charge cationic polymer or alum	Coagulation of fiber fines and fibrils.
Aim for near-zero zeta potential of solids	“ “ “ “
Use a high-mass polyelectrolyte flocculant	Attaching fiber fines so they can't choke channels
Use once-dried fibers, without more refining	Stiffer fibers forming a bulkier, more porous mat
Agitate flocculated fiber suspension	Breaking up fiber-to-fiber attachments
Employ microparticle retention chemistry	Optimizing reversible attachments, friction
Use filler having less structure	Less resistance to sheet compression
Use filler having less surface area	Less viscous resistance to water flow in web

In summary, most of the principles outlined in this review have the potential to be implemented during industrial operations. Though the mixtures of materials and the flow environments present in a paper machine system generally are too complex to be described in scientific detail, enough is known about the underlying mechanisms to allow an efficient search for new and better means of promoting the release of water. This kind of technology will continue to hold promise for further savings in evaporative energy, as well as for increasing the rates of production on existing papermaking equipment.

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