July 2006, Vol. 1, Issue 1

# BioResources

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

## *In this Issue …*



*And much more …*

Cover image: *Arundo donax* fibers and parenchyma cells, see article by Shatalov *et al.* in this issue.

***BioResources*** is supported by NC State University, with database retrieval and online hosting at www.***bioresources****journal*.com, and backup files and author instructions at ***ncsu.edu/bioresources.***

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

NC STATE UNIVERSITY
College of Natural Resources
Department of Wood and Paper Science
Campus Box 8005
Raleigh, NC 27695-8005
919.515.7707/919.513.3022
919.515.6302 (fax)

# *BioResources*

## Contents: Vol. 1, Issue 1, July 2006 (Inaugural issue)



*BioResources* is a peer-reviewed journal devoted to the science of lignocellulosic materials, chemicals, and applications. The journal is a service of North Carolina State University, College of Natural Resources (http://cnr.ncsu.edu). Articles can be down-loaded without charge as PDF files at www.bioresourcesjournal.com. The *BioResources* website is hosted by Scholarly Exchange, and articles become indexed for scholars through the Open Journal System (http://pkp.sfu.ca/). **Author instructions**, journal policies, an editorial board list, and article files (secondary source) can be found at http://ncsu.edu/bioresources. Correspondence should be directed to co-editors Martin A. Hubbe (hubbe@ncsu.edu, 919-513-3022) and Lucian A. Lucia (lucian.lucia@ncsu.edu, 919-515-7707).

# *BIORESOURCES* – AN ONLINE SCIENTIFIC JOURNAL DEVOTED TO LIGNOCELLULOSIC MATERIALS FOR NEW END USES AND NEW CAPABILITIES

M. A. Hubbe,[a] and Lucian A. Lucia [a]

In this inaugural issue, the Co-Editors of *BioResources* would like to welcome you. In your role as a reader, we welcome you to download scholarly articles and opinion pieces; this is an open-access journal, providing a maximum of potential impact. *BioResources* will deal with new and emerging uses of materials from lignocellulosic sources, including wood and crop residues. We also would like to welcome you as a prospective author. Our goal is to maintain very high standards of peer-review, as well as providing a mix of scholarly research articles, review articles, and editorials. By using an automated, online system of review and publication, we hope to accelerate scientific discourse. Our hope is to contribute to progress in the direction of a post-petroleum economy, taking advantage of the renewable, biodegradable, and relatively abundant nature of materials from lignocellulosic sources.

*Keywords: Bioresources, Online journal, Lignocellulosic, Materials, Biomass*

*Contact information: a: Department of Wood and Paper Science, NC State University, Campus Box 8005, Raleigh, NC 27695-8005 USA; *Corresponding authors: hubbe@ncsu.edu; lucian.lucia@ncsu.edu*

## THE NEED FOR A JOURNAL

In our opinion, rapid and meaningful scientific and technological progress requires a high level of discourse. Suitable forums are needed in which scientists and engineers can exchange ideas and challenge others to work at increasingly high levels of innovation and sound scientific judgment. Discourse is the key, we believe, and that is why we have chosen to encourage editorial opinion pieces and scholarly review articles, in addition to research articles. We also believe that the peer-review process is centrally important as a scientific community grapples with the challenge of establishing new scientific knowledge and separating what can be proven from what cannot.

As Co-Editors, we were motivated to start the online, peer-reviewed publication *BioResources* in response to a growing urgency of efforts to grow a bio-based economy. Because cellulose and lignin rank as the first and second most abundant chemicals resulting from photosynthesis, it is clear that these materials, as well as byproducts of lignocellulosic materials, will play a central role in efforts to create such an economy.

While there exist high-quality research journals dealing with the science and technology of lignocellulosic materials and their uses, we intend *BioResources* to encompass a unique set of features. Emphasis will be placed on consequences of science that lead in the direction of sustainable technology. Though there is a need for work over a broad range of biomass issues, our focus will be mainly on lignocellulosic materials, such as wood, paper, and crop residues. Our target audience will include scientists and policy makers, some of whom have considerable experience within fields such as wood

science, biomaterials, and papermaking science. Our target audience also will include people who are urgently looking to solve ecological problems or replace traditional manufacturing processes with more environmentally benign, green technologies.

## THE OPEN-ACCESS FORMAT

As we contemplated publishing a journal in the field of lignocelluosic science, the Co-Editors of *BioResources* considered the following hallmarks of high-quality scientific journals:

- Relatively prompt, efficient process of peer-review
- Emphasis on content, rather than form
- Distribution as broadly as possible, *i.e.* high impact

In our opinion, a web-based format can meets these objectives in an ideal way. Because the entire process takes place online, using an automated system for reviewing articles and adding articles to the journal, it is possible to avoid various delays associated with printing and distribution. The process can be further streamlined, as we have chosen to do in the case of *BioResources*, by asking the authors to format articles in a "ready-to-upload" condition. Though many journals are subscription-based, support from our university has made it possible for us to avoid the need for passwords or subscriptions. The open-access nature of *BioResources* is intended to maximize people's access to a body of scientific discourse that will have an impact on the future of all of us.

## HOW YOU CAN HELP

Regardless of the manner in which a scientific journal is produced, most of the work is a labor of love. Much of it is volunteer labor, pure and simple. Scholarly journals depend on the generosity of scholars, working as volunteers, to judge the overall quality and intellectual soundness of submitted articles. Authors, too, often are required to work for the hope of glory and progress, rather than expecting significant monetary rewards for their work in preparing scholarly articles. The Co-Editors of *BioResources*, though we don't consider ourselves to be volunteers, are likewise embarking on this venture as an added part of our work. We will be using advances in software, where we can, to streamline the process of reviewing and posting articles, but we are committed to a doing a lot of hard work to make this journal a success.

You can help. The first way you can help is by contributing high quality research articles that fit within the scope of our journal. More information about the journal's scope appears elsewhere on the http://www.ncsu.edu/bioresources website. We are especially enthusiastic when we receive an article that conveys scientific or technical information in an engaging way, *i.e.* telling a story that will capture the attention of other readers. Because of the Internet, as well as some databases to which we subscribe, you can be sure that your article will be picked up by literature searches.

Other ways that you can help are by volunteering to review articles, or by nominating a highly qualified person to serve on our Editorial Board. Our e-mail addresses appear below the title to this editorial. Even if your only role is as a reader of articles in *BioResources*, we want to express our deep appreciation to you and our commitment to the research community that we serve.

# COMPARATIVE STUDY OF LIPOPHILIC EXTRACTIVES OF HARDWOODS AND CORRESPONDING ECF BLEACHED KRAFT PULPS

Carmen S. R. Freire, Paula C. R. Pinto, Ana S. Santiago, Armando J. D. Silvestre, Dmitry V. Evtuguin and Carlos Pascoal Neto*

The lipophilic extractives of *Eucalyptus globulus*, *Eucalyptus grandis*, *Eucalyptus urograndis*, *Betula verrucosa* and *Acacia mangium* woods and of the corresponding ECF bleached kraft pulps, were characterized by GC-MS. The five hardwoods showed significant differences in the content and composition of the main families of extractives, namely fatty acids, long chain aliphatic alcohols and sterols. Significant differences in the composition persist after wood pulping and ECF bleaching of pulps. The fate of the various types of extractives during the wood and pulp processing is discussed. Long chain aliphatic acids and alcohols are quite stable during the pulp production and are retained to a great extent in the final bleached pulp; $\Delta^5$ sterols are mostly oxidized and partially retained in the pulps, while $\Delta^7$ sterols are completely degraded and/or dissolved. *B. verrucosa* and *A. mangium* bleached pulps show contents of fatty acids about 4 and 20 times higher than that of *Eucalyptus* pulps, respectively, while the content of long chain aliphatic alcohols in *A. mangium* pulp is of the order of 100 times higher than *Eucalyptus* and *B. verrucosa* pulps.

*Keywords: Lipophilic extractives; Hardwoods; Eucalyptus; Betula; Acacia; GC-MS analysis; ECF bleaching*

*Contact information: CICECO and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; *Corresponding author: cneto@dq.ua.pt*

## INTRODUCTION

Plantation hardwoods such as *Eucalyptus globulus*, *Eucalyptus grandis* and *Eucalyptus urograndis*, represent nowadays the major fiber sources for the pulp and paper industry in the Iberian Peninsula and South America (Hillman 2002). *Acacia* species, namely *Acacia mangium*, are also becoming important wood sources for the pulp-and-paper industry in the Asiatic region (Coleman 1998; Hillman 2002).

The lipophilic fraction of wood extractives, even present in small amounts in wood, may play an important role in pulp and paper production (Back and Allen 2000). The concentration and composition are determinants in the efficiency of pulping and bleaching processes. The extractives retained in the bleached pulp may affect significantly the physico-chemical properties of fibers, such us their surface energy, and contribute to pitch deposition in the papermaking process (Back and Allen 2000).

*E. globulus* wood extractives have been studied extensively (Swan and Åkerblom 1967; Santos et al. 1997; Wallis and Wearne 1997; Gutíerrez et al. 1998, 1999; Freire et

al. 2002a), as well as their behavior during kraft pulping and bleaching processes (Gutiérrez et al. 2001a; Gutíerrez et al. 2001b; Freire et al. 2003, 2005, 2006) and their relationship with pitch composition (Gutiérrez et al. 1998; del Río et al. 1998, 2000, Silvestre et al. 1999; Freire et al. 2002b, 2005). However, to our knowledge no significant investigations related to *E. urograndis* and *E. grandis* have been reported. In addition, only a few studies on the chemical composition of *Acacia mangium* wood (Pietarinen et al. 2003) have been published. Recently, a study of the surface and bulk composition of bleached kraft pulps of *Eucalyptus globulus*, *Eucalyptus grandis* and *Eucalyptus urograndis*, *Acacia mangium* and *Betula verrucosa* has been published (Pascoal Neto et al. 2004), pointing out significant differences in pulps compositions.

In the present study, the lipophilic extractives of these five hardwoods species were extensively characterized. The corresponding DEDED bleached kraft pulps were also analyzed for their lipophilic extractives, aiming to assess the fate of the different components during kraft pulping and ECF bleaching processes and the potential impact of extractives content and composition of bleached fibers on their properties and on the papermaking process.

## EXPERIMENTAL

### Samples

*Eucalyptus globulus* wood was selected from an industrial clone plantation cultivated in the region of Aveiro, Portugal. *Eucalyptus urograndis* and *Eucalyptus grandis* industrial woods were issued from Brazil. *Betula verrucosa* and *Acacia mangium* woods were obtained from pulp industries in Sweden and Indonesia, respectively.
Representative chip samples of the different wood species were ground and sieved, and the granulometric fraction of 40-60 mesh was used for analysis.

Wood chips of industrial size were kraft pulped to kappa number 15-16 in a laboratory circulation batch digester (Table 1); the pulps were then bleached (90% ISO brightness) by a conventional Elemental Chlorine Free (ECF) sequence (DEDED), using standard conditions, and then washed until pH ~7 and dried at room temperature before extraction.

**Table 1.** Conditions and Results of Kraft Pulping of the Different Hardwoods and $ClO_2$ Consumption During the ECF Bleaching of the Corresponding Kraft Pulps.

| | *E. globulus* | *E. urograndis* | *E. grandis* | *B. verrucosa* | *A. mangium* |
|---|---|---|---|---|---|
| **Kraft pulping*** | | | | | |
| Active alkali, % $Na_2O$ | 16 | 20 | 19 | 18 | 24 |
| Time at temperature, min | 50 | 65 | 60 | 65 | 65 |
| Pulp yield, %/ wood | 55.6 | 49.6 | 50.6 | 49.8 | 51.1 |
| Kappa number | 15.0 | 15.7 | 16.1 | 16.4 | 15.9 |
| Viscosity, ml/g | 1460 | 1130 | 1190 | 1340 | 990 |
| **ECF bleaching (90% ISO)** | | | | | |
| $ClO_2$ consumption, %/ pulp | 4.4 | 5.4 | 5.3 | 7.2 | 7.4 |

* Sulphidity=28%; Temperature=160 ºC.

## Extraction

The ground wood samples (three replicates of 20 g) were Soxhlet extracted with dichloromethane for 6 hours, while the bleached pulps (three replicates of 20 g) were Soxhlet extracted with acetone for 6 hours. Dichloromethane was selected as the solvent for wood instead of acetone because of its selectivity for lipophilic components, simplifying the analysis of the complex wood extracts. However, the nature and amount of lipophilic compounds extracted from the bleached pulps with the two solvents is similar, as confirmed by preliminary analysis, allowing the comparison of wood and bleached pulp lipophilic extractives composition. Acetone was used in the case of pulps in order to be consistent with the procedures used in a wider research project. The solvents were evaporated to dryness and the extracts were weighed. The results were reported as weight percent of the respective dry woods or pulps.

## GC-MS Analysis

The GC-MS analysis and quantification of the major lipophilic wood and bleached pulps extractives (before and after alkaline hydrolysis in the case of wood) were performed as previously described (Freire et al. 2002a). Nearly 20 mg of each dried sample were trimethylsilylated (Ekman 1983a) and injected three times. GC-MS analyses were performed using a Trace Gas Chromatograph 2000 Series equipped with a Finnigan Trace MS mass spectrometer, using helium as carrier gas (35 cm/s), equipped with a DB-1 J&W capillary column (30 m x 0.32 mm i.d., 0.25 μm film thickness). The chromatographic conditions were as follows: initial temperature: 80 ºC for 5 min; temperature rate: 4 ºC/min; final temperature: 285 ºC for 10 min; injector temperature: 290 ºC; transfer-line temperature: 290 ºC; split ratio: 1:100.

In order to verify the presence of esterified structures such as triglycerides, steryl esters, waxes and ferulic acid esters, the extracts were also analyzed by GC-MS, using a short length column (DB-1 J&W capillary column of 15 m x 0.32 mm i.d., 0.25 μm film thickness). The chromatographic conditions were as follows: initial temperature: 100 ºC for 3 min; temperature rate: 5 ºC/min; final temperature: 340 ºC for 12 min; injector temperature: 320 ºC; transfer-line temperature: 290 ºC; split ratio: 1:100. The partial chromatograms of the dichloromethane hardwoods extracts, obtained using this column, are presented in Fig. 1.

For quantitative analysis, the GC-MS was calibrated with pure reference compounds, representative of the major lipophilic extractives components, namely, hexadecanoic acid (97 % purity, Fluka Chemie, Madrid, Spain), 1-eicosanol (98 % purity, Fluka Chemie, Madrid, Spain),, 16-hydroxyhexacosanoic acid, 2-hydroxyoctadecanoic acid, stigmasterol (95 % purity, Sigma Chemicals Co., Madrid, Spain) and ferulic acid (99 % purity, Sigma Chemicals Co., Madrid, Spain), relative to tetracosane (99 % purity, Sigma Chemicals Co., Madrid, Spain), the internal standard used. The respective multiplication factors needed for the quantification of the peak areas were calculated as an average of six GC-MS runs.

Compounds were identified, as TMS derivatives, by comparing their mass spectra with the GC-MS spectral library, with data from the literature and in some cases, by injection of standards.

The approximate percentage of extractives removal from wood during the pulping and bleaching processes was calculated assuming the kraft pulping yields (Table 1) and neglecting the yield losses during the bleaching of pulps.

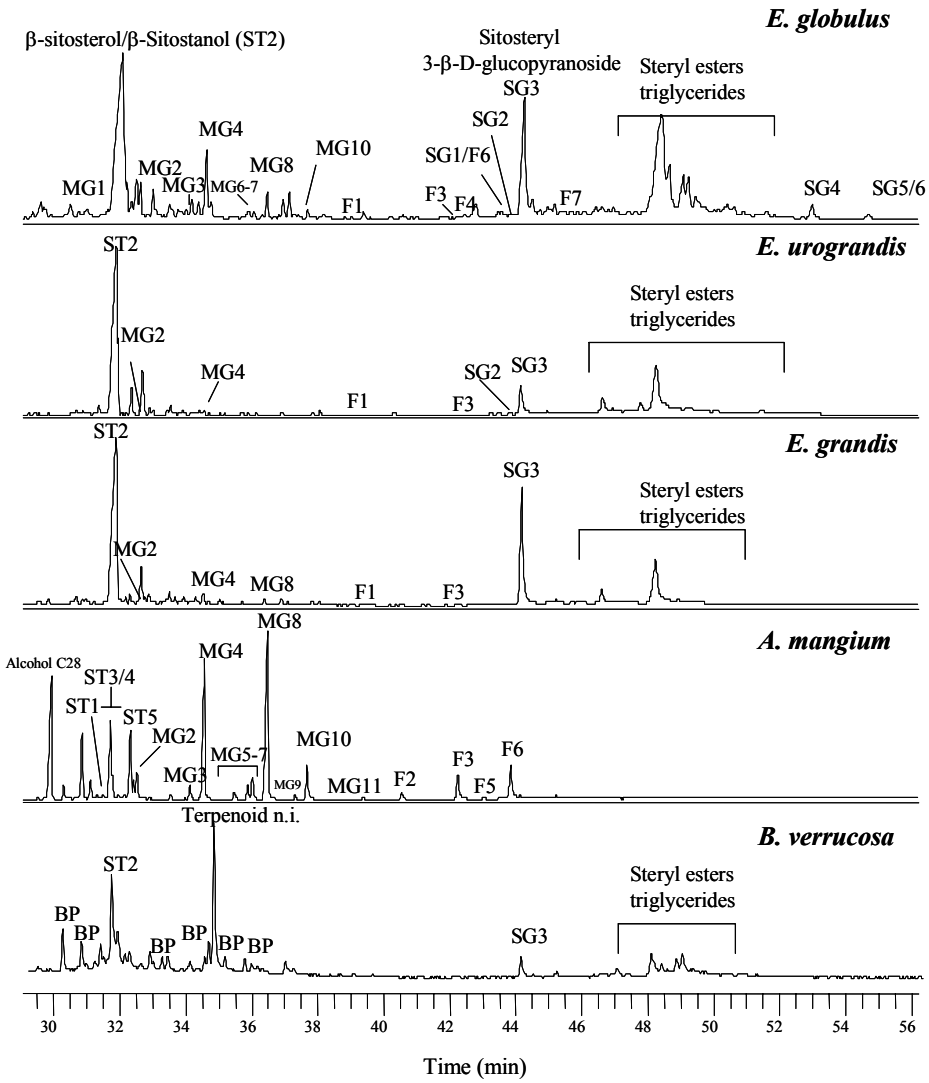



**Fig. 1.** Partial ion chromatogram, obtained using a short length column (15m), of the derivatized dichloromethane extracts of *E. globulus*, *E. urograndis*, *E. grandis*, *A. mangium* and *B. verrucosa* woods. **MG1**- 1-monodocosanoylglycerol, **MG2**- 1-monotetracosanoyl glycerol, **MG3**- 2-hexacosanoyl-glycerol, **MG4**- 1-monohexacosanoylglycerol, **MG5**- 2-mono(24-hydroxytetracosanoyl)glycerol, **MG6**- 1-mono(24-hydroxytetracosanoyl)glycerol, **MG7**- 2-mono-octacosanoylglycerol, **MG8**- 1-mono-octacosanoyl-glycerol, **MG9**- 2- mono(26-hydroxyhexacosanoyl)glycerol, **MG10**- 1-mono(26-hydroxyhexacosanoyl)-glycerol, **MG11**- 1-mono(28-hydroxyoctacosanoyl)glycerol; **F1**- docosanyl ferulate, **F2**- tetracosanyl ferulate **F3**-hexacosanyl ferulate, F**4**- feruloyloxydocosanoic acid, **F5**- heptacosanyl ferulate, **F6**- octacosanyl ferulate and **F7**- feruloyloxyhexacosanoic acid; **ST1**- 24-methyl-5α-cholest-7-en-3β-ol **ST2-** β-sitosterol + β-sitostanol, **ST3**- 24-ethyl-5α-cholest-7, trans 22-dien-3β-ol , **ST4-** ergosterol (or an isomer), **ST**5-24-ethyl-5α-cholest-7-en-3β-ol; **SG1**- campesteryl 3-β-*D*-glucopyranoside, **SG2**- stigmasteryl 3-β-*D*-glucopyranoside, **SG3**- sitosteryl 3-β-*D*-glucopyranoside, **SG4**- sitosteryl (6´-O-palmitoyl)-3-β-*D*-glucopyranoside, **SG5**-sitosteryl (6´-O-linoleoyl)-3-β-*D*-glucopyranoside, **SG6-** sitosteryl (6´-O-oleoyl)-3-β-*D*-glucopyranoside **BP**-betulaprenols.

**Table 2.** Families of Lipophilic Components Identified in the Dichloromethane Extracts of Woods and Their Contents Before (BH) and After (AH) Alkaline Hydrolysis of the Extract (mg of compound/kg o.d. wood.)

| Family | *E. globulus* | | *E. urograndis* | | *E. grandis* | | *B. verrucosa* | | *A. mangium* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **BH** | **AH** | **BH** | **AH** | **BH** | **AH** | **BH** | **AH** | **BH** | **AH** |
| **Fatty acids** | | | | | | | | | | |
| Saturated | 130.0 | 288.6 | 172.5 | 393.4 | 334.4 | 351.9 | 1426.1 | 1503.0 | 1379.9 | 4623.3 |
| Unsaturated | 88.8 | 121.1 | 106.0 | 185.9 | 90.5 | 111.2 | 490.4 | 454.0 | 40.6 | 66.5 |
| Diacids | 10.2 | 29.0 | 0.00 | 15.0 | 5.75 | 10.9 | 400.4 | 468.1 | 0.00 | 0.00 |
| α-hydroxy | 0.00 | 14.5 | 10.5 | 22.0 | 34.7 | 46.9 | 52.6 | 70.3 | 64.7 | 102.7 |
| ω-hydroxy | 0.00 | 67.7 | 0.00 | 155.8 | 7.27 | 98.4 | 54.4 | 61.8 | 221.2 | 1882.4 |
| Other hydroxy acids | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 301.4 | 214.5 | 0.00 | 0.00 |
| Total | **229.0** | **520.9** | **289.0** | **772.1** | **472.6** | **619.3** | **2725.3** | **2771.7** | **1706.4** | **6674.9** |
| **Aromatic compounds** | | | | | | | | | | |
| **Cinnamic acids (**ferulic and coumaric acids**)** | 0.00 | 59.4 | 0.00 | 223.4 | 1.17 | 86.5 | 8.98 | 15.8 | 7.50 | 572.1 |
| **Others** | 38.1 | 40.8 | 115.2 | 72.4 | 91.7 | 61.9 | 41.6 | 58.0 | 0.00 | 21.5 |
| **Total** | **38.1** | **100.2** | **115.2** | **295.8** | **92.87** | **148.4** | **50.58** | **73.8** | **7.50** | **593.6** |
| **Long Chain Aliphatic Alcohols** | | | | | | | | | | |
| < C20 | 38.9 | 43.4 | 8.29 | 5.30 | 6.14 | 11.9 | 10.6 | 14.9 | 9.17 | 39.3 |
| > C20 | 0.00 | 12.9 | 8.91 | 38.2 | 12.9 | 25.9 | 7.14 | 16.5 | 1616.8 | 1809.6 |
| Total | **38.9** | **56.3** | **17.20** | **43.50** | **19.04** | **37.8** | **17.74** | **31.4** | **1625.97** | **1848.9** |
| **Sterols** | **318.5** | **474.4** | **537.4** | **642.1** | **729.8** | **811.2** | **219.8** | **212.2** | **657.4** | **544.9** |
| **Betulaprenols/ other terpenes** | **10.8** | **9.02** | **0.00** | **0.00** | **0.00** | **16.9** | **174.01** | **109.9** | **0.00** | **0.00** |
| **Monoglycerides** | **22.0** | **0.00** | **26.4** | **0.00** | **15.6** | **0.00** | **0.00** | **0.00** | **962.6** | **0.00** |
| **Others/ Unidentified** | **202.2** | **90.3** | **512.0** | **183.9** | **113.7** | **52.7** | **723.4** | **364.0** | **1323.9** | **553.4** |
| **TOTAL** | **859.5** | **1251.0** | **1497.2** | **1937.4** | **1443.6** | **1686.3** | **3910.8** | **3563.0** | **6283.8** | **10215.7** |

## RESULTS AND DISCUSSION

### Lipophilic Extractives of Woods

The dichloromethane extractives of *E. globulus, E. grandis* and *E. urograndis* account, respectively, for 0.26%, 0.36% and 0.35 % of dry wood basis. These values are considerably lower than those found for *B. verrucosa* and *A. mangium* (1.31 and 1.32 % of dry wood basis, respectively). The lipophilic extractives content found for the *A. mangium* and *B. verrucosa* are in good agreement with the figures reported for *Acacia* (Pietarinen et al. 2003) and *Betula species* (Ekman and Holmbom 2000).

Sterols, fatty acids, long chain aliphatic alcohols and aromatic compounds are the main families of compounds found in the lipophilic extracts of these hardwood species. Sterols are among the major lipophilic components of *Eucalyptus* woods lipophilic extractives, particularly after hydrolysis of the extracts (Table 2). For the other hardwoods, although sterols were also found in significant amounts, they are minor components of the corresponding extracts.

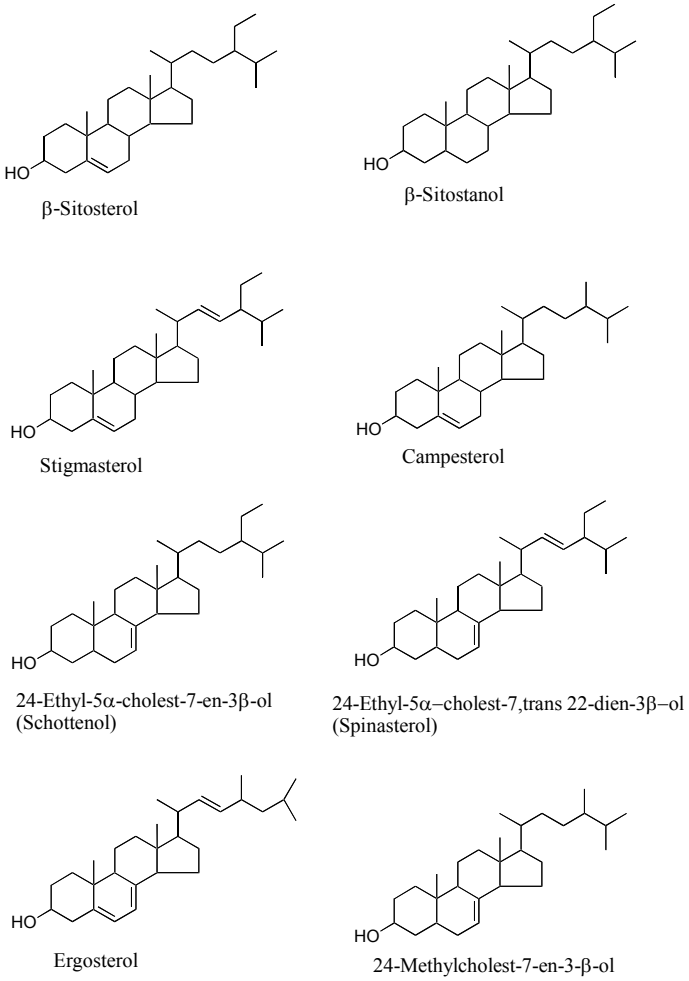



**Fig. 2**. Structures of the main sterols found in the investigated hardwoods.

β-Sitosterol and β-sitostanol (Fig. 2), the main components of the wood sterols group, were found in all *Eucalyptus* woods in both free and esterified forms, as confirmed by their increased content observed after alkaline hydrolysis of the extracts (Table 2) as well as by the analysis of the extracts by GC-MS with short columns (Fig. 1). *E. grandis* and *E. urograndis* woods contain sterols, particularly free sterols, in larger quantities than in *E. globulus.* β-Sitosterol and β-sitostanol, mostly in the free form, are also the main sterols found in *B. verrucosa* wood (Table 2, Fig. 1).

Among the major components of the *A. mangium* extract, two $\Delta^7$ sterols (Fig. 2) were identified by EI-MS as 24-ethyl-5α-cholest-7, trans 22-dien-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol (Artaut et al. 1984, Combaut 1986). In addition, small amounts of 24-methyl-5α-cholest-7-en-3β-ol and ergosterol (or an isomer) were also detected. The $\Delta^7$ sterols are not very common wood components, and only 24-ethyl-5α-cholest-7-en-3β-ol and ergosterol have been reported to occur in *Acacia* species (Pietarinen et al. 2003). The common $\Delta^5$ phytosterols, such as stigmasterol and β-sitosterol (Fig. 2), were not found in the *A. mangium* lipophilic extract. Recently, the identification of steryl esters in *Acacia mangium* and *Acacia crassicarpa* wood has been reported (Pietarinen et al. 2003). We have not detected any steryl esters in our *A. mangium* wood (Fig. 1). Such differences may be certainly assigned to specific plant growing conditions.

Fatty acids represent the major lipophilic components of the hardwoods investigated in this study (except for *E. grandis*, where sterols dominate). *A. mangium* and *B. verrucosa* have a content of these components (Table 2) much higher than in *Eucalyptus* woods. The qualitative compositions of fatty acids of the five hardwoods also show significant differences between them (Table 2, Fig. 3).

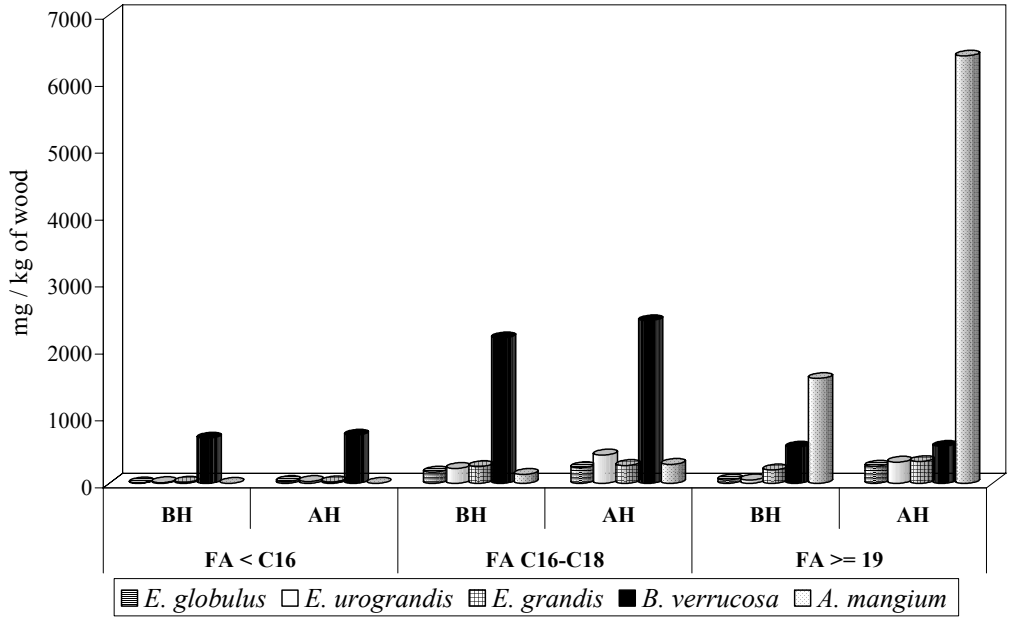



**Fig. 3.** Major fatty acids present in the dichloromethane extracts of the investigated hardwoods, before (**BH**) and after alkaline (**AH**) hydrolysis. **FA<C16** fatty acids with less than 16 carbon

atoms; **FA C16-C18** fatty acids with 16 to18 carbon atoms and **FA>=19** fatty acids with more than 19 carbon atoms.

Palmitic acid (C16) and the unsaturated oleic (C18:1) and linoleic (C18:2) acids are by far the most abundant fatty acids identified in *B. verrucosa* wood (Table 2, Fig. 3), as already reported (Ekman and Holmbom 2000). There are considerable amounts of eicosanoic (C20), heneicosanoic (C21) and docosanoic (C22) acids, and of several diacids and acids with less than 16 carbon atoms, such as heptanedioic, octanedioic, azelaic (nonanoic), decanedioic acids and hexanoic, heptanoic, octanoic and nonanoic acids, respectively, were also detected in *B. verrucosa* wood. All these fatty acids were found to occur almost exclusively in the free form (Table 2, Fig. 3), which is in close agreement with the small amounts of steryl esters and glycerides detected by GC-MS with short columns in this species (Fig. 1), as well as by the small increase observed on the amount of fatty acids detected after alkaline hydrolysis of the extract (Table 2).

On the other hand, *Eucalyptus* and *A. mangium* woods contain fatty acids in both free and esterified form as confirmed by the significant increases of fatty acids contents detected after alkaline hydrolysis of the extracts (Table 2, Fig. 3). The long chain tetracosanoic (C24), hexacosanoic (C26) and octacosanoic (C28) acids are the most abundant free and esterified fatty acids present in *A. mangium* wood. The esterified fraction of these fatty acids is largely due to 1-monoglycerides (1-hexacosanoyl glycerol and 1-octacosanoyl glycerol) (Fig.1), as will be discussed below. The common C16-C18 (palmitic, oleic and linoleic acids) fatty acids are minor components of *A. mangium* wood (Fig. 3). However, in the case of *Eucalyptus* woods extracts, the most abundant free fatty acids are palmitic, oleic and linoleic acids, whereas the esterified fraction is, to a great extent, ascribed to higher fatty acids, as recently reported for *E. globulus* (Freire et al. 2002a).

The long chain α- and ω- hydroxy fatty acids, such as 2-hydroxydocosanoic, 2-hydroxytetracosanoic and 22-hydroxydocosanoic, 24-hydroxytetracosanoic and 26-hydroxyhexacosanoic acids, recently identified in *E. globulus* wood (Freire et al. 2002a), were also detected in considerable amounts in *E. urograndis*, *E. grandis* and *A. mangium* woods (Table 2). However, in these hardwoods, although the hydroxy fatty acids were mainly detected after alkaline hydrolysis of the extracts, they were also found in free form, whereas in *E. globulus,* they were detected only after alkaline hydrolysis. The main ω- and α-hydroxy fatty acids found in the dichloromethane extract of *B. verrucosa* wood (before and after alkaline hydrolysis) were 8-hydroxyoctanoic, 9-hydroxynonanoic acids and 2-hydroxyoctanoic, 2-hydroxyhexadecanoic and 2-hydroxytetracosanoic acids, respectively. 2-hydroxydecanedioic and some unknown hydroxyoctadecanoic acids were also detected.

As recently reported by Pietarinen et al. (2003), monoglycerides with C24, C26 and C28 saturated acids represent an important fraction of *A. mangium* wood extracts. In addition, minor amounts of monoglycerides with 24-hydroxytetracosanoic, 26-hydroxyhexacosanoic and 28-hydroxyoctacosanoic acids were also detected in our *Acacia* wood. *Eucalyptus* woods, particularly *E. globulus*, also contain monoglycerides with long chain aliphatic acids (> C20) and ω-hydroxy fatty acids. Monoglycerides of C26, C28 and hydroxy fatty acids are reported here for the first time as *Eucalyptus* woods components and, in the case of *E. globulus*, can partially explain the high amounts of

fatty acids with more than C20 observed after alkaline hydrolysis of the extract. On the other hand, *B. verrucosa* wood contains very small amounts of monoglycerides.
There were diglycerides and triglycerides found in all wood species, comparatively in small amounts with respect to the other families of extractives.

Long chain aliphatic alcohols (free and esterified) represent only a small fraction of the total extractives of the three *Eucalyptus* woods and of *B. verrucosa*. However, they represent the second most abundant group of compounds present in the *A. mangium* wood extract, with 1-tetracosanol, 1-hexacosanol and 1-octacosanol as the main components. Around 12% of these fatty alcohols are esterified with ferulic acid as confirmed by the identification of considerable amounts of the corresponding ferulates, by GC-MS analysis with short columns (Fig. 1). Ferulic acid esters with fatty alcohols have already been reported to occur in *Acacia mangium* wood (Pietarinen et al. 2003).

Small amounts of ferulates with C22, C24 and C26 fatty alcohols were also detected in *Eucalyptus* woods. In addition, two ferulates with ω-hydroxy fatty acids, not identified in the other hardwoods, were also detected in *E. globulus* wood (Freire et al. 2002a). However, in *Eucalyptus* woods, ferulates were identified in very small amounts and consequently, could not explain the high amounts of ferulic acid detected after alkaline hydrolysis of the corresponding extracts. Coumaric acid (*cis* and *trans* forms) was also detected in considerable amounts, after alkaline hydrolysis of *E. grandis* and *urograndis* extracts, although coumaric acid esters were not detected before hydrolysis. The origin of the ferulic and coumaric acids in *Eucalyptus* woods is under investigation. Vanillic, syringic, vanillin and other aromatics (referred as *Aromatic compounds* in Table 2) were detected in all hardwoods.

*B. verrucosa* wood contains considerable amounts of a particular family of terpenes, namely betulaprenols (Table 2) (Ekman and Holmbom 2002). These compounds were not found in the other investigated hardwoods. Only some triterpenic acids, such as oleanolic acid, were identified in small amounts in *Eucalyptus* extracts.

Finally, sitosteryl 3-β-D-glucopyranoside, already identified in *E. globulus* (Gutiérrez et al. 2001b) and *Betula* species woods (Nilvebrant and Byström 1995), was also detected in considerable amounts in *E. urograndis and E. grandis* woods. However, no steryl glucosides were detected in *A. mangium* wood, at least in detectable amounts.

## Lipophilic Extractives of ECF Bleached Kraft Pulps

The acetone extractives found in *E. globulus*, *E. urograndis*, *E. grandis*, *B. verrucosa* and *A. mangium* ECF (DEDED) bleached pulps account for 0.37%, 0.16%, 0.27 %, 0.72% and 1.53% of dry pulp basis, respectively. Fatty acids, long chain aliphatic alcohols and sterols keep as the main lipophilic extractives present in the bleached pulps (Table 3). However, significant qualitative and quantitative differences may be pointed out when comparing the different pulps, between them, and with the corresponding wood extractives composition.

Sterols are extensively removed from hardwoods during pulping and ECF bleaching stages. Specifically, around 70%, 80%, 90%, 50% and 100% of sterols are removed from *E. globulus*, *E. urograndis*, *E. grandis*, *B. verrucosa* and *A. mangium* woods, respectively, during kraft pulping and the DEDED bleaching sequence.

The major fraction of sterols identified in the *Eucalyptus* woods and *B. verrucosa* bleached pulps is ascribed to several oxidation products of β-sitosterol (Table 3, Fig. 4), recently reported to be the major products from β-sitosterol oxidation with chlorine dioxide (Freire et al. 2003). β-Sitostanol is quite abundant in these ECF bleached pulps (Table 3).

**Table 3**. Families of Lipophilic Components Identified in the Acetone Extracts of the DEDED Bleached Pulps (mg of compound/kg o.d. pulp).

| | *E. globulus* | *E. urograndis* | *E. grandis* | *B. verrucosa* | *A. mangium* |
|---|---|---|---|---|---|
| **Fatty acids** | | | | | |
| Saturated | 502.1 | 340.9 | 262.3 | 2070.1 | 7069.7 |
| Saturated diacids | 8.90 | 20.2 | 8.67 | 38.1 | 146.4 |
| α-hydroxy | 5.61 | 29.7 | 24.1 | 0.0 | 102.3 |
| ω-hydroxy | 85.0 | 175.9 | 137.0 | 0.0 | 3184.1 |
| dihydroxiacids | 8.74 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | **610.35** | **566.7** | **432.07** | **2108.2** | **10502.5** |
| **Aromatic compounds** | **12.5** | **4.45** | **0.876** | **13.1** | **6.3** |
| **Long Chain Aliphatic Alcohols** | | | | | |
| < C20 | 17.1 | 12.4 | 11.8 | 12.9 | 18.7 |
| > C20 | 19.8 | 28.1 | 23.8 | 7.12 | 3295.1 |
| Total | **36.9** | **40.5** | **35.6** | **20.02** | **3313.8** |
| **Sterols** | | | | | |
| β-Sitosterol | 15.6 | 9.10 | 4.57 | 8.45 | - |
| β-Sitostanol | 76.1 | 97.3 | 43.1 | 88.3 | - |
| Oxidation products of sterols | 178.6 | 169.2 | 89.43 | 131.3 | - |
| **Total** | **270.3** | **275.6** | **137.1** | **228.1** | **0.0** |
| **Short Chain acids** | **27.1** | **12.0** | **8.78** | **68.4** | **383.2** |
| **Alkenes** | **87.8** | **31.7** | **14.1** | **79.9** | **0.0** |
| **Others/Unidentified** | **178.8** | **145.7** | **41.2** | **196.3** | **421.7** |
| **TOTAL** | **1223.8** | **1076.7** | **673.8** | **2714.0** | **14627.5** |

$\Delta^7$ sterols from *A. mangium* wood are completely degraded/removed during kraft pulping and ECF bleaching (Table 3). This behavior could be in part explained by the highest active alkali and $ClO_2$ consumptions observed during pulping and bleaching of *A. mangium* wood (Table 1). However, in the case of *B. verrucosa* wood, the active alkali and $ClO_2$ charge were also high and the percentage of removal of sterols is much lower than that observed for the *A. mangium* wood. Therefore, a complementary explanation might be related, with an easier removal of the oxidation products of $\Delta^7$ sterols formed during chlorine dioxide bleaching, when compared with the oxidation products of the corresponding $\Delta^5$ sterols (mainly β-sitosterol).

Fatty acids, particularly those <C20, are also extensively removed during pulping and ECF bleaching processes. The percentage of removal of fatty acids from the *A. mangium* wood was quite lower (around 20%) than that observed for the other hardwoods

(~60% for the *E. urograndis*, *E. grandis* and *B. verrucosa* wood and 40% for *E. globulus* wood), which can be directly related with the higher abundance of long chain fatty acids (> C20). Saturated fatty acids higher than C20 are the major fatty acids present in all bleached pulps (Fig. 5), which confirms that saturated fatty acids are very stable under the ECF bleaching conditions and that the fraction of fatty acids with less than C20 are relatively easily removed during pulping and bleaching stages.

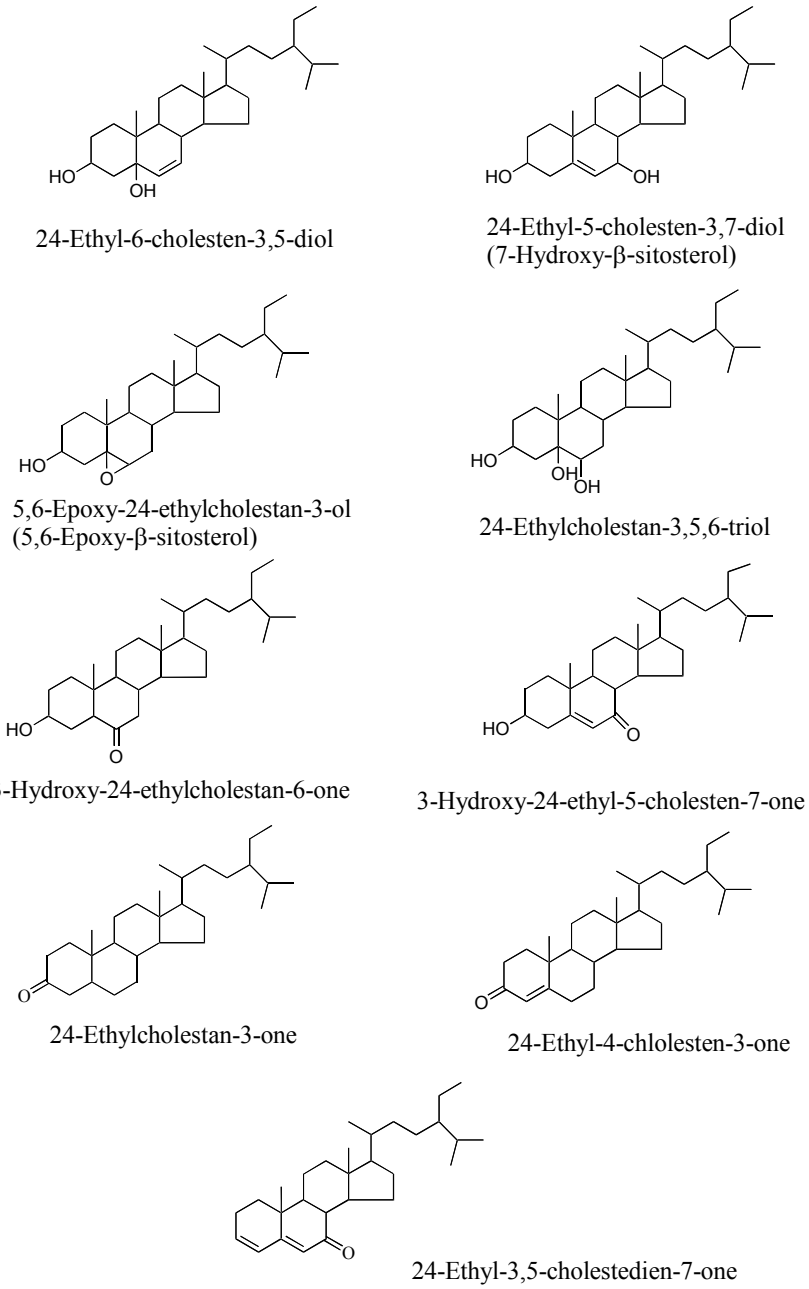



**Fig. 4**. Structures of the oxidized derivatives of sterols (mainly β-sitosterol) found in the DEDED bleached pulps.

Unsaturated fatty acids, such as oleic and linoleic acids, are minor components of bleached pulps since they are easily oxidized during $ClO_2$ bleaching (Freire et al. 2003). α- and ω-hydroxy fatty acids (> C20) are also very stable under chlorine dioxide bleaching and are especially difficult to remove from pulps (Freire et al. 2003). These fatty acids represent an important fraction of *Eucalyptus* and *A. mangium* bleached pulps lipophilic extractive components (Table 3). The identification of the long chain hydroxy fatty acids in these bleached pulps is of particular importance because of their tendency for pitch formation, recently demonstrated in a *Eucalyptus globulus* ECF bleached kraft pulp mill (Silvestre et al. 1999; Freire et al. 2002b). There were no hydroxy fatty acids detected in the *B. verrucosa* bleached pulp because the major components of this family present in the corresponding wood have carbon chains lower than 16 atoms and, therefore, are easily removed during pulping and bleaching processes.

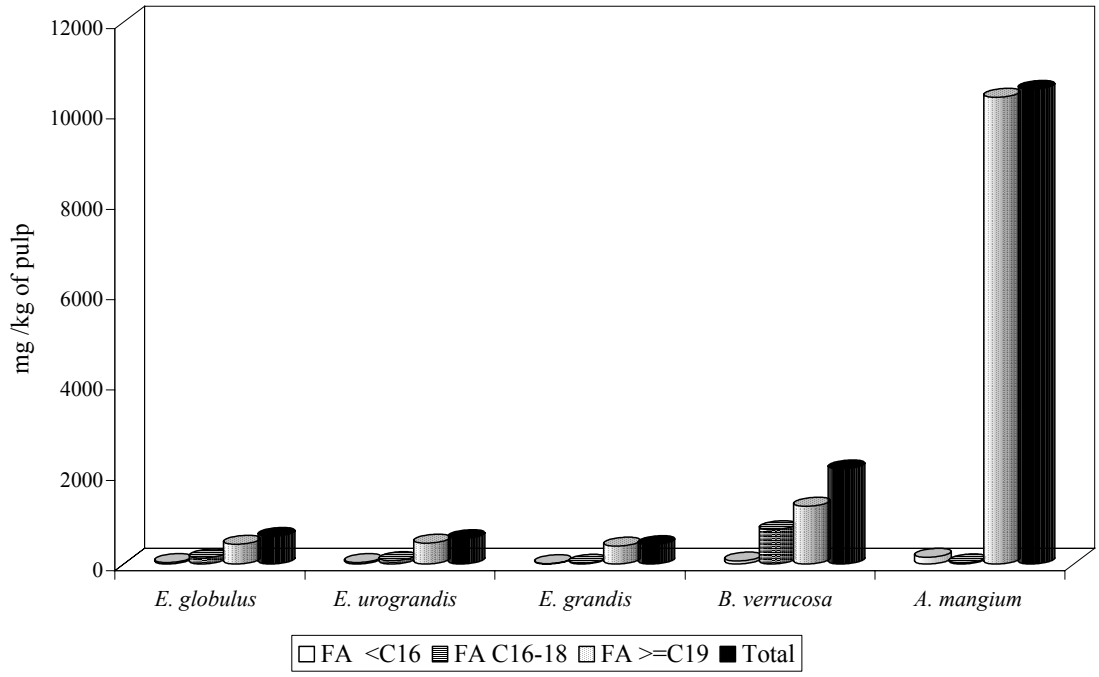



**Fig. 5.** Major fatty acids present in the acetone extracts of the hardwoods ECF bleached pulps. **FA< C16** fatty acids with less than 16 carbon atoms; **FA C16-C18** fatty acids with 16 to 18 carbon atoms and **FA>= 19** fatty acids with more than 19 carbon atoms.

As observed in woods, *Eucalyptus* and *B. verrucosa* bleached pulps contain small amounts of fatty alcohols, while in *A. mangium* bleached pulp, they represent the second most abundant family of lipophilic extractives. The removal of fatty alcohols from *A. mangium* wood (10%) is also much lower than that observed for *Eucalyptus* and *B. verrucosa* woods (around 70%, 50%, 50% and 70%, respectively, for *E. globulus*, *E. urograndis*, *E. grandis* and *B. verrucosa*). Again, this is assigned to the fact that fatty alcohols with 24, 26 and 28 carbon atoms dominate in the *A. mangium* wood, and are more difficult to remove from wood than the fatty alcohols with a lower number of carbon atoms.

Glycerides and other compounds such as ferulic acid and corresponding ferulates were not detected in the bleached pulps studied, certainly due to their complete removal and/or degradation during pulping and bleaching processes.

## CONCLUSIONS

1. The lipophilic extractives of the hardwoods investigated (*Eucalyptus globulus*, *Eucalyptus grandis*, *Eucalyptus urograndis*, *Betula verrucosa* and *Acacia mangium*) are composed essentially of fatty acids (including α- and ω-hydroxyacids), long chain aliphatic alcohols and sterols. The relative abundance and composition of these extractive families are significantly different in the various wood species. *B. verrucosa* is particularly rich in aliphatic acids (<C22), mainly in free form, while *A. mangium* wood is very rich both in long chain (>C20) aliphatic acids and aliphatic alcohols (in free and esterified forms).
2. During the kraft pulping of wood and pulp bleaching, using a chlorine dioxide based sequence, sterols are extensively removed (70-90% for *Eucalyptus*, 50% for *B. verrucosa* and 100% for *A. mangium*). The sterols fraction retained in bleached pulps are essentially β-sitosterol oxidation products and β-sitostanol. $\Delta^7$ sterols present in *A. mangium* are completely degraded and/or dissolved during the preparation of the bleached pulp. Fatty acids, particularly those <C20, are also removed in extents of 40-60%, except for *A. mangium* where about 80% are retained in bleached pulps. α- and ω-hydroxy fatty acids (>C20) are highly retained in the bleached pulps.
3. Globally, *B. verrucosa* and *A. mangium* bleached pulps have a concentration of fatty acids approximately 4 and 20 times higher, respectively, than that of *Eucalyptus* pulps while the content of long chain aliphatic alcohols in *A. mangium* pulp is of the order of 100 times higher than *Eucalyptus* and *B. verrucosa* pulps. Such different amounts of lipophilic components certainly will induce significant differences in the surface energies of fibers, affecting their behavior during the papermaking process and the physico-mechanical properties of the final paper product (Kokkonen et al. 2002). In addition, long chain fatty acids, long chain fatty alcohols, hydroxy fatty acids and several sterol oxidation products are the lipophilic extractives with high tendency for pitch deposition (Ekman and Holmbom 2000; Freire et al. 2002b). In this context, *B. verrucosa,* and particularly, *A. mangium,* are the pulps with higher potential for pitch formation during the papermaking process.

## ACKNOWLEDGMENTS

Thanks are due to FCT and ESF, within the Community Support Framework III, for the financial support of project POCTI CTM/34159/2000 and for the awarding of Ph.D. grants to C.S.R. Freire, P.C.R. Pinto and S. Santiago, to the Forest and Paper Research Institute RAIZ for supplying the *Eucalyptus* wood samples and collaboration in pulping and bleaching experiments and to Stora Enso and Kvaerner Pulping AB for supplying the *Betula verrucosa* and *Acacia mangium* woods, respectively.

## REFERENCES CITED


Artaud, J., Iatrides, M-C. and Gaydou, E. M. (1984). "Co-occurrence of $\Delta^5$- and $\Delta^7$-sterols in two *Gleditsia* species. A reassessment of the sterols composition in oils rich in $\Delta^7$ sterols," *Phytochem.* 23(10), 2303-2306.

Back, E. L. and Allen, L. H. (Eds.) (2000). *Pitch Control, Wood Resin and Deresination*, Tappi Press, Atlanta.

Coleman, M. J. (1998). "Tropical forestry: *Acacia* plantations in Indonesia," *Tappi J.* 81(12), 43-49.

Combaut, G. (1986). "GC-MS on plant sterol analysis," *Gas Chromatography/Mass Spectrometry*, H. F. Linskens and J. F. Jackson, eds., Springer-Verlag, Berlin.

del Río, J. C., Gutiérrez, A., González-Vila, F. J., Martín, F. and Romero, J. (1998). "Characterization of organic deposits produced in kraft pulping of *Eucalyptus globulus* wood," *J. Chromatog. A.* 823(1-2), 457-465.

del Río, J. C., Romero, J. and Gutiérrez, A. (2000). "Analysis of pitch deposits produced in kraft pulp mills using a totally chlorine free bleaching sequence," *J. Chromatog. A.* 874(2), 235-245.

Ekman, R. (1983). "Suberin monomers and triterpenoids from the outer bark of *Betula verrucosa* EhrH," *Holzforschung*, 37(4), 205-211.

Ekman, R. and Holmbom, B. (2000). "The chemistry of wood resin," *Pitch Control, Wood Resin and Deresination*. E. L. Back and L. H. Allen (Eds.) Tappi Press, Atlanta (Chapter 2).

Freire, C. S. R., Silvestre, A. J. D. and Pascoal Neto, C. (2002a). "Identification of new hydroxy fatty acids and ferulic acid esters in the wood of *Eucalyptus globulus*," *Holzforschung* 56(2), 143-149.

Freire, C. S. R., Silvestre, A. J. D., Pereira, C. C. L., Pascoal Neto, C. and Cavaleiro, J. A. S. (2002b). "New lipophilic components of pitch deposits from an *Eucalyptus globulus* bleached Kraft pulp mill," *J. Wood Chem. Technol.* 22(1), 55-66.

Freire, C. S. R., Silvestre, A. J. D. and Pascoal Neto, C. (2003). "Oxidized derivatives of lipophilic extractives formed during hardwood kraft pulp bleaching," *Holzforschung* 57(5), 503-517.

Freire, C. S. R., Silvestre, A. J. D. and Pascoal Neto, C. (2005). "Lipophilic extractives in *Eucalyptus globulus* Kraft Pulps. Behaviour during ECF bleaching," *J. Wood Chem. Technol.* **25**(1-2) 67-80.

Freire, C. S. R., Silvestre, A. J. D. and Pascoal Neto, C. and Evtuguin, D.V. (2006). "Effect of oxygen, ozone and hydrogen peroxide bleaching stages on the contents and composition of extractives of *Eucalyptus globulus* kraft pulps," *Biores. Technol.* 97(3), 420-428

Gutiérrez, A., del Río, J. C., González-Vila, F. J. and Martín, F. (1998). "Analysis of lipophilic extractives from wood and pitch deposits by solid-phase extraction and gas chromatography," *J. Chromatogr.* A 823(1-2), 449-455.

Gutiérrez, A., del Río, J. C., González-Vila, F. J. and Martín, F. (1999). "Chemical composition of lipophilic extractives from *Eucalyptus globulus* Labill wood," *Holzforschung* 53(5), 481-486.

Gutiérrez, A., Romero, J. and del Río, J. C. (2001a). "Lipophilic extractives from *Eucalyptus globulus* pulp during kraft cooking followed by TCF and ECF bleaching," *Holzforschung* 55(3), 260-264.

Gutiérrez, A. and del Río, J. C. (2001b). "Gas Chromatography/ Mass Spectrometry demonstration of steryl glycosides in Eucalypt wood, kraft pulp and process liquids," *Rapid Commun. Mass Spectrom*. 15(24), 2515-2520.

Hillman, D. C. (2002). "Single-species pulping. The world's preferred market pulps," *Solutions*, Nov., 27-28.

Kokkonen, P., Korpela, A., Sundberg, A, Holmbom, B. (2002). "Effects of different types of lipophilic extractives on paper properties," 17(4), 382-386.

Nilvebrant, N.-O and Byström, S. (1995). "Demonstration of glucosidic linked sterols in birch", *Proceedings of the 8th International Symposium on Wood and pulping Chemistry*, Helsinki, Finland, Vol. II, pp. 135-140.

Pascoal Neto, C., Silvestre, A. J. D., Evtuguin, D., Freire, C. S. R., Pinto, P. C. R., Santiago, A. S., Fardim, P. and Holmbom, B. (2004). "Bulk and surface composition of ECF bleached hardwood kraft pulp fibres" *Nord. Pulp Paper Res. J.* **19**(4), 513-520.

Pietarinen, S., Willför, S. and Holmbom, B. (2003). "Wood resin in *Acacia mangium* and *Acacia crassicarpa* wood and knots," *Appita J.* 57(2), 146-150

Santos, G. G., Alves, J. C. N., Rodilla, J. M. L., Duarte, A. P., Lithgow, A. M. and Urones, J. G. (1997). "Terpenoids and other constituents of *Eucalyptus globulus*," *Phytochem*. 44(7), 1309-1312.

Silvestre, A. J. D., Pereira, C. C. L., Pascoal Neto, C., Evtuguin, D. V., Duarte, A. C., Cavaleiro, J. A. S. and Furtado, F. P. (1999). "Chemical composition of pitch deposits from an ECF *Eucalyptus globulus* bleached kraft pulp mill: its relationship with wood extractives and additives in process streams," *Appita J.* 52(5), 375-382.

Swan, B. and Åkerblom, I.-S. (1967). "Wood extractives from *Eucalyptus globulus* Labill," *Svensk. Papperstidn.* 70: 239-244.

Wallis, A. F. A. and Wearne, R. H. (1997). "Analysis of resin in eucalypt woods and pulps," *Proc. 51st Annual General Conference. Appita*, Melbourne, Vol. I, pp. 45-50.

Article submitted: March 21, 2006; Accepted: April 29, 2006; Published: May 25, 2006

# IDENTIFICATION AND CHARACTERIZATION OF DIVERSE XYLANASES FROM THERMOPHILIC AND THERMOTOLERANT FUNGI

Sonia K. Ghatora,[a] Bhupinder S. Chadha,[a]* A. K. Badhan[a], H. S. Saini,[a] and M. K. Bhat[b]

Thirteen fungal isolates included in this study expressed multiple xylanase isoforms as observed by xylan zymograms of polyacrylamide gel electrophoresis (PAGE) and isoelectrofocussing (IEF) fractionated proteins. Eighty-three xylanases produced by these thermophilic and thermotolerant strains were detected using the IEF profiling technique. Xylanases identified on the basis of their isoelectric points (pI) were functionally diverse and exhibited differential catalytic activities against various xylan types (birch wood xylan, larch wood xylan, oat spelt xylan, rye arabino xylan and wheat arabino xylan) as well as debranched arabinan. Thermophilic isolates, *Chaetomium thermophilum*, *Humicola insolens*, *Melanocarpus* sp., *Malbranchea* sp. and *Thermoascus aurantiacus*, were found to produce alkaline active xylanases that showed a bleach boosting effect on Decker pulp resulting in increased brightness (1.60-2.04 ISO units).

*Keywords: Thermophilic and Thermotolerant fungi, Multiple xylanases, PAGE and IEF fractionated proteins, Specificities and catalytic activities, Alkaline active xylanases.*

*Contact information: a: Department of Microbiology, Guru Nanak Dev University, Amritsar-143005, INDIA; b: Food Materials Science Division, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK.*
**Corresponding Author: chadhabs@yahoo.com*

## INTRODUCTION

Xylan, the major hemicellulosic constituent of hardwood and softwood, is a branched heteropolysaccharide constituting a backbone of β-1,4 linked xylopyranosyl units substituted with arabinosyl, glucuronyl and acetyl residues (Shallom and Shoham 2003). The structure of xylan components from different sources depends upon extraction procedures as well as the frequency, number and type of substitutions (Viikari et al. 1994; Saha 2003). The hydrolysis of the xylan backbone is accomplished by endoxylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37) along with a variety of debranching enzymes, i.e. α-L-arabinofuranosidases, α-glucuronidases and acetyl esterases (Collins et al. 2005). Many of the xylanase-producing microorganisms express multiple isoforms (Wong et al. 1988) that have been ascribed to a variety of reasons, i.e., heterogeneity and complexity of xylan structure, genetic redundancy and post-translational modifications, etc. (Kormelink and Voragnen 1993; Li et al. 2000).

Thermophilic fungi, a unique group of microorganisms, that thrive at high temperatures are often associated with piles of agricultural and forestry products and other composting materials (Maheshwari et al. 2000). The distribution and colonization of thermophilic fungal population in compost is closely related to their ability to produce a variety of cell wall degrading enzymes (Sharma 1989). Since these fungal strains function in amelioration of xylan substrate present in lignocellulosic waste, each xylanase

produced may be biotechnologically important and show specialized function. There is need to isolate and identify such novel xylanases from diverse indigenous strains. Some of the thermophilic fungi, *Chaetomium thermophile, Humicola insolens* (syn. *Scytalidium thermophilum*), *Thermomyces lanuginosus* and *Thermoascus aurantiacus* have been reported to produce biotechnologically-important, thermostable xylanases. These xylanases are used in a variety of applications, i.e., clarification of juice and wine, starch separation and production of functional food ingredients, improving the quality of bakery products and in animal feed biotechnology (Saha 2003). Xylanases showing transglycosylation activities can also be used for tailoring drugs and in the preparation of neoglycoproteins (Eneyaskaya et al. 2003). Alkaline-active xylanases of thermophilic fungi find application in bleaching of pulp in paper industry obviating the need for chlorine (to some extent) in ecofriendly process (Subramanium and Prema 2002).

Due to their huge potential, xylanases with novel properties must be isolated and identified. This study highlights the identification and characterization of multiple and catalytically diverse xylanases produced by thermophilic and thermotolerant fungi isolated from composting soils, and their application in pulp bleaching.

## MATERIALS AND METHODS

### Microorganisms and Culture Conditions

Diverse thermophilic and thermotolerant fungi, namely, *Absidia corymbifera* MTCC 4620, *Acrophialophora nainiana Edward* MTCC 6662, *Aspergillus caespitosus* MTCC 6326, *Aspergillus terreus* MTCC 6335, *Chaetomium thermophilum* MTCC 4981, *Chrysosporium lucknowense* MTCC 3921, *Emericella nidulans* var. *lata* MTCC 6327, *Humicola insolens* MTCC 4520, *Humicola fuscoatra* MTCC 6329, *Melanocarpus* sp. MTCC 3922, *Malbranchea* sp. MTCC 4887, *Penicillium lagena* MTCC 6334 and *Thermoascus aurantiacus* MTCC 4890 were isolated from composting soils and grown and maintained on yeast-starch agar (YpSs, pH 7.0) of following composition (% w/v): starch 1.5, yeast extract 0.4, $K_2HPO_4$ 0.23, $KH_2PO_4$ 0.2, $MgSO_4.7H_2O$ 0.05, citric acid 0.057 and agar 2.0 (Cooney and Emerson 1964). The fungi were cultured at 45-50 $^oC$ for 7-10 days and stored at 4 $^oC$. These indigenous fungal strains were identified as described by Cooney and Emerson (1964) and have been deposited with Microbial Type Culture Collection (IMTECH, Chandigarh, India).

### Enzyme Production

The cultures were grown in 250 ml Erlenmeyer flasks that contained 50 ml of production medium of the following composition (% w/v): corn cobs 2.0; oat spelt xylan 0.1, yeast extract 1.0, $KH_2PO_4$ 0.3, $CaCl_2$, 0.05, $MgSO_4$ 0.05 and 1% v/v of trace element solution that contained (% w/v): $(NH_4)_2SO_4$ 0.2, KCl 0.5, $CaCl_2$ 0.1, $MgSO_4$ 0.5, $ZnSO_4$ 0.01 and $CuSO_4$ 0.005. The pH of the medium was adjusted to 6.0 prior to sterilization. The flasks were inoculated with 2 agar discs (2 mm in diameter) of 7-10 days old culture from YpSs agar plates and were incubated under shaking conditions (120 rpm) at 45/50 $^oC$ up to10 days (experiments performed in triplicates). The crude enzymes were filtered and centrifuged (11000 x g) for 10 min. The xylanase activity was estimated in these filtrates.

The xylanase assay of crude filtrate was determined using birch wood xylan as the substrate. The assay mixture which contained 500µl of 1 % birch wood xylan (Sigma, X0502) prepared in 50 mM Na-citrate buffer with pH 6.5 and 500µl suitably diluted enzyme was incubated at 50 $^{o}$C for 5 minutes. The reaction was stopped by the addition of 3 ml dinitrosalicylic acid (DNS) reagent and the contents were boiled for 15 minutes. The developed color was read at 540 nm using Novaspec II spectrophotometer (Pharmacia). The amount of reducing sugar liberated was quantified using the xylose standard. Xylanase activity is expressed in terms of units per ml of xylanase solution. A unit of activity is defined as the micromoles of xylose released per minute under assay conditions (Gomez de Segura et al. 1998; Sunna and Berguist 2003).

EGTA-EDTA mix (each @ 1 mM), PMSF (2 mM), and 0.02% sodium azide were added to the filtrate in order to avoid contamination and inhibit protease activity. Further, the clear supernatants were desalted using a PD-10 column (Pharmacia) and lyophilized (HETO, Drywinner 3) and the concentrated samples were used for fractionation on PAGE and IEF.

## Electrophoresis and Iso-Electric Focusing

The samples (protein-100µg) were fractionated by native-polyacrylamide gel electrophoresis (PAGE) that was performed in 7.5% gel with 4% stacking gel using the Mini-Protean II system of Biorad by the method of Laemmli (1970). Similarly, isoelectric-focusing (IEF) using 5% acrylamide gel containing 2.4% broad pH range (3.5-10.0) ampholine carrier ampholyte (Amersham Biosciences) was performed (Mini-Protean II system, BIORAD). Ethanolamine (0.4% v/v) and sulphuric acid (0.2% v/v) were used as cathodic and anodic electrolyte solutions, respectively (Bhat and Wood 1989). Isoelectrofocusing was carried out for 1 h each at a constant voltage of 100 V and 200 V followed by 500 V for 30 min.

## Activity Staining

Xylanase activity in PAGE and IEF gels was detected by activity staining (Zymogram technique) with 1% agarose replica containing covalently dyed RBB-Xylan prepared from oat spelt xylan (SIGMA). Upon completion of electrophoresis, the gels were incubated in sodium acetate buffer (50 mM, pH 6.0) for 30 min and then overlaid on RBB-Xylan containing gel for 30-60 min at 50 $^{o}$C. In order to avoid band diffusion, these gels were dried at 60 $^{o}$C.

## Quantification of Xylanases

After fractionating the proteins on IEF, the gel in each lane was sliced (1.25 mm thickness). Each slice was incubated in 500 µl sodium citrate buffer (50 mM, pH 6.0) for 72 h at 4 $^{o}$C. The eluted protein in each fraction was assayed for xylanase activity against 1% birch wood xylan.

## Substrate Specificity and Activity of Xylanase at Alkaline pH

The xylanase active fractions identified against birch wood xylan were also assayed for their catalytic action against different xylan types (oat spelt xylan, larch wood xylan, rye arabino xylan, wheat arabino xylan) and debranched arabinan. In order to

identify alkaline active xylanases, the assay under alkaline conditions using Tris-HCl buffer (50 mM, pH 9.0) was performed against birch wood xylan.

### Bleaching of Pulp

Decker pulp (10 % w/w), provided by ABC paper mills (Hoshiarpur, India), was subjected to alkaline extraction (NaOH, 6.3 % w/v) for 30 min. at 50 $^{o}$C, then was thoroughly washed with water and dried. A slurry of alkali treated pulp (6 % w/v pulp consistency) prepared in phosphate buffer (0.1 M, pH 7.0) was treated with 10 units xylanase/g (dry weight) at 65 $^{o}$C for 120 min under mild shaking (60 rpm). No enzyme was added in the control. A post-alkaline extraction (NaOH, 2 % w/v; 6 % w/v pulp consistency) was performed at 55 $^{o}$C under mild shaking (60 rpm).

The enzyme-mediated release of chromophoric material from pulp was measured spectrophotometrically at 237, 254, 280 and 465 nm in the enzyme filtrates after a 2 h incubation period using UV-VIS spectrophotometer (Shimadzu, UV mini 1240). Reducing sugars released from pulp were measured over the same incubation period according to the dinitrosalicylic acid method. The hand-sheets of bleached pulp were prepared according to standard TAPPI method (T 205 om-88) and their brightness was determined with an Elrepho 070 (Lorentzen and Wettre, Sweden).

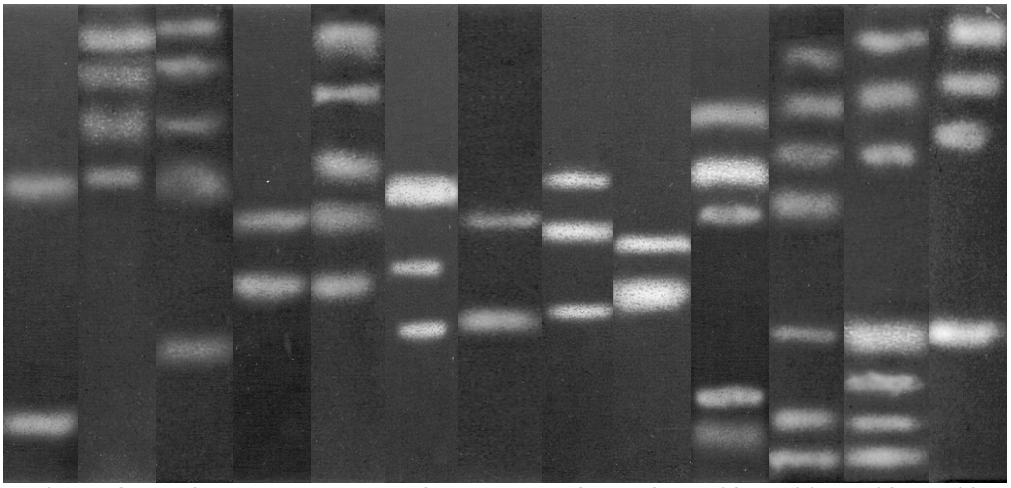
## RESULTS AND DISCUSSION

### Production and Multiplicity of Xylanases

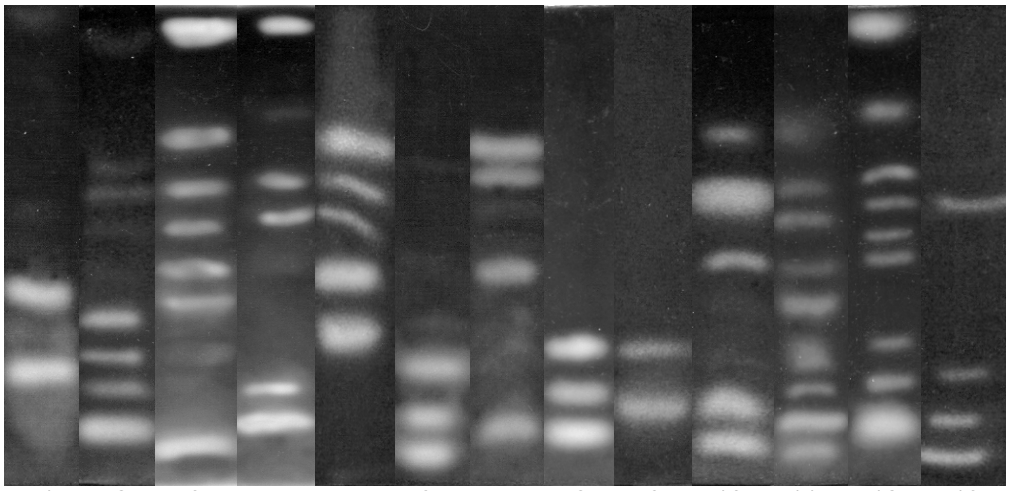
The fungal strains included in this study produced xylanases to a varied extent, where thermophilic fungal strains expressed high levels of xylanases as compared to thermotolerant fungi (Table 1). High xylanase titres produced by thermophilic fungi that are prevalent in composting soils possibly accelerate the process of natural decomposition. *Melanocarpus* sp. produced 264.2 units ml$^{-1}$ of xylanases followed by *Che. thermophilum* and *Malbranchea* sp. (162.2 and 141.7 units ml$^{-1}$), respectively. Among thermotolerant fungal strains, maximum xylanase production was observed with *A. terreus* (38.0 units ml$^{-1}$) followed by *P. lagena* (32.4 units ml$^{-1}$) and *E. nidulans* var. *lata* (31.2 units ml$^{-1}$).

The analysis of crude enzymes produced by these fungal cultures revealed the presence of multiple xylanases. Even though multiplicity of xylanases is widespread in microorganisms, it has been demonstrated in few fungi including *Trichoderma* sp., *Sclerotium rolfsii* and *Aspergillus* sp. (Wong et al. 1988; Sachslehner et al. 1998; de Vries and Visser 2001), the thermophilic fungi, *Melanocarpus albomyces* IIS 68 and *Myceliophthora s*p. (Saraswat and Bisaria 2000; Badhan et al. 2004), and in an anaerobic fungal strain, *Neocallimastix frontalis* (Gomez de Segura et al. 1998). Studies revealing functionally diverse multiple isoforms of xylanases from thermophilic fungi isolated from the same ecosystem have not been reported earlier. Thirteen fungal cultures isolated (in this study) from composting soils produced 51 diverse xylanases identified based on their electrophoretic mobilities using PAGE zymograms (Fig. 1-A). Both *A. terreus* and *E. nidulans* var. *lata* produced seven xylanase isoforms with very high to low electrophoretic mobilities. Whereas, *Ac. nainiana*, *Che. thermophilum*, *H. fuscoatra* and *T. aurantiacus* produced two xylanases each with distinct banding pattern. Furthermore,

the resolution of filtrates on IEF gels showed 65 xylanases with distinct pI. *E. nidulans* var. *lata*, *H. fuscoatra* and *H. insolens* produced xylanases of highly basic to acidic pI~9.7-3.4 with a high degree of multiplicity (Fig. 1-B). The production of a wide variety of xylanases by thermophilic and thermotolerant fungi isolated from composting soils suggests that these xylanases possibly exhibit overlapping yet dissimilar specificities to achieve superior xylan hydrolysis (Wong et al. 1988). Studies on xylanases from *Myceliophthora* sp. from our laboratory have clearly shown that the multiplicity was not a result of proteolytic post-translational modification (Badhan et al. 2006). Multiple xylanases may be the product of different alleles of the same gene (allozymes) or distinct gene products produced by a fungus to enhance the utilization of xylan (Wong et al. 1988; Uffen 1997).




**(A)**




**(B)**

**Fig. 1.** Zymogram of PAGE gel (A) and IEF gel (B) representing xylanases produced by thermophilic fungal strains: (1) *Che. thermophilum*, (2) *Chy. lucknowense*, (3) *H. insolens*, (4) *H. fuscoatra*, (5) *Melanocarpus* sp., (6) *Malbranchea* sp. (7) *T. aurantiacus*, and thermotolerant fungal strains: (8) *Ab. corymbifera*, (9) *Ac. nainiana*, (10) *A. caespitosus*, (11) *A. terreus*, (12) *E. nidulans* var. *lata* and (13) *P. lagena*.

**Table 1.** Xylanase Production by Different Thermophilic and Thermotolerant Fungal Cultures Grown on Corn Cobs and Oat Spelt Xylan under Submerged Conditions.

| ***Organisms*** | ***Xylanase Activity (units ml$^{-1}$)*** |
|---|---|
| **Thermophilic fungi** | |
| *Chaetomium thermophilum* | 162.2 ± 8.3 |
| *Chrysosporium lucknowense* | 96.6 ± 8.2 |
| *Humicola fuscoatra* | 12.9 ± 1.2 |
| *Humicola insolens* | 17.2 ± 2.5 |
| *Malbranchea* sp. | 141.7 ± 9 |
| *Melanocarpus* sp. | 264.2 ± 11 |
| *Thermoascus aurantiacus* | 18.3 ± 2.5 |
| **Thermotolerant fungi** | |
| *Absidia corymbifera* | 16.5 ± 1.5 |
| *Acrophialophora nainiana* | 9.7 ± 1.0 |
| *Aspergillus caespitosus* | 25.7 ± 2.1 |
| *Aspergillus terreus* | 38.0 ± 4.2 |
| *Emericella nidulans* var. *lata* | 31.2 ± 2.3 |
| *Penicillium lagena* | 32.4 ± 4.2 |

* Culture conditions: 250 ml Erlenmeyer flasks contained 50 ml of production medium; Incubation temperature - 45/50 $^{o}$C; Shaking at120 rpm; Incubation time upto10 days.

The differential expression of multiple xylanases by each strain was quantified using IEF profiling by slicing of gels and eluting proteins. By this procedure the major and minor xylanases were identified based on their relative proportions (Fig. 2.A-M). Most of the fungi produced major xylanases with neutral to acidic pI. However, *H. fuscoatra*, *H. insolens* and *E. nidulans* var. *lata* produced major xylanases of highly basic pI ~ 9.7, 9.5 and 9.7 with relative proportion of 29%, 33% and 30%, respectively (Fig. 2.L). Whereas, in *Che. thermophilum*, *Chy. lucknowense* and *Malbranchea* sp., xylanases of acidic pI ~ 4.5, 5.5 and 3.4, and 3.5 with high level of relative proportion i.e., 56%, 31%, 31% and 45% were expressed, respectively (Fig. 2.A, B and E). The observed levels of minor xylanases were 2-10%. Though minor xylanases are not produced in large quantities, they may be responsible for some specialized functions (induction, transglycosylation, etc.) or may work in tandem with the major xylanases to attain superior xylan hydrolysis (Wong et al. 1988; Thomson 1993).

## Substrate Specificity of Diverse Xylanases

In order to demarcate the observed xylanases as catalytically distinct and novel, various xylan types were used to analyze their substrate preferences. Most of the xylanases identified highly substituted wheat arabino-xylan as the preferred substrate for hydrolysis (Table 2 A and B, see Appendix). Xylanases from *H. fuscoatra* (HFX-VI), *Ac. nainiana* (ANX-VI) and *E. nidulans* var. *lata* (ENX-IV) showed 9.57, 9.71 and 8.26 fold higher activity, respectively, against wheat arabino xylan as compared to birch wood xylan (the usual substrate to assay xylanase activity). The activities of xylanases against

rye arabino xylan, another highly substituted form of xylan, were comparable to wheat arabino xylan. Xylanases identified from *H. insolens* (HIX-VI), *H. fuscoatra* (HFX-VI), *Ac. nainiana* (ANX-VI) and *E. nidulans* var. *lata* (ENX-IV) were highly active against rye arabino xylan and showed 7.76, 7.51, 8.38 and 6.26 fold higher activity, respectively, as compared to birch wood xylan. However, xylanases from *A. caespitosus* (ACSX-II and ACSX-VII), *A. terreus* (ATX-I and ATX-IX) and *E. nidulans* var. *lata* (ENX-I) showed exceptionally low activity against rye arabino xylan. Few of the xylanases of *H. fuscoatra* (HFX-I), *Ab. corymbifera* (ACX-V and ACX-VI), *A. caespitosus* (ASCX-II) and *E. nidulans* var. *lata* (ENX-I) identified oat spelt xylan as the most preferred substrate.

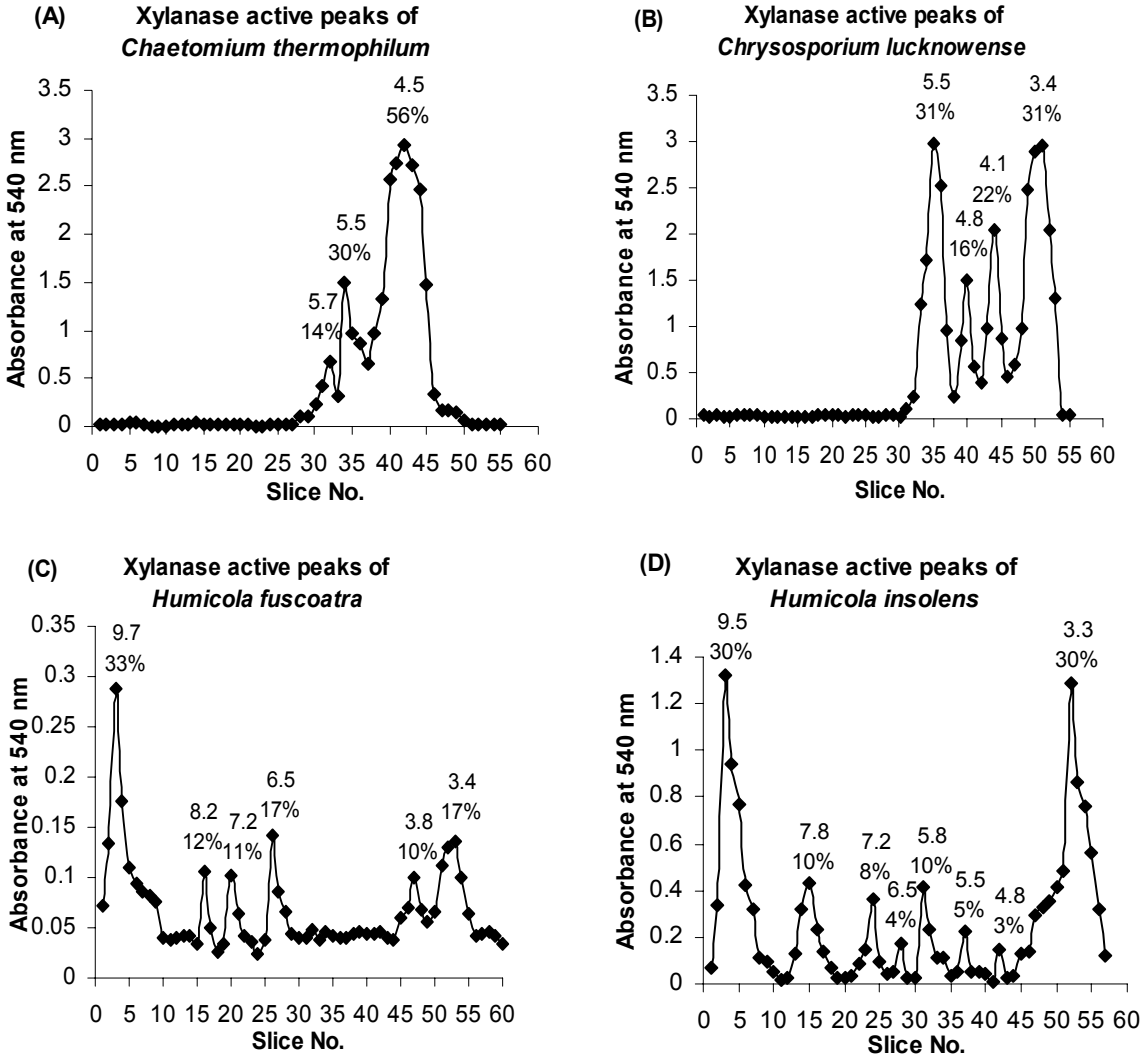
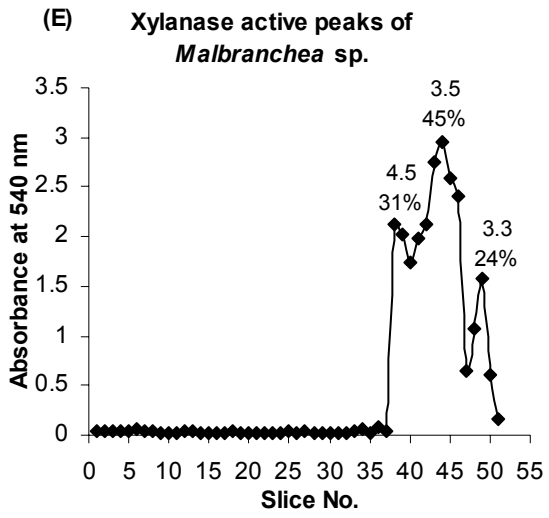
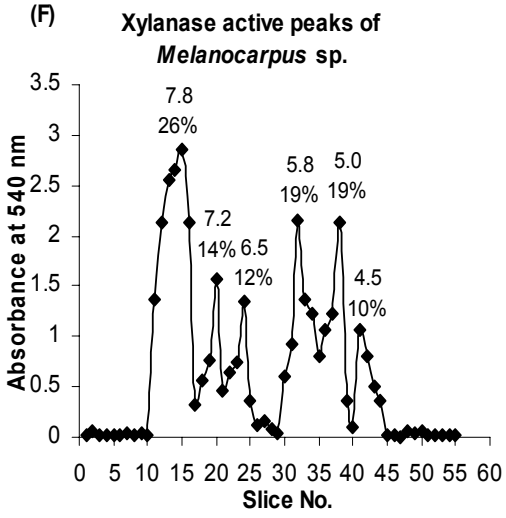



**Fig. 2.A-D.** Fractionation of thermophilic and thermotolerant fungi showing xylanase isoforms (pI and relative percent activity given at top of the each peak) using isoelectric focusing in polyacrylamide gel.

The activities against larch wood xylan were lower as compared to arabino-xylans, however, xylanases belonging to *H. fuscoatra* (HFX-II), *T. aurantiacus* (TAX-II), *A. terreus* (ATX-II) and *P. lagena* (PLX-I) used it as substrate of choice. The observed

differences in the activity of xylanases on structurally and chemically distinct xylan types (Table 2 A and B) suggest that they require the presence of a particular type of substituent in the vicinity to enhance their catalytic action (Silva et al. 2000). Furthermore, endoxylanases differ in their specificity towards the xylan polymer as some enzymes cut randomly between unsubstituted xylose residues, whereas the activity of other xylanases strongly depends on the substituents (arabinosyl, arabinofuranosyl, acetyl and glucuronyl residues) linked to the xylose residues neighboring the attacked residues (de Vries and Visser 2001). Minor xylanases produced by fungal strains showed appreciably higher activities (1.5-8.2 folds) against arabino-xylans, the main constituents in the straws (rice, oat, wheat, etc.), present in the composting soils and thus may be playing a significant role in its decomposition. Few of the xylanases belonging to *Ac. nainiana*, *A. terreus*, *P. lagena*, *Chy. lucknowense* and *H. fuscoatra* were catalytically versatile and also recognized debranched arabinan as substrate. Xylanases have cellulose binding domains with a few having the affinity to recognize xylan and cellulose (Subramanium and Prema 2002). These domains fold and function in an independent manner and may be responsible for such multi-functionality.



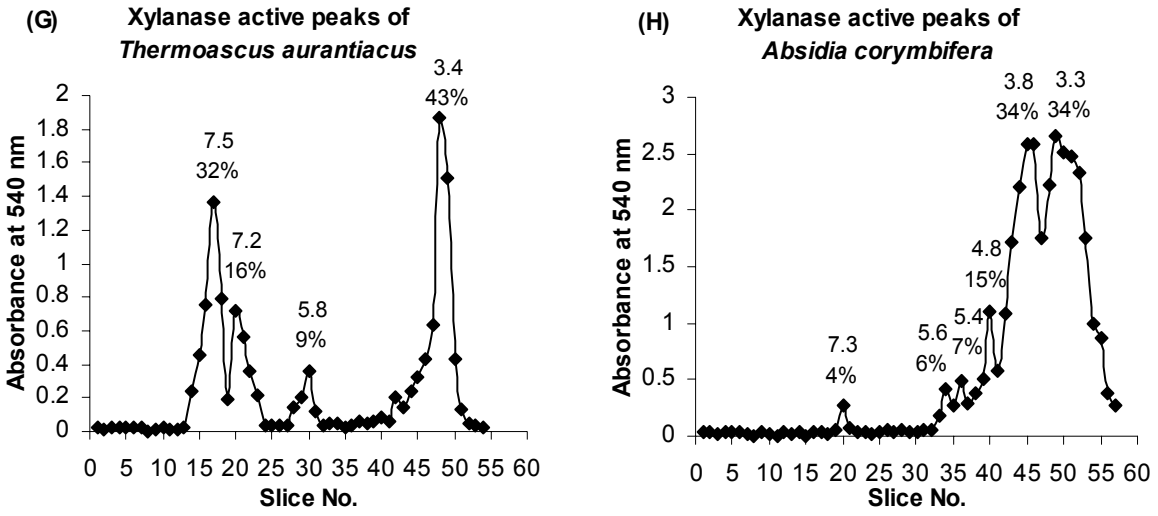
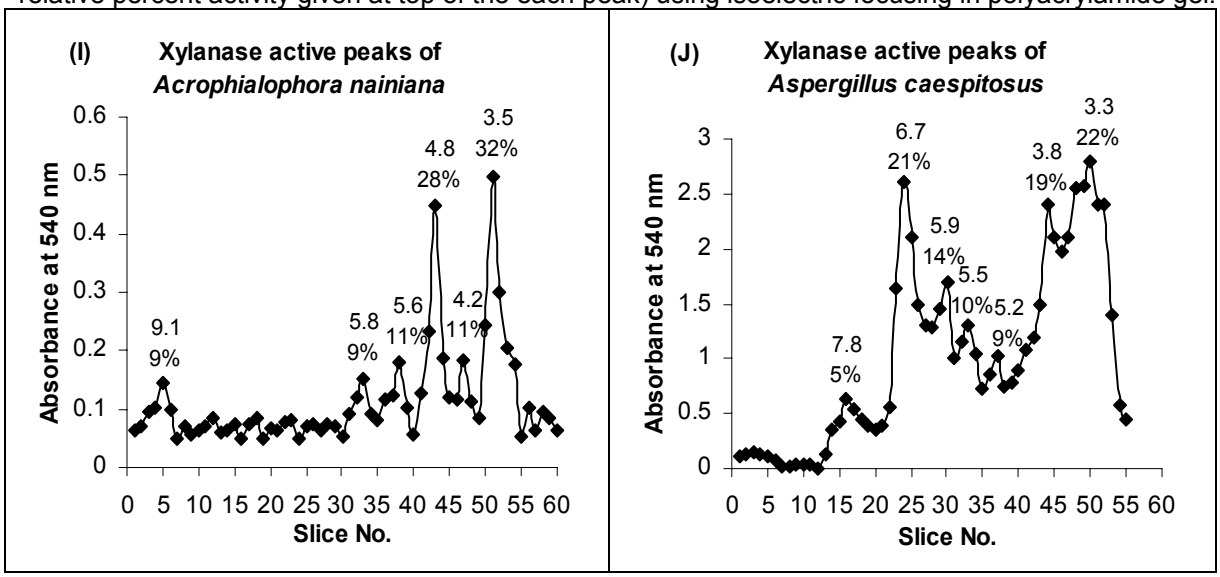



**Fig. 2.E-H.** Fractionation of thermophilic and thermotolerant fungi showing xylanase isoforms (pI and relative percent activity given at top of the each peak) using isoelectric focusing in polyacrylamide gel.



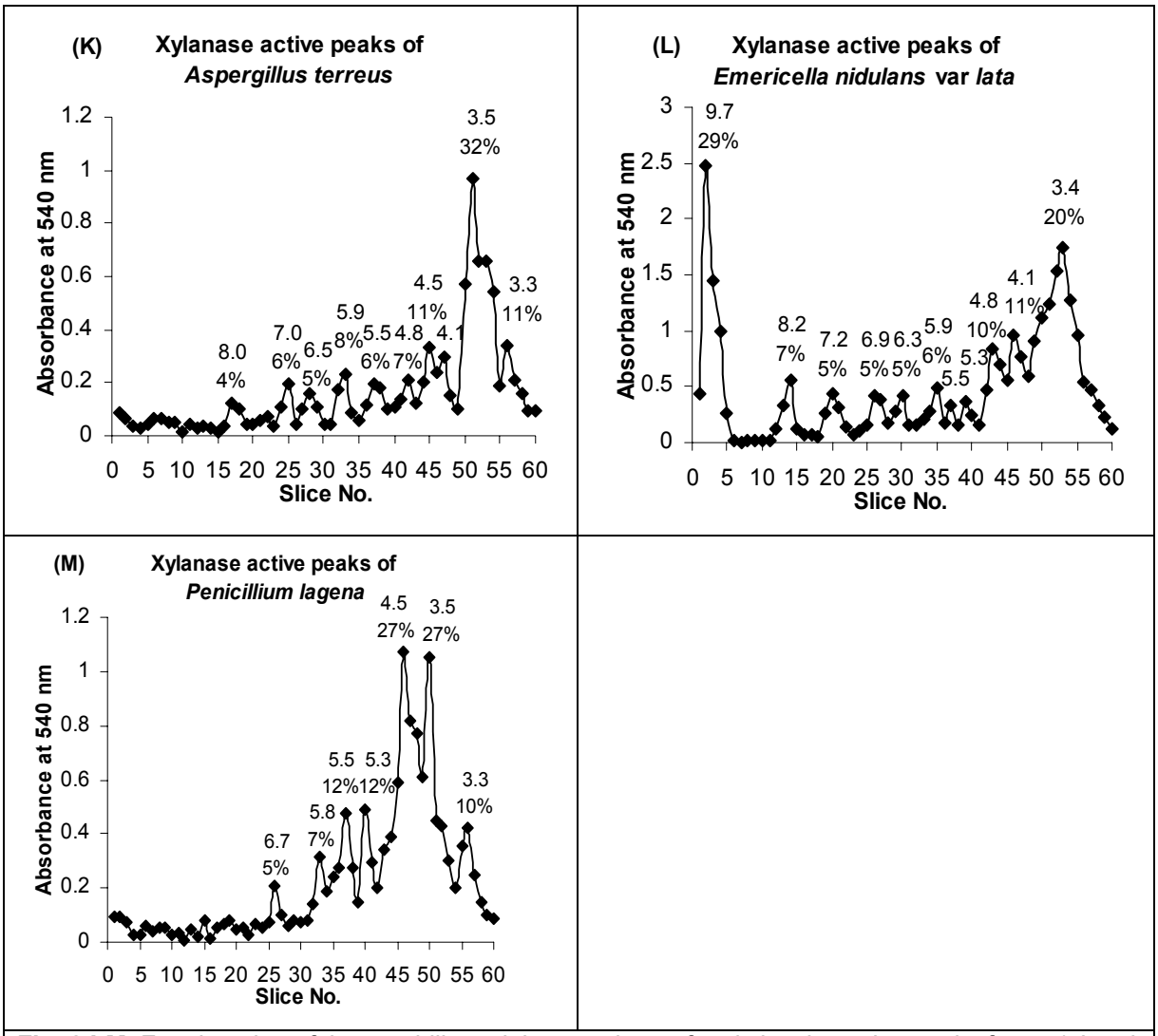



**Fig. 2.I-M.** Fractionation of thermophilic and thermotolerant fungi showing xylanase isoforms (pI and relative percent activity at top of the each peak) using isoelectric focusing in polyacrylamide gel.

## Alkaline Active Xylanases

The alkaline active xylanases were identified on the basis of relatively higher xylanase activity against birch wood xylan at pH 9.0 as compared to at pH 6.0 (Table 2). Interestingly, only the xylanases of thermophilic fungal strains were found to be active under alkaline conditions. All the xylanases of *Che. thermophilum*, but just five of *H. insolens* and two each of *T. aurantiacus* and *Malbranchea* sp. were active under alkaline conditions. Of the six isoforms produced by *Melanocarpus* sp., only one xylanase isoform (MAX-I) was active at alkaline pH. There are evidences to suggest that geographically distinct strains produce xylanases of different physicochemical characters as *Che. cellulolyticum* isolated in Pushchino, Russia, produced three xylanases with pI, 8.9, 8.4 and 5.0 that were active at neutral pH (Baraznenok et al. 1999), while we observed the expression of three alkaline active xylanases of acidic pI, 5.7, 5.5 and 4.5 by *Che. thermophilum*.

**Table 3.** Effect of Xylanase (Xylanase of Diverse Thermophilic and Thermotolerant Fungi) Pretreatment on the Release of Chromophoric Material and Reducing Sugars from Pulp, and Brightness of Pulp.

| *Organisms* | *Chromophore release* | | | | *Reducing Sugar* | *Brightness* |
|---|---|---|---|---|---|---|
| | *237nm* | *254nm* | *280nm* | *465 nm* | *(mg $g^{-1}$ Pulp)* | *(% ISO)* |
| **Thermophilic fungi** | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 32.40 ± 0.20 |
| *Che. thermophilum* | 0.604 | 0.497 | 0.408 | 0.083 | 0.379 | 34.01 ± 0.10 |
| *Chy. lucknowense* | 0.835 | 0.587 | 0.543 | 0.163 | 0.339 | 34.03 ± 0.10 |
| *H. fuscoatra* | 0.534 | 0.510 | 0.545 | 0.099 | 0.171 | 34.00 ± 0.10 |
| *H. insolens* | 0.546 | 0.536 | 0.524 | 0.173 | 0.209 | 34.04 ± 0.10 |
| *Malbranchea* sp. | 1.827 | 1.701 | 0.857 | 0.120 | 0.399 | 34.44 ± 0.15 |
| *Melanocarpus* sp. | 0.906 | 0.197 | 0.136 | 0.043 | 0.389 | 34.21 ± 0.10 |
| *T. aurantiacus* | 0.745 | 0.359 | 0.366 | 0.093 | 0.018 | 34.02 ± 0.10 |
| **Thermotolerant fungi** | | | | | | |
| *Ab. corymbifera* | 0.123 | 0.095 | 0.065 | 0.046 | 0.098 | 32.48 ± 0.12 |
| *Ac. nainiana* | 0.211 | 0.102 | 0.098 | 0.035 | 0.121 | 33.81 ± 0.10 |
| *A. caespitosus* | 0.809 | 0.717 | 0.521 | 0.110 | 0.298 | 34.10 ± 0.10 |
| *A. terreus* | 0.289 | 0.156 | 0.110 | 0.065 | 0.235 | 33.07 ± 0.10 |
| *E. nidulans* var. *lata* | 0.221 | 0.142 | 0.118 | 0.061 | 0.169 | 33.95 ± 0.10 |
| *P. lagena* | 0.245 | 0.152 | 0.121 | 0.055 | 0.175 | 33.78 ± 0.10 |

### Enzymatic Treatment of Pulp

Xylanase produced by thermophilic and thermotolerant fungal strains was used for the bleaching of Decker pulp. Interestingly, xylanases from all the thermophilic fungi showed a bleach boosting effect on the pulp, however, among thermotolerant fungi, only *A. caespitosus* xylanase was found to be effective. These biobleaching studies revealed that maximal chromophore release, reducing sugar release and brightness of pulp, was observed with alkaline active xylanases from novel strain of *Malbranchea* sp., with a gain of 2.04 ISO units (Table 3), and was much better as compared to other sources of alkaline active xylanases (1.63-1.81 ISO units) identified in this study. The observed increase in the pulp brightness by 2.04 units may result in decreased chlorine consumption by 20-25% as observed previously with commercial xylanases Novozyme 473 and VAI-Xylanase (Bajpai et al. 1994).

## CONCLUSIONS

This paper highlights the use of simple techniques like PAGE and IEF for high through-put screening of diverse xylanases from different fungal cultures. Furthermore, the techniques can also be used for identification of xylanases with novel properties.


## ACKNOWLEDGEMENTS

Financial support provided by Department of Biotechnology, New Delhi (India) for carrying out this study is duly acknowledged.

## REFERENCES


Badhan, A. K., Chadha, B. S., Ghatora, S. K., Saini, H. S., and Bhat, M. K. (2004). "Functionally diverse multiple xylanases of thermophilic fungus *Myceliophthora* sp. IMI 387099," *Enzyme Microb. Technol.* 35, 460-466.

Badhan, A. K., Chadha, B. S., Jatinder, K., Saini, H. S., and Bhat, M. K. (2006). "Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099," *Bioresource Technol.* (In press).

Bajpai, P., Bhardwaj, N. K., and Bajpai, P. K. (1994). "The impact of xylanases on bleaching of eucalyptus Kraft pulp," *J. Biotechnol*. 38, 1-6.

Baraznenok, V. A., Becker, V. A., Ankudimova, N. V., and Okanev, N. N. (1999). "Characterization of neutral xylanases from *Chaetomium cellulolyticum* and their biobleaching effect on eucalyptus pulp," *Enzyme. Microb. Technol.* 25, 651-659.

Bhat, M. K., and Wood, T. M. (1989). "Multiple forms of endo-1,4-β-D-Glucanase in the extracellular cellulase of *Pencillium pinophilum*" *Biotechnol. Bioeng.* 33, 1242-1248.

Collins T., Gerday, C., and Feller, G. (2005). "Xylanases, xylanase families and extremophilic xylanases," *FEMS Microbiol. Rev.* 29, 3-23.

Cooney, D. C., and Emerson, R. (1964). *Thermophilic fungi: An Account of Their Biology, Activities and Classification.* San Francisco: W.H. Freeman & Co.

De Vries, R. P., and Visser, J. (2001). "*Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides," *Microbiol. Mol. Biol. Rev.* 65, 497-522.

Eneyaskaya, E. V., Brumer III, H., Backinovsky, L.V., Ivanen, D. R., Kulminskaya, A. A., Shabalin, K. A., and Neustroev, K. N. (2003). "Enzymatic synthesis of β-xylanase substrates: transglycosylation reactions of the β-xylosidase from *Aspergillus* sp," *Carbohydr. Res.* 338, 313-325.

Gomez De Segura, B., Durand, R., and Févre, M. (1998). "Multiplicity and expression of xylanases in the rumen fungus *Neocallimastix frontalis*," *FEMS Microbiol. Lett.* 164, 47-53.

Kormelink, F. J. M., and Voragen, A.G. J. (1993). "Degradation of different [(glucurono) arabino] xylans by combination of purified xylan degrading enzymes," *Appl. Microbiol. Biotechnol.* 38, 688-695.

Laemmli, U. K. (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophage-T4," *Nature* 227, 680-685.

Li, K., Azadi, P., Collins, R., Tolan, J., Kim, J. S., and Eriksson K. L. (2000). "Relationships between activities of xylanases and xylan structures," *Enzyme Microb. Technol.* 27, 89-94.

Maheshwari, R., Bhardwaj, G., and Bhat, M. K. (2000). "Thermophilic fungi: Their physiology and enzymes," *Microbiol. Mol. Biol. Rev.* 63, 461-488.

Sachslehner, A., Nidetzky, B., Kulbe, D. K., and Haltrich D. (1998). "Induction of mannanase, xylanase and endoglucanase activities in *Sclerotium rolfsii*" *Appl. Environ. Microbiol.* 64, 594-600.

Saha, B. C. (2003). "Hemicellulose bioconversion," *J Ind. Microbiol. Biotechnol.* 30, 279-291.

Saraswat, V., and Bisaria, V. S. (2000). "Purification, characterization and substrate specificities of xylanase isozymes from *Melanocarpus albomyces* IIS 68," *Biosci. Biotechnol. Biochem.* 64, 1173-1180.

Shallom, D., and Shoham, Y. (2003). Microbial hemicellulases. *Curr. Opin. Microbiol.* 6, 219-228.

Sharma, H. S. K. (1989). "Economic importance of thermophilous fungus," *Appl. Microbiol. Biotechnol.* 31, 1-10.

Silva, C. H. C., Fonsêca, A. S., Lucena-Neto, S. A., Ximenes, E. A., Puls, J., and Filho, E. X. F. (2000). "Evaluation of hydrolysis products of xylan degrading enzymes from *Humicola grisea* var. *thermoidea* and *Aspergillus fumigatus* Fresenius," *World J. Microbiol. Biotechnol.* 16, 81-83.

Subramanium, S., and Prema, P. (2002). "Biotechnology of microbial xylanases: Enzymology, molecular biology and application," *Critical Rev. Biotechnol.* 22, 33-46.

Sunna, A. and Berguist, P. L. (2003). "A gene encoding a novel extremely thermostable 1,4-β-xylanase isolated directly from an environmental DNA sample," *Extremophiles* 7, 63-70.

Thomson, J. A. (1993). "Molecular biology of xylan degradation," *FEMS Microbiol. Rev.* 104, 65-82.

Uffen, R. L. (1997). "Xylan degradation: a glimpse at microbial diversity," *J Ind. Microbiol. Biotechnol.* 19, 1-6.

Viikari, L., Kantelinen, A., Buchert, J., and Puls, J. (1994). "Enzymatic accessibility of xylans in lignocellulosic materials," *Appl. Microbiol. Biotechnol.* 41, 124-129.

Wong, K. K. Y., Tan, L. U. L., and Saddler, J. N. (1988). "Multiplicity of β-1,4-xylanase in microorganisms: function and applications," *Microbiol. Rev.* 52, 305-317.

**Table 2A.** The Relative Percent Activities of Xylanase Isoforms (Peak Fractions) of Different Thermophilic Fungi against Different Types of Xylan Substrates (Substituted and Unsubstituted) and Debranched Arabinan.

| ***Substrate*** [a] → ***Isoforms***↓ | ***BWX*** [b] | ***LWX*** [b] | ***OSX*** [b] | ***RAX*** [b] | ***WAX*** [b] | ***DA*** [b] | ***BWX*** [c] |
|---|---|---|---|---|---|---|---|
| **Thermophilic fungi** | | | | | | | |
| ***Chaetomium thermophilum*** | | | | | | | |
| CTX-I (5.7)[d] | 100 | 103 | 213 | 165 | 232 | -* | 122** |
| CTX-II (5.5) | 100 | 127 | 143 | 162 | 209 | - | 114** |
| CTX-III (4.5) | 100 | 159 | 171 | 165 | 204 | - | 108** |
| ***Chrysosporium lucknowense*** | | | | | | | |
| CLX-I (5.5) | 100 | 128 | 228 | 217 | 231 | 50 | 41 |
| CLX-II (4.8) | 100 | 146 | 169 | 197 | 237 | 52 | 74 |
| CLX-III (4.1) | 100 | 107 | 153 | 138 | 183 | 50 | 80 |
| CLX-IV (3.4) | 100 | 192 | 263 | 233 | 307 | 43 | 58 |
| ***Humicola fuscoatra*** | | | | | | | |
| HFX-I (9.7) | 100 | 145 | 280 | 101 | 109 | - | 43 |
| HFX-II (8.2) | 100 | 140 | 121 | 115 | 115 | - | 0 |
| HFX-III (7.2) | 100 | 165 | 189 | 282 | 331 | - | 53 |
| HFX-IV (6.5) | 100 | 316 | 264 | 487 | 638 | - | 50 |
| HFX-V (3.8) | 100 | 250 | 142 | 415 | 669 | 12 | 63 |
| HFX-VI (3.4) | 100 | 228 | 321 | 751 | 957 | - | 56 |
| ***Humicola insolens*** | | | | | | | |
| HIX-I (9.5) | 100 | 121 | 218 | 292 | 322 | - | 189** |
| HIX-II (7.8) | 100 | 182 | 175 | 195 | 256 | - | 120** |
| HIX-III (7.2) | 100 | 192 | 180 | 126 | 259 | - | 115** |
| HIX-IV (6.5) | 100 | 151 | 170 | 215 | 286 | - | 97 |
| HIX-V (5.8) | 100 | 192 | 212 | 253 | 276 | - | 82 |
| HIX-VI (5.5) | 100 | 471 | 247 | 776 | 423 | - | 95 |
| HIX-VII (4.8) | 100 | 125 | 119 | 211 | 381 | - | 184** |
| HIX-VIII (3.3) | 100 | 149 | 224 | 197 | 259 | - | 149** |
| ***Malbranchea* sp.** | | | | | | | |
| MFX-I (4.5) | 100 | 142 | 171 | 146 | 204 | - | 116** |
| MFX-II (3.5) | 100 | 134 | 144 | 124 | 174 | - | 107** |
| MFX-III (3.3) | 100 | 112 | 149 | 147 | 167 | - | 80 |
| ***Melanocarpus* sp.** | | | | | | | |
| MAX-I (7.8) | 100 | 153 | 155 | 137 | 182 | - | 107** |
| MAX-II (7.2) | 100 | 123 | 155 | 188 | 185 | - | 71 |
| MAX-III (6.5) | 100 | 93 | 156 | 150 | 177 | - | 42 |
| MAX-IV (5.8) | 100 | 97 | 140 | 143 | 167 | - | 65 |
| MAX-V (5.0) | 100 | 87 | 137 | 137 | 159 | - | 87 |
| MAX-VI (4.5) | 100 | 100 | 96 | 168 | 200 | - | 46 |
| ***Thermoascus aurantiacus*** | | | | | | | |
| TAX-I (7.5) | 100 | 136 | 122 | 134 | 174 | - | 28 |
| TAX-II (7.2) | 100 | 139 | 127 | 116 | 129 | - | 74 |
| TAX-III (5.8) | 100 | 192 | 136 | 204 | 352 | - | 292** |
| TAX-IV (3.4) | 100 | 140 | 157 | 253 | 377 | - | 209** |

[a] Composition of each substrate:
BWX, Birch wood Xylan (> 90 % xylose);
LWX, Larch Wood Xylan (86 % xylose / 8.8 % arabinose / 1.2 % glucose / 3.2 % uronic acid);
OSX, Oat Spelt Xylan (75 % xylose / 10% arabinose / 15 % glucose);
RAX, Rye Arabino Xylan (49 % arabinose / 51 % xylose);
WAX, Wheat Arabino Xylan (41 % arabinose / 59 % xylose);
DA, Debranched Arabinan (arabinose).
Notes [b] through [d], *, ** See end of Table 2B

**Table 2B.** The Relative Percent Activities of Xylanase Isoforms (Peak Fractions) of Different Thermotolerant Fungi against Different Types of Xylan Substrates (Substituted and Unsubstituted) and Debranched Arabinan.

| ***Substrate*[a] → *Isoforms*↓** | ***BWX*[b]** | ***LWX*[b]** | ***OSX*[b]** | ***RAX*[b]** | ***WAX*[b]** | ***DA*[b]** | ***BWX*[c]** |
|---|---|---|---|---|---|---|---|
| **Thermotolerant fungi** | | | | | | | |
| ***Absidia corymbifera*** | | | | | | | |
| ACX-I (7.3) | 100 | 185 | 110 | 352 | 428 | - | 83 |
| ACX-II (5.6) | 100 | 216 | 110 | 462 | 529 | - | 52 |
| ACX-III (5.4) | 100 | 123 | 103 | 247 | 303 | - | 88 |
| ACX-IV (4.8) | 100 | 108 | 171 | 165 | 281 | - | 79 |
| ACX-V (3.8) | 100 | 101 | 175 | 147 | 156 | - | 84 |
| ACX-VI (3.3) | 100 | 105 | 180 | 112 | 137 | - | 89 |
| ***Acrophialophora nainiana*** | | | | | | | |
| ANX-I (9.1) | 100 | 200 | 171 | 557 | 666 | 66 | 7 |
| ANX-II (5.8) | 100 | 152 | 184 | 456 | 604 | 72 | 14 |
| ANX-III (5.6) | 100 | 215 | 162 | 587 | 575 | 34 | 62 |
| ANX-IV (4.8) | 100 | 278 | 232 | 450 | 685 | 60 | 55 |
| ANX-V (4.2) | 100 | 208 | 174 | 448 | 756 | 32 | 46 |
| ANX-VI (3.5) | 100 | 322 | 466 | 838 | 971 | 66 | 41 |
| ***Aspergillus caespitosus*** | | | | | | | |
| ASCX-I (7.8) | 100 | 107 | 109 | 108 | 158 | - | 25 |
| ASCX-II (6.7) | 100 | 94 | 142 | 67 | 100 | - | 90** |
| ASCX-III (5.9) | 100 | 75 | 70 | 101 | 135 | - | 32 |
| ASCX-IV (5.5) | 100 | 128 | 148 | 175 | 244 | - | 38 |
| ASCX-V (5.2) | 100 | 131 | 93 | 216 | 232 | - | 71 |
| ASCX-VI (3.8) | 100 | 104 | 120 | 162 | 214 | - | 98** |
| ASCX-VII (3.3) | 100 | 94 | 102 | 99 | 131 | - | 96** |
| ***Aspergillus terreus*** | | | | | | | |
| ATX-I (8.0) | 100 | 278 | 87 | 70 | 274 | 34 | 22 |
| ATX-II (7.0) | 100 | 403 | 150 | 146 | 300 | 53 | 7 |
| ATX-III (6.5) | 100 | 246 | 135 | 203 | 417 | 61 | 25 |
| ATX-IV (5.9) | 100 | 294 | 102 | 205 | 405 | 47 | 2 |
| ATX-V (5.5) | 100 | 212 | 201 | 398 | 306 | 30 | 17 |
| ATX-VI (4.8) | 100 | 277 | 120 | 283 | 368 | 18 | 21 |
| ATX-VII (4.5) | 100 | 185 | 99 | 179 | 273 | - | 17 |
| ATX-VIII (4.1) | 100 | 88 | 89 | 150 | 260 | - | 20 |
| ATX-IX (3.5) | 100 | 75 | 130 | 76 | 252 | - | 7 |
| ATX-X (3.3) | 100 | 226 | 96 | 234 | 321 | - | 13 |
| ***Emericella nidulans* var. *lata*** | | | | | | | |
| ENX-I (9.7) | 100 | 94 | 130 | 47 | 79 | - | 3 |
| ENX-II (8.2) | 100 | 134 | 123 | 125 | 156 | - | 36 |
| ENX-III (7.2) | 100 | 175 | 118 | 330 | 307 | - | 66 |
| ENX-IV (6.9) | 100 | 393 | 120 | 626 | 826 | - | 76 |
| ENX-V (6.3) | 100 | 226 | 140 | 463 | 200 | - | 66 |
| ENX-VI (5.9) | 100 | 163 | 103 | 343 | 350 | - | 70 |
| ENX-VII (5.5) | 100 | 179 | 63 | 401 | 384 | - | 68 |
| ENX-VIII (5.3) | 100 | 125 | - | 241 | 410 | - | 71 |
| ENX-IX (4.8) | 100 | 104 | 150 | 187 | 194 | - | 80 |
| ENX-X (4.1) | 100 | 134 | 153 | 219 | 293 | - | 58 |
| ENX-XI (3.4) | 100 | 96 | 145 | 173 | 211 | - | 44 |
| ***Penicillium lagena*** | | | | | | | |
| PLX-I (6.7) | 100 | 227 | 177 | 211 | 188 | - | 18 |
| PLX-II (5.8) | 100 | 307 | 115 | 456 | 433 | 28 | 26 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| PLX-III (5.5) | 100 | 165 | 130 | 146 | 176 | 7 | 24 |
| PLX-IV (5.3) | 100 | 126 | 146 | 260 | 288 | 4 | 22 |
| PLX-V (4.5) | 100 | 130 | 208 | 169 | 234 | 3 | 69 |
| PLX-VI (3.5) | 100 | 115 | 173 | 162 | 229 | 3 | 62 |
| PLX-VII (3.3) | 100 | 264 | 191 | 355 | 400 | 9 | 52 |

[a] Composition of each substrate:
BWX, Birch wood Xylan (> 90 % xylose);
LWX, Larch Wood Xylan (86 % xylose / 8.8 % arabinose / 1.2 % glucose / 3.2 % uronic acid);
OSX, Oat Spelt Xylan (75 % xylose / 10% arabinose / 15 % glucose);
RAX, Rye Arabino Xylan (49 % arabinose / 51 % xylose);
WAX, Wheat Arabino Xylan (41 % arabinose / 59 % xylose);
DA, Debranched Arabinan (arabinose).

[b] Relative activity against different xylan substrates at pH 6.0.
[c] Relative activity against birch wood xylan at pH 9.0.
[d] pI of xylanase isoforms expressed in respective fungal strain.
* Activity not detected.
** Active under alkaline conditions.

Article submitted: May 1, 2006; Accepted: May 31, 2006; Published: June 22, 2006

# ENZYMES IMPROVE ECF BLEACHING OF PULP

Pratima Bajpai,[a*] Aradhna Anand[a], Nirmal Sharma[a], Shree P. Mishra[b], Pramod K. Bajpai[a] and Dominique Lachenal[b]

The delignification efficiency of different laccase enzymes was examined on the eucalyptus Kraft pulp. The laccase enzyme from *Trametes versicolor* showing the highest delignification efficiency was selected and used in the elemental chlorine-free bleaching sequence for improving the pulp bleachability. An appreciable reduction in chlorine dioxide consumption was also obtained. Further reduction in chlorine dioxide consumption was obtained when the same laccase treated pulp was subjected to an acid treatment after the extraction stage followed by the $DE_PD$ sequence. Elemental-chlorine free bleaching was also performed using the xylanase-laccase treated pulp. Xylanase treatment was incorporated to the laccase mediator system in the elemental-chlorine free bleaching both sequentially and simultaneously. The bleaching sequence $DE_PD$ followed and in both the cases, the reduction in chlorine dioxide consumption was greater in comparison to the control. The chlorine dioxide consumption was reduced further when xylanase-laccase treated pulp was given an additional acid treatment. The final pulp properties of the treated pulps were comparable to the control pulp.

*Keywords: Laccase, Mediator, Delignification, ECF bleaching, Xylanase, Chlorine dioxide*

*Contact information: a: Thapar Centre for Industrial Research & Development, Patiala, 147004, India; b: École Française de Papeterie et des Industries Graphiques, BP 65, 38402, Saint Martin D'heres, France; *Corresponding author: pratima@thaparresearch.org*

## INTRODUCTION

Increasing awareness about environmental concerns has led the paper industry to look for cleaner production options aimed at the reduced consumption of chlorine and its compounds in the bleaching sequences which thereby minimizes the discharge of chlorinated organics in the effluent, i.e., AOX. (Mishra et al. 2001). These organo-chlorine compounds are produced mainly by the reactions between residual lignin present in wood fibers and the chlorine used for bleaching. Some of these compounds are found to be toxic, mutagenic, persistent, bioaccumulating, and harmful to biological systems (Bajpai and Bajpai 1996).

Elemental chlorine-free (ECF) bleaching for the pulp and paper industry, based on chlorine dioxide, offers a number of fundamental benefits over the traditional methods. The U.S. EPA's Cluster Rule for the pulp and paper industry has ECF as one of its core Best Available Technology (BAT) elements (Pryke 1997). In addition to producing the highest pulp quality, ECF bleaching has proven itself to be a pollution prevention process for the pulp and paper industry. Perhaps most important is the fact that the use of chlorine dioxide in the first stage of chemical pulp bleaching virtually eliminates dioxins and 12

priority chlorophenols proposed by the U.S. Environmental Protection Agency (EPA) for regulation to non-detect levels. The other benefits of ECF bleaching are that it decreases chloroform formation and total chlorinated organic compound (AOX) formation by 90%; efficiently utilizes forest resources; contributes to eco-system recovery; and is compatible with emerging minimum-impact mill technologies (Pryke 1997).

The use of biotechnology in pulp bleaching has attracted considerable attention and achieved interesting results in recent years (Bajpai and Bajpai 1996; Bajpai et al. 2005; Call 1999; Sariaslani 1989 and Paice et al. 1995a). The incorporation of enzymes into the ECF technology can be of further benefit in terms of consumption of bleach chemicals followed by the amount of pollution generation. Enzyme prebleaching using xylanase enzymes offers a solution by improving the effectiveness of bleaching chemicals in removing lignin (Bajpai and Bajpai 1996; Viikari 1994; Roncero 2005; Kansoh 2004; Sudha 2003). Xylanase enzymes are reported to partially hydrolyze the hemicelluloses portion of pulp. It is presumed that the enzyme hydrolyzes xylan into smaller fragments allowing lignin associated with these short hemicelluloses chains to be more easily removed during subsequent extraction stages in bleaching. Xylanase-aided bleaching is an indirect method which does not directly degrade lignin and thus, has a limited effect (Bajpai and Bajpai 1996; Kansoh 2004; Sudha 2003).

Another potential candidate is a laccase enzyme, which selectively decomposes the lignin in the fiber (Paice et al. 1995a; Call and Muck 1994; Call and Muck 1995a; Call and Muck 1995b; Paice et al. 1995b; Reid and Paice 1994; Kondo 1994; Kondo et al 1995; Bourbonnais and Paice 1996; Camarero et al. 2004; Fu et al. 2000; Kandioller and Christov 2001; Crestini et al. 2003; Nelson et al. 1998). Laccases belong to the multi-copper oxidases, which can reduce elemental oxygen to water in a four-electron step and simultaneously perform a one-electron oxidation of many aromatic substrates (Paice et al. 1995a; Reinhammer 1984). However, this enzyme alone is not able to delignify the pulp; it requires a mediator to become effective (Bourbonnais and Paice 1990). The mediator is a small redox molecule that acts as a "diffusible electron carrier" or "electron shuttle" between lignin and laccase. It is assumed that the mediator is needed because the large laccase molecule cannot enter the secondary cell wall and oxidize lignin directly. In the laccase mediator concept, the oxidized mediator acts directly on lignin and results in efficient delignification. The use of this enzyme is expected to have a lower impact on the environment by eliminating the use of chlorine and formation of organochlorine compounds (Call 2001). Its other expected benefits are a lower capital investment, a safe system for selectively removing lignin, and improved pulp yields (Reid and Paice 1994).

The successive combination of the two enzymatic methods, the hydrolytic xylanase and the oxidative laccase-mediator treatment, has previously been shown to increase the delignification efficiency (Herpoel et al 2002; Viikari et al. 1999). In light of the background cited here, attempts have been made to use xylanase and laccase enzymes in ECF bleaching for selective delignification of pulp for environment friendly bleaching.

## EXPERIMENTAL

Wood samples, infected with fungi, were collected from wood yards of pulp mills and other places. The sample pieces were inoculated in sterilized potato dextrose medium containing bacterial inhibitors in 250 ml Erlenmeyer flasks. The flasks were kept under agitation in a shaking incubator at 150 rpm and 30°C for 3-4 days. The fungus mycelium was then streaked on PDA plates. Fungal patches were further streaked on PDA plates many times to get the pure fungal colonies. The pure colonies were inoculated on slants for screening of laccase producing strains. The PDA slants were stored at 4°C until used.

Fungal cultures were inoculated in 50 ml sterilized potato dextrose medium in 500 ml Erlenmeyer flasks, incubated at 27-30°C and 60-70% relative humidity in an incubator. The laccase activity in culture broth of each culture was determined by monitoring the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as increased absorbance at 420 nm. The reaction mixture contained 2.5 ml of 0.1M sodium acetate buffer pH 5.0, 0.17 ml of the enzyme sample and 0.33 ml 5mM ABTS, in a final volume of 3.0 ml. 1 U is the amount of enzyme that converts 1 μmol substrate in one minute under the described conditions at 25°C.

Eucalyptus wood chips were procured from a pulp and paper mill in North India. Pulping was done in a rotary autoclave digester by Kraft process. Numerous experiments were performed at different temperatures, time, and active alkali doses to optimize the pulping conditions to obtain a pulp of kappa number 18-19.

The efficacy of laccase enzymes for delignification of eucalyptus pulp was studied at a pulp consistency of 15%, temperature 45$^{o}$C, pH 4.0 for 5.5 hours at different dose levels using HBT as mediator. The enzyme showing maximum delignification was selected and used for further studies. The xylanase treatments were carried out at a pulp consistency of 10%, temperature 50$^{o}$C, pH 8, time 2 hours and enzyme dose 0.075% (Pulpzyme HC from Novozymes).

ECF bleaching of laccase and xylanase-laccase treated pulps was conducted using a $DE_PD$ sequence. The bleaching sequence followed for the reference sample was $DE_PDD$. Acid treatment was conducted at pH 2, 90°C, for 2 hours at 10% consistency. Xylanase treatment was incorporated both sequentially and simultaneously. The effect of acid treatment in both the cases was also studied. The final pulps were characterized for optical properties.

Kappa number, a measure of residual lignin in the pulp, was determined as per Tappi test method T 236 om-99. The solution viscosity of a pulp gives an indication of the average degree of polymerization of the cellulose. The viscosity of pulp was determined by capillary viscometer method using Tappi test method T 230 om-99. Such a test gives a relative indication of the degradation (decrease in cellulose molecular weight) resulting from the pulping and/or bleaching process. The brightness and CIE whiteness of the pulp were measured using Technibrite TB 1c instrument as per Tappi test method T525 om-02 and T 560 pm-96, respectively. The brightness reversion of the pulp was estimated in terms of post colour (PC) number according to Tappi test method T 260 om-85.

## RESULTS AND DISCUSSION

Eucalyptus pulp of kappa number 18.2 was prepared in the laboratory. The unbleached pulp yield was 42% with a pulp viscosity of 10 cp. Where the pulp yield is defined as the mass of pulp (oven dry basis) divided by mass of wood chips (oven dry basis) and expressed as percentage.

Four enzymes, two of Trametes species (Laccase-1 & Laccase-2) and other two of Aspergillus species (Laccase-3 & Laccase-4) were selected. To determine the delignification efficiency of laccase enzymes, the treatment of eucalyptus pulp was done in a rotary autoclave digester with different laccase enzymes and mediator (HBT). Laccase-1 and Laccase-2 showed maximum delignification with 60 U enzyme/g of pulp whereas Laccase-3 and Laccase-4 showed maximum delignification with 120 U and 400 U enzyme/g pulp, respectively. Delignification efficiency was 48%, 50%, 15% and 27% in case of Laccase-1, Laccase-2, Laccase-3 and Laccase-4, respectively (Table 1). Laccase-1 was selected for the bleaching studies, as it showed the highest delignification rate. With Laccase-1 enzyme, maximum delignification occurred at 45°C, pH 4.0-5.5 (adjusted with sodium acetate buffer), retention time 3.0-5.0 hours, pulp consistency 15.0%, oxygen pressure 10 kg/cm$^2$, enzyme dose 60 U/g and mediator dose 3% (Table 2). Laccase-mediator treated pulps were then alkali extracted using hydrogen peroxide. The brightness and kappa number of the pulp were 48% ISO and 6.3, respectively. To know the effect of $E_P$ alone and $E_P$ followed by acid treatment, experiments were conducted without any enzyme. The kappa number of pulp dropped to 12.0 on extraction alone ($E_P$), in the beginning, which reduced further to 10.8 (results not shown in the Table) on acid treatment (after EPA).

**Table 1.** Delignification of Eucalyptus Kraft Pulp* with Different Laccase Enzymes

| Parameter | Laccase-1[a] | Laccase-2[b] | Laccase-3[c] | Laccase-4[d] | Control |
|---|---|---|---|---|---|
| $LE_P$ stage Kappa no. | 6.3 | 6.1 | 10.3 | 9.0 | 12.1 |
| Reduction in kappa no. (%) by laccase treatment | 48.0 | 50.0 | 14.9 | 27 | - |
| $LE_P$ stage brightness (% ISO) | 48.0 | 48.9 | 35.2 | 34.5 | 31.5 |

*Kappa number 18.2; Kappa number after only alkaline extraction (EP) 12.0
Conditions:
Laccase-1 & Laccase-2: Enzyme dose 60 U/g; HBT (mediator) dose 3%; pH 4.0; temp. 45 °C; residence time 5.5 h; consistency 15%; $O_2$ pressure 10 kg/cm$^2$
Laccase-3: Enzyme dose 120 U/g; HBT dose 3%; pH 5.5; temp. 45 °C; residence time 5.5 h; consistency 15%; $O_2$ pressure 10 kg/cm$^2$
Laccase-4: Enzyme dose 400 U/g; HBT dose 3%; pH 4.5; temp. 45 °C; residence time 5.5 h; consistency 15%; $O_2$ pressure 10 kg/cm$^2$
$E_P$ stage: NaOH 1.5%; $H_2O_2$ 0.5%; temp. 70 °C; residence time 2 h; consistency 10%
[a]Laccase-1: from *Trametes versicolor* TCIRD-2 [b]Laccase-2: from *Trametes versicolor* TCIRD-6, [c]Laccase-3: from *Aspergillus niger* TCIRD-10, [d]Laccase-4: from *Aspergillus niger* TCIRD-15

The results of ECF bleaching of laccase treated pulps are shown in Table 3. An appreciable reduction in chlorine dioxide consumption was observed. Chlorine dioxide demand reduced to 45.6% in comparison to the reference sample (Tables 3 and 4). When the same laccase treated pulp was subjected to an acid treatment (pH 2, temperature 90°C, time 2 h, consistency 10%) after the extraction stage followed by $DE_PD$ sequence, there was a remarkable effect of the acid treatment on the same pulp in the same conditions. The reduction in the chlorine dioxide dose increased to 58.1% in comparison to the control (Tables 3 and 4). This reflected the encouraging role of acid treatment on the laccase-mediator bleaching system.

**Table 2.** Optimum Conditions of Delignification by Laccase-1

| Parameter | Value |
|---|---|
| Enzyme dose (U/g) | 60 |
| Mediator (HBT) dose (%) | 3 |
| Consistency (%) | 15 |
| pH | 4.0 |
| Temperature ($^oC$) | 45 |
| Time (h) | 5 |
| $O_2$ pressure ($kg/cm^2$) | 10 |

**Table 3.** Effect of Laccase-Mediator System on ECF Bleaching

| Parameter | $D_OE_P*D_1D_2$ | LEp**DOEp***$D_1$ | $LE_P**AD_OEp***D_1$ |
|---|---|---|---|
| Kappa factor | 0.28 | 0.15 | 0.12 |
| Reduction in $ClO_2$ Dose (%) | - | 45.6 | 58.1 |
| LEp** Brightness (%ISO) | - | 46.4 | - |
| LEp**A Brightness (%ISO) | - | - | 47.0 |
| $D_O$ Brightness (%ISO) | 42.0 | 66.8 | 63.5 |
| $E_P$* Brightness (%ISO) | 65.2 | - | - |
| Ep***Brightness (%ISO) | - | 79.4 | 78.9 |
| $D_1$ Brightness (%ISO) | 84.5 | 88.0 | 88.2 |
| $D_2$ Brightness (%ISO) | 87.8 | - | - |
| Viscosity (cp) | 7.2 | 7.1 | 6.9 |
| Treatment conditions:<br>L stage conditions: Laccase dose 60 U/g pulp, HBT dose 3%, pH 4.0, temp. 45 °C, consistency 15% and retention time 5 h<br>A stage conditions: pH 2, temp. 90 °C, retention time 2 h, consistency 10%<br>Ep* stage conditions: NaOH dose 0.85%, H2O2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h<br>Ep** stage conditions: NaOH dose 1.5%, H2O2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h<br>Ep*** stage conditions: NaOH dose 0.8%, H2O2 dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h<br>$D_O$ stage conditions: pH 3.5, temp. 55 °C, retention time 30 min, consistency 10%<br>$D_1$ stage conditions: $ClO_2$ dose 0.8%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%<br>$D_2$ stage conditions: $ClO_2$ dose 0.4%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10% | | | |

The results of ECF bleaching of xylanase-laccase treated pulp are shown in Tables 4 and 5. The bleaching sequence followed for the reference sample and the xylanase-laccase treated sample was the same as followed for the laccase mediator

bleaching system. It was observed that laccase treated pulp shows a cumulative effect along with xylanase treatment.

When only laccase treated pulp was used, the chlorine dioxide demand was reduced to 45.6% in comparison to the control sample, which increased to 55% when xylanase treatment was also included to the same laccase mediator bleaching system (Tables 4 and 5). Both the simultaneous and sequential combination of xylanases with laccase-mediator bleaching systems showed the same reduction in the bleach chemical consumption. Similar results were reported by Herpoel et al. (2002) with wheat straw pulp where up to 60% reduction in kappa number was obtained after xylanase and laccase sequential treatments, followed by alkaline extraction.

The addition of the acid treatment stage to the xylanase-laccase treated pulp led to further reduction in the chemical consumption to 67.4% rather than 55% when no acid treatment was involved. There was no difference in the results of sequential and simultaneous treatments of xylanase application (Tables 4 and 5). As a parallel experiment for the sake of comparison, the pulp was acid treated without any laccase treatment. There was a decrease in kappa number by 1.5 points (about 8.2%) only but the reduction in chlorine dioxide consumption in $AD_0E_PD_1D_2$ bleaching sequence was about 15% (results not shown in Tables), which indicated the potential of acid treatment.

**Table 4.** Bleach Chemical Requirements

| Sample detail | Bleaching chemical (kg/TP) | |
|---|---|---|
| | $ClO_2$ | Reduction in $ClO_2$ dose (%) |
| $D_OEp^*D_1D_2$ | 32.2 | - |
| $LEp^{**}D_O\ Ep^{***}D_1$ | 17.5 | 45.6 |
| $LE_P{}^{**}AD_OEp^{***}D_1$ | 13.5 | 58.1 |
| $Sm.XLEp^{**}\ D_OEp^{***}D_1$ | 14.5 | 55.0 |
| $Sm.XLEp^{**}\ AD_OEp^{***}D_1$ | 10.5 | 67.4 |
| $Sq.XLEp^{**}D_OEp^{***}D_1$ | 14.5 | 55.0 |
| $Sq.XLEp^{**}\ AD_OEp^{***}D_1$ | 10.5 | 67.4 |

Treatment conditions:
X stage conditions: Xylanase dose 0.075%, pH 8.0, temp. 50°C, retention time 2h and consistency 10%
L stage conditions: Laccase dose 60 U/g pulp, HBT dose 3%, pH 4.0, temp. 45°C, consistency 15% and retention time 5 h
A stage conditions: pH 2, temp. 90°C, retention time 2 h, consistency 10%
Ep* stage conditions: NaOH dose 0.85%, $H_2O_2$ dose 0.5%, temp. 70°C, consistency 10% and retention time 2 h
Ep** stage conditions: NaOH dose 1.5%, $H_2O_2$ dose 0.5%, temp. 70°C, consistency 10% and retention time 2 h
Ep*** stage conditions: NaOH dose 0.8%, $H_2O_2$ dose 0.5%, temp. 70°C, consistency 10% and retention time 2 h
$D_O$ stage conditions: pH 3.5, temp. 55°C, retention time 30 min, consistency 10%
$D_1$ stage conditions: ClO2 dose in 1st, 2nd, 3rd sequence 0.8% & in others 1.1%, pH 3.5, temp. 75°C, retention time 3 h, consistency 10%
$D_2$ stage conditions: $ClO_2$ dose 0.4%, pH 3.5, temp. 75°C, retention time 3 h, consistency 10%

**Table 5.** Effect of Xylanase Treatment on the Laccase-Mediator Bleaching System

| Parameter | $D_OEp^*D_1D_2$ | Sm.XLEp** $D_OEp^{***}D_1$ | Sm.XLEp**A $D_OEp^{***}D_1$ | Sq.XLEp** $D_O$ $Ep^{***}D_1$ | Sq.XLEp**A $D_OEp^{***}D_1$ |
|---|---|---|---|---|---|
| Kappa factor | 0.28 | 0.12 | 0.09 | 0.12 | 0.09 |
| Reduction in $ClO_2$ dose | - | 55.0 | 67.4 | 55.0 | 67.4 |
| Ep* Brightness (%ISO) | 65.2 | - | - | - | - |
| XLEp** Brightness (%ISO) | - | 49.5 | - | 49.2 | - |
| XLEp**A Brightness (%ISO) | - | - | 50.3 | - | 50.0 |
| $D_O$ Brightness (%ISO) | 42.0 | 67.8 | 62.9 | 68.1 | 62.7 |
| Ep***Brightness (%ISO) | - | 79.7 | 77.5 | 77.0 | 77.3 |
| $D_1$ Brightness (%ISO) | 84.5 | 88.3 | 88.0 | 88.0 | 87.9 |
| $D_2$ Brightness (%ISO) | 87.8 | - | - | - | - |
| Viscosity (cP) | 7.2 | 7.0 | 6.8 | 7.0 | 6.8 |

Treatment conditions:
X stage conditions: Xylanase dose 0.075%, pH 8.0, temp. 50 °C, retention time 2h and consistency 10%
L stage conditions: Laccase dose 60U/g pulp, HBT dose 3%, pH 4.0, temp. 45 °C, consistency 15% and retention time 5 h
A stage conditions: pH 2, temp. 90 °C, retention time 2 h, consistency 10%
Ep* stage conditions: NaOH dose 0.85%, $H_2O_2$ dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep** stage conditions: NaOH dose 1.5%, $H_2O_2$ dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep*** stage conditions: NaOH dose 0.8%, $H_2O_2$ dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
$D_O$ stage conditions: pH 3.5, temp. 55 °C, retention time 30 min, consistency 10%
$D_1$ stage conditions: $ClO_2$ dose in 1st sequence 0.8% and in others 1.1%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%
$D_2$ stage conditions: $ClO_2$ dose 0.4%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%

The final pulps were characterized and it was found that the pulp properties were comparable to the reference sample, which shows that the laccase-mediator system does not affect the properties of the pulp (Table 6). Poppius-Levlin et al. (1999) have also reported that although laccase treatment temperature, laccase charge and mediator charge had pronounced effect on lignin reactions, their effect on pulp properties was insignificant.

About 43% reduction in kappa number of Eucalyptus kraft pulp after laccase treatment and alkaline extraction has been achieved by Fu et al. (2000), whereas bleaching of high kappa kraft pulps of different raw materials with a laccase mediator system provided 43-61% reduction in kappa number after E+P stage, using violuric acid as mediator (Chandra et al. 2001). Kandioller and Christopher (2004) have also reported various degrees of delignification depending on the pulp type, enzyme and mediator charge using laccase mediator treatment followed by alkaline extraction. By repeated laccase treatment and alkaline extraction (reinforced with oxygen and hydrogen peroxide), up to 80% reduction in kappa number was obtained by Sealey et al (1997).

This indicates that the laccase mediator treatment is capable of reacting with vestiges of residual lignin, which are typically very unreactive.

Further work on the effects of initial alkali extraction (E/ $E_P$/ $E_{OP}$ stage) and /or acid treatment (A stage) followed by conventional ECF, with and without the laccase mediator system under different conditions, is in progress.

**Table 6.** Bleached Pulp Properties

| Bleaching sequence | Final brightness (%ISO) | Final viscosity (cP) | CIE whiteness (%ISO) | P.C. number |
|---|---|---|---|---|
| $D_OEp^*D_1D_2$ | 87.9 | 7.2 | 80.0 | 0.28 |
| $LEp^{**}D_O$ $Ep^{***}D_1$ | 88.0 | 7.1 | 81.1 | 0.27 |
| $LE_P{}^{**}A$ $D_OEp^{***}D_1$ | 88.2 | 6.9 | 81.2 | 0.26 |
| $Sm.XLEp^{**}D_OEp^{***}D_1$ | 88.3 | 7.0 | 82.0 | 0.25 |
| $Sm.XLEp^{**}AD_OEp^{***}D_1$ | 88.0 | 6.8 | 82.0 | 0.26 |
| $Sq.XLEp^{**}$ $D_OEp^{***}D_1$ | 88.0 | 7.0 | 82.2 | 0.25 |
| $Sq.XLEp^{**}AD_OEp^{***}D_1$ | 88.0 | 6.8 | 82.3 | 0.25 |

Treatment conditions:
X stage conditions: Xylanase dose 0.075%, pH 8.0, temp. 50 °C, retention time 2h and consistency 10%
L stage conditions: Laccase dose 60 U/g pulp, HBT dose 3%, pH 4.0, temp. 45 °C, consistency 15% and retention time 5 h
A stage conditions: pH 2, temp. 90 °C, retention time 2 h, consistency 10%
Ep* stage conditions: NaOH dose 0.85%, $H_2O_2$ dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep** stage conditions: NaOH dose 1.5%, $H_2O_2$ dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
Ep*** stage conditions: NaOH dose 0.8%, $H_2O_2$ dose 0.5%, temp. 70 °C, consistency 10% and retention time 2 h
$D_O$ stage conditions: pH 3.5, temp. 55 °C, retention time 30 min, consistency 10%
$D_1$ stage conditions: $ClO_2$ dose in 1st, 2nd, 3rd sequence 0.8% and in others 1.1%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%
$D_2$ stage conditions: $ClO_2$ dose 0.4%, pH 3.5, temp. 75 °C, retention time 3 h, consistency 10%

## CONCLUSIONS

Based on the bleaching experiments performed in this study, it is concluded that the enzymes have an encouraging role in reducing the chemical consumption during elemental-chlorine-free bleaching. Upon implementation, the applications from the present findings can be expected to help reduce the amount of pollutants that are produced during future manufacture of bleached kraft pulp.

## ACKNOWLEDGMENTS

The authors thank Indo-French Centre for the Promotion of Advanced Research (IFCPAR) for providing financial support.

## REFERENCES CITED

Bajpai, P., and Bajpai, P. K. (1996). "Realities and trends in enzymatic prebleaching of Kraft pulp," *Adv. Biochem. Eng. Biotechnol*., T. Schepper, ed., Springer-Verlag, Berlin, 56, pp. 1-31.

Bajpai, P., Anand, A., Sharma, N., Bajpai, P. K., Mishra, S. P., and Lachenal, D. (2005). "Bleaching of eucalyptus Kraft pulp with laccase-mediator system," In: *Proc. 2005 International Pulp Bleaching Conference*, June 14-16, Stockholm, Sweden, pp 306-308.

Bourbonnais, R., and Paice, M. G. (1990). "Oxidation of non-phenolic substrates. An expanded role for laccase in lignin degradation," *FEBS Lett*. 267, 99.

Bourbonnais, R., and Paice, M. G. (1996). "Enzymatic delignification of Kraft pulp using laccase and a mediator," *Tappi. J*. 76(6), 199-204.

Call, H. P. (1999). "New enzymatically mediated delignification and bleaching systems," In: *10th International Symposium on Wood and Pulping Chemistry - 10th Biennial ISWPC- Main Symposium*, Yokohama, Japan, (1), 540-545.

Call, H. P. (2001). "New developments in enzyme assisted delignification and bleaching," In: *Proc. 8th Int. Conf. Biotech. in Pulp and Paper Industry*, Helsinki, Finland, 60.

Call, H. P., and Mucke, I. (1994). "Enzymatic bleaching of pulps with the laccase-mediator system," In: *Pulping Conference AIChE session*, San Diego, CA, USA, 38-52.

Call, H. P., and Mucke, I. (1995a). "Further improvements of the Laccase-Mediator-system (LMS) for enzymatic delignification and results from large scale trials," In: *Int. Non-Chlorine Bleaching Conf*., Amelia Island, Florida, USA, 16pp.

Call, H. P., and Mucke, I. (1995b). "The laccase-mediator-system (LMS)," *Biotechnology in the Pulp and Paper Industry: Recent Advances in Applied and Fundamental Research* (Proc. 6th Int. Conf. Biotech. Pulp and Paper Industry), E. Srebotnik and K. Messner, eds., Vienna, Austria, 27-32.

Camarero, S., Garcia, O., Vidal, T., Colom, J., Delrio, J. C., Gutierrez, A., Gras, J. M., Monje, R., Martinez, M. J., and Martinez, A. T. (2004). "Efficient bleaching of non-wood high-quality paper pulp using laccase-mediator system," *Enzyme and Microbial. Technol*. 35(2-3), 113-120.

Chandra, R. P., Chakar, F. S., Allison, L., Kim, D. H., Ragauskas, A. J., and Elder, T. (2001). "Delving into the fundamental LMS delignification of high kappa Kraft pulps," In: *Proc. 8th Int. Conf. Biotech. Pulp and Paper Industry*, 4-8 June 2001, Helsinki, Finland, 54.

Crestini, C., Jurasek, L., and Argyropoulos, D. S. (2003). "On the mechanism of the laccase-mediator system in the oxidation of lignin," *Chemistry* 9(21), 5371-5378.

Fu, S., Zhan, H., and Yu, H. (2000). "Preliminary study on biobleaching of Eucalyptus urophylla Kraft pulp with laccase-mediator system," *China Pulp Pap*. 19(2), 8-15.

Herpoel, I., Jeller, H., Fang, G., Petit-Conil, M., Bourbonnais, R., Robert, J.-L., Ashther, M., and Sigoillot, J.-C. (2002). "Efficient enzymatic delignification of wheat straw pulp by a sequential xylanase-laccase mediator treatment," *J. Pulp Pap. Sci*. 28(3), 67-71.

Kandioller, G., and Christov, L. (2001). "Efficiency of Trametes versicolor laccase-mediator systems in pulp delignification and bleaching," In: *Proc. 8th Int. Conf. Biotech. In Pulp and Paper Industry*, Helsinki, Finland, pp. 223-224.

Kandioller, G., and Christopher, L. (2004). "Delignification efficiency of Trametes Versicolor laccase in combination with various mediators," In: *9th Int. Conf. Biotech. Pulp and Paper Industry*. 10-14 Oct 2004, Durban, South Africa, Paper 05.4..

Kanosh, A. L., and Nagieb, Z. A. (2004). "Xylanase and mannanase enzymes from Streptomyces galbus NR and their use in biobleaching of softwood Kraft pulp," *Antonie Van Leeuwenhoek* 85(2), 103-114.

Kondo, R., Hirai, H., Harazono, K., and Sakai, K. (1995). "Biobleaching of Kraft pulp with lignin-degrading fungi and their enzymes," In: *Biotechnology in the Pulp and Paper Industry: Recent Advances in Applied and Fundamental Research* (Proc. 6th, Int. Conf. Biotech. Pulp and Paper Industry), E. Srebotnik and K. Messner, eds., Vienna, Austria, pp. 33-38.

Kondo, R., Kurashiki, K., and Sakai, K. (1994). "In vitro bleaching of hardwood Kraft pulp by extra cellular enzymes secreted from white-rot fungi in a cultivation system using a membrane filter," *Appl. Env. Microbiol*. 60, 921-926.

Mishra, D. K., Joshi, H. C., Bhatia, H. S., Chandarana, D. P., and Lakhotia, R. L. (2001). "Enzyme pre-bleaching towards ECF pulp: a successful attempt at Century Pulp and Paper," *IPPTA Convention Issue*, 5-14.

Nelson, P. J., Chin, C. W. J., Viikari, L., and Tenkanen, M. (1998). "The use of a laccase-mediator stage in bleaching of eucalypt Kraft pulps," In: *Proc. Int. Pulp Bleaching Conf*. Book 2, Helsinki, Finland, pp. 349-354.

Paice, M. G., Bourbonnais, R., Reid, I. D., Archibald, F. S, and Jurasek, L. (1995a). "Oxidative bleaching enzymes," *J. Pulp Pap. Sci*. 21, J280-J284.

Paice, M. G., Bourbonnias, R., and Reid, I. D. (1995b). "Bleaching Kraft pulps with oxidative enzymes and alkaline hydrogen peroxide," *Tappi J.* 78(9), 161-170.

Poppius-levlin, K., Tamminen, T., Rajanen, K., Jaaskelainen, A. S., and Viikari, L. (1999). "Suitability of laccase-mediator systems for hardwood Kraft pulp delignification," *Proc. Int. Symp. on Wood and Pulping Chemistry – 10th Biennial ISWPC – Main Symposium*, Yokohama, Japan, (1), 7-10 June, 1999: Vol. 1, 556.

Pryke, D. C. (1997). "Elemental chlorine-free (ECF): Pollution prevention for the pulp and paper industry," (http://www.ecfpaper.org/science/science.html).

Reid, I. D., and Paice, M. G. (1994). "Biological bleaching of Kraft pulps by white-rot fungi and their enzymes," *FEMS Microbiology Reviews*, 13, 369-376.

Rein Hammer, B. (1984). "Laccase," In: *Copper Proteins and Copper Enzymes*., R. Lontie, ed., CRC, Boca Raton, Florida, USA, 1.

Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2005). "The effect of xylanase on lignocellulosic components during the bleaching of wood pulps," *Bioresource Technol.* 96(1), 21-30.

Sariaslani, F. S. (1989). "Microbial enzymes for oxidation of organic molecules," *Crit. Rev. Biotechnol*. 9, 171–257.

Sealey, J. E., Ragaukas, A. J., and Runge, T. M. (1997). "Biobleaching of Kraft pulps with laccase and hydroxybenzotriazole," *Proc. Tappi. Biological Science Symp*. 339-342.

Sudha, B., Veermani, H., and Sumathi, S. (2003). "Bleaching of bagasse pulp with enzyme pre-treatment," *Water Sci. Technol*. 47 (10), 163-168.

Viikari, L., Kantelinen, A., Sundquist, J., and Linko, M. (1994). "Xylanases in bleaching: from an idea to industry," *FEMS Microb. Rev*. 13, 335-350.

Viikari, L., Oksanen, T., Buchert, J., Amann, M., and Candussio, A. (1999). "Combined action of hemicellulases and oxidases in bleaching," In: *10th International Symposium on wood and pulping chemistry - 10th Biennial ISWPC - Main Symposium*, Yokohama, Japan, (1), 504-508.

Article submitted: March 27, 2006; Accepted: May 8, 2006; Published: June 24, 2006

# PAPERMAKING FIBERS FROM GIANT REED (*ARUNDO DONAX* L.) BY ADVANCED ECOLOGICALLY FRIENDLY PULPING AND BLEACHING TECHNOLOGIES

Anatoly A. Shatalov * and Helena Pereira

The anatomical structure and chemical composition of the stem-wall material of giant reed is considered from the viewpoint of raw material characterization for industrial fiber production. The effect of stem morphology (nodes and internodes) on pulping results and general pulp properties is discussed. The advantages of application of modern organic solvent based (organosolv) pulping technologies to giant reed are shown in comparison with the conventional (kraft) method. The conditions optimization for Ethanol-Alkali pulping (a selected organosolv pulping process) is given, and the chemical kinetics of the principal macromolecular components during ethanol-alkali pulping is described. The bleachability of organosolv pulps by short totally chlorine free (TCF) bleaching sequences using hydrogen peroxide and ozone as the active bleaching chemicals without pulp pre-delignification is examined and compared with kraft pulps. The enzymatic pre-treatment of reed organosolv pulps by commercial xylanase preparation is considered as a possibility toward the improvement of pulp bleachability.

*Keywords: Arundo donax L.; Giant reed; Non-wood fibers; Organosolv pulping; Totally chlorine free (TCF) bleaching; Biobleaching; Bleach boosting*

*Contact information: Centro de Estudos Florestais, Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal; *Corresponding author: anatoly@isa.utl.pt*

## INTRODUCTION

Non-woody plants are an important alternative source of fibers for the pulp and paper industry. The role of agro-fiber biomass is particularly prominent in countries with limited wood resources. In some regions of Asia, Africa and Latin America this is the only source of industrial papermaking fibers (Atchison 1993). The need to prevent the fast global deforestation (particularly in North America) and/or to meet the re-orientation of the West European agriculture towards non-food crops, due to general food overproduction, stimulated a recent renewed interest in agro-fiber plants (Van Dam et al. 1994; Moore 1996). A number of new fiber crops (such as elephant grass and reed canary grass) and traditional long-fiber species (such as flax and hemp) were recently (re)investigated for prospective industrial utilization (Leminen et al. 1996).

A widely distributed, naturally growing perennial rhizomatous grass, giant reed (*Arundo donax* L.), is a well-known fiber crop, the papermaking potential of which is being intensively reconsidered now. It has specific features, such as an annual harvesting period, high biomass productivity (up to 37 t $year^{-1}$ $ha^{-1}$, Vecchiet et al. 1996), ability to be intensively cultivated (Dalianis et al. 1994) and easy adaptability to different climatic

and soil conditions (Perdue 1958) which make *A. donax* one of the more promising industrial crops.

The history of *A. donax* application for papermaking started in 1830, when the first pulps were made by the boiling of stem material in calcium hydroxide (Perdue 1958). The comprehensive study of the pulping and bleaching ability of giant reed using traditional kraft and soda processes and chlorine-based bleaching was carried out between 1930-1950. The pulps were produced with rather low yield, but with satisfactory strength properties and bleachability (Jayme et al. 1948; Bhat and Virmani 1951; Di Felippo 1955).

Recently, the Nile Fiber Group Inc. (Washington, USA) together with Samoa Pacific Cellulose (California, USA) announced a successful commercial pulp run using exclusively *A. donax* reed (Nile Fiber Group 2002). The totally chlorine free (TCF) bleached kraft pulps were produced from reed growing wild in southern California using existing chemical wood pulping facilities of the Samoa Pacific mill. The new wood-free *A. donax* pulp is planned to be marketed under the name Samoa Cane.

The environmental and economical concern of pulp and paper manufacture, related to conventional pollutant sulfur- and chlorine-based industrial pulping and bleaching technologies, led to a generation of new approaches with reduced negative ecological impact. The pulping methods based on organic solvents in the reaction solution (organosolv pulping) and the bleaching methods based on non-chlorine oxidative chemicals (TCF bleaching) are the real commercially proved alternatives to traditional technologies (Stockburger 1993; Reeve 1996).

In the present article, the possibilities of papermaking fiber production from *A. donax* using organosolv pulping technologies in combination with TCF bleaching are reported based on our accumulated research experience in this field.

## EXPERIMENTAL

### Materials

The stems of giant reed (*A. donax*), free of leaves, with origin from Athens, Greece were used in this study. *A. donax* was cultivated in a university experimental plantation (Agricultural Engineering Department, Agricultural University of Athens), without irrigation, and was harvested with an average stem height of 4 m. For pulping, the stems were manually cut up to the approximate size of industrial chips (or match size - for kinetic and pulping optimization studies) and the moisture content was determined according to TAPPI standards. For chemical analysis a small portion of chips was additionally ground and screened to uniform particle size of 40-60 mesh.

### Methods

Extractives were determined gravimetrically after extraction in a Soxhlet apparatus. Ash and silica (as $SiO_2$) were quantified according to TAPPI T15 os 58 and TAPPI T 245 om-94, respectively. Hemicelluloses were extracted from chlorite holocellulose by aqueous alkaline solutions and the insoluble residue was accepted as α-cellulose (Browning 1967). Carbohydrate composition was analyzed by GC as alditol-

acetate derivatives of monosaccharides after Saeman hydrolysis (Saeman et al. 1963). Lignin was determined as Klason and acid-soluble according to T 222 om-88 and UM 250 TAPPI, respectively.

The anatomical structure of the stem-wall material was examined by light microscopy of transverse sections (ca. 17 μm thickness, Reichert sliding microtome) and dissociated elements (macerated in acetic acid-hydrogen peroxide solution at 60ºC for 48 hours), as described elsewhere (Shatalov et al. 2001).

Pulping experiments were carried out in a 7-l laboratory-scale batch reactor with forced circulation of cooking liquor and automatic time-temperature and pressure control (Shatalov and Pereira 2001). Kinetic studies as well as cooking conditions optimization were performed in 100 ml stainless steel autoclaves rotated in an oil bath, using 10 g (on oven-dry base) material on each pulping (Shatalov and Pereira 2004a, 2004b). Kappa number of pulps was determined according to TAPPI T 236 cm-85. Pulp viscosity was measured in cupri-ethylenediamine (CED) solution according to SCAN-CM 15:88. Handsheet formation for physical and reflectance test of pulps was performed according to TAPPI T 205 om-88 and TAPPI T 272 om-92, respectively. Papermaking properties of pulp handsheets were examined according to TAPPI T 220 om-88. Pulp optical properties, i.e. ISO brightness and DIN 6167 C/2 yellowness index, were measured with a CM-3630 Spectrophotometer (Minolta).

Hydrogen peroxide bleaching (P-stage) as well as pulp chelating (Q-stage) and alkaline extraction (E-stage) were performed in sealed plastic bags plunged into an agitated water bath with temperature control. Low consistency ozone bleaching (Z-stage) was performed in a 2-l glass reactor (Fischer), equipped with a power stirrer and connected with a laboratory ozone generator (Fischer 502) (Shatalov and Pereira 2005, 2006a). Enzymatic pulp pre-treatment was carried out in the double-layer plastic bags incubated in a water bath under required temperature (Shatalov and Pereira 2006b).

## RESULTS AND DISCUSSION

### Giant Reed as a Raw Material for Fiber Production

Only the stems of *A. donax* are of interest for economically feasible fiber production on a commercial scale. The stem is morphologically heterogeneous and consists of hollow internodes and solid nodes, composed of nodal diaphragm (i.e., residual fragments of fundamental tissue – the pith) and adjacent transition regions (Fig.1).

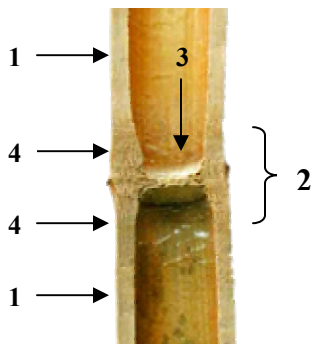
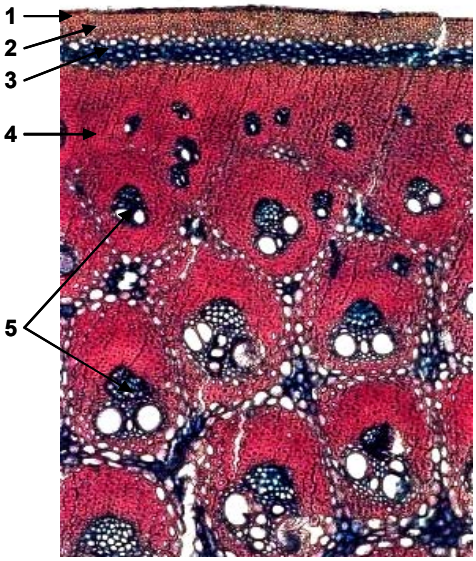



**Fig. 1.** Longitudinal cross-section of the *Arundo donax* L. culm: 1 - internode; 2 - node; 3 - nodal diaphragm; 4 - transition region (Shatalov and Pereira 2002a).

These botanically distinct parts can have different response on chemical processing. The anatomical and chemical analysis was therefore performed both with nodes and internodes, and the effect of stem morphology on general pulp properties has been examined using conventional kraft pulping, as a predominant process.

***Anatomical structure and fiber morphology***

An anatomical analysis showed similarity in the cellular structure of nodes and internodes (Shatalov and Pereira 2000a; Shatalov et al. 2001). The light microscopy of transverse sections revealed the prevalence of three tissue systems (Fig. 2): epidermal (or cortical parenchyma), fundamental (or ground parenchyma) and vascular (composed of fibro-vascular bundles).



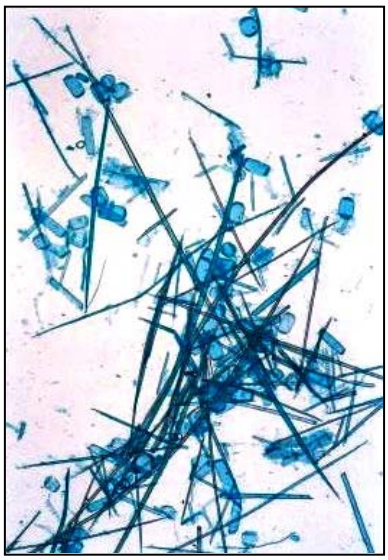



**Fig. 2. (left)** Transverse section of the internode of *A. donax* culm (100x): 1 - epidermis; 2 - strongly sclerified parenchyma cells; 3 - cortical parenchyma; 4 -fibers; 5 - fibro-vascular bundle.

**Fig. 3. (right)** Macerated sample of the internode of *A. donax* culm (100x) with fibers and parenchyma cells.

The average content of parenchyma in *A. donax* (57.8%) differs significantly from wood (7% and 20% for soft- and hardwood, respectively, Rydholm 1976) and resembles wheat straw and cornstalks (68% and 50%, respectively, Atchison 1993). The proportion of vascular tissue and fibers in *A. donax* (6.3% and 35.9%, respectively) is also different from woods (30% and 50% for hardwoods, Rydholm 1976), but close to other grasses, e.g., wheat straw (13.5% and 37.5%) and bamboo (11% and 38%). Thus, in comparison with woody raw materials, *A. donax* is poorer in fibers and richer in short parenchyma cells.

Biometric analysis of fibers (performed on macerated samples, Fig. 3) revealed some differences in fiber dimensions of nodes and internodes (Shatalov and Pereira 2000a; Shatalov et al. 2001). The fibers from internodes have equal length (1.2 mm) and smaller diameter (14.6 vs. 16.9 μm) and cell wall thickness (4.6 vs. 5.3μm), suggesting better papermaking properties, as compared to fibers from nodes. The average fiber

length of *A. donax* is fairly close to *Eucalyptus globulus* L. wood (0.7-1.3 mm) and resembles esparto (1.5 mm), wheat straw (1.0 mm) and bagasse (1-1.5 mm). The fiber width of *A. donax* is close to that reported for some eucalypts (13-19 μm) and resembles wheat straw (15 μm) and cornstalks (18 μm) (Atchison 1993). The fiber wall thickness of *A. donax* does not vary significantly from woods (2-8 μm) and is close to wheat straw (4 μm). Thus, the fiber biometry of *A. donax* (which directly correlates with strength properties of produced paper sheets) is very close to that of such world leaders of wood and non-wood pulp market as eucalypt wood and wheat straw, respectively.

**Table 1.** Results of Comparative Chemical and Anatomical Analysis of Nodes and Internodes of the *A. donax* Stem (Shatalov et al. 2001).

| | Node | Internode |
|---|---|---|
| Ash (% o.d. reed) | 4.77 | 6.14 |
| - silicates | 1.31 | 1.16 |
| Extractives (% o.d. reed) | 13.04 | 11.16 |
| - dichloromethane | 0.46 | 0.37 |
| - ethanol | 5.88 | 4.18 |
| - hot water | 6.70 | 6.61 |
| Lignin (% o.d. reed) | 20.92 | 21.31 |
| - Klason | 19.03 | 19.60 |
| - acid-soluble | 1.89 | 1.71 |
| Holocellulose (% o.d. reed) | 61.21 | 61.41 |
| - α-cellulose | 29.18 | 32.93 |
| - hemicelluloses | 32.03 | 28.48 |
| Parenchyma (%) | 55.8 | 59.8 |
| Fibre (%) | 37.9 | 33.9 |
| Vascular tissue (%) | 6.4 | 6.2 |
| Fibre length (mm) | 1.2 | 1.2 |
| Fibre width (μm) | 16.9 | 14.6 |
| Fibre wall thickness (μm) | 5.3 | 4.6 |

***Chemical composition***

The comparative analysis of chemical composition revealed some differences between nodes and internodes (Shatalov and Pereira 2000a; Shatalov et al. 2001). With close lignin content (ca. 21%), the nodes are richer in extractives (13.04 vs. 11.16%) and hemicelluloses (32.0 vs. 28.5%) and poorer in cellulose (29.2 vs. 32.9%), as compared with internodes. Generally, the chemical analysis resembled the typical characteristics for other grasses and deviations from wood. Obviously, *A. donax* contains considerably less lignin and cellulose than woods (24-34% and 38-50%, respectively, Rydholm 1976), but is comparable in hemicelluloses. Similar to other grasses, *A. donax* showed remarkable predominance of pentosans over hexosans (96% vs. 4% of hemicelluloses) and the prevalence of xylan over other non-cellulosic polysaccharide (ca 90% of total). One other difference from wood was found in the relatively high content of ash and extractives, which is also common for grasses.

***Effect of stem morphology on pulp and paper properties***

Different kraft pulping properties of nodes and internodes were established in accordance to the difference in chemical composition and anatomical structure (Shatalov and Pereira 2002a). The pulps with higher screened yield (44.5 vs. 38.6%) and lower content of residual lignin (Kappa number 25 vs. 33) were produced from internodes, as compared with nodes. The papermaking properties as well as brightness of unbeaten kraft pulps from internodes were also higher (Table 2). Thus, the internodes are more suitable for pulping and the presence of nodes has an adverse affect on pulp yield and properties.

**Table 2.** Results of Kraft Pulping and Papermaking Properties of Unbeaten Pulps from Nodes, Internodes and the Whole Stems of *A. donax* L. (Shatalov and Pereira 2002a).

| | Node | Internode | Whole stem |
|---|---|---|---|
| Yield (% o.d. reed)<br>- Screened yield<br>- Rejects | 43.0<br>38.6<br>4.4 | 44.7<br>44.5<br>0.2 | 43.8<br>42.1<br>1.7 |
| Klason lignin (% o.d. reed) | 4.2 | 3.2 | 3.3 |
| Kappa number | 33 | 25 | 26 |
| Viscosity number (ml $g^{-1}$) | 1054 | 1156 | 1135 |
| Burst index (kPa·$m^2$ $g^{-1}$) | 0.2 | 0.7 | 0.5 |
| Tensile index (N·m $g^{-1}$) | 5.2 | 25.2 | 17.4 |
| Tear index (mN·$m^2$ $g^{-1}$) | 4.4 | 13.3 | 10.5 |
| Brightness (% ISO) | 21.2 | 23.9 | 22.8 |

The results for the whole stem pulping (as the more useful option for practical reasons) are similar or somewhat lower than those for internodes, reflecting the mass proportion of nodes and internodes in the stem.

## Organosolv Pulping

***Comparison of different organosolv technologies***

Four different acid- and alkali-based organosolv pulping technologies, which are now under different stages of commercial development, were used to examine the pulping ability of *A. donax*: Alkali-Sulfite-Anthraquinone-Methanol (ASAM), Alkali-Anthraquinone-Methanol (Organocell), Ethanol-Alkali and Peroxyacids (Milox) (Shatalov and Pereira 2000a, 2000b, 2001). The autocatalyzed ethanol pulping of *A. donax* (Repap process) is under investigation now, and the results are not presented here. All methods were applied using standard conditions reported for pulping of similar crops or hardwood (Table 3). Conventional kraft pulp was used as a reference.

The comparative study showed a high accessibility of *A. donax* to organosolv delignification. Bleachable grade pulps with high yield (47-52% for organosolv vs. 44% for kraft), ISO brightness (25-37% ISO for organosolv vs. 23% ISO for kraft) and intrinsic viscosity (885-1192 ml $g^{-1}$ for organosolv vs. 1135 ml $g^{-1}$ for kraft), good mechanical properties and low content of residual lignin were produced (Table 4). The results of Ethanol-Alkali and ASAM pulping were particularly promising. The properties

of these organosolv pulps were superior to kraft pulps and comparable with those of industrial hardwood kraft pulp (e.g. *E. globulus*). The remarkably high brightness of Ethanol-Alkali and ASAM pulps (36.5% ISO and 37.1% ISO, respectively) suggested easy pulp bleachability using short bleaching sequences with reduced chemical charge.

**Table 3.** Pulping Conditions.

| | ASAM | Organocell | Ethanol-Alkali | Milox | |
|---|---|---|---|---|---|
| | | | | Stage 1 | Stage 2 |
| Alkali charge (% o.d.m.) | - | 20 | 25 | - | - |
| Chemical charge (% o.d.m.) | 20 | - | - | - | - |
| Active alkali (%, as $Na_2O$) | - | - | - | - | - |
| Sulfidity (%, as $Na_2O$) | - | - | - | - | - |
| Solvent content (% by vol.) | 30 | 30 | 40 | - | - |
| AQ (% o.d.m.) | 0.1 | 0.1 | - | - | - |
| Formic acid/acetic acid/water (% by vol.) | - | - | - | 60/20/20 | 60/20/20 |
| $H_2O_2$ charge (% o.d.m.) | - | - | - | 3 | - |
| Liquor-to-material ratio (ml $g^{-1}$) | 5/1 | 5/1 | 5/1 | 4/1 | 8/1 |
| Pulping temperature (ºC) | 175 | 170 | 140 | 90 | 100 |
| Pulping time (min) | 100 | 90 | 180 | 180 | 120 |

**Table 4.** Results of Organosolv Pulping of *A. donax* as Compared with Kraft.

| | ASAM | Organocell | Ethanol-Alkali | Milox | Kraft (ref.) |
|---|---|---|---|---|---|
| Yield (% o.d.m.) | 47.7 | 50.2 | 47.4 | 52.4 | 43.8 |
| - Screened | 46.8 | 46.6 | 47.0 | 51.9 | 42.1 |
| Brightness (% ISO) | 37.1 | 26.5 | 36.5 | 24.9 | 22.8 |
| Viscosity (ml $g^{-1}$) | 1192 | 885 | 1140 | 1042 | 1135 |
| Kappa number | 22 | 26 | 21 | 30 | 26 |
| Klason lignin (% o.d.m.) | 2.6 | 2.7 | 2.5 | 3.6 | 3.3 |
| Burst index (kPa $m^2$ $g^{-1}$) | 0.9 | 1.0 | 1.7 | 0.6 | 0.5 |
| Tensile index (N m $g^{-1}$) | 14.1 | 16.9 | 27.9 | 14.2 | 17.4 |
| Tear index (mN $m^2$ $g^{-1}$) | 10.5 | 9.2 | 11.4 | 7.1 | 10.5 |

Pulp beating using a laboratory PFI mill showed that the strength properties of reed organosolv pulps (particularly of Ethanol-Alkali pulp) can be substantially improved with minimal energy requirements on beating (Shatalov and Pereira 2000b, 2001). The maximal bursting and tensile strength can be easily reached at 1000 PFI rev. (2000 PFI rev. is required for kraft eucalypt pulp to reach the same values, Valente et al. 1991). At the same time, even moderate beating of organosolv reed pulps (up to 2000 PFI rev.) leads to a dramatic increase in drainage resistance to 60-70º SR, thereby causing serious dewatering problems (Shatalov and Pereira 2001).

***Ethanol-enhanced alkaline pulping***

Based on the results of comparative studies on pulping efficiency, the Ethanol-Alkali pulping (Ethanol-enhanced or Ethanol-reinforced alkaline pulping) was selected as a sulfur-free organosolv process having more potential for *A. donax*. The influence of process variables on yield and properties of Ethanol-Alkali pulps has been examined to

identify the optimal pulping conditions and the kinetics of lignin and carbohydrate degradation during Ethanol-Alkali delignification.

*Pulping conditions optimization.* The effect of such cooking variables as alkali charge (5-35% on o.d. reed), ethanol content (20-60% by vol.), liquor-to-reed ratio (5-8 ml/g), cooking time (5-240 min) and cooking temperature (130-150ºC) was examined (Shatalov and Pereira 2002b,c; 2004a,b). The alkalinity of the aqueous phase was found to be a controlling factor of Ethanol-Alkali delignification, strongly affecting pulping results. About 82% of lignin was removed and pulp yield fell to 49% with a rise in alkali concentration up to 25%. Ethanol addition to alkaline pulping solution improved substantially the selectivity of delignification (through suppression of degradation reactions of carbohydrates and prevention of lignin condensation reactions, Shatalov and Pereira 2002b). With a rise in ethanol content in the reaction mixture from 20 to 60% (by vol.), the yield of ethanol-alkali pulps increased by about 5%, while the content of residual lignin decreased by 10%. The intensity of these processes was more notable with ethanol content up to 40%. The change of the liquor-to-reed ratio (L/S) within an economically reasonable range of 5-8 ml $g^{-1}$ did not reveal any noticeable effect on pulping results, while the subsequent reduction of L/S ratio on pulping is undesirable because of an impaired diffusion of chemicals. The presence of ethanol in the alkaline pulping solution allowed using rather low temperatures for delignification. About 90% of lignin can be removed with 180 min pulping at 140ºC. Under these gentle conditions, the degradation of polysaccharides was reduced to a minimum, providing fairly high values of screened pulp yield of 45-48%.

*Kinetics of lignin and polysaccharide degradation.* A novel original approach for kinetic description of lignin and polysaccharide degradation during chemical pulping has been developed and applied (Shatalov and Pereira 2004c; 2005a,b,c).

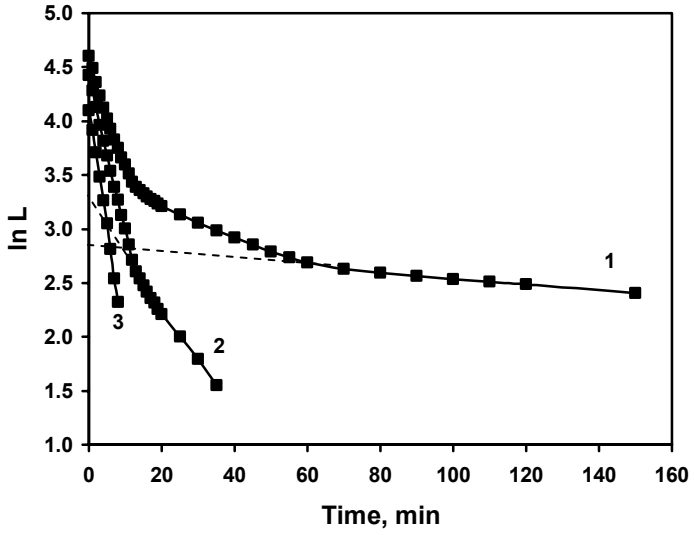



**Fig. 4.** Kinetic curves of ethanol-alkali delignification of *A. donax* (140ºC):1-experimental Ln$L$=Ln($L_1$+$L_2$+$L_3$)=f($t$); 2-calculated Ln($L$-$L_3$)=Ln($L_1$+$L_2$)=f($t$); 3-calculated Ln($L$-$L_3$-$L_2$)=Ln$L_1$=f($t$); $L_1$,$L_2$,$L_3$ - lignin fractions.

Based on properties of a multi-component reaction system, the degradation of lignin as well as carbohydrates was considered as a complex of *n*-parallel irreversible first-order reactions with similar final product. The successive elimination from the semi-logarithmic anamorphous of kinetic curve of the contributions from the individual polymer structures, or groups of polymer structures with close reactivity (as a kinetically homogeneous system) allowed estimating accurately the kinetic heterogeneity of delignification and quantifying the lignin or carbohydrate fractions with distinguishable reactivity.

Three lignin fractions of *A. donax* were revealed and quantified in proportions of approximately 61, 23 and 16% (Fig. 4). The proportion of lignin fractions was different from that reported for wood, but close to another crop – wheat straw, where the first more reactive lignin fraction was also found as a major fraction (about 90%). The values of apparent activation energy were estimated respectively as 64, 89 and 96 kJ $mol^{-1}$, and were generally within the range of those reported for wood kraft and organosolv pulping. The simulation of Ethanol-Alkali delignification using the calculated kinetic parameters showed the high reproducibility of experimental data on lignin removal. The data reproducibility was substantially higher in comparison with that obtained by conventional consecutive kinetic model (sum of square residuals (SQR) 0.0036 vs. 0.0856, Shatalov and Pereira 2005a) (Fig.5).

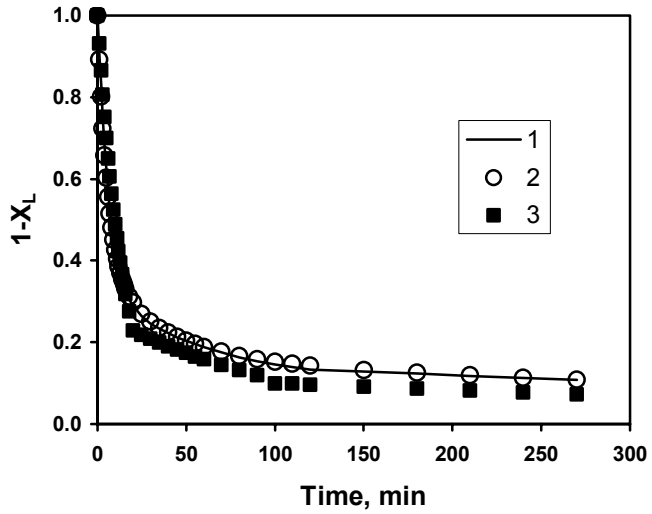



**Fig. 5.** Data fitting on lignin conversion: 1-experimental kinetic curve; 2-kinetic curve simulated by new model; 3-kinetic curve simulated by traditional model.

The degradation of polysaccharides was accurately described in terms of two kinetically homogeneous fractions. Total polysaccharide losses during Ethanol-Alkali pulping of *A. donax* result mainly from the fast removal of the first more reactive xylan and cellulose fractions (48 and 4%, respectively, Shatalov and Pereira 2005b). The degradation of the second fractions slowly proceeded through pulping with two to three orders lower rate. The apparent activation energies were estimated as 74.4 and 140.9 kJ $mol^{-1}$ - for xylan fractions and 105.2 and 106.5 kJ $mol^{-1}$ - for cellulose fractions. The degradation kinetics of minor *A. donax* carbohydrates (composed of arabinosyl,

galactosyl and mannosyl residuals) and uronic acid moieties during ethanol-alkali pulping was also studied (Shatalov and Pereira 2005c).

*Hexenuronic acids*. The unsaturated 4-deoxy-β-L-*threo*-hex-4-enopyranosyl-uronic acid (hexenuronic acid or HexA) formed from 4-*O*-methylglucuronic acid (MeGlcA) side groups of heteroxylan by β-elimination of methanol during alkaline pulping was shown to have harmful effect on subsequent pulp bleaching through the increased consumption of bleaching chemicals, decreased brightness and increased brightness reversion, poor metal removal, and formation of calcium oxalate deposits in the bleaching equipment. It was of special interest to examine the effect of organic solvent (ethanol) addition on the chemical behavior of HexA during alkaline pulping of *A. donax*. It was shown (Shatalov and Pereira 2003a, 2004d) that in ethanol-alkali reaction medium under pulping conditions about 90% of the initial uronic acid (UA) moieties of *A. donax* (composed mainly of MeGlcA side groups attached to heteroxylan) are degraded. At the end of the process, the residual MeGlcA in pulp are 84% converted to HexA. The maximal detected content of HexA in pulp was 30 μmol $g^{-1}$. No appreciable degradation of HexA during the course of pulping (under the temperature range of 130-150ºC) was observed. The overall rate of UA degradation was one order higher than the rate of UA conversion to HexA. The reaction medium alkalinity was found as a controlling factor for UA and HexA stability during pulping. The addition of organic solvent had a similar, but less notable, effect on UA and HexA stability. The kinetics of UA degradation as well as of HexA formation were accurately described in terms of three simultaneous first-order reactions, corresponding to three kinetically homogeneous fractions (Shatalov and Pereira 2003a, 2005c). The degradation of the first two uronic acid fractions (about 50% of total UA) as well as the formation of the first two hexenuronic acid fractions (about 63% of total formed HexA) proceeds with similar rates and is completed within the first-third of pulping time. The last (less reactive) HexA fraction is formed with one-order lower rate than the degradation rate of the last UA fraction.

## TCF Bleaching of Organosolv Pulps

Based on the results of the comparative study on organosolv delignification of *A. donax* (Shatalov and Pereira 2001), three alkali-based organosolv pulps, i.e., ASAM, Organocell and Ethanol-Alkali, were chosen for subsequent bleaching experiments. Kraft pulp from *A. donax* was used as a reference.

### *Peroxide bleaching*

All of the tested pulps were bleached with a simple three-stage peroxide sequence QPPP (where Q - chelating treatment and P - hydrogen peroxide bleaching stage), without oxygen pre-delignification (Shatalov and Pereira 2003b, 2005d). The conditions applied were identical for each P-stage and are summarized in Table 5. The pulp chelating with EDTA was done before bleaching to remove transition metals. There were some additional chemicals used (Epsom salt and DTPA) to prevent radical-induced degradation of carbohydrates.

The brightness level of ca. 76-78% ISO was reached for all organosolv pulps (Table 6), irrespective of the starting brightness. It was somewhat higher than that

reported for oxygen-delignified peroxide bleached soda-AQ reed pulp (75% ISO brightness, Basta et al. 2002) and oxygen-delignified QPPP-bleached acetosov pine and eucalypt pulps (67-70% ISO brightness, Vázquez et al. 2002). The peroxide consumption varied for different pulps and was directly related to starting pulp brightness.

**Table 5.** Conditions of hydrogen peroxide bleaching.

| | Q-stage | P-stage |
|---|---|---|
| Pulp consistency (%) | 3 | 10 |
| Temperature (°C) | 70 | 90 |
| Time (min) | 60 | 180 |
| $H_2O_2$ charge (% o.d. pulp) | - | 3.0 |
| NaOH charge (% o.d. pulp) | - | 1.5 |
| EDTA charge (% o.d. pulp) | 0.3 | - |
| DTPA charge (% o.d. pulp) | - | 0.2 |
| $MgSO_4$ charge (% o.d. pulp)* | - | 0.3 |
| pH initial** | 4.5 | - |
| pH final*** | - | 10.0-10.40 |
| * Magnesium sulfate was applied as $MgSO_4 \cdot 7H_2O$; ** pH was adjusted by diluted sulfuric acid | | |
| *** pH value varies with stage and pulp sample | | |

**Table 6.** Properties of peroxide bleached reed organosolv and kraft (ref.) pulps.

| | Ethanol-Alkali | ASAM | Organocell | Kraft (ref.) |
|---|---|---|---|---|
| Yield (%) | 93.2 | 90.2 | 90.6 | 91.9 |
| $H_2O_2$ consumption (%) | 60.0 | 68.9 | 82.2 | 74.4 |
| Lignin (% o.d. pulp) | 2.15 | 1.68 | 1.48 | 1.99 |
| - Klason | 0.78 | 0.72 | 0.50 | 0.89 |
| - Acid-soluble | 1.37 | 0.96 | 0.98 | 1.10 |
| Intrinsic viscosity (ml $g^{-1}$) | 1111 | 973 | 788 | 842 |
| Brightness (% ISO) | 76.4 | 77.0 | 77.6 | 75.4 |

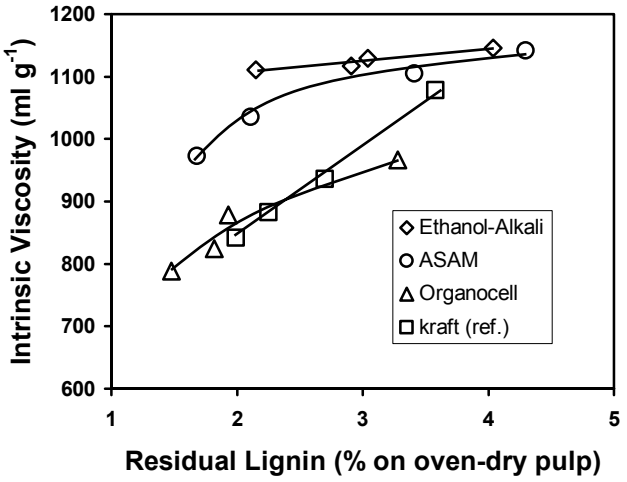



**Fig. 6.** Selectivity of peroxide bleaching of *A. donax* organosolv pulps (Shatalov and Pereira 2005d).

All organosolv pulps (particularly Ethanol-Alkali) showed substantially higher bleaching selectivity in comparison with kraft (Fig. 6). The viscosity loss of only 3% after complete bleaching was noted for Ethanol-Alkali pulp vs. 22% for kraft. The bleaching efficiency with respect to lignin removal, assumed as a measure of pulp bleachability, was also higher for reed organosolv pulps (Fig. 7). The delignification of ASAM pulp was more intensive (ca. 60% lignin removal), followed by Ethanol-Alkali and Organocell. The lignin removal from kraft pulp was poorer (ca. 40%), despite the highest brightness improvement per unit of bleaching chemical consumed (Shatalov and Pereira 2005d). Thus, no correlation between brightening and delignifying effect was observed during peroxide bleaching of *A. donax* pulps.

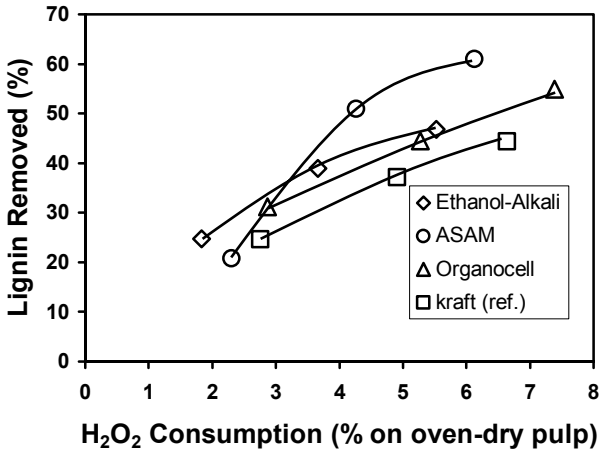



**Fig. 7.** Delignification efficiency of peroxide bleaching of *A. donax* organosolv pulps (Shatalov and Pereira 2005d).

The papermaking properties of bleached organosolv pulps after PFI beating were found superior (or close) to those of kraft pulp, in contrast to unbleached pulps where the improvement of kraft pulp properties with beating was definitely higher (Shatalov and Pereira 2001).

***Ozone-based bleaching***

To consider the possibility of brightness improvement, the introduction of an ozone-stage within a short TCF sequence was studied (Shatalov and Pereira 2005e, 2006a). The ASAM, Organocell and Ethanol-soda pulps were bleached by $AZE_RQP$ (actually ZEP) bleaching sequence without oxygen pre-bleaching, and compared with conventional kraft pulp (where A - acidic pulp pre-treatment, Z - ozone stage, $E_R$ - alkaline extraction in the presence of reducing agent, Q - pulp chelating, P - hydrogen peroxide stage). The specific bleaching conditions are listed in Table 7.

The introduction of an ozone-stage into the bleaching sequence resulted in an appreciable gain in brightness and degree of delignification in comparison with the three-stage peroxide bleaching (Table 8). The brightness was improved by 7.3; 5.8 and 2.0 points, respectively for Ethanol-Alkali, Organocell and ASAM pulps and showed the maximal values of 83.7% ISO; 83.4% ISO and 79.4% ISO, respectively, vs. 79.6% ISO

for kraft pulp. The removal of Klason lignin (which directly correlates with kappa number) by 83%, 77% and 69% was noted respectively for Organocell, Ethanol-Alkali and ASAM pulps, vs. 69% for kraft pulp. The reduction of ozonated pulps before alkaline extraction allowed limiting carbohydrate degradation with loss in intrinsic viscosity by only 11-18% for organosolv pulps, vs. 21% for kraft pulp (Shatalov and Pereira 2006b).

**Table 7.** Conditions of $AZE_RQP$ bleaching.

| | A | Z | $E_R$ | Q | P |
|---|---|---|---|---|---|
| Pulp consistency (%) | 3 | 3 | 10 | 3 | 10 |
| Temperature ($^oC$) | 20 | 20 | 60 | 50 | 90 |
| Time (min) | 30 | 20 | 60 | 50 | 90 |
| $O_3$ (% on oven-dry pulp) | - | 0.8 | - | - | - |
| $H_2O_2$ (% on oven-dry pulp) | - | - | - | - | 2.5 |
| NaOH (% on oven-dry pulp) | - | - | 1.0 | - | 1.5 |
| EDTA (% on oven-dry pulp) | - | - | - | 0.3 | - |
| DTPA (% on oven-dry pulp) | - | - | - | - | 0.2 |
| $MgSO_4$ (% on oven-dry pulp)* | - | - | - | - | 0.3 |
| $NaBH_4$ (% on oven-dry pulp) | - | - | 0.1 | - | - |
| pH ** | 2.0 | 2.0 | - | 4.5 | - |
| * Magnesium sulfate was applied as $MgSO_4 \cdot 7H_2O$; ** pH was adjusted by diluted sulfuric acid | | | | | |

**Table 8.** Properties of $AZE_RQP$-bleached organosolv and kraft (ref.) pulps from *A. donax.*

| | Ethanol-Alkali | ASAM | Organocell | Kraft (ref.) |
|---|---|---|---|---|
| Yield (%) | 93.4 | 91.0 | 90.9 | 92.1 |
| Lignin (% o.d. pulp ) | 1.65 | 1.89 | 1.26 | 1.66 |
| - Klason | 0.57 | 0.96 | 0.36 | 0.79 |
| - Acid-soluble | 1.08 | 0.93 | 0.90 | 0.87 |
| Intrinsic viscosity (ml $g^{-1}$) | 988 | 1009 | 789 | 852 |
| Brightness (% ISO) | 83.7 | 79.4 | 83.4 | 79.6 |
| Burst index (kPa $m^2$ $g^{-1}$) | 1.32 | 1.30 | 1.27 | 1.11 |
| Tensile index (N m $g^{-1}$) | 15.85 | 15.28 | 14.26 | 12.38 |
| Tear index (mN $m^2$ $g^{-1}$) | 9.16 | 9.02 | 8.54 | 8.84 |

The pulp bleachability, in terms of improvement in brightness or lignin removal per unit of applied chemicals, was found to be higher for Organocell pulp. The ASAM and Ethanol-Alkali pulps showed the highest bleaching selectivity, expressed by viscosity loss per unit of lignin removed or brightness improved. The development of strength properties of PFI beaten kraft pulp was poorer in comparison with ASAM, but close or somewhat better (as in the case of tearing strength) to Ethanol-Alkali and Organocell pulps. The overall bleaching results of organosolv pulps from *A. donax* were superior to those of kraft (Shatalov and Pereira 2006a).

***Enzyme-aided bleaching***

The effect of enzymatic pre-treatment on the bleachability of *A. donax* organosolv pulps has been examined. The ASAM, Organocell and Ethanol-Alkali pulps were treated

with the commercial xylanase preparation Ecopulp TX-200A (AB Enzymes) and bleached by simple one-stage hydrogen peroxide bleaching. The enzymatic bleach boosting effect was examined and compared with conventional kraft reed pulp (Shatalov and Pereira 2006c, d).

The bleaching effect (i.e., direct brightening and delignification) was already observed during the enzymatic stage and led to a brightness increase by 0.6-1.0% ISO (more for Ethanol-Akali and Organocell) and lignin removal by 11% for organosolv pulps vs. 0.7% ISO and 14%, respectively, for kraft pulp. The xylanase pre-treatment substantially improved the subsequent chemical bleaching of reed organosolv pulps with hydrogen peroxide (Table 9).

**Table 9.** Properties of enzyme-treated organosolv pulps from *A. donax* after one-stage hydrogen peroxide bleaching.

| | ASAM | Ethanol-Alkali | Organocell | Kraft (ref.) |
|---|---|---|---|---|
| Brightness (% ISO) | 63.4(1.5)* | 70.2(2.1) | 64.4(2.9) | 61.4(2.7) |
| Lignin (% o.d. pulp) | 2.65(0.22) | 2.55(0.29) | 1.75(0.30) | 1.59(0.33) |
| Intrinsic viscosity (ml $g^{-1}$) | 1144(29) | 1140(41) | 918(19) | 907(21) |
| Peroxide consumption (%) | 75.4(3.4) | 39.7(2.2) | 74.3(11.2) | 92.7(5.1) |
| * The property improvement in comparison with control sample (without xylanase treatment) is shown in brackets | | | | |

The gain in brightness by 1.5-2.9% ISO under reduced consumption of active bleaching chemical by 2.2-11.2% (as a maximum for Organocell and a minimum for ASAM pulp) was observed. The degree of delignification and intrinsic viscosity of enzyme-treated organosolv pulps increased after peroxide bleaching by 7.7-14.9% and by 2.1-3.7%, respectively (Shatalov and Pereira 2006c). Thus, commercial xylanase preparation specifically designed to improve bleachability of industrial wood kraft pulps can be successfully applied to untraditional reed organosolv pulps. This gives the possibility to incorporate enzymatic stage into the TCF bleaching sequences and to increase thereby the final brightness ceiling of bleached pulps. The brightness level of 85-90% ISO (the fully bleached reed organosolv pulps) can also be achieved by reinforcement of the enzyme-aided TCF bleaching by highly effective catalytic systems of oxidative delignification.

## CONCLUSIONS

*A. donax* (giant reed) possesses many of the qualities required for an ideal candidate to occupy leading positions on the world non-wood pulp market, and it provides an excellent alternative to wood fibers in meeting the rapidly growing demand for pulp and paper products. The high accessibility of *A. donax* to advanced ecologically friendly pulping and bleaching technologies makes it a particularly attractive and promising fiber source; especially in light of pressure against traditional industrial technologies toward more environmentally sound techniques.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Fundação para a Ciência e a Tecnologia (Portugal) within research contract SFRH/BPD/9376/2002.

## REFERENCES CITED

Atchison, J. E. (1993). "The future of non-wood plant fibers in pulp and papermaking." In: *Pulp and Paper Manufacture*, Vol. 3, Secondary Fibres and Non-Wood Pulping, M. J. Kocurek, ed., TAPPI, CPPA, 4-21.

Basta, J., Holtinger, L., Blom, C., Zhan, H. Y., Fang, H., Li, B. Y., and Li, J. J. (2002). "Overall aspects of $H_2O_2$ bleaching of non-wood fibres," In: *Emerging technologies of pulping and papermaking*, H. B. Liu, H. Y. Zhan and Y. I. Xie, eds., Guangzhou, China, 377-385.

Bhat, R. V., and Virmani, K. C. (1951). "Indigenous cellulosic raw materials for the production of pulp, paper and board. Part 1. Pulps for writing and printing papers from *Arundo donax* L.", *Indian Forest Leaflet* 123, 1-9.

Browning, B. L. (1967). *Methods of wood chemistry*. Wiley and Sons, New York.

Dalianis C. D., Sooter C. A., and Christou M. G., (1994). "Growing, biomass productivity and energy potential of giant reed (*Arundo donax*) and elephant grass (*Miscanthus sinensis "giganteus"*)." Proceedings of the 8th European Bioenergy Conference, P. Chartier, A. A. C. M. Beenackers, and G. Grassi, eds., Pergamon, UK, 575-582.

Di Felippo, J. (1955). "Twenty-five years of Argentine industrial experience in the pulping of straw and canes," In: *Pulp and Paper Prospects in Latin America*, United Nations, FAO, New York.

Jayme, G., Hindenburg, K. G., and Harders-Steinhauser, M. (1948). "Eignungsvergleich verschiedener Einjahrespflanzen als Rohstoff fur Papiersulfatzell-stoffe", *Das Papier* 2, 45-56.

Leminen, A., Johansson, A., Lindholm, J., Gullichsen, J., and Yilmaz, Y. (1996). *Non-wood fibres in papermaking.* VTT Research Notes. VTT, Finland. 3-9.

Moore, G. (1996). *Nonwood fibre applications in papermaking*. Pira International, UK. 1-4.

Nile Fiber Group (2002). Press release, http://www.nilefiber.com/press_release article 1.asp

Perdue, R. E. (1958). "Arundo donax: source of musical reeds and industrial cellulose," *Economic Bot.* 12, 368-404.

Reeve, D. W. (1996). "Introduction to the principles and practice of pulp bleaching." In: C. W. Dence and D.W. Reeve, eds., *Pulp bleaching. Principles and practice*. TAPPI Press, Atlanta, (Chapter 1).

Rydholm, S. A. (1976). *Pulping processes*. Interscience Publishers, NY, Chichester, Brisbane, Toronto.

Saeman, J. F., Moore, W. E., and Millet, M. A. (1963). In: *Methods in Carbohydrate Chemistry*, R. L. Whistler, ed., Academic Press, New York, 54-69.

Shatalov A. A., and Pereira H. (2000a). "*Arundo donax* L. (giant reed) as a source of fibres for paper industry: Perspectives for modern ecologically friendly pulping technologies," Proceedings of the 1st World Conference on Biomass for Energy and Industry, Sevilla, Spain, James & James Ltd, UK, 2, 1183-1186.

Shatalov A. A., and Pereira H. (2000b). "Organosolv pulping of *Arundo donax* L. as an alternative to the kraft process for production of high quality non-wood fibres," Proceedings of the 6th European Workshop on Lignocellulosics and Pulp (EWLP), Bordeaux, France, 571-574.

Shatalov, A. A., Quilho, T., and Pereira, H. (2001). "*Arundo donax L.* reed: New perspectives for pulping and bleaching – 1. Raw material characterization", *TAPPI J.* 84(1), 1-10.

Shatalov, A.A., and Pereira, H., (2001). "*Arundo donax* L. reed: New perspectives for pulping and bleaching – 2. Organosolv delignification," *TAPPI J.* 84(11), 1-12.

Shatalov A. A., and Pereira H. (2002a). "Influence of stem morphology on pulp and paper properties of *Arundo donax* L. reed," *Industrial Crops and Products* 15(1): 77-83.

Shatalov A. A., and Pereira H. (2002b). "Ethanol-enhanced alkaline pulping of *Arundo donax* L. reed: influence of solvent on pulp yield and quality," *Holzforschung* 56(5): 507-512.

Shatalov A. A., and Pereira H. (2002c). "Carbohydrate behaviour of *Arundo donax* L. in ethanol-alkali medium of variable composition during organosolv delignification," *Carbohydrate Polymers* 49(3): 331-336.

Shatalov A. A., and Pereira H. (2003a). "Hexenuronic acids in ethanol-alkali pulping," Proceedings of the 12th Int. Symp. on Wood and Pulping Chemistry (ISWPC), Madison, USA, 113-116.

Shatalov A. A., and Pereira H. (2003b). "Influence of organosolv pulping process on TCF bleachability of *Arundo Donax* L. pulps," Proceedings of the 12th Int. Symp. on Wood and Pulping Chemistry (ISWPC), Madison, USA, 109-112.

Shatalov, A. A., and Pereira, H. (2004a). "*Arundo donax* L. reed: New perspectives for pulping and bleaching – 3. Ethanol-reinforced alkaline pulping," *TAPPI J.* 3(2), 27-31.

Shatalov A. A., and Pereira H. (2004b). "Papermaking fibres from giant reed (*Arundo donax* L.) by sulfur-free pulping and chlorine-free bleaching," Proceedings of the 2nd World Conference on Biomass for Energy, Industry and Climate Protection, 10-14 May 2004, Rome, Italy, 1939-1942.

Shatalov A. A., and Pereira H. (2004c). "Improved delignification kinetics of *Arundo donax* L. as a way to increase the quality of non-wood papermaking fibres," Proceedings of the 2nd World Conference on Biomass for Energy, Industry and Climate Protection, 10-14 May 2004, Rome, Italy, 1974-1977.

Shatalov A. A., and Pereira H. (2004d). "Uronic (hexenuronic) acid profile of ethanol-alkali delignification of giant reed *Arundo donax* L.," *Cellulose* 11(1): 109-117.

Shatalov, A. A., and Pereira, H. (2005a). "Kinetics of organosolv delignification of fibre crop *Arundo donax* L.," *Industrial Crops and Products* 21(2): 203-210.

Shatalov A. A., and Pereira H. (2005b). "Kinetics of polysaccharide degradation during ethanol-alkali delignification of giant reed – Part 1. Cellulose and xylan," *Carbohydrate Polymers* 59(4): 435-442.

Shatalov A. A., and Pereira H. (2005c). "Kinetics of polysaccharide degradation during ethanol-alkali delignification of giant reed – Part 2. Minor carbohydrates and uronic acids," *Carbohydrate Polymers* 61(3): 304-313.

Shatalov A. A., and Pereira H. (2005d). "*Arundo donax* L. reed: New perspectives for pulping and bleaching - 4. Peroxide bleaching of organosolv pulps," *Bioresource Technology* 96(8): 865-872.

Shatalov A. A., and Pereira H. (2005e). "Ozone based TCF bleaching of non-wood organosolv pulps", Proceedings, 13th Int. Symp. On Wood Fibre and Pulping Chemistry (ISWFPC), Auckland, New Zealand, 609-613.

Shatalov A. A., and Pereira H. (2006a). "*Arundo donax* L. reed: New perspectives for pulping and bleaching - 5. Ozone-based TCF bleaching of organosolv pulps," *Bioresource Technology*, submitted paper.

Shatalov A. A., and Pereira H. (2006b). "Polysaccharide degradation during ozone based TCF bleaching of non-wood organosolv pulps," *Carbohydrate Polymers*, in press.

Shatalov A. A., and Pereira H. (2006c). "Enzyme-aided TCF bleaching of non-wood organosolv pulps," accepted for the 9th European Workshop on Lignocellulosics and Pulp (EWLP), 20-30 August, 2006, Vienna, Austria.

Shatalov A. A., and Pereira H. (2006d). ""Xylanase pre-treatment of giant reed organosolv pulps: bleach boosting effect", *Industrial Crops and Products*, submitted paper.

Stockburger, P. (1993). "An overview of near-commercial and commercial solvent-based pulping processes," *TAPPI J.* 76(6), 71-74.

Valente, C. A., Mendes de Sousa, A. P., and Furtado, F. P. (1991). In: *ESTUDOS 3, Colectânia de estudos de I&D publicados em 1990 e 1991*, TECHNOCEL, S.A., Portugal, 37-45.

Van Dam, J. E. G., Van Vilsteren, G. E. T., Zomers, F. H. A., Shannon, W. B., and Hamilton, I. T. (1994). *Industrial fibre crops*, Agro-Industrial Research Division, EC.

Vázquez, G., Freire, S., González, J., and Antorrena, G., (2002). "Oxygen delignification as a pretreatment for acetosolv pulps bleaching," *TAPPI J.* 85(2), 1-5.

Vecchiet, M., Jodice, R., and Schenone, G., (1996). "Agronomic research on giant reed (Arundo donax L.). Management system and cultivation of two different provenance," In: *Biomass for Energy and the Environment*, P. Chartier, G. L. Ferrero, U. M. Henius, S. Hultberg, J. Sachau, M. Wiinblad, eds., Proceedings of the 9th European Bioenergy Conference, 24-27 June 1996, Copenhagen, Denmark. Pergamon, UK. 1, 644-648.

Article submitted: May 23, 2006; Revised version accepted: June 16, 2006; Published July 10, 2006

# DECAY RESISTANCE OF CEMENT-BONDED ORIENTED STRAND BOARD

Antonios N. Papadopoulos

The objective of this study was to evaluate the decay resistance of cement bonded oriented strand board (OSB) against brown (*Coniophora puteana)* and white rot (*Termites versicolor )* fungi. Overall, both fungi failed to attack the cement-bonded OSB. Boards made with 3.0 cement-to-wood ratio showed weight gain instead of weight loss. Therefore, it is recommended that cement-bonded OSB is technically suitable for exterior use where both moisture and favourable conditions for fungi development are present.

*Keywords: Oriented strand board, OSB, Cement, Decay resistance, Coniophora puteana, Termites versicolor*

*Contact information: Technological Educational Institute of Kavala, Branch of Drama, Department of Forestry and Management of Natural Environment, Laboratory of Wood and Wood Products, Drama, TK 66100, Greece. Email: antonios1974@hotmail.com, antpap@teikav.edu.gr*

## INTRODUCTION

Cement-bonded wood composite panels are not a novel concept, having been on the market for over a century. In the past, these panels have consisted of excelsior and magnesite and have been used primarily as low-density insulating materials. By the early 1960's, a high-density cement-bonded structural flakeboard was developed, leading to expanded applications (Deppe 1974). Today, wood-cement panels have found acceptance in a number of countries as a result of certain desirable characteristics. The development and use of wood-cement panels attest to their attraction as building materials. In addition to their resistance to fire, these materials have a special attraction for use in warm, humid climates where decay and termites are a major concern (Jorge et al. 2004). The cement binder provides a durable surface as well as one that can be easily embossed and colored for an alternate, low maintenance finished product. The raw materials used are compatible with a range of processing methods to provide a variety of products that are easily machined with conventional wood-working tools. Although heavier than resin-bonded panels, they are lighter than concrete and, therefore, wood-cement panels can replace concrete in construction, namely prefabricated construction, in elements that are not subjected to loads, like walls. These attributes appeal to engineers, architects and contractors for use in public and multifamily residential buildings.

The majority of research in this field has been carried out on particleboards and flakeboards. The focused topics include the problem of the compatibility between cement and wood and ways of overcoming the problem, methods of manufacture and the properties exhibited by common wood-composites, special techniques to accelerate the curing of cement and to improve the properties, and finally manufacture of non-wood raw

materials – cement composites. An excellent review can be found elsewhere (Jorge et al. 2004).

Recently, oriented strand board (OSB) has been successfully manufactured at laboratory scale, using cement as a binder (Papadopoulos, in press). It was found that an increase of cement-wood ratio resulted to an increase in all strength values, with the exception of rupture. The modulus of elasticity, internal bond strength and thickness swelling values obtained with 2.0 wood-cement ratio conformed to the more stringent requirements of EN 300 for OSB/4. An important observation was that a lower cement-wood ratio was required in order to manufacture acceptable OSB than particleboards and this may be due to the geometry of the strands.

The objective of this paper was to examine the decay resistance of cement-bonded OSB against brown and white rot fungi. This is the first study, as far as the author is aware, that examined the decay resistance of cement-bonded oriented strand board. The issue of durability of cement-bonded particleboard has been recently addressed. Okino and co-workers (2004), found no measurable wood degradation (weight loss) in cement-bonded particleboards made with 2.0 or higher cement-to-wood ratio, exposed to the attack of *Gloeophyllum trabeum* and *Trametes versicolor.* Tests conducted by Pirie et al. (1990) suggested that conventionally made cement-bonded particleboards are very resistant to the attack of the white rot fungus *Pleurotus ostreatus* and to the brown rot fungus *Coniophra puteana*.

## EXPERIMENTAL

### OSB Manufacture

Aspen ring-cut strands (*Populus alba*) were used in this study, with average strand size of 75 mm x 20 mm x 0.75 mm (length x width x thickness). The strands were air-dried to approximately 10% moisture content (MC). The bonding agent employed was commercial grade Portland cement, type I. Ammonium chloride ($NH_4Cl_2$ - 2% based on weight of cement) was introduced into cement slurry to accelerate cement set during hydration. A predetermined amount of air-dried strands and an ammonium chloride (anhydrous) distilled water solution were thoroughly blended. Cement was subsequently added and the constituents were mixed until the cement paste completely hydrated. The quantity of distilled water added, was calculated using a relationship developed by Simatumpang (1979) and applied by other researchers as well (Jones et al. 1985; Moslemi and Pfister 1987; Fuwape 1995; Sudin et al. 1995). In his formulation, the water requirement was determined as follows:

$$\text{water (litres)} = 0.35\ C + (0.30 - MC)\ W$$

where:

C = cement weight (kg)
MC = wood strands moisture content (oven-dry basis)
W = oven-dry wood strand weight (kg).

After 15 minutes of manual mixing, the cement-wood water mixture was screened onto a caul. The mat was evenly distributed to provide as uniform a density as possible and pre-pressed to a thickness of approximately 50 mm. Cold pressing took place under an initial pressure of 2 – 5 MPa, depending on the cement-to-wood ratio to a 16mm thickness, after which the board was retained in compression for 24 hours. Target board density was 1000 Kg/m$^3$. Two replicates of each board were made at cement-wood ratios of 3.0, 2.0, and 1.5 (by weight), giving a total of 6 boards. To minimize cement capillary desiccation and enhance hydration, boards were misted with distilled water, then wrapped in cellophane before storing for curing at 20$^0$C and 65% relative humidity for a month.

### Decay Tests

Samples were packed in an argon atmosphere and sterilised by irradiation (2.5 Mrad.) prior to decay tests, using the methods described in DD ENV 12038:1996. Laboratory pure strains of the brown rot fungi *Coniophora puteana* (No FPRL 11E) and white rot fungi *Termites versicolor* (CTB 863A) were used, grown on malt agar. Blocks were planted on sterile specimen supports placed on the cultures of the test fungus actively growing on 5% malt agar in 500ml capacity jars. An additional set of sterile control samples were used to assess operational control losses. The closed jars were incubated for 16 weeks, at 22 +/-1$^0$C and 75 +/-5% relative humidity to evaluate the efficacy of the treatments. After incubation, the samples were removed from the jars, cleaned, weighed, conditioned to constant weight as above and re-weighed. Weight loss (WL) was expressed as a percentage of the initial weight of the sample. Weight losses from sterile controls were subtracted from the decay results to give corrected data.

## RESULTS AND DISCUSSION

The results obtained after a 16-week incubation period are presented in Table 1. Overall, both fungi failed to attack the cement-bonded OSB. Visual examination revealed a slight presence of mycelium in the surface of the tested samples. Similar observation has also been made by Okino et al. (2004) in cement-bonded particleboards made from eucalypt and rubber wood particles. Boards made with 3.0 cement-to-wood ratio showed weight gain instead of weight loss. This, according to de Souza and co-workers (1997), was a consequence of the final curing process of the cement, at these high cement-wood ratios. Investigation of cement-bonded panels is not very common. Tests conducted by Dinwoodie and Paxton (1991) and Pirie et al. (1990) suggested that conventionally made cement-bonded particleboards are very resistant to the attack of the white rot fungus *Pleurotus ostreatus* and to the brown rot fungus *Coniophra puteana*. Similar results were also reported by Okino et al. (2004; 2005) in cement-bonded boards made from eucalypt or cypress particles. However in that case, fungi tests were conducted according to the ASTM D 2017-8, where the exposure to fungi period is 12 weeks. The present study was the first study, as far as the authors are aware, that examined the decay resistance of OSB using cement as a binder.

**Table 1.** Weight loss (WL) of cement-bonded OSB. (Standard deviations in parentheses – Each value is the mean of eight samples).

| | Brown rot | White rot |
|---|---|---|
| Cement : Wood | WL (%) | WL (%) |
| 1.5 | 5.25 | 7.33 |
| 2.0 | 0.72 (0.06) | 3.24 (0.55) |
| 3.0 | -3.21 (0.59) | -2.22 (0.7) |
| Commercial OSB | 11.22 (2.11) | 28.25 (3.21) |

## CONCLUSIONS

1. Both fungi used in this work failed to attack the cement-bonded oriented strand boards.
2. Boards made with 3.0 cement-to-wood ratio showed weight gain instead of weight loss.
3. It is recommended that cement-bonded OSB is technically suitable for exterior use where both moisture and favourable conditions for fungi development are present.

## REFERENCES CITED

de Souza, M. R., Geimer, R. L., Moslemi, A. A. (1997). "Degradation of conventional and $CO_2$ injected cement-bonded particleboard by exposure to fungi and termites," *J. Tropical Forest Products* 3, 63-69.

Deppe, H. J. (1974). "On the production and application of cement-bonded wood chipboards", *Proceedings of the 8th Washington State Symposium on Particleboard*, 267-286.

Dinwoodie, J. M., Paxton, B. H. (1991). "The long term performance of cement-bonded particleboard," in: *Inorganic Bonded Wood and Fiber Materials*, 2nd International Conference, Moscow, 45.

Fuwape, J. A. (1995). "The effect of cement-wood ratio on the strength properties of cement-bonded particleboard from spruce," *J. Tropical Forest Products* 1, 49-58.

Johns, W. E., Rammon, R. M., Youngquist, J. (1985). "Chemical effects of mixed hardwood furnish on panel properties," *Proceedings of the 19th Washington State Symposium on Particleboard*, 363-377.

Jorge, F. C., Pereira, C., Ferreira, J. M. F. (2004). "Wood-cement composites: a review," *Holz als Roh-und Werkstoff* 62, 370-377.

Moslemi, A. A., Pfister, S. C. (1987). "The influence of cement-wood ratio and cement type on bending strength and dimensional stability of wood-cement composite panels," *Wood Fiber Sci.* 19, 165-175.

Okino, E. Y. A. , de Souza, M. R., Santana, M. A. E., Alves, M. V., de Souza, M. E., and Teixeira, D. E. (2004).“Cement-bonded wood particleboard with a mixture of eucalypt and rubberwood,” *Cement & Concrete Composites* 26, 729-734.

Okino, E. Y. A., de Souza, M. R., Santana, M. A. E., Alves, M. V., de Souza, M. E., and Teixeira, D. E. (2005). “Physico-mechanical properties and decay resistance of *Cupressus spp* cement bonded particleboards,” *Cement & Concrete Composites* 27, 333-338.

Papadopoulos, A. N. (in press). “Mechanical and physical properties of cement-bonded OSB”, *Holz als Roh-und Werkstoff*.

Pirie B. J., Glasser, F. P., Schmitt-Henco, C., and Akers, S. A. S. (1990). “Durability studies and characterisation of the matrix and fibre-cement interface of asbestos-free fibre-cement products,” *Cement & Concrete Composites* 12, 233-234.

Simaturpang, M. H. (1979). “The water requirements of manufactured cement-bonded particleboard,” *Holz als Roh-und Werkstoff* 37, 379-382.

Sudin, R., Shaari, K., and Selamat, S. (1995) “Cement-bonded particleboard from pre-soaked oil palm trunk: effects of particle size and chemical additive,” *J. Tropical Forest Products* 1, 71-77.

Article submitted: May 16, 2006; Revision accepted: July 14, 2006; Published: July 15, 2006

# CHEMICAL MODIFICATION OF PINE WOOD WITH PROPIONIC ANHYDRIDE: EFFECT ON DECAY RESISTANCE AND SORPTION OF WATER VAPOUR

Antonios N. Papadopoulos

The purpose of this paper is to show the effects of level of substitution with a linear chain anhydride (propionic anhydride) on decay resistance and on water vapour sorption of modified Scots pine sapwood. The work described herein has demonstrated that chemically modified Scots pine sapwood with propionic anhydride afforded substantial bioprotection against *Coniophora puteana*. It required a weight gain of approximately 17% following reaction to ensure complete protection. The sorption of water vapour of propionic anhydride modified wood was greatly reduced.

*Keywords: Chemical modification, Propionic anhydride, Sorption, Water vapour, Decay resistance, Coniophora puteana, Scots pine.*

*Contact information: Technological Educational Institute of Kavala, Branch of Drama, Department of Forestry and Management of Natural Environment, Drama, TK 66100, Greece. Email: antonios1974@hotmail.com, antpap@teikav.edu.gr*

## INTRODUCTION

Two goals of chemical modification of wood are to improve dimensional stability and decay resistance. Various chemical modification reactions have been studied for these purposes (Rowell, 1983). Acetylation is the most studied area of wood modification. For example, Stamm and Tarkow (1947) measured the equilibrium moisture content (e.m.c) of unreacted and vapour phase acetylated (30% acetyl content) Sitka spruce at various humidities and found a 67% reduction in water sorbed at 95% relative humidity. It has been shown that the dimensional stability of wood can be effectively improved by esterification with anhydrides (Rowell et al. 1988). There is limited work reported on the water vapour sorptive properties of such modified woods. A number of authors have investigated the sorption isotherms of acetylated wood specimens at only one level of substitution (Risi and Arseneau 1957; Spalt 1958; Popper and Bariska 1972; Yasuda et al. 1995). Although the effect on overall stabilisation in response to liquid water soaking is well documented (Stamm 1964; Rowell 1983; Hill and Jones 1996), there is little evidence of how sorption is influenced by esterification with anhydrides. Peterson and Thomas (1978) acetylated loblolly pine, green ash and yellow poplar using acetic anhydride in xylene. The modified samples were tested against the brown rot fungus *G. trabeum* and the white rot fungus *Coriolus versicolor*. It was found that the white rot was generally easier to control than the brown rot, with levels of acetylation as low as 7% being able to provide protection against rot.

Although of all the methods investigated, modification using acetic anhydride has received by far the most attention, there have been a number of papers published

concerning modification of wood with other linear chain anhydrides (Rugevista and Embrehsha 1988; Hill and Jones 1996; Hill and Papadopoulos 2002). Stamm and Tarkow (1947) reported that they propionylated and butyrylated whole wood and veneer, using pyridine as a catalyst, in the vapour or liquid phase using the corresponding anhydrides. It was reported that modified samples showed no sign of decay after three months exposure to *Poria versicolor,* whereas the unmodified samples showed weight losses of up to 50%. Suttie et al. (1999), modified Scots pine with acetic, propionic, butyric, or hexanoic anhydrides and determined decay resistance against the brown rot fungi *Coniophora puteana*, *Gloeophyllum trabeum*, *Poria placenta* and a white rot fungus (*Coriolus versicolor*) using European Standard method EN113 and a vermiculite overlay method. Resistance to soft rot attack was also determined using a modified ENV 807 stake test in unsterile soil. The effect of different levels of reaction upon decay resistance was only tested with the soft rot experiment. In this, it was found that a threshold of *ca*. 23% was required to ensure protection, regardless of the anhydride used.

The purpose of this paper is to show the effects of level of substitution on dimensional stability and decay resistance of a linear chain anhydride (propionic anhydride) modified Scots pine sapwood. The choice of propionic anhydride was based on (i) the limited information that exists in the literature, especially as far as the decay resistance of propionylated wood is concerned and (ii) its lower cost compared to other linear chain anhydrides (i.e. butyric, valeric, hexanoic). The reaction between wood and propionic anhydride is a single site reaction as depicted in Figure 1 and yields the corresponding carboxylic acid (propionic acid) as a by-product of its reaction with wood.

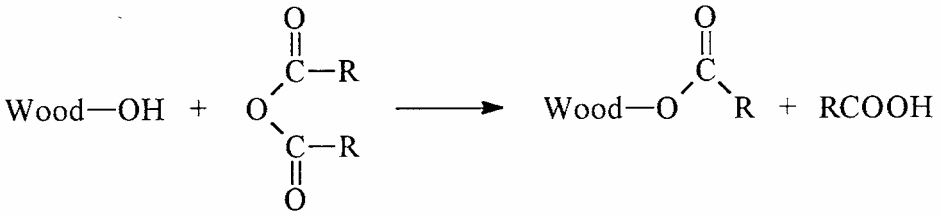



**Fig. 1.** Reaction of wood with propionic anhydride (R= $C_2H_5$ propionic anhydride).

## EXPERIMENTAL

### Chemical Modification of Wood Samples

Scots pine (*Pinus sylvestris*) sapwood samples of dimension 20 mm x 20 mm x 5 mm (radial x tangential x longitudinal) were cut from freshly felled kiln dried logs. Samples were carefully sanded to remove loosely adhering fibres, then placed in a Soxhlet extractor for solvent extraction using toluene/methanol/acetone (4:1:1 by volume) for eight hours. Samples were dried in an oven for eight hours at 105°C. Prior to weighing (four figure balance), samples were transferred to a vacuum desiccator and allowed to cool to ambient temperature over silica gel. Weighed samples ($W_1$), were vacuum impregnated with pyridine for one hour then transferred to a flask containing pyridine set in an oil bath at 100°C. Pyridine swells the wood and acts as a catalyst for the modification reaction. Samples were allowed to equilibrate in the hot pyridine for

one hour. Sets of hot samples (twenty-eight replicates) were added to a flask containing a one molar solution of the propionic anhydride in pyridine set in an oil bath at 100°C for a specific length of time to achieve the desired level of modification. At the end of the reaction period, the flask was removed from the oil bath, the hot reagent decanted off and ice cold acetone added to quench the reaction. Samples were allowed to sit in the acetone for one hour before being transferred to the Soxhlet apparatus for solvent extraction as detailed previously. Samples were re-weighed ($W_2$) after oven drying as detailed previously. Samples were free of reagent, by-product and pyridine after this procedure.

The extent of reaction was calculated as weight percent gain (WPG) determined by the differences in oven dry weight of the sample before modification ($W_1$) and after modification ($W_2$) according to the equation [Weight percent gain (WPG) = ($W_2$ - $W_1$) / $W_1$ x 100].

## Infra-red (IR) Analysis

For Infra-red (IR) analysis, the treated samples were ground up by using a microdismembrator (20.000 rpm for 6 min). The fibre flour was then mixed with oven-dry potassium bromide (KBr) powder (the fibre flour/KBr ratio was 1:100) and placed in a vibratory ball mill capsule. The mixture was ground for about 2 min. The ground mixture was then transferred to a press and the bolts of press screwed down. The bolts were tightened with a spanner to press the disk. After a few minutes, the bolts were loosen and removed. The press was placed directly into a sample beam of a Mattson FTIR spectrometer, Nicolet 750, series II.

## Decay Tests

The samples were packed in an argon atmosphere and sterilised by irradiation (2.5 mrad) prior to decay tests. Each set of 16 modified and unmodified control samples at each level of treatment was subdivided into four groups of four replicates. Laboratory pure strains of the brown rot fungi *Coniophora puteana* (No FPRL 11E) were used, grown on malt agar. Four unmodified control or modified wood samples were planted on sterile specimen supports placed on the cultures of the test fungus actively growing on 5% malt agar in 500ml capacity jars (EN 113, 1996; the only modification was the sample size, see 2.1). An additional set of sterile control samples were used to assess operational control losses. The closed jars were incubated for 16 weeks, at 22 +/- 1°C and 75 +/- 5% relative humidity to evaluate the efficacy of the treatments. After incubation, the samples were removed from the jars, cleaned, dried overnight at 105°C and weighed. Weight loss was expressed as a percentage of the initial oven dried weight of the sample. Weight losses from sterile controls were subtracted from the decay results to give corrected data.

## Equilibrium Moisture Content

The method for controlling relative humidity, as described by Stamm (1964), has been widely used and was selected for being simple, economical and reasonably precise. Test samples were kept above saturated solutions of various salts in containers stored in a controlled temperature room set at 20°C (variation +/- 1°C). Pure water results in the saturated vapour pressure corresponding to 100% relative humidity. The addition of a

solute to water reduces its vapour pressure in proportion to its mole fraction in the case of diluted solutions. When a saturated solution at a controlled temperature is used, a constant relative humidity is maintained (Siau 1996).

Six salts were chosen and these are listed in Table 1, along with the relative humidity of the atmosphere above each saturated solution at 20°C (according to Kaye and Laby 1966). They were chosen on the basis of giving minimum relative humidity variation with changes in the temperature (Stamm 1964). Excess salt was always present within each solution to ensure saturation was maintained. The solution and air in the container were agitated by bubbling air through the solution.

**Table 1.** Saturated salt solutions used and their resultant relative humidities at 20 $^0$C.

| Salt | Relative humidity (%) |
|---|---|
| Potassium nitrate ($KNO_3$) | 93 |
| Sodium chloride (NaCl) | 76 |
| Sodium dichromate ($Na_2Cr_2O_7$) | 55 |
| Potassium carbonate ($K_2CO_3$) | 44 |
| Potassium acetate ($CH_3COOK$) | 23 |
| Lithium chloride (LiCl) | 12 |

The oven dry wood samples (two replicates) were placed in the containers above saturated salt solutions. They were left to equilibrate for 4 weeks and then weighed once a week, using a four-place analytical balance until it became obvious that no significant weight change had occurred since the last weight was recorded (and e.m.c had been attained). Equilibrium moisture content (e.m.c) was reached within 6 weeks for all but the two highest humidities, which required longer exposure times.

## RESULTS AND DISCUSSION

### Infra-red (IR) Analysis

Scots pine samples were satisfactorily reacted with propionic anhydride at 100°C under the pyridine catalysis. The esterification of wood was established by infra-red spectroscopy (Figure 2). The infra-red spectra confirmed the occurrence of wood-anhydride reaction. The strong vibration obtained in the region of 1736 and 1730cm$^{-1}$ (C=O) was a distinct pattern present in modified samples, which indicates ester bond formation. As expected, such absorption was not present in unmodified wood.

### Decay Resistance of Modified Wood

The chemical modification of wood with propionic anhydride afforded substantial bioprotection of Scots pine against *Coniophora puteana* (Table 2). The data (weight loss *vs* weight percent gain) were plotted and the fit (dash line in Figure 3) shown by linear

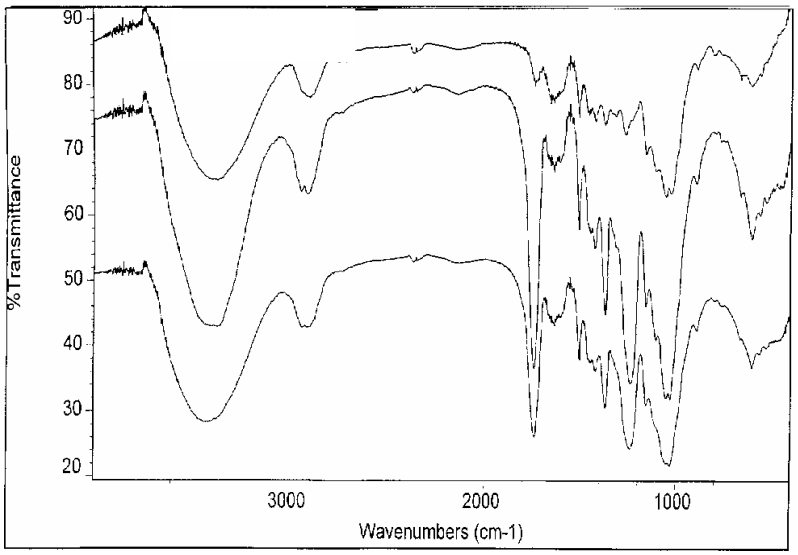



**Fig. 2.** FTIR spectra of unmodified control (top), propionic anhydride modified at 25.4% WPG (middle), and propionic anhydride modified at 5.6% WPG (bottom).

regression analysis indicates a positive relationship between extent of modification and decay resistance ($R^2$= 0.88). Next, the sterile control data were plotted against the weight percent gain data to give a linear fit (dot line in Figure 3). The point that one line crosses the other determines the threshold value (Figure 3). The threshold value of protection of propionic anhydride was 17%; it is suggested therefore that a WPG of approximately 17% is required following reaction to ensure complete protection. Earlier soil block decay tests by Goldstein et al. (1961) and Peterson and Thomas (1978) showed adequate decay resistance against brown rot fungi in wood treated with acetic anhydride to 17%, whereas Forster (1998) reported a higher value (24%). Soft rot soil bed tests by Suttie et al. (1998) showed that a value of approximately 23% is required to ensure complete protection of wood treated with the series of anhydrides used in the present study against *Coniophora puteana.*

**Table 2.** Weight percent gain (WPG) and weight losses of Scots pine sapwood modified with propionic anhydride. (Standard deviation in parentheses).

| Treatment | Reaction Time (min) | WPG (%) | Weight Loss (%) | | |
|---|---|---|---|---|---|
| | | | C. puteana | Sterile controls | Corrected |
| Control | 0 | 0 | 64.64 (2.5) | 0.29 (0.1) | 64.35 |
| Propionic | 45 | 5.6 (0.3) | 41.14 (8.3) | 0.6 (0.3) | 41.08 |
| | 105 | 14.8 (0.2) | 4.10 (2.3) | 0.45 (0.2) | 3.65 |
| | 300 | 20.5 (0.7) | 1.89 (0.4) | 0.37 (0.3) | 1.52 |
| | 420 | 25.4 (0.4) | 1.60 (0.3) | 0.54 (0.2) | 1.06 |

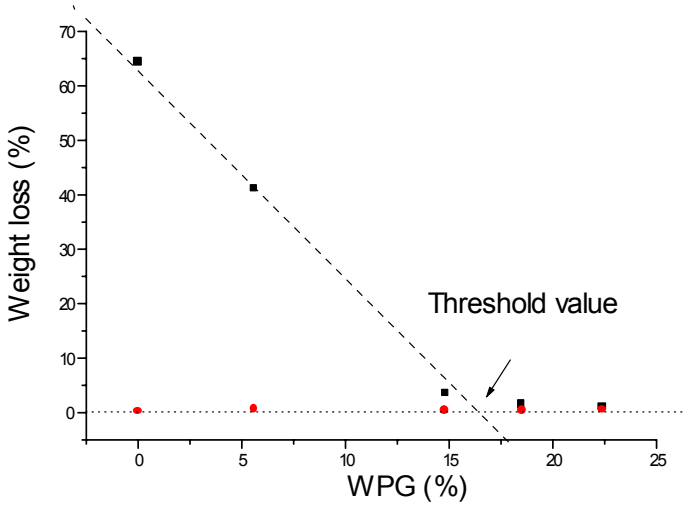



**Fig. 3.** Determination of threshold value of protection.

## Sorption of Water Vapour of Modified Wood

The e.m.c's at various levels of relative humidity for Scots pine sapwood modified with propionic anhydride are presented in Table 3. Table 3 indicates an obvious trend of reducing water vapour sorption with increasing WPG, which is true for the whole range of relative humidity. This is in agreement with the results reported earlier on various lignocellulosic fibres modified with acetic anhydride (Rowell and Rowell1989).

**Table 3.** Mean values for experimentally derived equilibrium moisture contents (e.m.c's) at various levels of relative humidity for Scots pine sapwood modified with propionic anhydride. (Standard deviation in parentheses).

| Reagent | WPG(%) | | | EMC (%) | | | |
|---|---|---|---|---|---|---|---|
| | | 12 | 23 | 44 | 55 | 76 | 93 |
| Control | 0 | 2.33 (.05) | 4.19 (.09) | 7.02 (.02) | 8.38 (.09) | 12.68 (.19) | 18.95 (.28) |
| Propionic | 6.1 | 2.01 (.08) | 3.45 (.08) | 5.62 (.14) | 6.88 (.05) | 10.45 (.05) | 15.62 (.24) |
| | 10.9 | 1.81 (.08) | 2.71 (.09) | 5.19 (.05) | 6.15 (.14) | 9.33 (.14) | 13.86 (.15) |
| | 15.6 | 1.33 (.05) | 2.1 (.12) | 4.05 (.14) | 5.01 (.15) | 7.62 (.12) | 12.01 (.18) |
| | 19.7 | 1.19 (.09) | 2.11 (.05) | 3.32 (.09) | 4.01 (.11) | 6.55 (.08) | 9.98 (.15) |
| | 24.5 | 0.9 (.12) | 1.62 (.05) | 2.77 (.15) | 3.65 (.05) | 5.71 (.08) | 9.42 (.12) |

## CONCLUSIONS

1. A catalysed reaction between Scots pine sapwood and propionic anhydride was established by increase in weight and by infra-red spectra.
2. Chemically modified Scots pine sapwood with propionic anhydride afforded substantial bioprotection against *Coniophora puteana.*

3. It required a weight gain of approximately 17% following reaction to ensure complete protection against *Coniophora puteana*.
4. The sorption of water vapour of propionic anhydride modified wood was greatly reduced.

## REFERENCES CITED

EN 113 Wood Preservatives (1996). *Determination of the toxic values against wood destroying Basidiomycetes cultured on an agar medium*, British Standards Institution. EN 113 (1982), BS 6009 (1982), 15.

Forster, S. (1998). *Decay resistance of modified softwood*, Ph.D thesis, University of Wales, Bangor.

Goldstein, I. S., Jeroski, E. B., Lund, A. E., Nielson, J. F., and Weaver, J. W. (1961). "Acetylation of wood in lumber thickness," *Forest Products Journal* 11(8), 363-370.

Hill, C. A. S., and Jones, D. (1996). **"**The dimensional stabilisation of Corsican pine sapwood by reaction with carboxylic acid anhydrides,**"** *Holzforschung* 50, 457-462.

Hill, C. A. S., and Papadopoulos, A. N. (2002). **"**The pyridine-catalysed acylation of pine sapwood and phenolic model compounds with carboxylic acid anhydrides. Determination of activation energies and entropy of activation,**"** *Holzforschung* 56, 150-156.

Kaye, G. W. C., and Laby, T. H. (1966). *Tables of physical and chemicals constants and some mathematical functions*, Longmans.

Peterson, M. D., and Thomas, R. J. (1978). **"**Protection of wood from decay fungi by acetylation. An ultrastructural study,**"***Wood and Fiber* 10(3), 149-163.

Popper, R., and Bariska M. (1972). "Acylation of wood. Part I: The sorption behaviour of water vapour," *Holz Roh Werkstoff* 30, 289-294.

Risi, J., and Arseneau D. F. (1957). "Dimensional stabilisation of wood. Part I: Acetylation," *Forest Products Journal* 7, 210-213.

Rowell, R. M. (1983). "Chemical modification of wood," *Forest Products Abstracts* 6(12), 366-382.

Rowell, R. M., and Rowell, J. S. (1989). Moisture sorption properties of acetylated lignocellulosic fibres. In: *Proceedings of the 10th cellulose conference,* C. Schuerch, ed., John Wiley and Sons, New York, 343-355.

Rowell, R. M., Youngquist, J.A., and Montrey, H.M.. (1988). "Chemical modification: adding value through new FPL composite technology," *Forest Products Journal* 38, 67-70.

Rugevista, A. A., and Embrehsha, A. E. (1988). "Modification of wood with acylating agents and polyethylene glycol," *Trudy-Latviiskoi-Sel'skokhozyaistevennoi-Akad.* 253 39-44.

Siau, J. F. (1996). *Wood: influence of moisture on physical properties,* Department of Wood Science and Forest Products, Virginia Polytechnic Institute and State University.

Spalt, H. A. (1958). "The fundamentals of water vapour sorption by wood," *Forest Products Journal* 8, 288-295.

Stamm, A. J. (1964). *Wood and Cellulose Science,* The Ronald Press, New York.
Stamm, A. J., and Tarkow, H. (1947). "Dimensional stabilisation of wood," *Journal of Colloid Chemistry* 51, 493-505.
Suttie, E. D., Hill, C. A. S, Jones, D., and Orsler R. J. (1999). "Chemically modified solid wood. I. Resistance to fungal attack,"*Material und Organismen* 32, 159-182.
Yasuda, R., Minato, K., and Norimoto, M. (1995). "Moisture adsorption thermodynamics of chemically modified wood," *Holzforshung 49*:548-554.

Article submitted: May 16, 2006; Revision accepted: July 14, 2006; Published July 15, 2006

# LASER-INDUCED PLASMA EMISSION SPECTROSCOPY (LIPS): A USEFUL ANALAYTICAL TOOL FOR THE SURFACE CHEMICAL CHARACTERIZATION OF COATED PAPER MATERIALS

Lucian A. Lucia,[a,*] Brian Willett,[b] Jouko Korppi-Tommola[c]

A nontraditional method for chemical analysis of light weight coated (LWC) grade paper is described. Laser-induced plasma emission spectrometry (LIPS) produces three-dimensional profiles of coatings and fillers. The technique ablates the coating from the top to the paper base with a laser beam. LIPS provides spatial resolution and characterization of the chemical homogeneity of the paper coating and relates that information to the efficiency of the coating method. It provides information on binder migration which is a hindrance for improved stable coatings. Herein, several commercial LWC paper grades and their base papers were analyzed. Pigment identification of light weight paper coatings, coat weight distribution maps, and profiles were obtained. Blade and film coating generate different coating distribution profiles that are detected by LIPS. The phenomenon of binder migration was demonstrated by observing its masking effects on Si spectra. An investigation of filler content in base papers involved analysis of base paper used in LWC grades. The papers represented two different types of formers, but because of confidentiality issues, little else was revealed. A significantly higher amount of filler was observed at the surface of the base stock in Former A than in Former B, as indicated from Mg spectra of the filler.

*Keywords: Laser ablation, Light weight coating, Spectroscopy, Plasma, Binder*

*Contact information: a: Department of Wood and Paper Science, North Carolina State University, Campus Box 8005, Raleigh, North Carolina 27695-8005 b: Institute of Paper Science and Technology, Georgia Institute of Technology, 500 Tenth Street NW, Atlanta, Georgia 30332-0620; c: University of Jyväskylä, Department of Chemistry, P.O. Box 35, Jyväskylä, Finland 40014; *Corresponding author: lucian.lucia@NCSU.edu*

## INTRODUCTION

LIPS is a nontraditional surface analytical technique for coated paper substrates, whose capacity to perform surface profilometry is among the best available. It is essentially a surface analytical technique that uses laser light to thermally ablate the top layer of a substrate to identify the chemical constituents (both organic and inorganic) of the ablated layer. Its appeal is evident in the simplicity of its functionality, its ability to probe almost any surface, and in the quality of the data it provides.

The issues of coating formation, homogeneity, and stability have plagued paper coatings for many years and do not have an efficient technology or method to gauge their level of quality. Therefore, methods to probe these issues improve the economics and quality of coatings. In general, the goals of applying a coating to a base paper stock are to cover the surface of the base paper with a microporous layer and to smooth out any unevenness in the base (Kartovaara 1989). Paper coatings are applied to paper in order to

improve its smoothness, opacity, gloss, and printability. They comprise a wide and varied array of inorganic pigments, organic binders, and adhesives and additives. Many coating formulations are of a proprietary nature, although the composition of most coating formulations is quite similar. These components are all amenable to chemical investigation as a function of spatial location in the coating by LIPS. This method may allow the paper coatings sector to develop a greater appreciation of which coating technologies lend themselves to better, smoother, glossier applications, and which are most stable. The work presented here was conducted to provide preliminary data on the technique, its application to commercial paper coatings, and to elucidate differences in the commercial coatings under study.

## Coating Technologies

Fig. 1 illustrates simplified, magnified cross sections of paper coatings prepared by three different techniques. The air knife coater provides a uniform coating layer that follows the contour of the sheet. The roll coater provides good coating coverage, but patterning defects are introduced as a result of film splitting (Smook 1992). Roll coaters do not provide the same high degree of smoothness as that provided by blade coaters. The blade coater sacrifices uniform coating thickness across the sheet by distributing thicker coating applications into the low relief areas of the sheet, resulting in a smoother sheet.

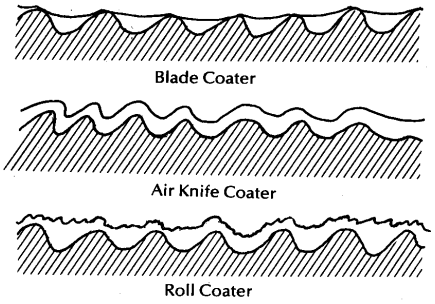



**Fig. 1.** A representation of the cross section of paper coated by three different methods.

The success of a commercial coating operation requires that the coating formulation be stable, evenly colored, and not tend to weep or scratch during application to paper. Fig. 2 is a highly simplified representation of how a cationic polymer additive can assist in the preparation of a homogeneous formulation through charge-charge interactions. The left-hand image represents the anionic charge character of various surfaces and colloidal matter within a coating formulation (excluding the necessary monoatomic cations for electroneutrality) with emphasis on the charged internal periphery of the coating. In general, such an anionically-stabilized formulation has only a limiting ability to resist particle-to-particle sticking collisions, especially when under mechanical stress during a coating operation. The middle image is a cartoon representation of a general polycationic additive. The final result, as shown in the right-hand image, represents a way in which a polycationic polymer can enhance the overall flow of the formulation by adsorbing in a three-dimensional manner onto the surfaces of the dispersed materials. The colloidal stability of the coating formulation is enhanced due

to the somewhat extended conformation of the adsorbed polycation chains; close approach of such particles is unfavorable due to a reduction in conformational entropy when the adsorbed layers become squeezed. An expected result is more uniform flow, yielding a more homogeneous application of coating materials. In addition, the ability of cationic polyelectrolytes eventually to form macromolecular bridges between negatively charged materials is expected to favor gradual immobilization of the coating formation, reducing a tendency for weeping after the coating has been applied to paper.

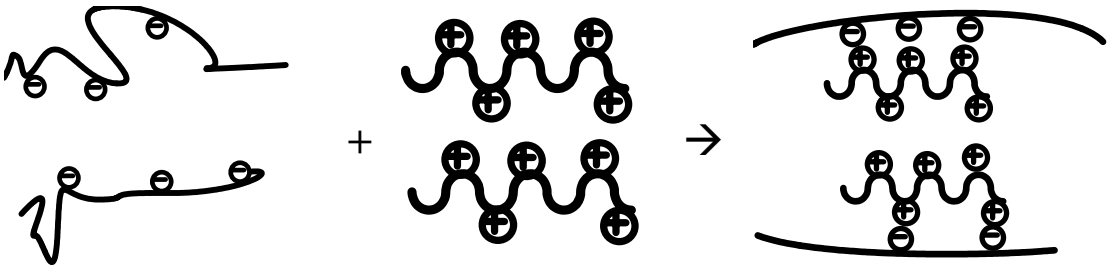


**Fig. 2.** A simplified representation of an enhanced flow condition induced in a coating formulation via a cationic polyelectrolyte-bulk anionic coating charge interaction.

## Laser Induced Plasma Emission Spectrometry (LIPS)

Laser-induced plasma emission spectroscopy (LIPS) or Laser-induced breakdown spectroscopy (LIBS) is an analytical tool that utilizes elemental spectral emission lines generated from a laser-induced plasma. Laser ablation technologies have steadily developed over the past 40 years, starting with the construction of the first ruby laser by Maiman (1960). Today's lasers are far more sophisticated, powerful, and portable, but the principles governing laser ablation spectroscopy remain unchanged. The method has been employed in the elemental analysis of materials, including, among others, metals (Autin et al. 1993), metal ores (Grant et al. 1991), aerosols (Radziemski 1983), ceramics (Heitz 1997), soils (Eppler et al. 1996), human tissue (Samek et al. 2001), artwork (Anglos et al. 1997), detection and identification of bacteria (Baudelet et al. 2006), hazardous materials (Whitehouse 2006), aerosol compositions (Chen et al. 2005), and paper (Hakkanen 1998; Hakkanen and Korppi-Tommola 1998; Hakkanen and Korppi-Tommola 1995).

A representation of the system which was used in the present study is shown in Fig. 3. As shown, the spectrometer used for this type of analysis consists of three components: (i) a light source (LASER in block diagram) and a computer-controlled system to control the laser pulse energy (ii) a sample translation stage (all intermediate components between laser and collection at the ICCD/SPECTROGRAPH), and (iii) a spectrometer with a time resolved multi-channel detection system (ICCD & Spectrograph) (Hakkanen 1998a). The light source is an ArF excimer laser (193 nm), which is capable of generating a cold plasma. Cold plasmas consist mainly of neutral atoms that provide high emission intensities of spectral lines and maintain a good signal-to-background ratio.

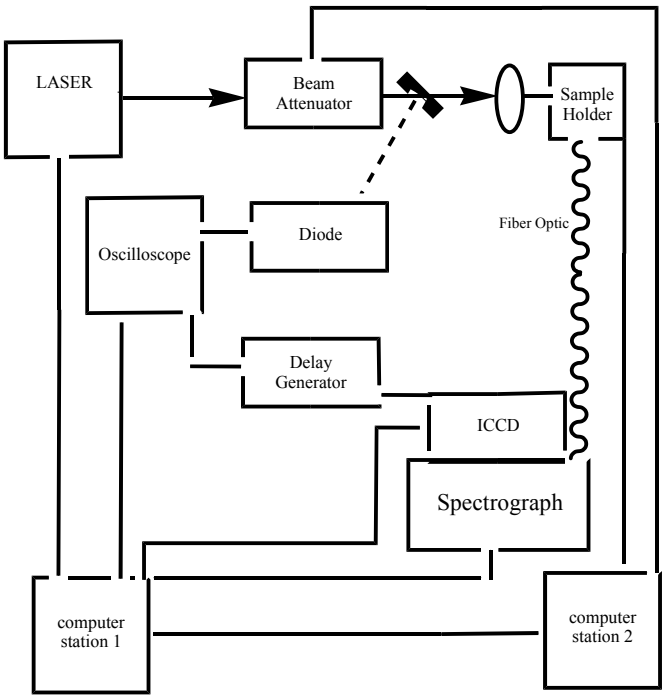



**Fig. 3.** A simplified block diagram representation of the LIPS system that was used in this study which is available at the Department of Chemistry, University of Jyväskylä (Finland).

Analyses are carried out while blowing ambient air at atmospheric pressure onto the focal area in the sample holder. Pulse durations are normally of a 15 ns full width half height (fwhh) spectral window. A computer controlled beam attenuator is used to control both laser energy and intensity. The attenuator adjusts the beam by tilting two filters (wg 280 Schott) simultaneously in opposite directions. Depending on the spot diameter, laser pulse energies are varied from 0.2 mJ to 0.9 mJ. For this work, pulse energies were maintained at 5 mJ. Laser pulse energy is continuously computer monitored throughout the analysis. The laser beam was focused at normal incidence to the sample using 50mm long quartz lenses. The sample holder consists of an aluminum cylinder upon which the paper sample is mounted. The cylinder and the sample are translated in both the X and Y direction using a computer controlled step motor. Light emissions from the plasma plume are carried via a fiber optic bundle to a spectrograph. A gated Intensified Charged Coupling Device (ICCD) using a spectral range of 185-215 nm, the optimal wavelength range for paper coatings, detected the spectrally resolved light. Finally, a digital delay pulse generator with a 50 ns delay time was used to optimize the emission signals from the spectrally resolved light.

It is the analysis of these neutral atom spectral lines that provides the signature elemental composition of the ablated material. Plasma formation is dependent on wavelength, with absorption of laser radiation as the mechanism for the near infrared (NIR) region, and multi-photon ionization as the mechanism in the ultraviolet (UV) region.

A number of factors affect the nature of the generated plasma plume These include laser light wavelength and energy, plasma temperature, physical properties of the target material and atmospheric operating conditions. These factors, in turn, determine the reliability of the emission spectra for analytical purposes. Calibrations and corrections are normally built in to most laser hardware and software set-ups to account for or correct these factors. Several considerations should be discussed regarding the specifics of the hardware used in the LIPS set-up. In optimizing the LIPS method, it was decided to minimize the amount of ablated material while maximizing the signal-to-background noise. This was accomplished by using a low pulse energy (less than 60 mJ) and short pulse durations (less than 100 ns). Laser light wavelength in the lower UV range was also used, since this mainly generates neutral atom ions.

Another parameter that was considered in the LIPS set-up was matrix effects. Paper is, by its very nature, an inhomogeneous solid, consisting of fibers, fines, fillers, coating pigments and various additives. Similarly, a mineral pigment, such as kaolin, is not homogeneous throughout the sample, as minor impurities within the crystal lattice can cause variable signal intensities. For quantitative results, a calibration is therefore necessary for each sample which was obtained.

LIPS has a number of advantages for purposes such as analysis of paper samples. One of the most important is providing rapid 3-dimensional analysis of substrates. A 10 cm profile of LWC paper with measurements taken every 100 um, using 20 shots/point, takes approximately 30 minutes. Relatively large areas of coated paper (4 $cm^2$) can be analyzed in less than an hour. There is no sample preparation and no contamination, since the analysis is strictly of an optical nature. Both quantitative and qualitative data can also be obtained from this analysis.

Some of the more common pigments used in paper coatings and fillers are kaolin and calcium carbonate. LIPS spectra for a coated paper sample containing mixed kaolin and calcium carbonate is shown in Figure 4 (Hakkanen 1998a). The spectrum was detected using the same LIPS setup as was used in this work. Carbon and calcium lines are from the calcium carbonate and binder. Silicon and aluminum lines originate from the kaolin. Magnesium lines come from impurities in both pigments. Another potential source of magnesium is talc, which is often added at the wet end of paper machines for control of pitch deposition. It should be noted that a narrower wavelength range can and should be used for most paper coating analyses since relevant elements show useful emission lines in this range, and a better resolution of line intensities can be obtained.

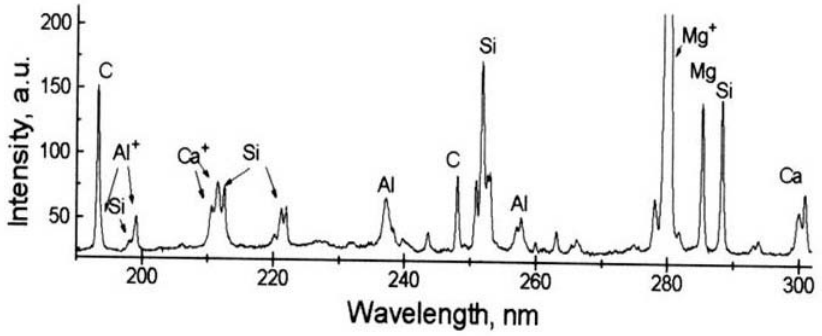



**Fig. 4.** LIPS spectra of coating with mixed calcium carbonate and kaolin pigment.

## EXPERIMENTAL

Base paper samples used in this study were produced on commercial paper machines. They were then pilot-coated using both pilot blade and film coaters. Base and LWC data are tabulated in Table 1.

**Table 1.** Pertinent Data for the LWC Paper Used in this Study

| Base paper | Original basis weight ($g/m^2$) | LWC paper | Forming method | Coat weight ($g/m^2$) |
|---|---|---|---|---|
| P2 | 37 | Kp 404 | film | 9 |
| P14 | 37 | Kp 436 | blade | 9 |
| P5 | 36 | Kp 419 | film | 5 |
| P17 | 36 | Kp 445 | blade | 5 |
| P3 | 40 | Kp 410 | film | 9 |
| P15 | 40 | Kp439 | blade | 9 |
| Former A | NA | Kp 404A | film | NA |
| Former B | NA | Kp 421 | film | NA |

Variables that were investigated in this work include coat weight, coating methodology, and impact of the former used in base paper production on coating characteristics. The LIPS method was used as the principle method of compositional analysis.

### Generation of LIPS Spectra

For each coated sample, 3-dimensional spatial maps of coating distributions were produced using the previously described home-built laboratory-grade LIPS system at the Department of Chemistry, University of Jyväskylä in Finland. This was accomplished by analyzing a 4 $cm^2$ area of each sample. Coating layers were completely removed by 20 laser pulses in each xy-position. In each experiment, sample-to-sample collection from center was 500 μm for a total of 1600 points for each $4cm^2$ area. The top and bottom side of each sample was analyzed. Additionally, each coated sample was analyzed along a line [either MD or CD] at 100 μm centers, both top and bottom side, to provide a 10 cm coating distribution profile. Again, coatings were completely removed by directing 20 pulses at each xy-position. Similarly, base papers were analyzed to provide 10 cm filler profile distributions. The base papers were measured at 200 μm centers and 5 pulses were directed at each xy-position. Top and bottom sides of each base paper were measured. The spectral emission data was saved in a matrix format and the data were then manipulated using MATLAB graphics software to produce graphic images shown in this manuscript.

## SEM Analyses

Before the actual spectroscopy was carried out, it was decided to characterize the LWC paper using scanning electron microscopy (SEM). Following the spectroscopic analysis, further characterization of both the base papers and their coated equivalents was also conducted in order to compare the LIPS results with SEM. The samples were embedded in spur resin, polished and etched. A plasma coating of Au/Pd was applied to provide electrical conductivity. The instrument used was a JEOL JSM-6400 SEM with LaB6, operated at 10 kV accelerating potential.

## Paper Testing

Both LWC and base paper were characterized by physical tests. There were several reasons for doing this. Foremost was the fact that two different formers were used to make base paper that was later film-coated. Therefore a comparison of these base papers, and by inference, the actual formers, required characterization of the samples. Secondly, it should be noted here that this study was part of a larger study on light-weight coatings carried out by a company. Apart from the proprietary nature of a number of the results, certain data were not available to the author. For both formers, neither the basis weights nor coat weights for the base papers and the LWC papers, respectively, were known. Finally, by conducting surface smoothness tests on both base papers and LWC papers, a comparison with LIPS results could be made. Base paper properties and characteristics determined included average basis weight, Sheffield smoothness of both top and bottom sides, Gurley porosity, hard and soft platen caliper, and hard and soft density. For the coated papers, an average coat weight and surface smoothness were determined. Paper testing was done in accordance with TAPPI Standard Methods.

## Burnout Testing

The burnout test, also known as binder burnout, is a traditional test used by papermakers to characterize the coating coverage of a coated sheet of paper by using image analysis to scan the sheet (O'Neill and Jordan 2000). The burnout test was used to characterize both coated and uncoated sheets. Image analysis was then used to make both quantitative and qualitative assessments of coating characteristics of the samples. The results obtained were useful in assessing the validity of the LIPS application to coated paper.

The procedure for the test, as used in this work, is as follows. A salt solution is prepared consisting of 25 g of ammonium chloride dissolved in 1 L of 50/50 deionized water/methanol. The paper sample is completely immersed for several minutes in the solution and removed and allowed to fully dry in air. The sample is then charred in a muffle oven for 6 min. at 225ºC. Chemical pulp fibers turn black, while mechanical pulp fibers turn brown; the inorganic pigment remains largely unaffected.

For the image analysis, a 2.5 x 2.5 cm square of paper was scanned using a 1200 dpi UMAX PowerLook Scanner. A scanning program, Scion Image-Beta 4.02, was then used to measure the area of dark (fibers) against a white (pigment) background. The Scion Image program has 256 gray levels, and assigns 0 to white and 255 to black.

## RESULTS AND DISCUSSION

### Paper Testing Results

The results of physical tests conducted on both base papers and their coated equivalents provided a context in which to compare these results with analyses obtained from LIPS. Paper testing data are reported in Table 2.

**Table 2.** Base Paper Data

| Sample # | Average basis weight ($gm/m^2$) | Smoothness (Sheffield units) | | Porosity (Gurley sec) | Hard platen caliper (mm) | Soft platen caliper (mm) | Hard density ($kg/m^3$) | Soft density ($kg/m^3$) |
|---|---|---|---|---|---|---|---|---|
| | | top | bottom | | | | | |
| P5/P17 | 36.66 | 214 | 168 | 31.94 | .0627 | .0497 | 584.7 | 737.6 |
| P2/P14 | 38.00 | 183 | 180 | 50.00 | .0630 | .0494 | 603.2 | 769.2 |
| P3/15 | 41.39 | 208 | 177 | 86.34 | .0716 | .0569 | 578.1 | 727.4 |
| Former A | 41.51 | 161 | 200 | 59.02 | .0673 | .0549 | 616.8 | 756.1 |
| Former B | 43.92 | 173 | 200 | 49.5 | .0741 | .0600 | 592.7 | 732.0 |

The labels used for sample identification for both coated and uncoated papers were based solely on locations within the reel of paper.

Because coat weights were not provided for the LWC papers using Former A and Former B base papers, they were calculated from measurements obtained from the samples. The mean measured basis weight of the base paper was subtracted from the mean measured basis weight for their coated equivalents. Basis weight data was available for base papers P5/P17, P2/P14 and P3/15. Coat weight data was also available for their coated equivalents. Mean measured coat weights were also determined for these papers by the same method to check the validity of the measurements. Coat weight data measurements are tabulated in Tables 3 and 4.

**Table 3.** Coated Paper Data

| LWC # | Base paper equivalent | Mean LWC basis weight ($g/m^2$) | Mean base paper weight ($g/m^2$) | Mean coat weight per side ($g/m^2$) | Reported coat weight ($g/m^2$) | Coat weight used ($g/m^2$) |
|---|---|---|---|---|---|---|
| Kp404/436 | P2/P14 | 56.66 | 38.00 | 9.328 | 9 | 9 |
| Kp419/445 | P5/P17 | 46.39 | 36.66 | 4.87 | 5 | 5 |
| Kp410/439 | P3/P15 | 58.43 | 41.39 | 8.52 | 9 | 9 |
| Kp404A | Former A | 52.40 | 41.51 | 5.45 | NA | 5 |
| Kp421 | Former B | 61.33 | 43.92 | 8.705 | NA | 9 |

**Table 4.** Smoothness Data of Coated Papers

| Coated Sample | Sheffield smoothness (S.U.) | |
|---|---|---|
| | Side 1 | Side2 |
| Kp 404 | 38 | 34 |
| Kp 436 | 20 | 21 |
| Kp 419 | 48 | 54 |
| Kp 445 | 43 | 39 |
| Kp 410 | 35 | 34 |
| Kp 439 | 18 | 14 |
| Former A | 43 | 33 |
| Former B | 35 | 34 |

## Coating Pigment Analysis

The results from the analyses indicated that the coating pigment and filler used in all samples was kaolin. Since no differences were noted in plasma emission spectra from layer to layer, it was concluded that a single coating was applied to each of the base papers. Analyses were conducted for Si and Mg once the filler and coating pigment was determined to be kaolin. Specifically, the Si line of wavelength 288 nm was used to detect the presence of the coating. Since the coating contains a high proportion of kaolin, the composition of which is directly proportional to Si, the Si emission intensity must therefore be a semi-quantitative analysis of pigment distributions (since Si impurities can affect the signal correlation).

## Coat Weight Distributions

By producing 3-dimensional spatial distribution maps of coating coverage, a visual analysis of coat weight distributions was possible. Histogram plots showing frequency distributions of spectral line intensities provide a semi-quantitative treatment of the same data.

Fig. 5 illustrates the difference between film-coated paper with a coat weight of 9 $gm/m^2$ and one with a coat weight of 5 $gm/m^2$. The spatial distribution maps illustrate variations in coat weight across the sample area. Fig. 6 is a coating distribution profile for the same papers. The Si line intensities are color scaled in accordance with the visible light spectrum. For all results discussed herein pertaining to both spatial distribution maps and coating profile maps, the most intense oranges reflect the highest spectral line intensities. Conversely, the most intense blues reflect the lowest spectral line intensities.

In the paper with the 9 $gm/m^2$ coat weight, approximately 10 pulses were required to completely remove the coating. In the paper with the 5 $gm/m^2$ coat weight, 6 to 7 pulses were needed. Visual inspection of the maps indicate that base paper is exposed through the lower coat weight after the first laser shot, and it is very likely that base paper is exposed through the unablated surface.

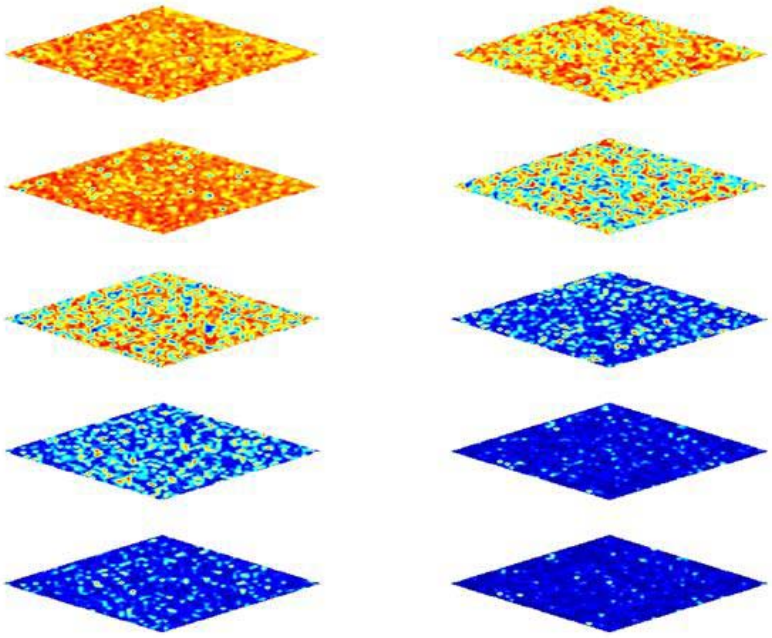


**Fig. 5.** Coating distribution in film-coated paper. Left image: coat weight 9 gm/m$^2$; Right image: coat weight 5 m/m$^2$: 1, 2, 5, 10, and 15 pulses.

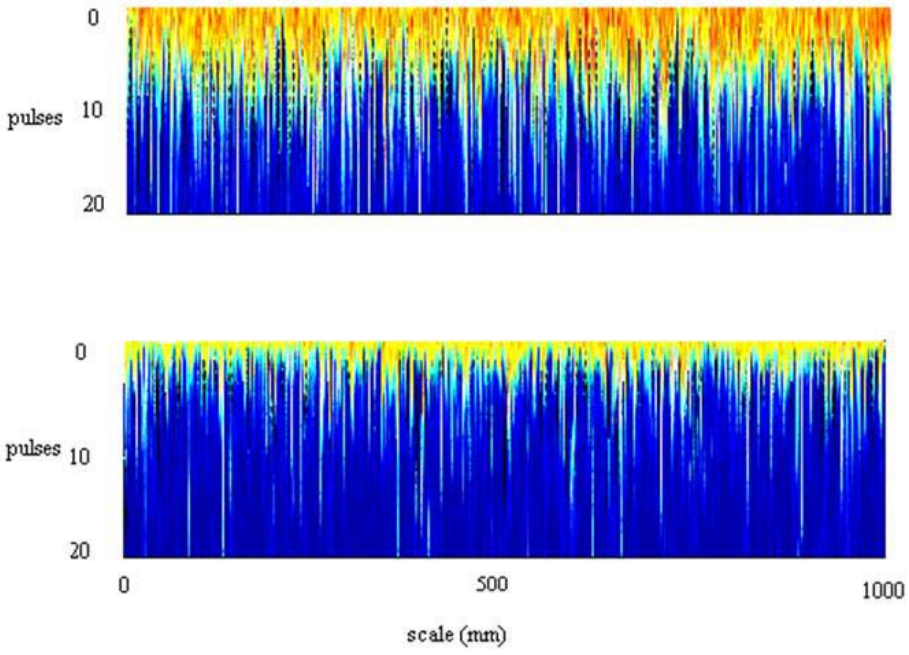



**Fig. 6.** Coating distribution profile of 9 g/m$^2$ (top) and 5 g/m$^2$ (bottom) film-coated paper.

Burnout images of the coated papers support the idea that the base stock is at least partly exposed through the unablated surfaces of coated samples. Fig. 7 contains burnout images of 9 gm and 5 gm coat weight papers, respectively. By thresholding the images, surface coating coverage can be estimated. Coating coverage for the 9 g/m$^2$ paper was 93%, while coating coverage for the 5 g/m$^2$ paper was 84%. Visual inspection of both burnout images shows clearly that there are more fibers exposed at the surface of the 5 gm coat weight paper.

Studying histograms that show frequency distributions of Si line intensities can also aid in assessing coating coverage. Fig. 8 shows three-dimensional spatial distributions of the same two film-coated papers. The coating distribution maps reflect the removal of the second coating layer in each case. Their associated frequency distribution plots of Si emission intensities are indicative of the coating coverage and coat weight distribution.

It is evident that the coating coverage and coat weight distribution is less uniform for the lighter coat weight, with a skew of distributions towards the lower Si emission intensities. Film coating is essentially contour coating, in that the application follows the naturally undulating surface of the base sheet. It is therefore reasonable to assume that the coating thickness should be fairly uniform and, by inference, that the Si line intensities should have a tight distribution. This is clearly evident in the higher coat weight. At coat weights as low as 5 g/m$^2$, it is not unreasonable to see a broader distribution.

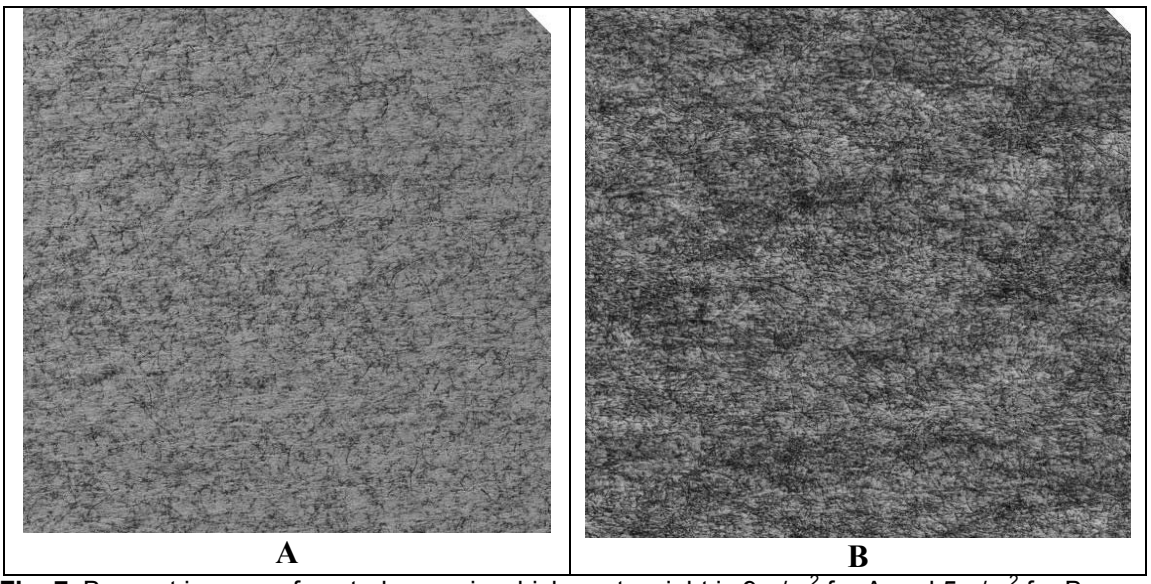



**Fig. 7.** Burnout images of coated paper in which coat weight is 9 g/m$^2$ for A and 5 g/m$^2$ for B.

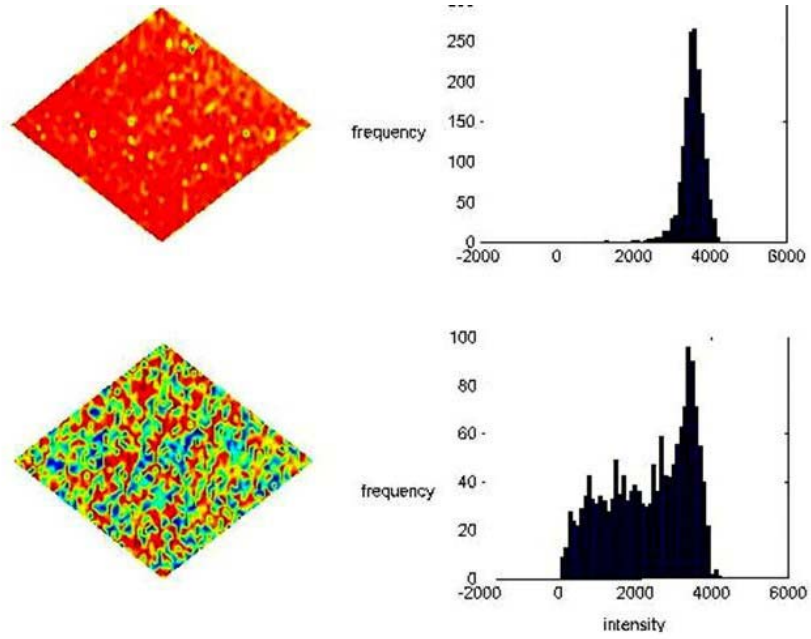



**Fig. 8.** Frequency distribution plots of Si line intensity for 9 gm and 5 gm coat weights, film-coated paper; second ablated layer.

## Film Versus Blade Coating

Two traditional methods used to coat papers are the film coating and blade coating methods. With film coating, coating color is metered onto the coating roll and transferred onto the paper in a roll nip. With blade coating, an excess of coating color is first applied to the paper and then scraped off with a blade. Both coating methods result in different surface properties or characteristics of the coated paper. Fig. 9 illustrates this difference.

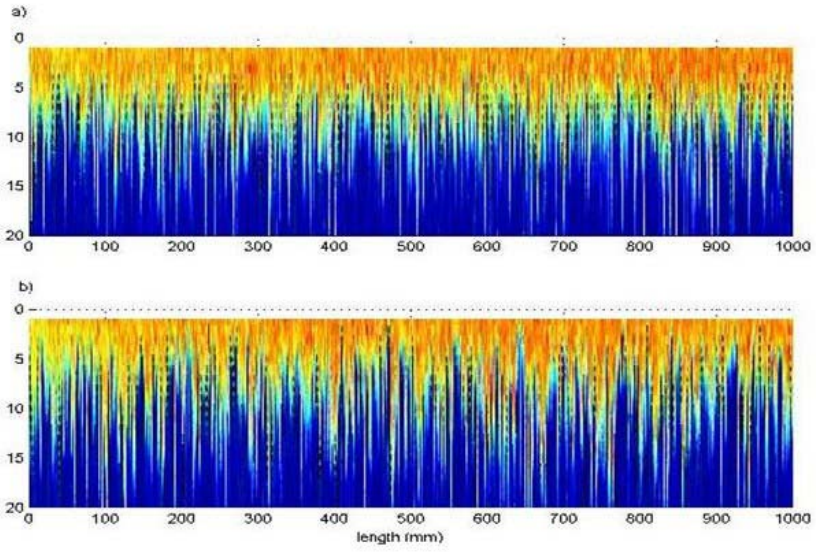



**Fig. 9.** Coating distribution profile in (top-a) film-coated paper and (bottom-b) blade-coated paper. Coat weights are 9 g/m$^2$.

Blade coaters trade greater surface smoothness of the coated paper in exchange for a less even coat weight distribution. Low relief areas in the naturally undulating base paper surface receive a thicker coating than high relief areas. This is evident in the coating distribution profile, where area and points of low Si emission intensity represent those high relief areas and thus a very thin coating layer. The film-coated papers, according to their coating distribution profile, display a more even coat weight distribution, which in turn is a trade-off for the smoother sheet surface provided by the blade coater. Smoothness data for the coated papers support this interpretation of LIPS. In each case, the blade-coated versions of the base papers yield lower Sheffield unit values.

Histogram plots for the same film and blade coated papers are illustrated in Fig. 10. Three-dimensional spatial distribution maps of the same coated papers are shown in Fig. 9. The distribution for the blade-coated paper shows a tailing of low line intensities that do not appear in the film-coated paper.

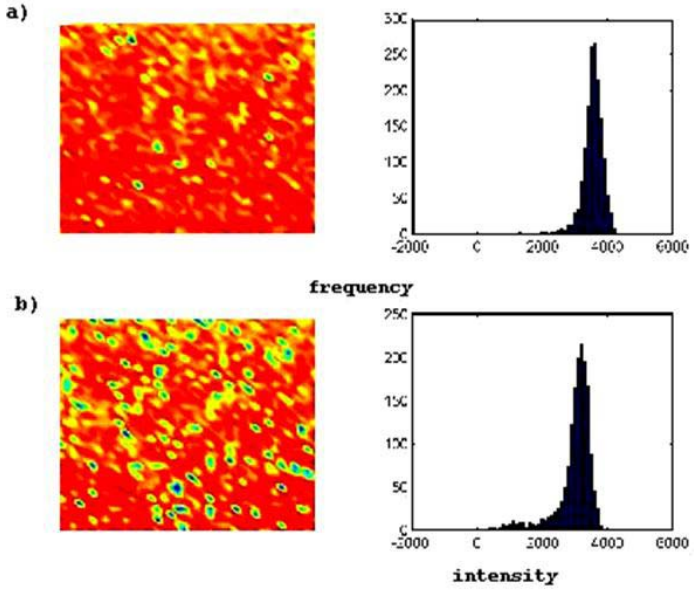



**Fig. 10.** Frequency distribution plots of Si line intensity in a) film-coated paper and b) blade- coated paper. Coat weight 9 gm/m$^2$.

A high frequency of strong Si signals was noted in the case of blade-coated paper. The higher frequency of lower coat weights for the blade coated papers are indicative of small or shallow voids in the paper surface that are leveled in the blade coating process. This is consistent with what LIPS is indicating: blade coating provides a smoother sheet. By contrast, the film-coated paper shows a normal distribution in a tight range of signal intensities, consistent with a more even coat weight.

## Binder Migration

Binder migration is the adventitious movement of binder after application of the coating color to the base paper. It may be caused by surface tension force pulling liquid

(mainly water) into pores, carrying some binder with it or by evaporative drying in the dryer section (Casey 1985).

Binder migration is evident, however, when looking at Si emission signals from the first laser pulse to the second laser pulse of ablated coating layers. Si emission intensity is larger for the second pulse because binder migration to the surface of the coating effectively reduces the amount of coating pigment in the volume of ablated material. This results in lower Si emission intensities in the first ablated layer relative to the second one. This effect is illustrated in Fig. 11.

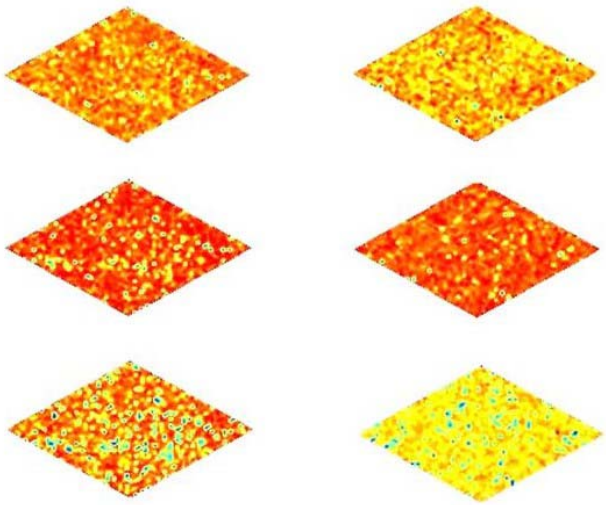


**Fig. 11.** Binder migration effect illustrated in coating distribution maps of film-coated (left) and blade-coated (right) paper. First (top), second (middle) and third (bottom) pulses. Note the difference between second and third pulse.

## Basis Weight Effects

Figure 12 shows coat weight distributions for two film-coated papers with equal coat weights of 9 g/m$^2$.

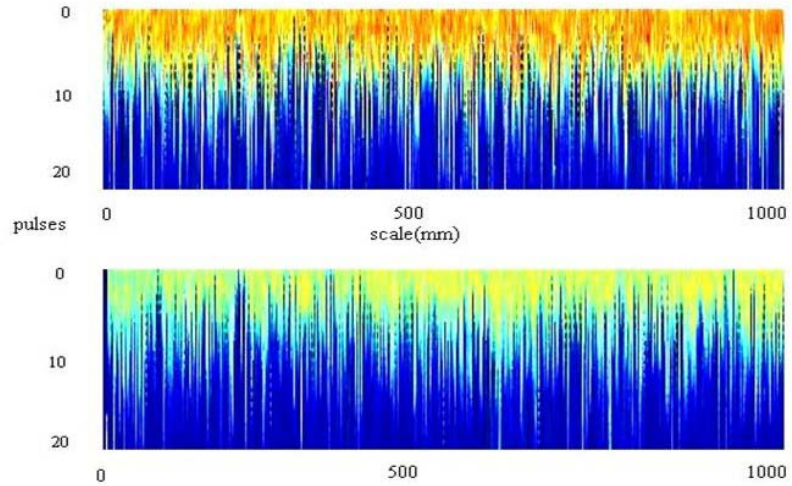



**Fig. 12.** Coating weight distribution profiles of film-coated paper. Base paper basis weights are 37 g/m$^2$ (top) and 40 g/m$^2$ (bottom). Coat weight 9 g/m$^2$.

The base paper basis weights for the top and bottom profiles are 37 g/m$^2$ and 40 g/m$^2$, respectively. From the coating distribution profiles, it is evident from the LIPS signal that there is a higher coat weight distribution *at the surface* of the lower grammage paper. From paper testing results, the heavier paper appears less porous than the lighter one (porosity of 86.34 Gurley sec compared to 31.94 Gurley sec). Since the coat weights are the same (9 g/m$^2$), the LIPS signal should be slightly higher, if anything, for the less porous sheet since coating color is *less likely to penetrate as deeply when applied*. Frequency distribution plots for both papers (Fig. 13) show that there is a greater frequency of higher emission line intensities for the lower basis weight paper, again consistent with a *higher surface* coat weight compared to the heavier paper.

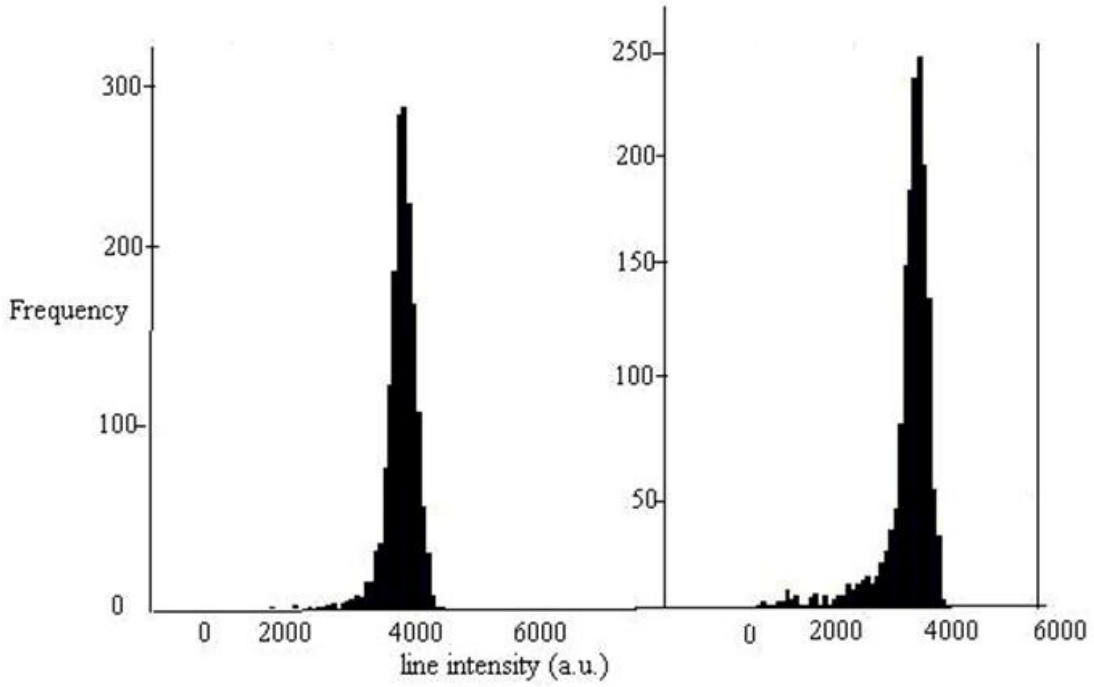



**Fig. 13.** Frequency distribution of Si line intensity for different basis weight papers (left plot is 37 g/m$^2$ while the right is 40 g/m$^2$) with the same coat weight (9 g/m$^2$).

The same results were obtained with blade-coated papers using the same base papers and coat weights. It was thought that binder migration in the heavier sheet might be responsible for the lower signals, since, if the pigment could not penetrate the base paper, it was possible that the binder may have concentrated at the surface as well. Fig. 14 shows spatial distribution maps for both papers. The maps indicate that there does not seem to be any effect in signal intensity due to binder migration.

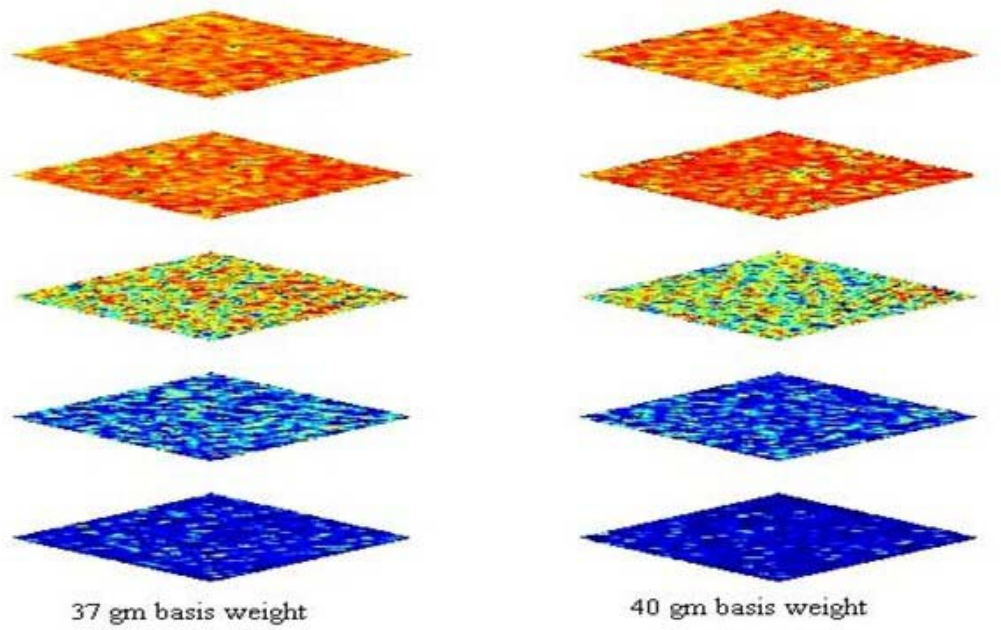



**Fig. 14.** Spatial coating distribution maps for high and low basis weight papers.

## CONCLUSIONS

1. Pigment identification within light-weight paper coatings is possible by identifying the elemental composition of the pigment. Since each element emits spectra at its characteristic wavelengths, the elemental composition of the pigment can quickly be determined. Coat weight distribution maps and profiles can be created based on spectral emission intensities of the signature elements. This is made possible by coupling the LIPS data with suitable software graphics.
2. Coating technologies, such as blade and film coating, generate noticeably different coating distribution profiles that can be detected by LIPS. Interpretation of coating distribution maps and profiles, coupled with smoothness data of the coated sheet allowed a qualitative assessment of the coating technologies, to the point of determining differences in distribution of coating in the substrates.
3. Coat weight variations are characterized by the relative intensities of spectral emission lines of its elements. Coat weight distribution maps and profiles were used to identify areas of poor coating coverage in very light coatings. Histogram plots of Si line intensities proved useful in interpreting variations in coating coverage for different coat weights. Burnout images were useful in confirming the results.

## ACKNOWLEDGMENTS

The authors are grateful to the Institute of Paper Science and Technology Center at the Georgia Institute of Technology whose research funding made this work possible. This research allowed BW to partially fulfill his requirements for the M.Sc. degree.

## REFERENCES

Anglos, D., Couris, S., and Fotakis, C. (1997). "Laser diagnostics of painted artworks: Laser induced breakdown spectroscopy in pigment identification," *Appl. Spec.* 51(7), 1025-1030.

Autin, M., Briand, A., Mauchien, P., and Mermet, J. M. (1993). "Characterization by emission spectroscopy of a laser-produced plasma from a copper target in air atmospheric pressure," *Spectrochem. Acta* 48(6/7), 851-862.

Baudelet, M., Guyon, L., Yu, J., Wolf, J.-P., Amodeo, T., Frejafon, E., and Laloi, P. (2006). "Femtosecond time-resolved laser - induced breakdown spectroscopy for detection and identification of bacteria: A comparison to the nanosecond regime." *J. Appl. Phys.* 99(8), 084701/1-084701/9.

Casey, J. P. (1985). *Pigment Coated Papers - A Critical Assessment of the Processes, Technical Developments, and Economics.* Marcel Dekker, Inc., New York.

Chen, Y., Bulatov, V., Singer, L., Stricker, J., and Schechter, I. (2005). "Mapping and elemental fractionation of aerosols generated by laser - induced breakdown ablation." *Anal. Bioanal. Chem.* 383(7-8), 1090-1097.

Grant, K., Paul, G., and O'Neill, J. (1991). "Quantitative elemental analysis of iron ore by laser induced breakdown spectroscopy," *Applied Spec.* 45(4), 701-705.

Hakkanen, H. (1998). "Development of a method based on laser induced plasma emission spectrometry for rapid spatial analysis of material distributions in paper coatings," Ph.D. dissertation, University of Jyväskylä, Jyväskylä, Finland.

Hakkanen, H. J., and Korppi-Tommola, E. I. (1998). "Laser-induced plasma emission spectrometric study of pigments and binders in paper coatings: matrix effects," *Anal. Chem.* 70(22), 4724-4729.

Hakkanen, H. J. and Korppi-Tommola, E. I. (1995). "UV-plasma study of elemental distributions of paper coatings," *Appl. Spec.* 49(12), 1721-1728.

Hakkanen, H. J., and Korppi-Tommola, J. E. I. (1993). "Laser-induced fluorescence imaging of paper surfaces," *Appl. Spec.* 47(12), 2122-2125.

Heitz, J., Pedarnig, J. D., Baurle, D., and Petzow, G. (1997). "Excimer-laser ablation and micro-patterning of ceramic $Si_3N_4$," *Appl. Phys.* A65, 295-261.

Kartovaara, I. (1989). "Coatweight distribution and coating coverage in blade coating," *Paperi ja Puu* 9, 134-140.

Lee, Y., Song, K., and Sneddon, J. (1996). *Lasers in Analytical Atomic Spectroscopy*. Chapter 5. John Wiley & Sons, New York.

Maiman, T. H. (1960). "Optical maser action in ruby," *Brit. Commun. & Electron.* 7, 4674-675.

O'Neill, M., and Jordan, B. (2000). "The burnout test revisited," *J. Pulp Pap. Sci.* 26(4), 131-134.

Peterson, R. A., and Williams, C. L. (1992). "Determining paper-coating thickness with electron microscopy and image analysis," *Tappi J.* 75(10), 122-126.

Radziemski, L., Loree, T., Cremers, D. and Hohhmann, D. (1983). "Time-resolved laser-induced breakdown spectroscopy of aerosols," *Anal. Chem.* 55, 1246-1252.

Eppler, A. S., Cremers, D. A., Hickmott, D. D., Ferris, M. J., and Koskelo, A. C. (1996). "Matrix effects in the detection of Pb and Ba in soils using laser-induced breakdown spectroscopy," *Appl. Spec.* 50(9), 1175-1181.

Samek, O., Beddows, D. C. S., Telle, H. H., Kaiser, J., Liska, M., Caceres, J. O., and Gonzales Urena, A. (2001). "Quantitative laser - induced breakdown spectroscopy analysis of calcified tissue samples." *Spectrochim. Acta, Part B: Atom. Spectrosc.* 56B(6), 865-875.

Sheppard, J. C., and Grant, G. (1974). "Use of positive ion backscattering in the analysis of paper coatings and filler," *Tappi J*. 57(3) 151-154.

Sinclair, A. R. (1975). *Synthetic Binders in Paper Coatings*, TAPPI Monograph Series, No. 36, TAPPI Press, Atlanta.

Smook, G. A. (1992). *Handbook for Pulp and Paper Technologists* (2nd Ed.) Angus Wilde Publications, Vancouver, BC, Canada.

Wang, S., Brown, R., and Grey, J. D. (1994). "Application of laser ablation-ICPMS to the spatially resolved micro-analysis of biological tissue," *Appl. Spec.* 48(11), 1321-1325.

Whitehouse, A. I. (2006). "Laser - induced breakdown spectroscopy and its application to the remote characterisation of hazardous materials." *Spectrosc. Europe* 18(2), 14, 16-21.

Article submitted: April 17, 2006; Revision accepted: June 29, 2006; Published July 15, 2006

# AN OVERVIEW OF THE AUSTRALIAN BIOMASS RESOURCES AND UTILIZATION TECHNOLOGIES

* Behdad Moghtaderi, Changdong Sheng, and Terry F. Wall

Information on Australian biomass resources including bagasse, black liquor from paper pulp production, wood waste and forestry residues, energy crops, crop wastes, food and agricultural wet waste, and municipal solid wastes is provided in the review. The characteristics of the Australian biomass are typical of those of other countries, i.e., high moisture and volatile matter, low heating value and density, and low sulfur and nitrogen content, but high Ca and Mg for woody biomass. The characteristics influence biomass utilization. Biomass is used extensively at present within Australia, primarily for domestic heating, as bagasse in the sugar industry, and for electricity generation. Biomass usage for electricity generation is increasing and is expected to reach 5.2 Mt/year by 2019-20. Exports, as wood chips, are approximately 10 Mt/year in 2000-01. Forestry residues have been estimated to be 23 Mt/year. Current technologies that utilize biomass in Australia include those for electricity and heat by direct combustion, cofiring with coal and fluidized bed combustion), for biogas generation (from landfills, and aerobic digestion, and as bio-liquids. Related to bio-liquid fuels, ethanol production from molasses and wheat is making progress. The resultant ethanol is used as a petrol extender, and a bio-diesel process is under development.

*Keywords: Biomass, Australian resources, Utilization technologies*

*Contact information: Discipline of Chemical Engineering, School of Engineering, Faculty of Engineering & Built Environment, The University of Newcastle, Callaghan, NSW 2308, Australia; *Corresponding author: Behdad.Moghtaderi@newcastle.edu.au*

## INTRODUCTION

Coal energy is facing increasingly heavy pressure related to concerns of global warming and reducing greenhouse gas $CO_2$ emission. One of the biggest contributors to greenhouse gas emissions is fossil fuel coal (Davidson 1999; Werther et al. 2000; Sami et al. 2000; Williams et al. 2001). Among the nations that use this fossil fuel, Australia has a major dependence on coal energy and in addition is the preeminent coal exporter. Domestically, coal supplies a 41% share of primary energy consumption and a 79% share of electricity generation, and coal will continue to be a crucial part of the energy mix in the foreseeable future (Dickson et al. 2002). However, due to environmental concerns associated with greenhouse gas emissions and global warming, the reliance on coal is on a decline despite the availability of coal and efforts being directed towards clean coal technologies (Dickson et al. 2002). The implication is that other energy resources are required to fill the gap. Biomass is expected to play a significant role in this context

because of its $CO_2$ neutrality and renewable nature, as well as its relatively low cost and availability.

Currently, Australia obtains 5.9% of its primary energy requirements from renewable resources, of which biomass together with biogas, is predominant and holds a 78.3% share. About 4.5% of the primary energy consumption in Australia is from biomass, and this percentage is expected to increase to 4.8% in 2019-20 (Dickson et al. 2001). Of this, nearly 2.0% is in the form of bagasse (cane residue used in the sugar industry), and 2.8 % in the form of firewood (Eucalyptus and pine used for domestic heating) and wood waste (both soft- and hard-woods such as pine and Eucalyptus, respectively used in the wood products industries) (Bush et al. 1997). Biomass has the potential to be an increasingly cost competitive renewable energy source in Australia, and still to make a valuable contribution to the overall energy system mainly because of its very low price and the fact that it is renewable. There is still considerable scope for making better use of the existing biomass energy supplies and also for developing new supplies.

Biomass is particularly well positioned to play a major role in Australia's effort to reduce greenhouse emissions. Following the government's Mandatory Renewable Energy Target (AGO 2003) adopted on April 1, 2001, the role of renewable energy, mainly biomass, in electricity generation has substantially increased. The energy target requires electricity retailers and large electricity purchasers to source an additional 2% of their electricity from renewable energy sources by 2010. As a result, the market share of electricity generation for coal is anticipated to shrink from 79.3% (1998-99 data) to 71.1% by 2019-20 while the share of biomass will is expected to rise to about 2.0% from its current 0.6% level (Dickson et al. 2001). The present review provides an overview of the Australian biomass resources and utilization technologies in an attempt to promote and facilitate the use of biomass in the energy mix of Australia.

## BIOMASS RESOURCES IN AUSTRALIA

### Background

Biomass is a natural resource. Biomass refers to materials derived from photosynthesis that are not fossilized, such as wood from forest, residues from agricultural and forestry processes, and industrial, human or animal wastes. Australia has a substantial biomass resource, which could provide feedstock for substantial biomass energy and chemical and chemicals industries. The biomass resources that are considered here are the eligible biomass resources under the Renewable Energy (Electricity) Act 2000 (ORER, 2000):

- Bagasse
- Black liquor from paper pulp production
- Wood wastes and forestry residues
- Energy crops
- Crop wastes
- Food and agricultural wet waste
- Municipal solid waste (MSW)

Note that MSW is predominantly biomass resources and may also have 30-40% fossil fuel-based hydrocarbons. Therefore, it is also included here.

It must be highlighted that Australia has substantial resources of biomass and wastes. However, it is not easy to estimate the potential amount of all these biomass resources available for utilization, mainly because of wide distribution of these resources across the country, difficulty in biomass collection, species classification and inaccuracy of availability estimation methods (i.e., calculation). The available data on Australia's biomass resources are summarized in Table 1, which is reported based on the amount (Mt/year), or energy production (PJ/year), or potential electricity capacity (MW), or electricity generation (GWh/year). Each resource is discussed in more detail in the following sections.

**Table 1.** Summary of the Main Biomass Resources in Australia

| Resource | Amount (Mt/year) | Energy (PJ/year) | Electricity capacity (MW) | Electricity Generation (GWh) | Reference |
|---|---|---|---|---|---|
| Bagasse | 10.6 | 100 | 1000 | 4,000 | REM,1999 |
| Cane trash | 9.25 | - | - | - | |
| Black liquor | 0.25[a] | - | 49 | 90 | REM,1999 |
| Wood wastes | 6.9 | 120 | - | 10,000 | REM,1999 |
| Forest wastes | 23 | - | 1,500 | - | |
| Crop wastes | 44[b] | - | - | - | RIRDC, 2002b |
| Wet waste | 1.8[a] | - | 200 | - | REM,1999 |
| MSW | 8.7 | - | 330 | - | Glover, 2002 |
| C&I | 10.3 | - | 400 | - | |
| C&D | 13.5 | - | 200 | - | |
| Energy crop[c] | | | | | ERDC, 1994 |
| Cereals | 30 | - | - | - | |
| others | 8 | - | - | - | |

a. Estimated value based on reported electricity capacity or generation
b. Estimated from the reported in-field agriculture resources reduced by cane wastes
c. Potential energy crops, but agroforestry crops not included.

## Bagasse and Cane Trash

Bagasse is the residual fibre waste from raw sugar processing. It is currently the largest source of renewable energy after hydroelectric, and provides 90 Peta Joules (PJ), or about 2% of Australia's total primary energy demand (Bush et al. 1997). Bagasse has the advantage over some other forms of biomass (such as woody weeds or forestry thinnings) in that the sugar processing requires the bagasse to be brought to a central location at the mill, so there are no additional transport costs. Bagasse is actually and potentially a major source of biomass energy in Australia, as it can be used as boiler feedstock to generate steam for processing heat and electricity as well. The steam raised from bagasse is used to work the machines that shred and crush the cane, for processing heating, and increasingly to cogenerate heat and electricity. The Australian bagasse cogeneration industry is already quite well developed and mature. Bagasse is available for about half of each year (June to November) from the 25 sugar mills in the State of Queensland (Qld), 3 in the State of New South Wales (NSW) and 1 in the State of Western Australia (WA). These mills have an installed electricity-generating capacity of

about 250 MW, fueled almost entirely with bagasse in cane crush season and cofiring other biomass such as wood wastes in non-crush season (Redding Energy Management, 1999). However, the bagasse resource could potentially supply a much greater capacity. Sugar Research Institute estimated that 10.6 million tonnes (Mt) are available annually, equivalent to 100 PJ. This could fuel 1000 MWe of capacity, producing around 4000 GWh/year (Redding Energy Management 1999).

Cane waste is comprised of the leaves and tops of the sugar cane plant. This material is not milled for sugar extraction, and in the past, was burnt in the fields either prior to or after the cane had been harvested. Research has indicated that a typical sugar cane crop, when harvested as green or unburnt cane, is composed of 75% sugar cane stalk and 25% leaves and tops (Sunshine Electricity 2002). This waste provides a huge potential fuel resource. Considering an average harvest of 37 Mt of cane per year for the five years from 1994 to 1998 (RIRDC 2002a), it is estimated that around 9 Mt cane waste per year is produced in Australia. Technologies have been developed in NSW and QLD for harvesting the cane trash (Burnard 2002) so as to use it as fuels for cogeneration. For example, in a proposed project, Sunshine Electricity proposes to develop a cogeneration plant adjacent to the Broadwater Sugar Mill that is fueled with bagasse blended with sugar cane waste and will be using annually up to 250,000 tonnes of bagasse and 230,000 tonnes of cane leaves and cane trash. (Sunshine Electricity 2002).

## Black Liquor from Paper Pulp Production

Black liquor is produced as a combustible by-product of the paper and pulp making processes. In Australia, the available black liquor resource is already effectively utilized on-site for cogeneration of electricity and processing steam. Waste wood and coal are also commonly used in addition to the black liquor. Currently, 0.15 Mt of black liquor is produced per year with a total capacity of 49 MW, which is equivalent to around 90 GWh electricity and processing heat (Redding Energy Management 1999). There is still a potential capacity of black liquor from new paper pulp plants, which is expected also to be used for on-site cogeneration. About 20% more capacity is anticipated to come on-line in the next decade, primarily using the conventional combustion-based strategies rather than gasification strategies.

## Wood Waste and Forestry Residues

Australia has 1,581,000 $km^2$ forest area. Over 17 Mt of products are produced annually from forests and plantations across Australia, of which 6-7 Mt is sold as firewood in the domestic market for home and industrial use (Driscoll 2000) and 10 Mt is exported in the form of wood chips (Yainshet 2002). The huge amount of forest residues and wood waste generated from forestry and plantation industry is believed to be sufficient to meet the federal government's 2% renewable energy target (Redding Energy Management 1999). Although there are a few plants in Australia generating electricity from resources such as sawdust at sawmills, the current level of electricity production from forestry residues and wood waste is not significant at all.

Australian sawmills only utilize 45% of the wood. Of the remaining off-cuts, 30% is used to produce woodchips, and the rest is sawdust and chip rejects. The only current use for this is in the horticultural industry. Australia produces 1.25 million tonnes of

sawmill waste per year, and many mills pay to have this removed. Timber residues from sawmills and from material that is not chipped for export or used for board products is estimated to be equivalent to about 120 PJ/yr (Redding Energy Management 1999). It is equivalent to about 6.9 Mt wood waste per year. At a conversion efficiency of 30% this would generate about 10,000 GWh of electricity annually. However, in practice only a fraction of this resource could be economically used for electricity generation.

The ERDC report estimated total forestry residues at 23 million tonnes per year. It was estimated that up to 1500 MW of capacity could be installed to utilize this resource (nominally comprised of 150×10MW projects) (Redding Energy Management 1999). The practice, however, is not sustainable at the current rate, as resources are anticipated to decline within a timeframe of 100-150 years. Innovative harvesting practices are being researched in order to extend the sustainability of forestry residues utilization. One of the more interesting developments in this context is the research activities being conducted as part of the "Joint Venture Agroforestry Program (JVAP)", a partnership among Rural Industries Research & Development Corporation, Forest and Wood Products Research & Development Corporation, and Land and Water Australia (RIRCD 2002c). The idea is the incorporation of trees into a farming system. While for farmers, tree growing boosts their productivity and offers an additional source of income, processors benefit from a more sustainable source of timber with an estimated timeframe of several hundreds of years (RIRCD 2002c).

## Energy Crops

Energy crops refer to biomass (e.g., planted wood sugar cane, corn, wheat, sorghum, etc.) planted specially for direct utilization as energy fuel (i.e., power generation) or as feedstock for production of biofuels (e.g., biodiesel, bioethanol, biomethanol, etc.). There is widespread semi-commercial scale trialing of this resource overseas, especially for power generation, such as coppicing willow, switchgrass, and miscanthus in the UK, and switchgrass in the USA. In Australia, growing energy forestry crops on a large scale has received renewed interest, mainly in woody biomass, but not currently for electricity generation. The Commonwealth Scientific and Industrial Organization (CSIRO) has conducted trials on intensively managed, short rotation bioenergy plantations grown on effluent, saline water, and sludge at Wagga Wagga, NSW (CSIRO FFP 2000). These plantations demonstrate potentially very short rotation times of 2-3 years for fuel reduction. Growth rates for selected eucalypts were shown to be around 15 tonnes per hectare per annum. A full-scale integrated wood processing plant is being built at Narrogin, Western Australia, by Western Power (RIRDC 2001). The demonstration plant will use planted mallee eucalyptus to produce eucalyptus oils, activated carbon, and renewable electricity. To date, approximately 9000 hectares of oil mallee have been planted, and this is set to increase when the Narrogin plant comes on-line. Also, GreenEco has a long-term target of 1000 MW of capacity from crop-based resources producing 1800 GWhr/yr. The target will be achieved by utilizing a combination of crop wastes or crops grown specifically for electricity production (Redding Energy Management 1999).

In Australia, some 237 million hectares (Mha) of land can potentially be used for producing additional biomass feedstock. Of the area, 132 Mha is not suitable because of

the difficult terrain. Soil quality reduces the total down to 77 Mha, of which 51 million has already been utilized (Stewart et al. 1979 & 1982), leaving just over 26 Mha available for new developments, which is relatively small (Foran and Mardon 1999). The challenge is therefore to select, or develop, suitable species, which grow well in the medium-to-low rainfall areas and produce good yields.

Although energy crops are expected to be a more expensive resource than forestry and wood wastes and are yet to be proven worldwide as a commercially viable resource, energy crops are expected to make a substantial contribution to meeting the federal government's 2% subsequent renewable target.

### Crop Waste

There is theoretically a very large resource of crop wastes available in the form of straw and stubble from broadacre cropping. For example, in NSW alone there are 200,000 t/year cotton trash and 300,000 t/year of rice hull (Burnard 2002). RIRDC has estimated that the potential in-field agricultural resources are around 55 Mt (RIRDC 2002b). Besides nearly 20 Mt bagasse and cane waste, there is 35 Mt crop wastes per year. However, the availability of the resource is still uncertain. There is doubt that collection of the resource is viable, not only because of the associated cost or the need in the field production cycle for cropping, but also because the resource may not be available in a collectible form. Currently, this resource is only used commercially for generating biogas, primarily through projects under Sustainable Energy Development Authority (SEDA) of NSW (Burnard 2002). These include a cotton trash gasifier (25,000 t/year) at Auscott gin Narrabri, and a rice hull gasifier at Ricegrowers Co-operative Ltd (Sunrice).

### Wet Wastes from Agriculture and Food Processing

This resource includes wastes from intensive animal production, the possibility of collecting waste from field animal production, and waste streams from processing of fruit and vegetables, grains, meat and meat products, beverages, and milk products. The potential resource from field animal production is theoretically large but it is doubtful that the field collection of the resource is practically viable. In total, the potential resource associated with wet wastes from agriculture and food processing could be of the order of 200 MW (1,500GWh/year), made up of: 50 MW from piggeries, a further 50 MW from other animal husbandry, and 100 MW from food processing wastes (Redding Energy Management 1999).

### Municipal Solid Wastes

Municipal solid waste (MSW) generally refers to post consumer discards and spent and surplus materials, such as council collected garbage. It consists of approximately 60-70% biomass (lignocellulosic) originated materials and 30-40% fossil based hydrocarbons. Additionally, the unwanted 'byproducts' of the productive/manufacturing sector (i.e., commercial and industrial, C&I, wastes) and the residuals from the construction and demolition sector (C&D) can also be included in MSW, since these wastes are usually disposed of in landfill. It has been reported that 8.7-13.5 Mt of MSW, 3 Mt of C&I, and 10.3 Mt of C&D are annually generated nationwide (Glover 2002;

Wootton 2002). Considering utilization of 30% MSW and C&I and 13% C&D, a total capacity of 930 MW electricity may be potentially produced, which is equivalent to 2.2% of total installed capacity and 3.6% total coal-fired installed capacity of Australia (Glover 2002). The potential capacity is sufficient to meet the federal government's 2% renewable electricity target.

## BIOMASS CHARACTERISTICS

When used as a fuel for thermal chemical conversion process, biomass is generally characterized by conventional fuel analyses, including proximate analysis, ultimate analysis, ash analysis, heating value, etc. The characteristic data of typical Australian biomass species, including bagasse and cane trash, eucalyptus, and pine, used for electricity generation have been collected from the open literature (Sunshine electricity 2002; Gupta et al. 2002; DET CSIRO 2003). These are summarized in Table 2 compared with US biomass (Jenkins et al. 1998). Note that biomass composition varies from sample to sample even for same biomass species, such as bagasse, because of biomass resources varying in species, region, plantation, processing, and so on.

The compositions of main organic elements (C, H, O) of Australian biomass are compared with those of international biomass in van Krevelen diagrams, shown in Figure 1. It can be seen that the organic compositions of Australian biomass are located in the composition range of international biomass. There also are no considerable differences between Australia's and overseas biomass in terms of other properties (see Table 2). However, a closer examination of Figure 1 reveals that the average H/O ratio (i.e., obtained by dividing H/C by O/C) for overseas biomass is about 2.28 while for the Australian biomass the H/O ratio drops to about 2.07. This is an indication that, in general, the Australian biomass species (considering only lignocellulosic biomass) have slightly higher carbon contents, which make them quite attractive particularly for power generation applications.

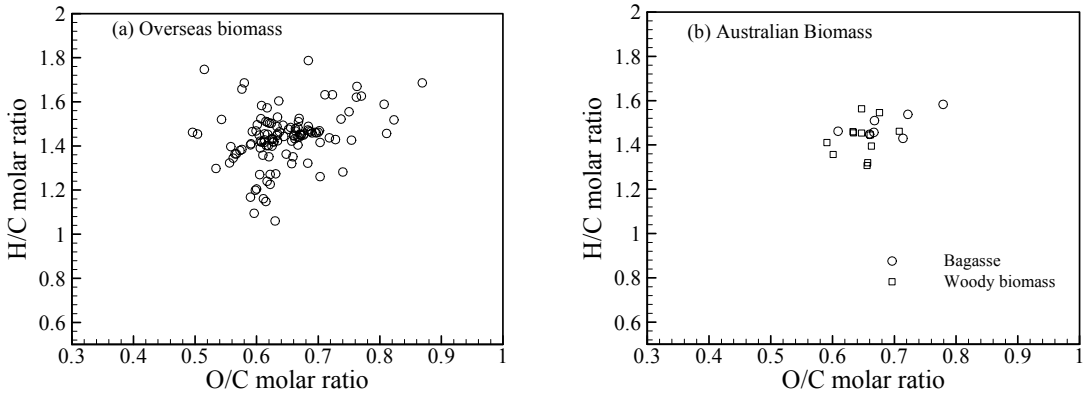



**Figure 1.** van Krevelen diagram for biomass materials (a) International biomass; (b) Australian biomass.

**Table 2.** Characteristics of Biomass Materials

| | Australian biomass | | | | | | | | US biomass | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bagasse | Cane Trash | Camphor | Eucalyptus | Pine | Pine Sawdust | Pine Chips | Camphor | Rice Hulls | Rice Straw | SwitchGrass | Bagasse | Poplar |
| Proximate Analysis | | | | | | | | | | | | | |
| Total Moisture (%ar) | 44.0 - 53.0 | 10.0-60 | 20-50 | | | | 41.2 | | N/A | N/A | N/A | N/A | N/A |
| Ash %db | 4.0 - 30.0 | 4.0-20 | 0.4-2.0 | 0.52 | 0.3 | 0.6 | 1.1 | 0.8 | 20.26 | 18.67 | 8.97 | 2.44 | 2.7 |
| Volatile Matter %daf | 75 - 87 | 80 - 85 | 84-91 | 82.55 | 86.5 | 85 | 81 | 85 | 63.52 | 65.47 | 76.69 | 85.61 | 84.81 |
| Fixed carbon %daf | N/A | 15-20 | 9.0 - 16 | 16.93 | 13.2 | 14.4 | 17.9 | 14.2 | 16.22 | 15.86 | 14.34 | 11.95 | 12.49 |
| Gross calorific value,MJ/kg | 18 - 21 | 18-21 | 19.22 | N/A | N/A | 19.52 | 20.55 | 19.95 | 15.84 | 15.09 | 18.06 | 18.99 | 19.02 |
| Physical Properties | | | | | | | | | | | | | |
| Bulk Density | 120 - 200 | 70-150 | 160-490 | | | | | | N/A | N/A | N/A | N/A | N/A |
| Ultimate Analysis (%daf) | | | | | | | | | | | | | |
| Carbon | 43 -52.05 | 43-51 | 50.7-51.3 | 48.33 | 49.3 | 49 | 48.6 | 50.3 | 38.83 | 38.24 | 46.68 | 48.64 | 50.18 |
| Hydrogen | 5.2 - 6.9 | 6.1-6.3 | 6.0-6.2 | 5.89 | 6.42 | 5.9 | 6.26 | 6.1 | 4.75 | 5.2 | 5.82 | 5.87 | 6.06 |
| Nitrogen | 0.2 - 0.4 | 0.45-1.15 | 0.1-0.15 | 0.15 | 0.2 | 0.3 | 0.2 | 0.1 | 0.52 | 0.87 | 0.77 | 0.16 | 0.6 |
| Sulphur | 0.02 - 0.11 | 0.1-0.3 | 0.08-0.2 | 0.01 | 0.04 | 0.1 | 0.01 | 0.2 | 0.05 | 0.18 | 0.19 | 0.04 | 0.02 |
| Oxygen | 40.8 - 52.0 | 39-45 | 42.2-43.0 | 45.62 | 42.5 | 44.1 | 43.8 | 42.5 | 35.47 | 36.26 | 37.38 | 42.82 | 40.43 |
| Chlorine | N/A | N/A | | 0 | 0 | N/A | 0.11 | N/A | 0.12 | 0.58 | 0.19 | 0.03 | 0.01 |
| Ash Analysis (%) | | | | | | | | | | | | | |
| $SiO_2$ | 45 - 75 | 55-73 | 10.0-25.0 | 5.90 | 14.67 | 39.20 | 17.30 | 8.12 | 91.42 | 74.67 | 65.18 | 46.61 | 5.9 |
| $Al_2O_3$ | 7. 0 - 22 | 3-12 | 2.5-6.5 | 0.84 | 4.89 | 9.09 | 5.71 | 4.04 | 0.78 | 1.04 | 4.51 | 17.69 | 0.84 |
| $Fe_2O_3$ | 4.0 - 25 | 0.8-4 | 4.0-12.0 | 1.40 | 7.20 | 6.57 | 5.78 | 5.10 | 0.14 | 0.85 | 20.3 | 14.14 | 1.4 |
| CaO | 1.5 - 7 | 2.5-9 | 19-40 | 49.92 | 30.30 | 17.60 | 52.50 | 46.10 | 3.21 | 3.01 | 5.6 | 4.47 | 49.92 |
| MgO | 1.5 - 8 | 1.5-6 | 20-38 | 18.40 | 11.01 | 8.74 | 6.78 | 21.80 | <0.01 | 1.75 | 3 | 3.33 | 18.4 |
| $Na_2O$ | 0.2 - 1.0 | 0.1-0.7 | 0.6-1.6 | 0.13 | 1.63 | 1.12 | 1.43 | 0.76 | 0.21 | 0.96 | 0.58 | 0.79 | 0.13 |
| $K_2O$ | 1.0 - 6.5 | 7-18 | 3.0-12.0 | 9.64 | 18.61 | 13.50 | 8.00 | 7.04 | 3.71 | 12.3 | 11.6 | 0.15 | 9.64 |
| $TiO_2$ | 0.1 - 2.0 | 0.5-1.0 | 0.15-0.35 | 0.3 | 0.41 | 0.90 | 0.66 | 0.16 | 0.02 | 0.09 | 0.24 | 2.62 | 0.3 |
| $Mn_3O_4$ | 0.1 - 1.0 | 0.1-0.5 | 0.4-1.7 | N/A | N/A | 0.34 | 1.50 | 0.64 | N/A | N/A | N/A | N/A | N/A |
| $SO_3$ | 0.2 - 2.0 | 1.0-4.0 | 2.0-9.0 | 3.95 | 4.62 | N/A | N/A | N/A | 0.72 | 1.24 | 0.44 | 2.08 | 2.04 |
| $P_2O_5$ | 1.0 -3 | 1.3-10 | 2.0-7.5 | 1.34 | 2.31 | 2.40 | 0.39 | 5.28 | 0.43 | 1.41 | 4.5 | 2.72 | 1.34 |
| BaO | N/A | 0.02-0.09 | 0.25-0.6 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| SrO | N/A | 0.02-0.04 | 0.25-0.6 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| ZnO | N/A | 0.02-0.04 | 0.03-0.12 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| $CO_2$ / other | N/A | N/A | N/A | 8.18 | 4.35 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 8.18 |

## BIOMASS UTILIZATION TECHNOLOGIES IN AUSTRALIA

The conversion of biomass resources into useful energy services and products can be undertaken using a wide range of technological pathways. In Australia, various technologies for biomass utilization are being used or are under commercial development because of the availability of considerable existing and potential biomass resources. In particular, Australia has substantial experience in landfill gas, sewage gas, and bagasse power plants. Biomass utilization projects vary in scale from simple combustion in domestic open fires to fully commercial combined heat and power stations typically comprising complex 100 MWe thermo-chemical reactors. The technical and commercial status of biomass utilization technologies together with other renewable energy technologies has been summarized in the *Renewable Energy Action Agenda* Discussion Paper (DITR 1999) and Renewable Energy Roadmap (DITR 2002). Based on bioenergy types produced from biomass, the technologies and their application are also summarized in Table 3. The recent developments on these technologies are discussed in more detail in the following sections.

**Table 3.** Status of Modern Biomass Utilization Technologies in Australia

| Bioenergy type | Technology | Biomass used | Status | Application examples |
|---|---|---|---|---|
| **Electricity** | Co-firing biomass and coal | All biomass | Mature and fully commercial | Macquarie Generation and Delta Electricity |
| | Direct combustion for cogeneration electricity and heat with conventional boiler | Bagasse (and cane trash); wood wastes; black liquor | Mature and fully commercial | In sugar mills and wood processing plants (Sunshine Electricity, 2002) |
| | Direct combustion in Fluidized bed boiler | Green wastes | Mature and commercial | EnviroStar (2003), Energy Equip (2003) |
| | Biomass Integrated Gasification Combined Cycle | Bagasse and cane trash | Research & development, demonstrating and pre-commercial | Sugar Research Institute (Hobson and Dixon 2002) |
| **Biogases** | | | | |
| Landfill gas | Landfill/gas recovery | MSW | Established and commercial | Energy Development Limited, EDL, (2003) |
| Sewage gas | Digestion | Sewage | Mature and fully commercial | Malabar Sewage Treatment Plant, NSW (DITR 2000) |
| Biogas | Digestion | Wet wastes and food wastes | Digestor –commercial demonstration | EarthPower Technologies Sydney Pty Ltd |
| Syngas | Gasification for producing syngas | Crop wastes (cotton trash and rice hull) | Transition between R&D and commercialization | Methanex Pty Ltd 5,000 t/y Syngas Factory in Darwin |
| **Biofuels** | | | | |
| Bio-ethanol | Hydrolysis/Fermentation / distillation | Sugar, Molasses, starch cellulose, wood | Established Commercial with subsidy. Ethanol exempt from fuel excise to encourage production | Various companies, see Burnard (2002) for a complete list. |
| Bio-oil | Pyrolysis, gasification | MSW, wood waste (mallee) | Established and demonstration for | Western Power (see RIRDC 2002a |

| | | | | |
|---|---|---|---|---|
| | | | commercialization | report), SWERF (see Wooton 2002 for more details) |
| Bio-methanol | Gasification | All biomass | Research & development | Various organizations and companies, see RIRDC (2002a) report for more details |

## Electricity and Heat

As summarized in Table 3, within the Australian context there are four potential technologies for utilizing biomass as fuel to generate electricity and heat (i.e., processing heat). Biomass can be used: (1) through direct combustion in conventional steam boiler to cogenerate processing steam and electricity, (2) through cofiring coal and biomass in a pulverized coal-fired boiler, (3) through directly combusting in a fluidized bed combustor (FBC), or (4) through gasification in a gasifier to produce low calorific value gas for running a gas turbine / generator for electricity generation. The biogases produced from biomass (see Table 3), can also be burnt or cofired with other fuels in boilers or gas engines further to generate electricity and heat, but which is described separately in the following biogas section.

*Direct Combustion*. Direct combustion is a commercialized technology, which is widely used to generate electricity and/or processing heat for processes of the industries related to the biomass resources, such sugar industry and wood products industry. In these cases, the biomass resources are generally available on-site and burned in conventional steam boilers. Bagasse has been extensively used as main fuel to generate electricity and heat in sugar mills of New South Wales and Queensland (Redding Energy Management 1999). Most sugarcane mills utilize bagasse to cogenerate steam and electricity for their own needs, but recently some plants have been expanded and upgraded to allow the exportation of large quantities of electricity to the grid. New relatively large cogeneration plants have been also established recently. Examples include: cogeneration projects of Condong Sugar Mill (30 MW) and Broadwater Sugar Mill (38 MW) in NSW (Sunshine Electricity 2002; Burnard 2002) and Rocky Point Sugar mill in QLD (Burbidge 2000) where sugarcane (both bagasse and cane trash) is utilized as the base fuel and other locally available biomass fuels (e.g., wastes from Camphor Laurel and saw mill residues) as supplement fuels.

The Australian bagasse cogeneration industry is already quite well developed. Benefits to the reduction of greenhouse gas and to generation of a second revenue stream for the sugar industry imply a significantly potential role of cogeneration in the electricity market (Dixon 2000). The 2% renewable measure is evolving as the primary driver for the development of cogeneration projects in the sugar industry.

Technologies are essentially the same for other biomass fuels, such as wood wastes including bark, chips, sawdust, mill sludge, fiber and scrap timber, which are used to generate processing steam and electricity in the timber product industry (Allsopp 2002). Australia has a great deal of experience in designing and building such plants.

*Co-firing*. Co-firing biomass and coal in an existing pulverized coal-fired power plant is a low cost option to reduce greenhouse gas emissions by partly substituting coal with $CO_2$ neutral biomass fuels. High combustion efficiency can be achieved with only minor or no modification to existing systems. It has been demonstrated that up to 15% of total energy output can be substituted from biomass fuel sources. In Australia, the *Renewable Energy (Electricity) Act 2000* (ORER 2000) is the major driver behind electricity generation companies to adopt the practice of coal/biomass cofiring. In August 1999, Macquarie Generation's Liddell Power Station became the first coal-fired power station in Australia licensed to carry out cofiring for electricity production (Macquarie Generation 1999) with the aim of generating 2% of energy through the burning of renewable biomass. The cofiring program was then expended to the company's Bayswater power station. The biomass supplied for Macquarie Generation's co-firing program includes sawdust and shavings from saw mills, forest thinnings, and laminate and MDF plant wastes. The 2% of energy output translates to 5% of biomass fuel by mass because of the lower calorific value of the biomass fuel compared to Australian black coal. At full capacity, the two stations consume about 900,000 tonnes per annum of biomass fuel. Other electricity generators are also burning biomass fuels. For example, Delta Electricity is cofiring biomass in its plants, aimed at reducing greenhouse emissions by up to 20,000 tonnes each year (Delta Electricity 1999). Other examples include CS Energy in the state of Queensland and Western Power in the state of Western Australia. The sources of biomass fuel for these companies include saw mill residues (a by-product of sustainable plantation operations), construction and demolition wood wastes, as well as urban green waste (UGW). Despite some technical difficulties, the cofiring technology appears to be fully adopted as a full-scale commercial operation by the electricity generation sector.

*Fluidized Bed Combustion*. A fluidized bed combustor (FBC) utilizes biomass fuels also through direct combustion. FBC involves the combustion of waste in a fluidized bed of sandy material with high thermal inertia but low combustion temperature. This form of combustion is highly tolerant to low heating value wastes with variable quality, and produces much lower pollutant emissions than other conventional forms of combustion (e.g., the low combustion temperature results in low thermal NOx production). Several green waste to energy projects are under way, using FBC technology of Energy Equipment Australia (EEA, 2003), including those in Nowra (18 MWt) of NSW, Bell Bay (65 MWt) of Tasmania, Kemerton (65 MWt) of Western Australia, Gold Costal (65 MWt) of Queensland and Morwell (65 MWt) of Victoria. For example, the energy facility being constructed in Stapylton, Gold Costal, Queensland (Stapylton Green Power Plant) by EnviroStar Energy Ltd., uses bubbling FBC technology which is fueled by UGW collected by urban councils within the greater Brisbane region. The first stage is 5 MWe and the second stage is 20 MWe. Approximately 240,000 tonnes of UGW will be converted into 145 GWh of green power annually using FBC and steam turbine (EnviroStar Energy, 2002). A similar plant (20 MWe) will be also built in Morwell, La Trobe Valley, Victoria, which will be fueled by household green wastes as well as sawmilling residues from plantation timbers (EnviroStar Energy, 2002).

*Biomass Integrated Gasification Combined Cycle (BIGCC)*. BIGCC is an advanced technology for utilizing biomass to generate electricity, in which biomass is

gasified with air to produce syngas, which is then combusted to drive a gas turbine / generator for production of electricity. The residual heat of exhaust gas from the gas turbine is also used to produce steam and run a steam turbine / generator to generate more electricity. The combined cycle insures a very high energy efficiency. A 5 Mwe BIGCC system fueled by bagasse and cane trash is under development for a commercial scale demonstration plant in Queensland (Hobson and Dixon 2002).

## Biogas

Biogas generation technologies include biochemical technologies such as anaerobic digestion, which breaks down organic material into methane and carbon dioxide, and thermal/chemical technologies such as pyrolysis and gasification, which converts biomass wastes into syngas and/or other products.

*Landfill Gas Technology*. Landfill gas technology is commercialized technology in Australia for generation of low calorific gas from landfilled MSW. Landfill gas is generated by the anaerobic decomposition of organic refuse deposited in landfills. It is primarily a mixture of carbon dioxide and methane in roughly equal proportions. Small quantities of water vapor and minor organic compounds are also present in the landfill gas. The substantial methane content of landfill gas enables it to be utilized as a fuel for power generation. Typically, generation of landfill gas begins within weeks of the organic MSW being deposited at a landfill site and continues at a gradually decreasing rate for over twenty years after filling ceases.

The commercial utilization of landfill gas as a fuel requires the gas to be extracted from landfill sites with a reasonably consistent flow and quality. In Australia, Energy Developments Ltd (EDL 2003) has developed a process that meets this requirement by drilling a pattern of vertical gas production wells across the landfill area. These wells are linked by an underground piping network to a central gas collection facility. The entire system is maintained under a vacuum, inducing the flow of landfill gas into the collection facility, where gas processing is undertaken to reduce moisture levels and filter out fine particles. The processed landfill gas is then used as a fuel in either gas engine or gas turbine generator sets. The power generation facilities are interconnected with a utility grid to enable the sale of electricity produced. The installed capacity for landfill gas in Australia was reported about 80 MW at the end of 1997 (39 MW in Victoria, l3 MW in WA, l2 MW in SA, 13 MW in NSW and l MW in Queensland) and increased to 100 MW by 2000. The installed capacity of landfill gas is expected to steadily grow to about 250 MW by 2010 (Redding Energy Management 1999).

*Anaerobic digestion*. Anaerobic digestion technology is being demonstrated in Australia mainly to convert sewage and wet wastes from agriculture and food processing into biogas. In Australia, the amount of sewage feedstock for biogas production using the anaerobic digestion technology is relatively small, although the electricity production from this source has potential for expansion from its current level of 20 GWh/year to an upper limit of about 200 GWh/year. The Malabar Sewage Treatment plant in NSW now generates 3 MW of electricity from digester gas (DITR 1999). The wet wastes are potentially a larger resource. For example, over 100 MW of power for use in NSW can be produced from biogas generated from wet waste sources. Projects, such as Orange City

Beef Biogas Plant under SEDA (Bartle 2002), have been developed to generate biogas from the wet wastes for firing boilers for cogeneration.

*Gasification*. Gasification technology is being demonstrated and commercialized in Australia mainly to gasify agricultural wastes into biogas. In NSW, two projects are under development, one for gasification of cotton trash and the other for gasification of rice hull to produce biogas (Bartle 2002). One project is developed using a technology (i.e., swept-drum pyrolyser and char gasifier) from Biomass Energy System Technologies (BEST) to convert cotton trash into syngas, which is aimed at 200,000 t/year cotton trash mostly in NSW. The first stage of the project is to replace LPG gas in ginning season (8,500 tones, 2,200 h/yr) and the second stage is to develop 2.5 MW cogeneration system together with cotton trash collection and storage (25,000 tonnes, 8,000 h/yr). In another project, the same gasification technology is applied for utilizing 300,000 t/year of NSW rice hull to generate syngas. Gasification technology is quite well established and current operations do not face any safety related challenges.

## Biofuels

In Australia, generating biofuels (Bio-oil, biodiesel, bioethanol, and biomethanol) from biomass, particularly from planted wood, is claimed to offer several advantages on a broad national scale. These include (ABA 1999; RIRDC 2002a):

- Environmental benefits (reduced greenhouse emissions, reduced vehicle exhaust emissions, improved urban-air quality, salinity abatement, improved soil stability and fertility and weed control)
- Economic benefits (related to the opportunities to provide viable economic alternatives to existing agricultural/forestry industries)
- Regional benefits (The majority of biomass resources are located in rural and regional Australia and their development could be expected to provide a major economic, employment and social stimulus to these areas.)

Therefore, biofuels have been attracting attention from the government and industries. Several R&D and demonstration projects have been conducted recently in Australia.

*Ethanol ($CH_3CH_2OH$)*. Bio-ethanol, a renewable fuel, is known as an "oxygenate" because it contains 37% oxygen by weight (Klass 1998). Oxygen enhances the combustion of petrol in engines, and therefore contributes to reductions in exhaust emissions such as carbon monoxide. Ethanol is currently the most widely used biofuel in the transport sector in Australia (38% ethanol production in 2000 used as transport fuel, the other 30% and 28% used as chemical in industry and for export, respectively) (Naughten 2001). Commercial sales of ethanol to the transport sector commenced in 1992, and sales of the 10% ethanol/petrol blend fuel (E10) were expected to comprise 7% of annual petrol usage by the NSW/ACT vehicle transport fleet by the end of 2000. The Commonwealth Government is providing significant specific support for the development of ethanol as a transport fuel. In addition to providing an excise exemption for ethanol of 44 cents/lit, the Commonwealth and New South Wales Governments are currently supporting the development of new technologies to convert lignocellulose (woody waste products) into ethanol.

Ethanol is alcohol produced commonly from fermentation of sugar, using yeasts or other microorganisms to convert sugars into ethanol with $CO_2$ as a by-product. There

is little difference between the processes in fermentations for potable alcohol (beers, wines) and those for industrial or fuel grade ethanol. The feed for all ethanol fermentations is sugar, such as the sugars present naturally in sugarcane. In Queensland, ethanol is already made from molasses, a low-value, sugar-containing by-product of crystal sugar manufacturing.

In Australia, fuel ethanol research and trials on the production from biomass have been undertaken intermittently over the past twenty-five years. Previously, ethanol was produced mainly from molasses, a by-product of sugar industry (Naughten 2001; ABA 2003). Recently, fuel ethanol is produced as part of the value-added processing of wheat starch by Manildra Energy Australia Pty Ltd. at its plant at Nowra, NSW (Burnard 2003), which decreased the share of sugarcane based ethanol to 56% (of total 113 million litres ethanol) in 2000 and 63% (of total 173 million litres) in 2001 (Naughten 2001). Using wheat starch as feedstock in Western NSW, the Manildra plant will have the capacity to produce up to 700 million litres of blended ethanol fuel annually. According to industry estimates, use of this fuel could reduce transport emissions by up to 7 per cent in NSW and the Australian Capital Territory (ACT). The plant has sourced wheat from farms in Western NSW, milled at the company's Manildra plant. It incorporates two technological innovations developed and proven in an existing pilot plant. These are Manildra's continuous fermentation technology and a molecular sieve dehydration system; both will deliver significant energy and cost efficiencies. The project represents a major advance in the commercialization of ethanol fuel in Australia in terms of meeting current and future demands for renewable transport fuel.

Sugarcane and wheat are food crops. Their use for ethanol production is limited by their costs as human and animal feeds, and it is typically the by-products of the manufacture of food grade products that are used for ethanol production. Therefore, the cost of ethanol from these materials is higher, mostly because of the cost of the feedstock. Biomass, in the form of wood and agricultural residues, is viewed as a low cost alternative feed to sugar and starch. However, only part of the biomass (cellulose and hemicellulose) can be converted into sugar using the fermentation process. Nevertheless, because it is potentially available in far greater quantities than sugar and starch feeds, it receives significant attention as a feed material for ethanol production. Technical and economic research related to ethanol production from wood plantation materials (Foran and Marden 1999; RIRDC 2002a) has been carried out in Australia.

*Biodiesel*. Biodiesel is the name for a variety of ester-based oxygenated fuels made from vegetable oils or animal fats (Klass 1998). Vegetable and animal oils and fats, like soybean, rapeseed/canola, recovered vegetable and animal fats, can be used to produce biodiesel. Biodiesel is the only alternative fuel that can be used directly in any existing, unmodified diesel engine. Because it has similar properties to petroleum diesel fuel, biodiesel can be blended in any ratio with petroleum diesel fuel. Biodiesel has many advantages as a transport fuel. For example, biodiesel can be produced from domestically grown oilseed plants such as canola. Producing biodiesel from domestic crops reduces Australia's dependence on foreign petroleum, increases agricultural revenue, and creates jobs.

The production of biodiesel is well known and carried out using a series of catalytic reactions. There are a small number of outlets providing biodiesel currently in

Australia. There are also quite a number of companies around Australia in the planning stages or nearing completion of their biodiesel production facilities (BAA 2003). A bio-diesel pilot plant, using acid esterification, glycerine recovery technologies to generate up to 10 ML/year was recently developed under the administration of SEDA (Burnard 2002).

*Bio-oil*. Bio-oil refers to the oils, including benzene and toluene derived either from biomass directly by pyrolysis or indirectly by separating/processing tar compounds formed during biomass gasification. In Australia, bio-oil technology is undergoing commercialization. For example, EDL has been demonstrating a technology called Solid Waste to Energy & Recycling Facility (SWERF®) (Wooton 2002), which integrates waste to energy and recycling processes and uses advanced thermal conversion to convert waste into useful energy forms including electricity and bio-oil. The waste material used is mainly MSW and also includes waste biomass and commercial and industrial waste. The SWERF® process steps are as follows (Wooton 2002):

- Sterilisation and pulping of the waste in a rotating pressurized autoclave.
- Materials separation to recover recyclable materials.
- Washing of the "pulp" to remove dirt, sand and glass fragments.
- Drying of the pulp in a pressurized flash drying device.
- Gasification of the pulp to produce Syngas, and/or Bio-oil.
- Generation of electricity from Syngas and/or sale of Bio-oil as refinery or petrochemical.

It is reported that in Bio-oil mode, SWERF® generates about 130 kg of bio-oil together with approximately 300 kWh of electricity for each tonne of municipal solid waste (MSW) processed.

Pilot-scale studies on an integrated oil mallee processing and electricity cogeneration plan is underway in Western Australia (RIRDC 2001), which is aimed at cogenerating mallee oil, activated carbon, and electricity.

*Methanol ($CH_3OH$)*. In addition to ethanol, the other well known oxygenate fuel is methanol (Klass 1998). It is also one of the most commonly used chemicals in the world today. Methanol has been used as the feedstock for the production of methyl tertiary butyl ether (MTBE), a widely used additive to boost octane levels in petrol (RIRDC 2002a). In 1997, 27% of the world's methanol production was used to make MTBE. However, recent problems with contamination of groundwater by MTBE in the USA have led to reductions in its use and expanded production of ethanol as an alternative oxygenate for petrol. Methanol can also be used in fuel cells. Commercial production of methanol is from synthesis gas (a mixture of $H_2$ and CO) by a catalytic process. Now almost all the methanol used world-wide come from the processing of natural gas.

Methanol can also be derived from biomass. Modern methods proposed for the production of methanol from biomass involve the conversion of the biomass to a suitable synthesis gas through gasification and pyrolysis, after which the processing steps are very similar to those developed for methanol from natural gas. There is adequate technology available for all stages except for gasification. The production of synthesis gas by gasifying biomass, such as wood, straw, bagasse, has yet to be demonstrated in a large-scale plant but it has been implemented in small demonstration plants overseas.

Technologies such as the Institute of Gas Technology pressurised direct oxygen fired gasification and the Battelle-Columbus indirect gasification have been used for biomass gasification (RIRDC 2002a). There is no commercial or demonstration unit of methanol from biomass, although there are many research projects concerning production of methanol from biomass (e.g. Hamelinck and Faaij 2002; Dong and Steinberg 1997; Borgwardt 1997).

Nevertheless, there are several studies reported in Australia (Stewart et al. 1979, 1982; Foran and Mardon 1999; RIRDC 2002a) detailing technical and economic analysis of generating methanol from biomass, particularly from wood. In addition, prospects for an Australian economy based on ethanol and methanol fuels have been considered (Foran and Mardon 1999). The major conclusions from the research are:

1. Australia has land for biomass feedstock production. There is potential to generate 14.4 Mt of methanol and 4.4 Mt of ethanol per annum according to the estimation of Stewart et al. (1979, 1982).

2. Research has focused on a number of linked problems in Australian farming lands, such as dryland salinity. This has led to wide-scale research and development of deep-rooted plant production systems, in particular, the oil mallee production system, such as in the wheat belt of Western Australia (RIRDC 2001). Biomass yields of 5 tonnes per hectare dry matter per year are feasible and, with spaced planting of 20% of total land area of wheat belt, 15 million dry tonnes would be available per annum. It is estimated that control of the dryland salinity problem may require a landscape cover of 80%, and this potentially could produce 75 million tonnes per annum, enough to make the Western Australian State self-sufficient in liquid fuels and allow for some export (RIRDC 2001).

3. Methanol is estimated to cost 62 cents per litre in a 390 ML plant built today in Australia using the world's best technology (RIRDC 2002a), where 1.34 Mt fresh biomass is required annually. The assumed cost of the delivered raw material is $30 per tonne of fresh woody biomass. The price depends on plant size (RIRDC 2002a; Foran and Mardon 1999) and raw material price (Foran and Mardon 1999). Using high price raw material (164 $/tones on dry weight) and assuming a relatively small plant (100,000 tonnes methanol per annum, i.e., 127 ML per annum, and 0.26 Mt dry biomass used), Foran and Mardon (1999) estimated a price of 81 cents per litre for methanol. Using the same price of raw material and considering 50% moisture content of fresh biomass, the price of methanol is estimated to be 64 cents per litre, consistent with the results of the RIRDC study (2002a). A decrease in the price may be possible due to some improvements to technology over the next fifteen years. Nevertheless, the cost gap between bio-methanol and synthetic methanol is anticipated to exist until 2015. The methanol from biomass is estimated to cost 50 cent/litre versus the methanol from natural gas at 24 cent/litre (RIRDC 2002a). The benefits to the environment (i.e., benefits related to reductions in production of greenhouse gases, valued at 9.5 cent/litre, and on-site benefits of salinity reduction, valued at 1.4 cent/litre) will further narrow the gap and achieve the best price of about 39 cent/litre) (RIRDC 2002a).

4. Unlike the production of ethanol, the production of methanol from biomass is relatively insensitive to biomass quality or species (Foran and Mardon 1999) since the whole biomass substance is converted into carbon oxides, hydrogen, water, and so on. Table 4 presents the amount of biomass (dry weight) needed for generating one tonne of methanol. The amount varies depending on biomass moisture content.

**Table 4.** Quantities of Raw Biomass Material Used in Methanol Production (Foran and Mardon 1999)

| Raw material | % dry matter | Dry weight of biomass (tones) for producing per tones of methanol | |
|---|---|---|---|
| | | Without pre-drying | With pre-drying* |
| Wood | 80 | 2.2 | |
| Wood | 50 | 3.0 | 2.2+0.4 |
| Bagasse | 50 | 3.1 | 2.3+0.4 |
| Sugarcane trash | 50 | 3.3 | 2.4+0.4 |
| Cereal straw | 80 | 2.4 | |

* Pre-drying materials of 50% dry matter requires 0.4 units of raw materials as fuel.

5. Compared to ethanol production, methanol is cheaper (Foran and Mardon 1999; RIRDC 2002a) since all biomass material is converted into methanol. Moreover, the capital costs of methanol plants are also lower, primarily because no acids are used in the processes so there is no need to construct the plant from more expensive acid-resistant materials.

Currently, there is no project in Australia considering methanol from biomass, either from an R&D standpoint or as a production facility. This is despite the fact that all related technologies for the production are available. The reasons are the high price and lack of market interest. Ethanol can be blended into petrol as green fuel, whereas methanol cannot. If new applications and markets can be exploited, then there is potential for significant growth in the use of methanol, since it has a comparable price to ethanol but higher conversion efficiency. Possibilities include the use of methanol for generating clean fuels from coal. Methanol also might be considered for use in fuel cells.

## BIOMASS PRICES

The prices of bioenergy from various technologies compared with the price of energy generated from fossil fuels are summarized in Table 5. As noted in the table, bioenergy is more expensive than conventional energy. However, because great environmental contributions can be made by biomass utilization (e.g., $CO_2$ and dryland salinity reductions), a considerable decrease in bioenergy price can be achieved, should appropriate tax incentives become set in place (RIRDC 2002a). The social benefit, e.g., the contribution to the regional economy and employment, is also important but difficult to estimate. After considering the benefits of greenhouse abatement and on-farm salinity reduction, biomass electricity is cost competitive with conventional electricity (RIRDC

2002a). The government promotions and community awareness also has lead bio-ethanol to be considered an acceptable transportation fuel in the Australian market.

**Table 5.** Cost of Bioenery Technologies Compared to Other Renewable Energy Technologies and Fossil Fuels – Current and Expected Trend (Redding Energy Management 1999)

| Energy source | Technology | Cost, $/MWh[a] | Expected trend | Comment |
|---|---|---|---|---|
| Coal | Coal fired steam | 30-40 | Stable | |
| Gas | | 35-60 | Small decrease | |
| Wind | Wind turbine/generator<br>Wind RAPS | 75-90<br>150-400 | Decrease 15-30 by 2010 | Site (wind resource) variation is reason for the range in costs |
| Hydro | Hydro turbine/generator<br>Micro hydro RAPS | 40-100<br>70-250 | Increase (attractive site are used)<br>Remain constant | Cost is very site specific |
| Fuel wood | Boiler<br>Pyrolysis furnace | 70-110<br>0.45-0.85 per litre | | Cost assumes biomass is provided at a cost of 20-50$/tonnes |
| Bagasse | Boiler (cogeneration)<br>Gasification | 50-60<br>30-100[b] | Decrease expected with efficient increase | Embedded generator network cost savings |
| Various wastes | Boiler (cogeneration)<br>Gasifier/gas engine | 80-200[b] | Decrease 25% by 2010 | |
| Sugar, starch cellulose | Hydrolysis/fermentation /distillation | 0.28-0.69 / lit ethanol | Competitive with oil by 2010 | Worldwide cost has decreased 50% over past 10 years |
| Wet waste | Biogas digestion/<br>Gas engine | 30-200 | Increase beyond 2005 | Economics depend on negative cost of fuel and value of by-products |
| Landfill gas<br>Sewage gas | Gas engine | 50-99 | No change to 2010 | Most of resource recoverable at 65$/MWh |

a. Unit is Australian $/MWh except where other unit is specific
b. Estimated cost once technically viable

## SUITABILITY OF BIOMASS FOR LOCAL USE AND EXPORT

In Australia biomass, comprising mainly bagasse and wood waste, is the dominant feedstock in the renewable energy consumption (Bush et al. 1997). Unlike other renewable energy sources (e.g., wind, solar, geothermal, etc.) biomass produces $CO_2$ emissions when it is processed (e.g., combustion, gasification, etc.). Nevertheless, biomass is considered to be a $CO_2$ neutral energy resource, because the carbon dioxide produced from biomass is absorbed by growing plants in a relatively short cycle. In Australia there is substantial land area for biomass production, and there are substantial biomass resources. Biomass utilization not only provides renewable energy and reduces

greenhouse gas emission for sustainable energy development, but also benefits the sustainable agricultural and forestry industries. Therefore, much attention has been directed to it from governments, industries and communities.

On November 20, 1997, the Prime Minister announced a package of measures designed to address climate change, including measures aimed at reducing the impact of the energy sector on the environment in a document called "Safeguarding Our Future: Australia's Response to Climate Change." From then on, extensive initiatives were put forth for reducing the greenhouse gas emissions and utilizing renewable energy on both the supply and demand sides.

Renewable energy use is expected to expand significantly in Australia in the next decade. This is due to market forces and government initiatives. Perhaps the most important initiative is the Mandatory Renewable Energy Target (MRET) through the Renewable Energy (Electricity) Act 2000 and its Regulation (ORER 2000), which involves a mandatory target for electricity retailers and large electricity purchasers to source an additional 2% of their electricity from renewable energy sources by 2010. This is equivalent to an extra 9,500 GWh per annum of electricity generated from renewable energy (ORER 2003). This regulation enables the biomass electricity to be cost-competitive in the electricity market.

Another important driver is the Renewable Energy Action Agenda (DITR 2002) initiated by the federal government in 1999. This entails renewable energy industry participants working with government, primarily through the Department of Industry Tourism and Resources and the Australian Greenhouse Office, to move the renewable energy industry to sustainable and international competitiveness with a target of annual sales of $4 billion by 2010. The industry participants have identified five overarching strategies to achieve this goal. These strategies relate to:

- Market Development
- Building Community Commitment
- Building Industry Capability
- Setting the Policy Framework
- Encouraging a Culture of Innovation.

The Renewable Energy Action Agenda (DITR 2000) launched by the then Minister for Industry, Science and Resources in June, 2000, identifies nine priority initiatives to implement the strategies. This agenda and the related "roadmap" (DITR 2002) include several initiatives aimed at promoting utilization of renewable energy, such as Initiative 2 "Promote the Development of the Renewable Transport Fuel Industry," and at promoting development of the domestic market and better exploitation of the export market for Australian renewable energy.

Additionally, state governments have also established a wide range of programs that can stimulate the development and uptake of sustainable energy technologies. These programs have been classified in the "Commonwealth and State Government Support to Sustainable Energy in Australia: an Overview"(AGO 2002) as:

- Programs that specifically target Renewable Energy.
- Programs that target overall greenhouse emissions abatement including through waste management, and energy efficiency.

- Programs that support innovation in firms including in the field of Sustainable Energy.

Due to the significant role that biomass plays in the Australian renewable energy market, such programs are motivating biomass energy utilization and related technology research and development in Australia.

Another key driver of growth of the renewable energy industry will be the level of community awareness and commitment to the environment and the community's acceptance of renewable energy industry as a solution to environmental concerns regarding energy use (DITR 2000).

For consumers, Green Power schemes represent the first convenient means by which they can exercise choice over the source of their electricity and support the development of renewable energy. Green power schemes offer electricity customers the opportunity to support grid-connected renewable energy systems. However, the household connection to the Green Power schemes is very low (just over 3% of Australian households, CSIRO 2000), compared with those in other parts of the world particularly in the Scandinavia (e.g. the uptake in Sweden is about 13%, Bird et al. 2002). This relatively low uptake is likely to be significantly affected by the low level of consumer awareness. Almost 80% of households not connected to Green Power reported that they were not aware of the scheme.

In the Renewable Energy Action Agenda (DITR 2002), an initiative, Initiative 4 (Increase community commitment to renewable energy) focus primarily on the provision of independent, accurate information and other awareness raising activity to increase the community's commitment to renewable energy and Green Power. The government and industry will be jointly responsible for the implementation of the initiative through various activities. Additionally, technology improvement and contribution to environment protection and sustainable development will lead to the decrease of renewable energy price, thus acceptable to the community.

## SUMMARY

- Bioenergy technology is extensively developed and commercially used in Australia. Australia has substantial experiences in cogeneration of electricity and heat from biomass material, particularly from bagasse, and also in landfill gas and sewage gas generation.
- For generation of biofuels from biomass, technology is under demonstration and commercialization for large-scale plants. Moreover, much attention is being put on bioethanol, driven by substituting petrol as renewable transportation fuels.
- In the case of bio-ethanol, there has been little technology development effort, due to a lack of market interest. However, technical and economic analysis indicated that methanol is at least economically equivalent to ethanol production and even higher conversion efficient because there is no limit on biomass raw material. If new markets can be exploited using bio-methanol, such as using methanol for clean coal and for fuel cells, then the prospect of bio-ethanol is bright. This is because methanol technology not only generates green energy, but also can promote biomass plantation.

In this way bio-methanol has potential to significantly benefit sustainable energy, sustainable agriculture, the forestry industry, and rural economies.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance provided to them by Prof. Raj Gupta (University of Alberta, Canada), Mr. Steve Richarson (University of Newcastle), Dr. Emre Cetin (University of Sydney) and Dr. Vladimir Strezov (Macquarie University).

## REFERENCES CITED

Allsopp, B. A. (2001). "Timber by-products as biomass fuels: materials handling issues," http://www.ghd.com.au/papers/

Australian Greenhouse Office (AGO) (2002). "Commonwealth and state government support to sustainable energy in Australia: An overview," http://www.greenhouse.gov.au/renewable/

Australian Greenhouse Office (AGO) (2003). "Renewable energy website," http://www.greenhouse.gov.au/renewable/

Australian Biofuels Association (ABA) (1999). "Multi-benefit of a national biofuel industry," Policy paper from the Australian Biofuel Association, http://www.australianbiofuelsassociation.org.au/

Bartle, J. (2002). "Achieving sustainability through bioenergy," Bioenergy Australia 2002 Conference, Sydney, NSW.

Bird, L., Wustenhagen, R., and Aabakken, J. (2002). "Green Power Marketing Abroad: Recent Experience and Trends," *National Renewable Energy Laboratory Technical Report, NREL/TP-620-32155*, USA.

Borgwardt, R. H. (1997). "Biomass and natural gas as co-feedstocks for production of fuel for fuel-cell vehicles," *Biomass and Bioenergy* 12, 333-345.

Burbidge, D. (2000). "Renewable energy boosts rural Queensland," *Proceedings of Solar 2000 from Fossils to Protons*, CD-ROM, (D. Mills, ed.), Australian and New Zealand Solar Society Conference, Brisbane, Nov 29 – Dec 1.

Burnard A. (2002). "Sustainable Energy Development Authority (SEDA), New bioenergy developments in New South Wales," *Australian Bioenergy Conference 2002*, Manly, NSW, 2-3 December.

Bush, S., Harris, J., and Ho Trieu, L. (1997). "Energy 1997 projections- Australian energy consumption and production," *ABARE Research Report* 97.2, Canberra.

CSIRO Forestry and Forest Products (2000). "The Wagga Wagga effluent plantation project," www.ffp.csiro.au/pff/effluent_guideline/.

Delta Electricity (1999). http://www.del.com.au/

Department of Industry, Tourism and Resources (DITR) (2000). "Renewable energy action agenda," June, http://www.industry.gov.au/

Department of Industry, Tourism and Resources (DITR) (1999). "Renewable energy action agenda discussion paper," December, http://www.industry.gov.au/

Department of Industry, Tourism and Resources (DITR) (2002). "Renewable energy roadmap," October, http://www.industry.gov.au/

DET (Division of Energy Technology) (2003). CSIRO, Australian Biomass Database, at http://www.det.csiro.au/.

Davidson, R. M. (1999). "Experience of Co-Firing Waste with Coal," International Energy Agency (IEA) Coal Research report, 1999.

Dickson, A., Thorpe, S., Harman, J., Donaldson, K., and Tedesco, L. (2001). (ABARE), "Australian energy outlook to 2019-20," Australian Institute of Energy, National Conference *Energy 2001 – Exploring Australia's Energy Future*, Sydney, 22-33 November.

Dong, Y., and Steinberg, M. (1997). "HYNOL – An economical process for methanol production from biomass and natural gas with reduced $CO_2$ emission," *International Journal of Hydrogen Energy*, 22, 971-977.

Driscoll, D. A., Milkovits, G., and Freudenberger, D. (2000). *Impact and use of firewood in Australia*. CSIRO Sustainable Ecosystems report to Environment Australia.

Energy Development Ltd. (2003). http://www.edl.com.au/

EnviroStar Energy Ltd. (2002). http://www.envirostar.com.au/projects/.

Energy Equipment Australia (2003). http://www.energyequipment.com.au/

Energy Research and Development Corporation (ERDC). (1994). "Biomass in the energy cycle study," ERDC 94/234, Canberra, Australia.

Foran, B., and Mardon, C. (1999). "Beyond 2025: Transition to the bio-alcohol economy using ethanol and methanol," *Report to National Dryland Salinity Program of the Land and Water Resources Research and Development Corporation*, December.

Gupta, R. P., Beacher, C., Bhargava, A., and Wall, T. F. (2002). "The fate of inorganic matter in biomass during combustion," *Pittsburgh Coal Conference*.

Hamelinck, C. N. and Faaij, A. P. C. (2002). "Future prospects for production of methanol and hydrogen from biomass," *Journal of Power Source* 111, 1-22.

Hobson, P. A., and Dixon, T. F. (2002). "Gasification technology development for the sugar industry in Queensland," *Bioenergy Australia 2002 Conference*, Sydney, NSW, 2 –3 December.

Jenkins, B. M., Baxter, L. L., Miles, T. R., Jr., and Miles, T. R. (1998). "Combustion properties of biomass," *Fuel Processing Technology* 54, 17-46.

Klass, D.L. (1998). "*Biomass for Renewable Energy, Fuels, and Chemicals*," Academic Press, San Diego, USA, ISBN: 0124109500.

Macquarie Generation (1999). http://www.macgen.com.au/

Glover, M. (2002). "The application of urban waste streams for sustainable energy recovery," *Bioenergy Australian 2002 Conference*, Sydney, NSW, 2 –3 December.

Naughten, D. (2001). "Viability of sugar cane based fuel ethanol," *ABARE Report to AFFA*, Canberra, Australia, October.

Office of the Renewable Energy Regulator (ORER) (2000). *Renewable Energy (Electricity) Act*, No.170, 2000, at http://www.orer.gov.au/

Redding Energy Management (1999). "2% renewables target in power supplies – potential for Australian capacity to expend to meeting the target," Report to Australian Greenhouse Office, January, www.greenhouse.gov.au

Renewable Energy (Electricity) Act 2000 (2000). www.orer.gov.au

RIRDC (Rural Industries Research & Development Corporation) (2002a). *Wood for Alcohol Fuels, Status of Technology and Cost/Benefit Analysis of Farm Forestry for Bioenergy*," RIRDC Publication No.02/141, Barton ACT: Rural Industries Research and Development Corporation, November 2002.

RIRDC (Rural Industries Research & Development Corporation) (2002b). *Biomass Atlas of Australia*, RIRDC Publication No.02/137, November 2002.

RIRDC (Rural Industries Research & Development Corporation) (2002c). *Agroforestry and Farm Forestry*, A Report for Joint Venture Agroforestry Program, RIRDC Publication No.02/63, June 2002.

RIRDC (Rural Industries Research & Development Corporation) (2001). *Integrated Tree Processing of Malle Eucalypts*, A Report for Joint Venture Agroforestry Program by Enecon Pty Ltd, RIRDC Publication No.01/160, November 2001.

Sami, M., Anamalai, K., and Wooldridge, M. (2000), "Cofiring of Coal and Biomass Fuel Blends," *Progress in Energy and Combustion Science* 27, 171-214.

Stewart, G. A., Gartside, G., Gifford, R. M., Nix H. A., Rawlins, W. H. M., and Siemon, J. R. (1979). *The potential for liquid fuels from agriculture and forestry in Australia*, CSIRO.

Stewart, G. A., Hawker, J. S., Nix, H. A., Rawlins, W. H. M., and Williams, L. R. (1982). *The potential for production of 'hydrocarbon' fuels from crops in Australia*, CSIRO.

Sunshine Electricity (2002). *Broadwater Biomass Cogeneration Proposal, Environmental Impact Statement*, February 2002.

Werther, J., Saenger, M., Hartge, E. U., Ogada, T., and Siagi, Z. (2000). "Combustion of Agricultural Residues," *Progress in Energy and Combustion Science* 26, 1-27.

Williams, A., Pourkashanian, M., and Jones, J. M. (2001). "Combustion of Pulverised Coal and Biomass," *Progress in Energy and Combustion Science* 27, 587-610.

Wootton, P. (2002). "Development of SWERF® technology," Bioenergy Australia 2002 Conference, Sydney, NSW, 2-3 December.

Yainshet, A., Nelson, R., and Love, G. (2002). "Rising domestic plantation wood supplies – Implications for Australian production and use of forest products," ABARE Current Issues, 02.7., August 2002, at: http://www.abareconomics.com/

Article submitted: June 2, 2006; Revision accepted: July 23, 2006; Published August 1, 2006

# SENSING THE ELECTROKINETIC POTENTIAL OF CELLULOSIC FIBER SURFACES

Martin A. Hubbe[a]

The charged nature of a cellulosic fiber surface is expected to play major roles in such phenomena as fiber dispersion, flocculation, adhesion, and adsorption of polyelectrolytes. This review focuses on the evaluation of such charges by means of electrokinetic measurements, with emphasis on the fiber-pad streaming potential technique. Results of recent experiments suggest that a continuous network or networks of pores below the outer surface of a kraft fiber can significantly contribute to observed streaming potential data. At present it is not clear whether the main subsurface contributions to the observed electrokinetic effects come from fibrillar layers on the fiber surfaces or from systems of nanopores within the cell walls of fibers. Based on the literature it is possible to suggest two conceptual models to account for the fact that the streaming potential of polymer-treated fibers can change in sign, dependent on the concentration of salt. Additional research is needed to clarify various theoretical and practical points. There may be opportunities to make more effective use of streaming potential tests in the future by carrying out such tests at reduced salt levels.

*Keywords: Electrokinetics, Zeta potential, Streaming potential, Microelectrophoresis, Cellulose, Fibers, Papermaking, Cationic demand, Polyelectrolytes, Adsorption*

*Contact information: Department of Forest Biomaterials Science and Engineering, Campus Box 8005, Raleigh, NC 27695-8005 USA; hubbe@ncsu.edu*

## THE CHARGED NATURE OF CELLULOSIC FIBERS

### Why Study the Electrokinetics of Fibers?

Electrical charges on surfaces can play a dominant role in the interactions of lignocellulosic materials (Davison and Cates 1975; Lindström 1996). This review focuses on sensing and quantifying effects of such charges by means of electrokinetic tests. The word "electrokinetics" implies that an electrical current or potential arises due to relative movement between two phases, as in the case of cellulosic fibers or fines suspended in water. From a practical standpoint, papermakers have used a variety of electrokinetic procedures to control and optimize the levels of additives (Hubbe 2000). Electrokinetic data can help to predict the dosages of highly charged materials that are needed to achieve different balances of stability *vs*. coagulation of suspensions (Verwey and Overbeek 1948; Hunter 1987; Lindström 1996), adsorption of strength-promoting additives (Strazdins 1980; Brouwer 1991; Spence et al. 1997; Thiele and Kopp 1997), retention of fine materials (Lindström et al. 1974; Tanaka 1984; Kumar 1991; Beck 1998), and maximum rates of water release during paper's formation (McKague et al. 1974; Davison and Cates 1975; Horn and Melzer 1975). In addition, the creping

performance of tissue (Stitt 1998) and the performance of hydrophobic sizing agents (Poppel 1992) tend to be highly correlated with electrokinetic potential data.

In addition to the practical benefits, electrokinetic data have the potential to shed new light on the fundamental nature of cellulosic surfaces. After an overview of the most widely used electrokinetic test methods for samples of papermaking interest, this review will consider what the test data may be trying to tell us about the topography and nanoporosity of fibers.

Likely benefits of future work related to cellulose fiber charge may involve new composite technology, scaffolds for biological cell growth, membranes, and high-tech applications of paper. It makes sense that as our understanding of the ionic and morphological nature of fibers becomes clearer, we will be in a better position to pursue new technological approaches. At the same time it is important to bear in mind certain limitations of electrokinetic methods, making it necessary to combine a variety of analytical approaches to interpret what the data are trying to tell us about fiber surfaces.

## Factors Affecting Electrokinetic Potential of Cellulosic Fibers

Chemical composition is perhaps the best place to begin in order to understand the charged nature of lignocellulosic surfaces exposed to aqueous solution. Previous studies, based on potentiometric, conductometric, and polyelectrolyte titrations, have done much to quantify the charge contributions of carboxylic acid groups and other functional groups that are accessible to different kinds of probe molecules (Lloyd and Horn 1993; Räsänen et al. 2001; Lindgren et al. 2002; Fardim and Holmbom 2005). For example, the kraft pulping and bleaching of wood fibers tends to decrease the relative amounts of extractives and lignin-related chemicals, thereby decreasing the negative character of the remaining solid material (Goulet and Stratton 1990; Lloyd and Horne 1993). Despite the fact that bleaching agents such as oxygen, ozone, and chlorine dioxide tend to convert various lignin moieties to carboxylic acids, much of this material tends to be solubilized and washed free of the pulp during subsequent alkaline extraction and washing operations (Goulet and Stratton 1990; Laine and Stenius 1997; Laine 1997).

Further changes in the observable charged nature of pulp fibers occur during mechanical processing. Papermakers often use the words "opening up" to describe what happens to fibers during refining, a process in which fiber suspensions are passed between grooved plates or conical surfaces, one of which is rotating. The repeated compression and shearing effects of the "bars" on the refiner plates cause cellulosic fibers to become internally delaminated, and the surfaces become fibrillated (Baker 1995). Though the area that is accessible to high-mass probes, such as cationic polyelectrolytes, tends to be increased by refining (Strazdins 1972), the zeta potential sometimes becomes less negative (Jacquelin and Bourlas 1964). The latter effect is due to the lesser proportions of lignin and extractives in the inner parts of the fiber cell wall (*e.g.* S2 sub-layer), as compared to the outer (P and S1) layers. At the same time, the increased effective surface area of the fibers means that a greater amount of cationic polymer needs to be added in order to achieve a neutral electrokinetic potential (Strazdins 1972; Davison and Cates 1975).

The drying of fibers can have effects that are almost the opposite of refining, in many respects. A high proportion of pores in the cell walls tend to close when kraft

fibers are dried, and many of these pores remain closed if the fibers are subsequently rewetted (Stone and Scallan 1966). The once-dried fibers have a reduced ability to adsorb cationic polymers, compared to never-dried fibers of the same origin (Gruber et al. 1996).

The effects of solution pH on fiber charge and electrokinetic potential are complex. Not only does pH affect the dissociation of various different types of carboxylic acid (and other) groups within cellulosic fibers (Lloyd and Horne 1993; Herrington and Petzold 1992; Bygrave and Englezos 1998), but also there can be substantial changes in swelling, *i.e.,* the relative amount of water held within the nanopores of the cell wall (Grignon and Scallan 1980; Scallan 1983; Lindström 1992). The latter effect would be expected to change the accessible surface area, depending on the size of the soluble ionic species used to evaluate the charged nature of the substrate.

Though it has been widely understood that increases in the concentrations of salts such as NaCl and $Na_2SO_4$ generally ought to decrease fiber swelling (Lindström 1992), as well as the overall importance of electrical charge interactions at fiber surfaces (Hunter 1987; van de Steeg et al. 1992), recent studies suggest that more attention needs to be paid to these issues. For instance, Fält and Wågberg (2003) observed that the amount of water held within fibers went through a maximum with respect to sodium sulfite concentration at approximately 0.05M. This observation will be considered in more detail later in this article, since it provides some evidence regarding the nature of cellulosic surfaces. Various studies have shown that the amount of cationic polymer required to titrate cellulosic fiber suspensions to neutral charge tends to increase moderately with increasing salt. Test methods used in such analyses are discussed in the next section.

Some electrolytes have been found to affect the charged nature of cellulosic fibers to a much greater degree than others. Such differences have been attributed to the relative abilities of ions to form complexes with ionic groups at the cellulose surface (Grignon and Scallan 1980; Scallan 1983; Fält and Wågberg 2003) or to adsorb specifically within the condensed part of the layer of counter-ions (Hiemenz and Rajagopalan 1997). Such interactions have been found to follow a lyotropic series (Eagland and Allen 1977). Räsänen and Stenius (1997) concluded, however, that whereas protons interact specifically with fiber surfaces, the other ions are governed by a non-specific Donnan equilibrium.

Multivalent cations tend to have a much more dominant effect of fiber surface charges, compared to low-valence ions (Lindström and Söremark 1975). Polyelectrolytes often display high-affinity adsorption behavior on cellulosic fibers (Balodis 1967; Gruber et al. 1996), leading to dramatic changes in electrokinetic behavior.

## ELECTROKINETIC TESTS FOR PAPERMAKING APPLICATIONS

Over the years a variety of electrokinetic methods have been developed, and many of them have been applied to the study of lignocellulosic materials. A common feature of such tests is some kind of movement of liquid relative to the solid surfaces (Sennett and Olivier 1965; Hunter 1981; Hidalgo-Alverez 1991; Müller 1996; Hiemenz

and Rajagopalan 1997). The relative motion causes a flow of counter-ions adjacent to the surface, resulting in an electrical potential or a current. The structure of electrical double layers, *i.e.* the distribution of ions versus distance relative to charged surfaces, is described in well known textbooks (Verwey and Overbeek 1948; Hunter 1987; Hiemenz and Rajagopalan 1997).

In the following sections the most important techniques for measuring electrokinetic properties of fiber suspensions are presented, this includes micro-electrophoresis, streaming current and streaming potential. The roles of external and internal surfaces are discussed in the context of recent streaming potential measurements.

## Micro-electrophoresis (ME)

Of the various electrokinetic tests that have been applied to suspensions of cellulosic materials, microelectrophoresis (ME) probably has the best-established theoretical foundation (O'Brien and White 1978; Hunter 1981; Hidalgo-Alverez 1991; Farley 1992; Strazdins 1995). Briefly stated, one applies an electric field to a suspension of very small particles and evaluates the resulting velocity of particle motion. The experiment is typically carried out in a capillary tube or rectangular channel, using a strategy that helps to maintain laminar flow conditions.

Though many papermakers have used micro-electrophoresis methods, especially during the 1970s (Strazdins 1972; Melzer 1972; McKague et al. 1974; Davison and Cates 1975; Lindström and Söremark 1975), the method has developed a reputation as (a) being tedious, and (b) not always providing useful information. An inherent consequence of doing tests within a capillary cell is that an applied electric field will act upon counter-ions adjacent to the capillary walls, inducing electro-osmotic flow of the electrolyte solution. As long as the flow in the cell remains laminar, it is relatively straightforward to calculate the positions in the cell ("stationary levels") at which the net flow due to electro-osmosis equals zero. The adjective "tedious" probably arose due to the difficulty of viewing tiny points of lights (the particles) through a microscope. In addition, erroneous results can be expected if the operator fails to properly calibrate the focal plane. Settling of particulate debris within capillary cells (Tanaka 1984), affecting the calibration, has been a further source of frustration. Pelton et al. (1993) came up with a more robust procedure, measuring particle velocities at various points across the width of a capillary cell, and eliminating the need to separately determine the stationary levels.

Another possible criticism of the ME method, with respect to evaluation of cellulosic materials, is the fact that cellulosic fibers are much too large to fit into the types of capillary cells that are typically used. Rather, the common procedure involves filtering a suspension of papermaking stock with a coarse screen and placing the filtrate in the test device. Strazdins (1972) asserted that the zeta potential of fine materials tends to be representative of all of the surfaces within such a suspension. Data presented by Jaycock and Pearson (1976) is often cited in support of this statement; in the cited study the results of streaming potential tests of fibers agreed closely with micro-electrophoretic test results for fines. The cited study also demonstrated a mechanism whereby colloidal materials originating from the cellulosic pulp adsorbed onto various mineral surfaces, causing the respective zeta potentials to become closer to that of the cellulosic material. At the limits of short mixing times, and when using highly charged chemical additives,

there are many examples in which surfaces of differing zeta potential have been found to coexist (Strazdins 1972; Sanders 1994; Wang and Hubbe 2001; Leiviskä et al. 2005). Possible sources of zeta potential difference among fine particles and fibers include differing content of extractives, hemicellulose, *etc.* (Rundlöf et al. 2000; Fardim and Holmbom 2005), differing affinities for potential adsorbates (Marton 1980), and different rates at which the suspended matter becomes saturated with a given adsorbate. While it is sometimes said that adsorption of materials onto fines is "more rapid," compared to adsorption onto larger fibers (Lindström and Söremark 1976), Strazdins (1994) is probably correct in stating that rates of arrival of adsorbate at various surfaces are almost independent of the size of a given particle or fiber. Rather, it appears that the fibers, being porous and covered by fibrils, can act like a sort of sponge, taking up greater quantities of adsorbate per unit of external area. This "sponge model" of the cellulosic surface will be considered in greater detail later in this review.

## Streaming Current (SC)

Streaming current (SC) test methods have received considerably less theoretical analysis (Gerdes 1966; Cardwell 1966; Kenaga et al. 1967; Dentel and Kingery 1989; Ojala 1993; Walker et al. 1996; Phipps 1999), compared to the micro-electrophoresis methods discussed in the previous section. Nevertheless, the simple operation and robust nature of the equipment has led to the widespread use of SC methods both in the laboratory and online for the evaluation of aqueous samples from paper machines. The most commonly used SC devices employ a plastic piston, which reciprocates within a dead-ended plastic cylinder. The reciprocal motion, having a frequency of about 4 Hz, causes a rapid movement of aqueous solution back and forth within the annular space. Electrode probes located at two points along the axis of the cylinder sense the electrical consequence of counter-ions, adjacent to the plastic walls, becoming moved back and forth by the liquid flow. A review of the SC method has appeared recently (Hubbe and Chen 2004).

The word "indirect" is perhaps the best adjective to describe how SC tests can be applied to the evaluation of lignocellulosic materials in suspension. SC methods have achieved great practical success as a means of detecting endpoints of polyelectrolyte titrations (Bley 1992; Kaunonen and Springer 1988; Hubbe and Chen 2004). In other words, one relies upon the assumption that whatever sign of polyelectrolyte is in excess in the aqueous phase of the sample will tend to dominate the charge at the plastic surfaces. The titration method is easily automated, and online SC titration systems have been installed on many paper machines (Kaunonen 1989; Bley and Kästner 1992; Bley and Bischof 1994; Baumgartner and Bley 1994; Gratton and Pruszynski 1995; Veal 1997; Denbrok and Peacock 1999; Berger et al. 2002). In many applications the test results are used to control the addition rate of a highly charged cationic polymer, keeping the cationic demand at a more constant level. The benefits of online charge control can include reduced overall costs of retention chemicals, more rapid dewatering, more efficient use of sizing agents, and reduced variability of paper properties.

Though it is hard to deny the practical utility and mechanical reliability of SC test methods, when used in paper mills, caution is required with respect to using SC procedures to gain information about what is happening at cellulosic surfaces. As noted

by Jaycock (1995), it is difficult to be sure whether or not the plastic surfaces of the device have become completely covered by colloidal materials from the sample, as is required for the analysis to be valid. This issue is especially problematic in the case of samples that contain mainly hydrophilic polyelectrolytes. SC devices are often constructed of poly-tetrafluoroethylene (PTFE), a polymer that has a reputation for not sticking to anything. Certain combinations of low-charge, hydrophilic polyelectrolytes and moderate to high salt levels can cause highly misleading results of SC titrations or make it impossible to make the SC device reach a zero signal (Chen et al. 2001). Further deviations, relative to an assumed 1:1 stoichiometry of titration, have been attributed to the existence of charge-stabilized polyelectrolyte complexes (Chen et al. 2003).

Though the test surfaces of SC devices are plastic, rather than cellulosic, it is still possible to use SC titrations as a means of studying adsorption phenomena onto fibers (Cardwell 1966; Kenaga et al. 1967; Sezaki et al. 2006). Cardwell (1966) was the first to employ SC titrations in this manner, adding an excess of polyelectrolyte to a fiber slurry, then back-titrating aliquots of filtrate, to find the concentration of the initial polyelectrolyte remaining in the solution phase. Sezaki et al. (2006) extended this approach to cases involving polyampholytes, polymers containing both positive and negative dissociable groups. By adjusting the pH of the filtrate to either high or low pH it was possible to convert either the positive or negative groups to a neutral form and to titrate the polyampholyte as if it were a simple polyelectrolyte.

## Streaming Potential (SP)

Quinke (1859) is credited with the earliest observation that an electrical potential results when liquid is passed though a porous plug or mat. Three wood fiber species were included among his samples. Helmholtz (1879) derived an expression showing that the voltage differences observed in such tests were more or less independent of the length, size, and form of the packed material through which the liquid was forced at a specific pressure, given a prescribed composition of electrolyte (Li and de Bruyn 1966). Thus,

$$\zeta = 4 \pi \eta \Omega E / ( \varepsilon P ), \quad (1)$$

where $\zeta$ is the zeta potential (usually expressed in millivolts), $\eta$ is the viscosity of the solution, $\Omega$ is the electrical conductivity, $E$ is the measured change in electrical potential resulting from the application of pressure, $\varepsilon$ is the dielectric constant of the solution, and $P$ is the differential pressure applied across the sample. In his derivation, Helmholtz made two simplifying assumptions, as follows:

- The electrical double layers are thin relative to the dimensions of the passages through the porous material; and
- The solid materials and the surface of those materials are non-conductive under the conditions of testing.

As will become clearer in later parts of this review, neither of these assumptions is strictly true with respect to cellulosic fibers. A mat of wet cellulosic fibers has at least two classes of pore sizes – and probably three – as will be described in a later section. Since the pore sizes associated with a wet fiber mat can range from below 1 nm up to

almost 1 mm, the first assumption is likely to be violated, especially at the limit of low salt concentration. Wet, cellulosic fibers are known to be conductive (Briggs 1928; Scallan 1989; van de Ven 1999), in violation of Helmholtz's second assumption, though the effects of fiber conductance are often ignored when the bulk electrolyte concentration is higher than about 5 mM (Goring and Mason 1950a; van Wagenen and Andrade 1980; Hunter 1987). The following form of the Helmholtz-Smoluchowski equation is often employed in order to account for fiber conductivity (Van Wagenen and Andrade 1976),

$$\zeta = 4 \pi \eta (\Omega_B + 2\Omega_S/a) E / ( \varepsilon P ), \quad (2)$$

where $\Omega_B$ is the bulk solution conductance, $\Omega_S$ is the surface conductance, and $a$ is the radius of a pore, treated as being a cylinder. Equations (1) and (2) can be applied not only to packed beds of fibers, but also to capillary cells having various configurations (Anderson and Koh 1979; Schurz et al. 1989; Scales et al. 1992; Barron et al. 1994). In the case of a pad of fibers, it is often most practical to measure the electrical conductance through the pad, and then to use this value to determine a correction term equivalent to $2\Omega_S/a$ in Eq. (2).

As mentioned above, streaming potential tests have a very long history when applied to lignocellulosic materials (Quinke 1859). Briggs (1928) was apparently the first to apply the method in the case of pulp fibers intended to be used in paper production. Interestingly, Briggs was also the first to notice an issue that has concerned many subsequent investigators; that is, the ratio of potential drop to pressure drop across the fiber pad was not constant. Rather, the ratio depended on the applied pressure (see also Ball and Fuerstenau 1973). Briggs concluded, with considerable foresight, that the effect might involve the conductive nature of fiber surfaces. Remarkably, Briggs also observed that the streaming potential of fibers became increasingly negative with the addition of small amounts of KCl, up to a concentration of 0.1 M. Both of these observations will be considered in more detail later in this review, since they provide clues to the nature of cellulosic fiber surfaces.

Despite the long and distinguished history of streaming potential analyses, papermakers have not adopted such tests to nearly as great an extent that they earlier adopted microelectrophoresis measurements, and more recently have come to rely on streaming current tests. At face value, this circumstance seems odd. After all, fiber-pad streaming potential tests are direct, sensing electrokinetic events occurring directly on the fiber surfaces under study (Hubbe and Wang 2004). No visual observations are required. It is not necessary to assume that the electrokinetic nature of the fiber surfaces is the same as that of the fines. Furthermore, it is not necessary to wait for the system to equilibrate with a second surface, such as plastic, in order to interpret the results. It will be argued, later in this review, that the relative unpopularity of the fiber-pad streaming potential method may stem from a well-founded, but misplaced reluctance on the part of papermakers to carry out any experiments with distilled water.

Online applications of streaming potential tests can involve even greater challenges. As noted by Winters (1998), online fiber-pad streaming potential tests can suffer from air bubbles, plugging of the screens, and build-up of material on the electrode probes. An extensive system of rinsing may be required in order to prevent fouling of the

wetted parts of the device, especially if it is to be used continuously in a titration mode (Hubbe 1999).

It has long been recognized that subtle differences may exist between the environments of the two electrode probes that are used to evaluate the potential difference across a porous mat (Korpi and de Bruyn 1972; Ball and Fuerstenau 1973). To overcome this problem, some researchers have insisted upon using symmetrical devices in which the flow of liquid could be passed in either direction through a plug of porous material constrained between a pair of porous barriers (Fuerstenau 1956; Hoffman 1975; Jain et al. 1993). But such procedures require that a porous plug be individually prepared before each test. For practical use in evaluation of fibrous slurry samples, it is generally preferred to use flow of liquid to gather a plug or mat adjacent to a single screen, through which filtrate is pushed or drawn (Penniman 1991, 1992, 1994; Hand et al. 1993; Thiele and Kopp 1997; Wang and Hubbe 2001). Differences in the condition of the two electrode probes are dealt with, in such cases, by measuring a reference voltage in the absence of flow through the porous sample (Ball and Fuerstenau 1973; Penniman 1991; Wang and Hubbe 2001). The single-screen design for measuring streaming potential lends itself well to automated, online operation (Sack 1976; Evans et al. 1985; Ericksson 1987; Richter et al. 1989; Crill 1991; Sack et al. 1993; Rohloff and Höschle 1993; Nazir 1994; Penniman 1994; Miyanishi 1995a,b; Padovani and Colasurdo 1995; Petzold and Allen 1996; Hubbe 1999).

Despite the length of the above list of publications and patents pertaining to online use of streaming potential for paper machine slurry samples, the fact remains that usage of such devices is much less common in comparison to, for instance, streaming current (SC) methods, both online and in the lab. Paper machine systems employing online streaming potential measurements are the exception, rather than the rule (Crill 1991; Sack et al. 1993; Miyanishi and Shigeru 1997; Hubbe 1999; Miyanishi 1999). While practical issues, such as deposits and bubbles, may help explain why the method is not used more widely, the question arises as to whether or not papermakers have been satisfied that they have achieved meaningful correlations of the measurements with paper machine operating variables**.**

## Other Tests

Although the test methods mentioned in the above three subsections are perhaps the best known, they don't exhaust the possible ways in which investigators have probed the electrokinetic nature of lignocellulosic surfaces. For example, one can characterize zeta potentials of suspended matter by either applying an alternating electrical field and sensing the intensity and phase of the resulting ultrasound (Springer and Taggart 1986) or, conversely, by applying an inaudible sound and detecting the resulting electrical fluctuations (Marlow et al. 1988). Both of these approaches work best when considering particles which have a compact shape and a large difference in density, relative to the suspending medium. Unfortunately, cellulosic fibers do not fulfill either of these requirements very well.

Two kinds of dye adsorption methods for fiber charge characterization deserve mention. Tanaka et al. (2000) developed an innovative method involving a strongly colored form of high-charge cationic polyelectrolyte. Measurements of the strength of

coloration of filtrate solution, after mixing a known amount of polymer with a sample of interest, were used to calculate the adsorbed amounts, assuming an approximate 1:1 interaction of the polymer with accessible charged groups. Another straightforward strategy employs a basic dye such as methylene blue to quantify the accessible bound anionic groups in a mixture (Fardim and Holmbom 2003). Such dye molecules can be expected to interact with sites that are inaccessible to polyelectrolytes, depending on the molecular mass of the latter. Recently, a fluorescent dye has been used in order to quantify acidic groups at the surfaces of single fibers (Matthews et al. 2004).

A second dye-related method became quite popular, for a while, among papermakers seeking an alternative to microelectrophoresis tests (St. John and Gallagher 1992). Terayama (1952) observed that dyes such as toluidine blue-O changed their color when in the presence of an excess of strongly charged anionic polyelectrolyte, such as polyvinylsulfate. The primary use of the method, as originally conceived, has been to determine the endpoint of titrations involving polyelectrolytes. As in the case of SC tests, as described earlier, one can evaluate the amounts of polyelectrolyte adsorbing onto cellulosic fibers, based on evaluation of the concentration remaining in the bulk solution. The method was adapted and popularized, for paper industry applications, by Halabisky (1977). Halabisky modified the method such that titrations were carried out in both the positive and negative directions, each time adding a known excess of positive or negative polyelectrolyte to an aliquot of fiber suspension. Then, as a means of obtaining a single number to represent the system, a ratio was computed from the two endpoint quantities, and the log of the "colloid titration ratio" (CTR) was obtained. Values of the logarithm of CTR were often found to correlate well with zeta potential data (Springer and Taggart 1986; Carrasco et al. 1998). However, taking a ratio entails some loss of information pertaining to the ability of the sample to take up cationic and anionic polyelectrolytes. An alternative reporting system and procedure were proposed to avoid such loss of information (Hubbe 1979). More recently, the toluidine blue-O titration method has been incorporated into online determinations of cationic demand (Pursiheimo and Thomas 1998).

All of the charge evaluations mentioned so far have been based on an implicit assumption of an approximately 1:1 stoichiometry between polymer-bound ionic groups and surface-bound ionic groups. The assumption appears to be reasonably accurate at the limit of low ionic strength of the solution and in the absence of significant excess polyelectrolyte in the bulk solution (Wågberg et al. 1989; Laine et al. 1996; Koljonen et al. 2004; Horvath et al. 2006). Recently, Mocchiutti and Zanuttini (2005) published an elegant analysis of what can be expected when different levels of cationic polymer are added to a net-negative sample of papermaking fiber slurry. Though the initial polymer added to the system is expected to lie flat on the surface, higher amounts are expected to result in a three-dimensional adsorbed conformation, with loops and tails of polyelectrolyte extending outwards from the surface (see Fleer et al. 1993). The predicted deviations from 1:1 stoichiometric interaction between the polyelectrolyte and fiber surfaces were consistent with experimental data.

## WHAT ELECTROKINETIC DATA CAN TELL US ABOUT FIBER SURFACES

After having discussed various electrokinetic methods, the following question remains: "What can the test data tell us about fiber surfaces?" As pointed out near the beginning of this review, some of the equations most often used in interpreting electrokinetic test results appear to be based on unrealistic simplifying assumptions. Also, there seem to be some inconsistencies in the data, relative to what we think we know about cellulosic fiber surfaces. This section will take a different approach to these issues, making the tacit assumption that various sets of reported electrokinetic data are accurate. Instead, questions will be considered relative to what descriptions of fiber surfaces can best account for observed electrokinetic behavior.

As an example, consider the relative charge densities of cellulosic surfaces, in comparison with the charge densities of cationic polymers that are most commonly used in charge titration work. Chen et al. (2004) estimated that the charge density of such polymers is about one hundred times higher than the charge density of a cellulosic fiber surface, based on the proportion of bound carboxylic acid groups on the latter. Nevertheless, these authors detected a one-to-one stoichiometric interaction, based on an analysis of released counter-ions on the polyelectrolyte. It was concluded that the polyelectrolyte must be able to interact, somehow, with carboxylate groups within a three-dimensional volume, not just at the outer surfaces of the fibers.

Further evidence that cellulosic surfaces are somehow different from other materials that have been studied by electrokinetic methods date back to some of the earliest work in which the streaming potential method was applied to papermaking fiber suspensions (Briggs 1928; Bull 1934). Bull found that various different electrokinetic methods agreed well with one another when applied to protein-covered substrates. However, the methods failed to give consistent results when applied to cellulose. Briggs observed that the magnitude of negative zeta potential of cellulose fibers, as calculated from streaming potential measurements, increased with increasing salt concentration, reaching a maximum at 0.1M KCl.

### Towards More Realistic Descriptions of Cellulosic Surfaces

Earlier in this article brief mention was made of a "sponge" model for the cellulose fiber. In other words, it was proposed that cationic polyelectrolytes diffusing towards a cellulosic fiber surface may gradually continue diffusing into sub-surface regions of the fiber. Such regions may involve, for instance, fibrillation at the fiber surfaces or pores within the cell walls. Such diffusion may account for at least part of the observed decay of zeta potentials of cellulosic surfaces freshly treated with cationic polyelectrolytes (Strazdins 1977; Penniman 1992; Koethe and Scott 1993; Farley 1997; Wang and Hubbe 2002; Hubbe et al. 2006a). Hostetler and Swanson (1974) confirmed the general concept of the mechanism by studying the adsorption of cationic polymers of differing molecular mass onto silica gel suspensions of differing known pore size. Figure 1 provides a cartoon representation of such a mechanism.

As shown, if the amount of adsorbed polyelectrolyte is sufficient to reverse the inherent negative zeta potential of the cellulosic fiber surfaces, then one expects there to be an excess of negatively charged counter-ions, such as chloride or sulfate, adjacent to

the treated fiber surfaces. Flow past the freshly-treated surfaces is expected to push some of these counter-ions, those which are located in the diffuse parts of electrical double-layers outside of a plane of shear (Hunter 1987). The expected result is a positive streaming potential. A decay of streaming potential is expected if the polyelectrolytes are able to adsorb into dead-ended pores (see Part B of figure), causing the external surfaces to revert to a net-negative condition.

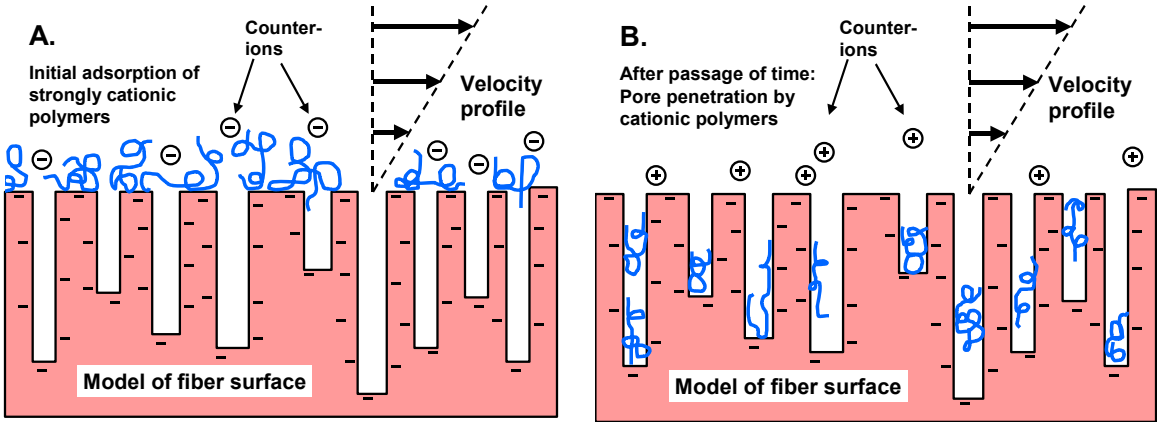



**Fig. 1.** Model of cellulose fiber surface in which pores are considered as being discontinuous, open at one end, and sufficiently large to permit diffusion of polyelectrolytes. Flow past the outer surface pushes counter-ions, resulting in an electrokinetic current and potential. Part A: Initial situation following adsorption of high-charge cationic polymer. Part B: After diffusion of polymer into pores.

Various authors have considered the effects of loops and tails of macromolecules extending outwards from substrates (Jones 1979; Vernhet et al. 1994; Ohshima 1997; Bauer et al 1998). The consensus of such work is that extended macromolecules are expected to shift the location of a shear plane further outwards from a surface. Such effects are expected to be important in the case of cellulose fibers, due to the presence of fibrils and microfibrils (Pelton 1993; Rojas et al. 1998, 2000; Rojas and Neuman 1999).

Estimates of the pore sizes within the cell walls of chemically pulped fibers from wood were first obtained by a solute exclusion method (Stone and Scallan 1968). Briefly stated, a known mass of never-dried fibers was suspended in a known total amount of water with a known amount of dissolved dextrin, having a narrow distribution of molecular mass. Depending on the molecular mass of the polymer, the authors observed that the concentration in the bulk solution was higher than would have been expected, had the polymer been able to penetrate into all of the pores that were accessible to water. Though the results of solute exclusion tests have indicated pore widths of at least 5-20 nm in the case of sulfite and kraft pulps (Stone and Scallan 1968; Berthold and Salmén), it has been pointed out that the initial analysis did not account for excluded volume effects, which can result from a restriction in the degrees of freedom of motion of dissolved polymer segments when confined to a very small pore (Alince and van de Ven 1997). Alince (2002) concluded, based on adsorption experiments with cationic polyelectrolytes, that typical pores in kraft fibers have widths of about 100 nm. The latter figure also tended to agree with electron micrographs given in the same article.

Further evidence to support a pore-diffusion model, including the model depicted in Fig. 1, can be found in the results of various adsorption tests of cationic materials onto cellulosic fibers. For instance, Öhman et al. (1997) observed relatively high apparent charge of the fiber surfaces when the analysis was based on pH titrations, *i.e.* the adsorption of $H_3O^+$ ions. By contrast, analyses based on adsorption of cationic polymers yielded lower apparent charge, especially in the case of the highest-mass polymer used as probes. Such results are consistent with the existence of a class of pores that are accessible to simple ions, but are too small to allow ready diffusion of the polyelectrolytes. Unfortunately, this type of test result can be questioned due to the strong reversal of electrokinetic charge often observed after solid substrates are exposed to an excess of high-mass polyelectrolyte (Hoogeveen et al. 1996; Sukhorukov et al. 1998; Schwarz et al. 1998; Wågberg and Ödberg 2000). The reversal appears to result due to the existence of loops and tails extending towards the solution phase, where they are expected to have a dominating effect on the electrostatic interactions of those surfaces (Varoqui 1982; Ohshima 1997).

Though both zeta potential decay and the results of solute exclusion tests can be explained in terms of the diagram in Fig. 1, the photographic evidence just cited (Alince 2002) suggests that it would be more accurate to think in terms of continuous networks of pores below the outer surfaces of cellulosic fibers. Figure 2 shows two kinds of pores that ought to be considered in order to account for the electrokinetic behavior of cellulosic fibers. Part A of the figure assumes that the main observable effects arise due to flow within layers of fibrils and microfibrils at fiber surfaces. Such layers may or may not be accessible to polyelectrolytes, depending on such factors as molecular mass, time, degree of refining of the fibers, and depth of location at the fiber surface. Part B of the figure assumes that the main observable effects arise due to flows within the fiber cell walls, and that the electrokinetic contribution of such flow is greater than that of flow within fibrillar layers at fiber surfaces. It is assumed in both parts A and B that the pore networks are continuous, at least during the time that the fibers remain wetted and swollen with water.

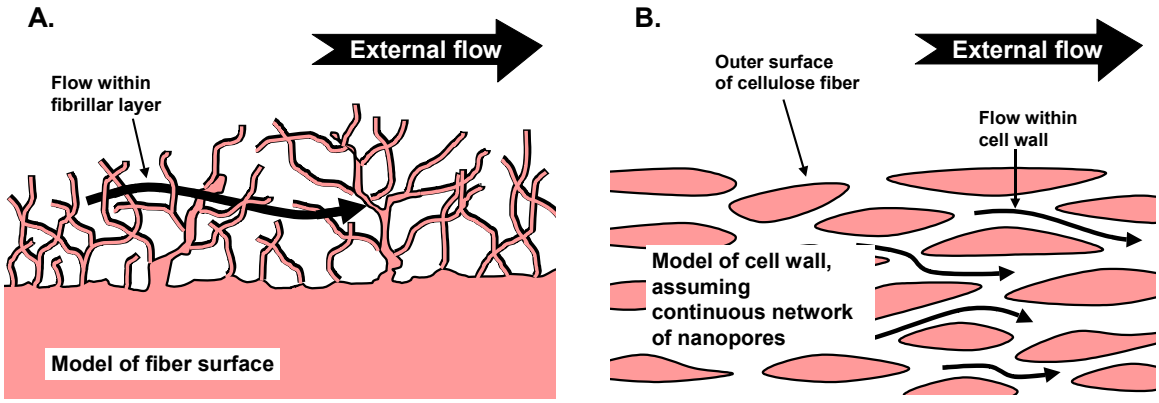



**Fig. 2.** Two types of continuous pores networks associated with cellulosic fibers. Part A: Pore network within a layer of fibrils and microfibrils at a fiber surface. Part B: Pore network within the cell wall of a fiber, especially after chemical pulping to remove lignin.

The degree of fibrillation of fiber surfaces is expected to depend on the degree to which the fibers have been refined (Baker 1995). As noted by Pelton (1993), fibrils can range all the way down to molecular dimensions, making it difficult even to define the location of a true outer surface of cellulosic fibers. Given the wide range of sizes of different fibrillar elements at the surfaces of refined fibers, it makes sense to expect void spaces as large as about 100 μm within a layer of such fibrils. This estimate is about a factor of 1000 larger than what has been estimated for the size of pores in the cell walls of chemically pulped fibers. The lower limit of pore size within a layer of fibrils might depend on such factors as packing pressure of a fiber mat, as well as electrical double-layer forces of repulsion between adjacent fibrils. Pang and Gray (1998) demonstrated that the degree of extension of fibrils outward from fiber surfaces tended to decrease with the addition of salt, consistent with the reduced influence of electrostatic repulsion between the surfaces. Forsström et al. (2005) concluded that water within fibrillar layers on kraft fibers can account for a substantial fraction of the water remaining with the fibers following centrifugation, according to the water retention value (WRV) procedure.

## Evidence Based on Fiber Electrical Conductance

When considering which of the figures presented so far best accounts for physical reality, one of the first types of evidence to consider is the conductivity of wet fibers. According to Scallan (1989), wetted fibers are about 20 times more conductive of electricity in comparison to a $10^{-4}$ molar salt solution. As mentioned earlier, various authors have used corrections for "surface conductance" of fibers, especially in cases where the concentration of salt in the bulk solution was below about 1 to 5 x $10^{-4}$ M (Goring and Mason 1950a; Ghosh and Pal 1961; van Wagenen and Andrade 1980; Hunter 1987; Revil et al. 1999; van de Ven 1999). The conductivity of wet fibers is said to be too high to be due only to the counter-ions at the outer surface of the fibers (van de Ven 1999). Rather, it would appear that a flow of current passes through the cell wall (as in Fig. 2B) or within layers of fibrils at the fiber surfaces (as in Fig. 2A). In this regard, the term "surface conductance" maybe ought to be replaced by a more general term such as "conductance of wet fibers." Goring and Mason (1950b) discussed a model roughly equivalent to Fig. 2A, hypothesizing that cellulosic chains, bearing negatively charged groups, were "partially dissolved" at the fiber surfaces.

## Evidence based on Effects of Fiber Pad Compression

Earlier, when introducing Helmholtz's (1879) analysis of streaming potential, it was mentioned that Helmholtz made two simplifying assumptions. Not only did he assume that the packed materials (including their surfaces) were non-conductive, but he also assumed that the double layers were thin relative to the dimensions of the pore spaces. Evidence pertaining to the latter assumption comes from many experiments in which streaming potential was evaluated over a range of different pad densities of cellulosic fibers. Pad density was found to have a significant effect on calculated zeta potentials (Neale 1946; Goring and Mason 1950b; Chang and Robertson 1967a; Melzer 1972), though none of the cited studies offered a mechanistic explanation. Interestingly, Briggs (1928) already had observed an effect of applied pressure on zeta potentials

calculated from streaming potential data; higher applied pressures of water would be expected to have effects similar to that of mechanical compression of a fiber mat.

A possible explanation for the observed effects of packing density is proposed as follows: With increasing compression of a fiber pad, the various pores within the pad are shifted towards smaller sizes. Assuming that the condition of the aqueous solution remains constant, the ratio of the pore size to the thickness of the ionic double layer is shifted. In the case of very small pores, such as those within the cell wall (rather than between adjacent fibers), a decrease in pore diameter would be expected to increase the degree of overlap of ionic double layers on adjacent pore surfaces. A suppression of electrokinetic phenomena is expected in such cases (Hunter 1981; Miller et al. 1992; Wan 1997; Bernabé 1998; Ricq et al. 1998; Revil et al. 1999; Alkafeef et al. 2001). One way to explain the suppression effect is that a double-layer, of the type expected at an outside surface, "cannot fit" within the available space. Equivalently, it is expected that the local pH within very narrow pores, at low ionic strength, can be shifted relative to its bulk value (Grignon and Scallan 1980; Fält and Wågberg 2003). A greater proportion of carboxylate groups within the pore would be in their protonated, uncharged state. The following expression provides an estimate of the ratio between streaming current within a nanopore, versus that of an equivalent unbounded surface (Alkafeef et al. 2001),

$$r = 1 - \tanh(\kappa h) / (\kappa h), \quad (3)$$

where $r$ is the ratio of streaming current in a narrow pore of width $h$ in comparison to the streaming current in a pore many times larger than the Debye length $\kappa^{-1}$ corresponding to the ionic strength of the flowing aqueous solution. Figure 3 illustrates the dependency of $r$ on the product $kh$, according to Eq. 3.

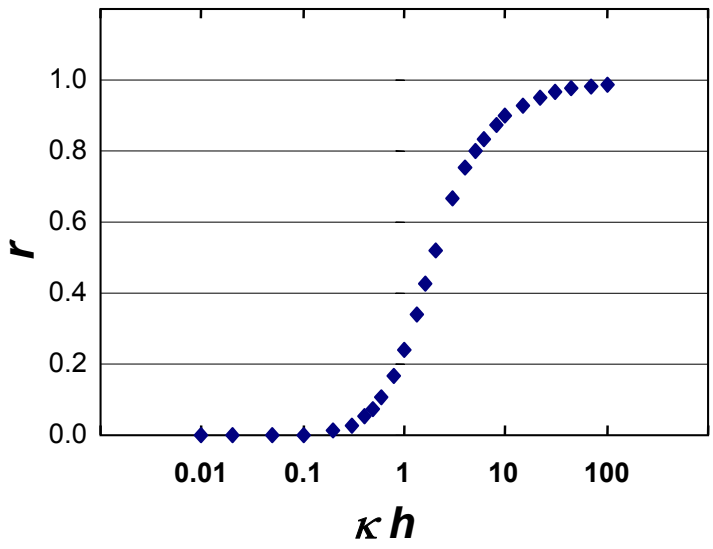



**Fig. 3.** Factor $r$ describing relative magnitude of streaming current within a small capillary having a thickness of $\kappa h$, where $\kappa$ is the Debye-Hückel reciprocal length parameter describing the double layer thickness.

Let's suppose that the explanation just given is valid, accounting for the observed dependency of streaming potential on pad compression. It does not necessarily follow, however, that pores within the cell wall of a fiber provide a continuous passage for the flow of liquid, in response to an applied external pressure. It is alternatively possible that a class of extremely small pores exists within the compressed layers of fibrils and microfibrils at the fiber surfaces. These two alternatives are basically the same as was represented earlier in Fig. 2. Tentatively, it is proposed that nano-sized pores exist both within the cell wall itself and within fibrillar layers. At the same time, there may be larger pores between adjacent fibers within a mat.

Another competing or parallel explanation for the effect of pad compression on zeta potential values calculated from streaming potential data involves the conductivity of the fibers or of the fiber surfaces (Ghosh and Pal 1961; van Wagenen and Andrade 1980; van de Ven 1999; Revil et al. 1999). There are two ways in which compression of a fiber pad can be expected to change the relative contribution of fiber conductance to the net, observed streaming potential. First, by forcing the pore openings to become smaller, on average, there is a lower conductance of electricity via the liquid phase. Second, it might be expected that pressing conductive fibers together would facilitate the flow of current from one fiber surface to the next. In support of such a mechanism, Scallan (1989) showed increasing conductance with increasing solids contents of fiber pads, especially in the case of fibers that were rich in ionic groups.

## Evidence Based on Reversible Sign of Streaming Potential

A recent study provided further evidence that a continuous network of pores exists below the outer surface of cellulosic fibers (Hubbe et al. 2006a). The experimental situation is shown schematically in Fig. 4.

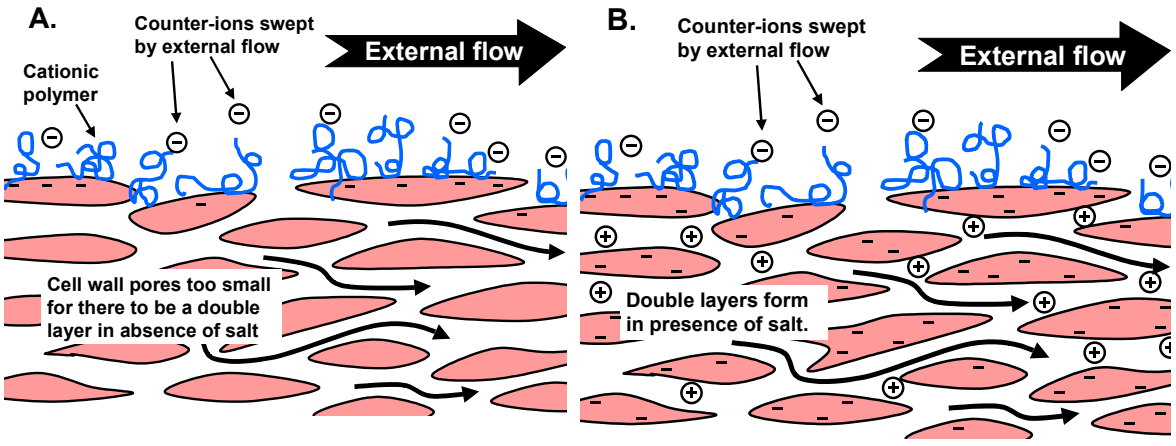



**Fig. 4.** Model of fiber surface showing contrasting contributions to the sign of streaming potential. Part A: In the absence of salt, most of the electrokinetic signal is expected to come from external surfaces, which are accessible to adsorption by high-mass cationic polyelectrolyte; the surfaces within nanopores don't contribute significantly, since there is not enough space to accommodate a double layer and the pH is shifted. Part B: In the presence of salt the double layers within nanopores develop more fully, but the electrokinetic contribution is negative, based on the assumption that the polyelectrolytes cannot reach those surfaces.

Before forming the fiber pad, a slurry of bleached hardwood kraft fibers in $10^{-4}$ M $NaHCO_3$ was treated with high-mass poly-diallyldimethylammonium chloride (poly-DADMAC). The amount of poly-DADMAC was selected so that it was about twice the amount needed in order to achieve a neutral streaming potential. A strongly positive streaming potential was observed. Upon addition of sodium sulfate to the mixture, the sign of the observed streaming potential became negative. Then, by a series of dewatering, dilution, and salt addition steps, the fibers were alternated between salt-free and salt-containing conditions. The sign of streaming potential accordingly switched back and forth, in a quasi-reversible manner.

Referring again to Fig. 4, the reversible switching of sign of streaming potential is consistent with the expectation that the electrokinetic contribution of nano-sized pores will be suppressed in the absence of salt (Alkafeef et al. 2001). Then, upon addition of salt (part B of figure), the double-layers become compressed. When the double layers are thin, relative to the size of a certain class of pores, then Helmholtz's (1879) assumptions are more closely fulfilled. Thus, the electrokinetic phenomena within the smallest pores begin to approach what would be expected in the case of large pores or adjacent to external surfaces exposed to solution. The situation depicted in Fig. 4 happens to be a very effective way of demonstrating this kind of behavior, since the polyelectrolytes are expected to be too large to diffuse easily below the outer surfaces of the fibers.

Based on the accumulated evidence, it is now possible to form a conceptual picture of cellulosic fibers, relative to their interactions with charged polymers. In summary, it appears that fibers have continuous networks of pores below their outer surfaces. At least some of these pores appear to be smaller than about 50 nm, small enough that their effect on the observed electrokinetics of the system become suppressed when the electrical conductivity is reduced to about 60 μS/cm. The data also imply that the very fine pores can be too small to be efficiently covered by the high-mass poly-DADMAC molecules, and that they can remain negative in character, even while the outer surface of the fibers can become positive, following the adsorption of high-charge cationic polymer. The basic mechanisms were confirmed recently by similar experiments with suspensions of silica gel (Hubbe et al. 2006b), a material having more narrowly defined pore dimensions.

## Pictorial Models

Figure 5 shows two ways in which the concepts just described can be reconciled with features of cellulosic fibers already discussed in this article. Part A considers a case in which it is assumed that relatively large pores exist between fibers in a compressed mat. It is assumed that any pores in the fibers, existing below the level accessible to polyelectrolytes, must be similar in size or smaller than the thickness of the double layer, as expressed by the Debye $\kappa^{-1}$ parameter, especially in the absence of added salt. It is reasonable to expect that such nanopores will be present not only within the cell walls, but also among microfibrils at fiber surfaces, especially if the latter have become mechanically squeezed together. Though there is evidence that high-mass cationic polymers initial adsorb only on the outermost surfaces of kraft fibers (Tatsumi and Yamauchi 1997; Hubbe et al. 2006a), there is also evidence suggesting that gradual

diffusion into the fiber takes place (Strazdins 1977; Penniman 1992; Koethe and Scott 1993; Farley 1997; Wang and Hubbe 2002; Hubbe et al. 2006a). Given the tiny dimensions of pores in the cell wall, relative to the size of typical polyelectrolytes used in papermaking, it is reasonable to expect that such diffusion is mainly limited to penetration below layers of fibrils.

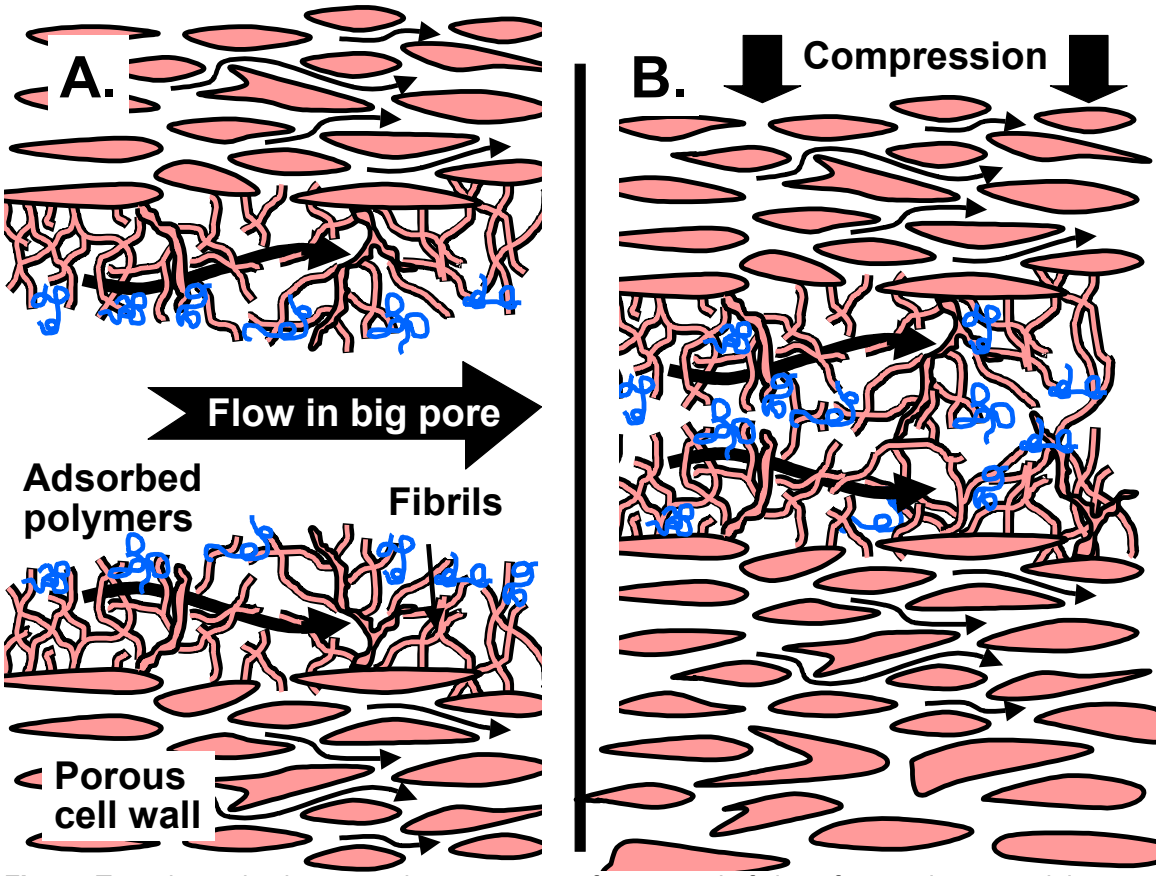



**Fig. 5.** Two alternative interpretations to account for reversal of sign of streaming potential upon addition or removal of salt from cellulosic fiber suspension treated with high-mass cationic polyelectrolyte. Part A: Model in which positive streaming potential, in the absence of salt, is assumed to arise mainly due to flow in macro-pores past the outer surfaces of the fibers, to which the polyelectrolyte is adsorbed. Electrokinetic effects due to nanopores within the fibrillar layer and cell walls are assumed to be significantly suppressed in the absence of salt. Part B: Model in which it is assumed that large pores are not the main contribution to streaming potential, due to compression of the mat. Polyelectrolytes are assumed to diffuse within the fibrillar layers at the fiber surface, but not significantly into the cell wall pores. In either case, addition of salt is expected to increase the relative importance of the electrokinetic effects arising within the smaller pores.

Part B of Fig. 5 shows an alternative interpretation to describe what has been learned from the recent experimental work. In this model it is assumed that the fibers in the mat, during an analysis of streaming potential, have been sufficiently compressed so that fibrillar layers on adjacent fibers are pushed against one another. Because streaming potential is directly proportional to applied pressure of a fluid, it follows that a majority of the electrokinetic signal will arise as the fluid passes through points of greatest

resistance within the mat, *i.e.,* fibrillar layers and fiber cell walls, rather than the open volumes between such features. In contrast to the model shown in Part A, one has to assume that the pores within the fibrillar layers are mainly accessible to polyelectrolyte adsorption, and that their typical size is substantially larger than that within the cell walls. These assumptions are needed in order to account for the observed changes in the sign of streaming potential, upon addition or removal of salt (Hubbe et al. 2006a). Because Fig. 4 presents two possible models, rather than one, it is clear that there is a need for further research to be able to answer such questions as whether fibrillar layers or nanopores within cell walls tend to have a larger effect on observed electrokinetic effects.

## Reappraisal of some Early Data

Much time has passed since the earliest detailed measurements of the streaming potential of pulp fibers (Briggs 1928). Certain aspects of that study have long remained unexplained. As noted earlier in this review, Briggs observed that zeta potential values calculated from the observed streaming potentials first increased in absolute value as the salt concentration was increased from zero. Such a trend is contrary to what would be expected based on a simple double-layer concept, involving a planar surface (Hiemenz and Rajagopalan 1997). Two hypotheses to explain those results can be attempted now, in light of the concepts presented in Fig. 5.

First, let us assume that the observed effect was due to the "suppression" of electrokinetic effects represented by Eq. (3). Thus, in the absence of salt, only the flow around the fibers, by way of relatively large passages, is expected to contribute significantly to the streaming potential. If that were the only component of flow, then, neglecting the conductivity correction, one might use Eq. (1) as an approximation to calculate the streaming potential. However, because the suppression term in Eq. (3) needs to be applied to the electrokinetic effect within the tiny pores within the cell wall, one expects that the overall effect will be lower in absolute magnitude. Though the direction of the trend is consistent with the observations (Briggs 1928), it is not possible, in the absence of detailed knowledge of pore structures, to give a quantitative estimate. A detailed analysis would need to include the conductivity correction.

The explanation just given also can help account for the observation of Fält and Wågberg (2003), who observed a maximum in the degree of fiber swelling at a certain concentration of salt. Likewise, Li and Dai (2004) observed increasing water retention of bleached eucalyptus pulp upon addition of 0.03 to 0.1% of either NaCl or $CaCl_2$ in different cases. In the absence of salt, the double layers within the smallest pores are expected to be strongly suppressed, as already noted, so the expected osmotic pressure should be correspondingly weak. With increased salt, the double layers will have sufficient room to become well established, leading to a maximization of the effect. Further salt is expected to make the double layers thin relative to the pore dimensions, leading to a reduction of swelling effects, as shown in many studies (Grignon and Scallan 1980; Lindström and Carlsson 1982; Scallan 1983; Fält and Wågberg 2003).

A second explanation for Briggs' observation can be based on expected effects of fiber electrical conductance. In the absence of salt, the effect of such conductance can be attributed to the presence of counter-ions adjacent to acidic groups within the pore structures of fibers. If one assumes that these groups remain dissociated, regardless of

the changes in salt concentration, then it follows that fiber conductivity will contribute less and less to the net streaming potential as the salt concentration is raised. Applying Eq. (3), it follows that the calculated zeta potential may increase with increasing salt. Further increase in salt is expected to reduce the thickness of the double layer sufficiently that a lower proportion of the counter-ions are located outside of the hydrodynamic slip plane, so that the calculated zeta potential is expected to decrease as high levels of salt are approached. Though these effects are, once again, in the correct direction to account for Briggs' (1928) observations, a detailed analysis would need to relax the assumption of a constant degree of dissociation (Lindgren et al. 2002).

The foregoing discussion has attempted to deal separately with each of Helmholtz's main assumptions, *i.e.,* non-conducting solids and thin double layers related to pores sizes. When considering the conductance of cellulosic fiber cell walls, however, one can combine the two concepts as follows: At low ionic strength the double layers cannot be accommodated within the space of nanopores in the cell walls, and the local pH becomes depressed on the nanopore surfaces, relative to the bulk pH. It follows that the counter-ions can be greatly reduced, in comparison to what might be expected based on the surface concentration of dissociable groups. With increasing ionic strength of the bulk solution, the double layers become more fully developed, the pH within nanopores becomes closer to the bulk pH, and the carboxyl groups within the nanopores become more substantially dissociated. This description is consistent with the observed increasing specific conductance of cellulosic fibers with increasing salt concentration, within a low concentration range (Scallan 1989). Likewise, when Chowdiah et al. (1983) considered the interpretation of streaming potential data within narrow pores wetted by non-aqueous media, they concluded that a correction for conductivity (*e.g.* Eq. 3), was accurate only for pores that are large relative to $\kappa^{-1}$. For narrower pores, the analysis also needed to account for the overlap of double-layers from the adjacent solid surfaces.

## Practical Limitations

Any detailed discussion of papermaking's electrokinetic aspects runs the risk of neglecting other important factors. Such factors, which often play a dominant role in various papermaking operations, include polymeric bridging attachments, hydrodynamic shear forces, and mechanical sieving of fine materials. Though many practical studies have shown strong correlations between electrokinetic measurements and the optimization of paper machine operations (Chang and Robertson 1967b; Strazdins 1977, 1980; Stratton and Swanson 1981; Kumar et al. 1991; Poppel 1992; Vanderhoek 1994; Miyanishi and Montegi 1996), such correlations can be hard to discern when looking at routine data from paper mills. For instance, it has been found that zeta potential has little correlation with fines retention in cases where the fiber slurry is formed into paper in the presence of high levels of hydrodynamic shear (Horn and Linhart 1991; Stratton and Swanson 1981; Stark and Eichinger 1989; Tripattharanan et al. 2004). In such cases, it is necessary to use very high-mass retention aids which possess the capability of physically bridging between surfaces in order to achieve high levels of retention.

Caution also is needed when considering electrokinetic events within a papermaking fiber suspension are dominated by materials in the colloidal phase (Smith 1992). In other words, the net charges associated with the fiber surfaces may be

insignificant relative to the surface charge contribution of materials that cannot be filtered. In such cases, it makes sense to focus on the cationic demand of the mixture, not on the streaming potential of the fibers. A previous review deals with the selection of the most appropriate electrokinetic test to use, depending on the type of sample, and also depending on the kind of question that one wants to answer (Hubbe 2000).

## PATHS FORWARD

### Cellulose Characterization

When one considers the results of various studies cited in this review, it is clear that both theoretical and practical questions remain. On the theoretical side, although there is evidence that pores of different sizes are influencing electrokinetic signals in distinct ways, the electrokinetic data alone cannot tell us the location of these pores. In this regard, it would make sense to run experiments in which nanofibrillar material has been optionally removed from fiber surfaces by means of enzymes (Jackson et al. 1993; Özdil et al. 2003; Buschle-Diller et al. 2005). Then, matched sets of enzyme-treated and untreated fiber suspensions could be treated with sufficient cationic polymer to strongly reverse the streaming potential to positive in the absence of salt. If the tests were to show a reversible sign of potential for both types of fiber, then the results would be consistent with a significant contribution to electrokinetic signals coming from continuous network of pores within the cell wall of fibers. If, however, the test results were to show substantially less reversibility in the case of the enzymatically treated fibers, then the results would indicate a substantial contribution of the fibrillar layers to the observed electrokinetic effects.

As illustrated by some of the studies cited in this article, complementary test protocols can be a great help to understand phenomena that are only partially revealed by electrokinetic measurements. As in the work of Tatsumi and Yamauchi (1997), depth analysis methods might be used to test some theories regarding the extent to which polyelectrolytes of different molecular mass can penetrate either into a fibrillar layer or into the cell wall of fibers prepared by different pulping methods. Adsorption isotherms would be a second way to probe differences in accessible surface area before and after such processes as refining, enzymatic treatments, and drying of the fibers.

Another approach to this kind of research might involve mathematical models of porosity within packed beds of fibers, and determining whether reasonable models of porosity are consistent with expected effects of streaming potential and electro-osmotic effects within a network of pores. In particular, it would be interesting to carry out fluid dynamic calculations, finding out the extent to which electro-osmotic effects, induced by streaming potential, might cause local flows within nanopores to be in the opposite direction from applied external pressure. The results of a related study suggest that such work could yield interesting findings (Erickson and Li 2001).

Another type of needed research concerns the conductivity of fibers. Though the word “surface” has been used to describe the type of fiber conductivity that affects streaming potential results (Ghosh and Pal 1961), the work cited in the present review points in the direction of a three-dimensional effect. On the one hand, one may picture a

set of bound carboxylic acid groups existing within a "gel" of hemicellulose or oxidized cellulose chains that fill the volume beneath an outermost surface of the fibers (Goring and Mason 1950b; Pelton 1993). Alternatively, one may picture those same kinds of charges existing on exposed surfaces within nanopores or larger pores (Stone and Scallan 1968; Alince and van de Ven 1997). Since it has been found that some of the intermediate to larger pores within cell walls tend to close preferentially when chemically pulped cellulosic fibers are dried (Stone and Scallan 1966), it would make sense to measure the effects of drying on subsequent fiber conductivity, when the fibers are rewetted. Similar experiments could be carried out on fibers that have been "polished" by mild enzymatic treatment (Jackson et al. 1993; Özdil et al. 2003; Buschle-Diller et al. 2005).

## Fiber Modification

Electrokinetic measurements also have potential for monitoring and control of certain chemical modifications of fibers. In particular, it is well known that cellulosic fibers can be treated to increase their negative charge (Waleka 1956; Lindström and Carlsson, 1982; Roberts and Tatham, 1992; Laine 1997; Hubbe et al. 1999). In principle, such strategies have the potential to make the fibers more receptive to cationic starch (Marton and Marton 1976), ketene dimer size formulations (Isogai et al. 1997), and other cationic additives. Curing reactions of polyamidoamine-epichlorohydrin type wet-strength agents, in particular, are expected to be enhanced if the fibers are first treated to increase their carboxyl group content (Wågberg and Björklund 1993; Espy 1994, 1995; Saito and Isogai 2005). In addition, it has been shown that an enhanced negative charge of fiber surfaces can promote inter-fiber bonding (Walecka 1956; Didwania 1969; Roberts and Tatham, 1992), even without the addition of cationic polyelectrolytes.

It would make sense to carry out streaming potential titrations in the absence of salt in order to evaluate the net charge of the outermost surfaces of fibers, which had been treated in different ways to vary their negative character. By selection of treatment conditions it is possible to direct fiber modification reactions either to the bulk of the fiber cell wall or just to the outer exposed surface. For example, by suspending fibers in a non-swelling solvent, chemical derivatization reactions of cellulose mainly are excluded from the bulk of the fiber (Ehrnrooth et al. 1977). By contrast, similar reactions in the presence of a swelling solvent resulted in a pervasive reaction, throughout the cell wall thickness. In the case of aqueous phase fiber treatments, the location of chemical reaction depends on the fiber's history of pulping and drying (Barzyk et al. 1997). Because a large proportion of the pores in the kraft pulp fiber tend to close irreversibly during drying (Stone and Scallan 1966), the penetration of various reagents tends to be limited. Thus, when recycled kraft fibers are chemically treated in the aqueous phase, the effects are expected to be more surface-specific, compared to never-dried fibers. A number of studies have shown advantages, such as combinations of increased bonding strength and relatively low apparent density resulting from such treatments (Barzyk et al. 1997). Streaming potential tests at contrasting salt levels, as described earlier in this article could be a powerful way to determine whether the effects of fiber modification mainly affect just the outsides of the fibers, or whether the effects of treatment have permeated the nanoporous structure of the cell wall.

## Online Control

Automation has become almost an expectation when electrokinetic tests are to be applied to modern industrial processes. In the case of papermaking, it would seem that significant information can be obtained by at least three classes of electrokinetic tests, as follows:

**Ionic demand:** Streaming current titrations, and their automated applications, were already described earlier in this review (see also Hubbe and Chen 2004). Such tests appear to give reliable information about the cationic demand of various aqueous samples that contain dissolved polyelectrolytes and colloidal substances. Despite the fact that some of the online streaming current titrating systems can employ automatic filtration of fiber slurries, there does not appear to be any publication describing online titration of whole furnish suspensions. To carry out such an analysis, all that is needed is to pretreat the fiber suspension with an excess of polyelectrolyte, filter the suspension, and then back-titrate an aliquot of the filtrate with polyelectrolyte solution of the opposite charge.

**Zeta potential of fines:** Micro-electrophoresis tests of fiber fines and other particulate materials tend to work well in a laboratory setting, but there may be a considerable challenge to apply such tests online in paper mills. Automation itself is not the main problem, since laser-Doppler technology can be used to obtain distributions of particle velocities almost instantaneously (Sanders 1994; Leiviskää et al. 2005). The contamination of capillary cells is a much more serious issue, especially when one considers the high levels of dissolved and colloidal materials present in typical paper machine process waters. However, because the zeta potential of fine materials taken from a paper machine system can change rapidly upon storage (Strazdins 1995), there is a great need for online testing. A resurgence in zeta potential analysis of fines would appear to be very likely, dependent on development of truly robust micro-electrophoresis equipment, not dependent on the use of capillary cells.

**Streaming potential of fibers:** As has already been described, it would appear that fiber-pad streaming potential tests can offer considerable advantages in terms of online monitoring and control of outcomes related to the fiber surfaces. The measurements are direct, meaning that the electrokinetic events one is detecting take place immediately on the fiber surfaces that comprise the greatest part of the mass of dry paper.

Modern automation methods make it increasingly reasonable to consider a wide range of analytical procedures, not limited to conventional titrations or straightforward measurements of streaming potential. For instance, one could envision a device that automatically carries out streaming potential titrations at two or more defined levels of solution conductivity. Laboratory results from this kind of testing suggest that the low-conductivity tests would provide a much more accurate means of optimizing paper machine systems to maximize drainage rates and improve the efficiency of retention (Hubbe et al. 2006a). In adopting such a practice, papermakers would be asked to violate a long-standing taboo against carrying out tests in deionized water. For instance, it does not make sense to evaluate retention aids or other wet-end additives in the absence of salt, since such experimental results are less likely to predict the performance of those additives on the paper machine, where salts are present. But in cases where one is

primarily interested optimizing the performance of a paper machine, there is an incentive to try something unconventional. The required procedure requires initial filtration of fiber slurry samples, followed by dilution with salt-free solution, followed by evaluation of streaming potential. By this means the influence of subsurface nanopores can be selectively excluded, making it possible to sense charge effects due to the outermost surfaces of fibers.

## REFERENCES CITED

Alince, B. (2002). "Porosity of swollen pulp fibers revisited," *Nordic Pulp Paper Res. J.* 17(1), 71-73.

Alince, B., and van de Ven, T. G. M. (1997). "Porosity of swollen pulp fibers evaluated by polymer adsorption," in C. F. Baker, ed., *The Fundamentals of Papermaking Materials,* Pira Intl., Leatherhead, Surrey, UK, 771-788.

Alkafeef, S., Gochin, R. J., and Smith A. L. (2001). "The effect of double layer overlap on measured streaming currents for toluene flowing through sandstone cores," *Colloids Surf. A* 195(1-3), 77-80.

Anderson, J. L., and Koh, W. (1979). "Electrokinetic parameters for capillaries of different geometries," *J. Colloid Interface Sci.* 59(1), 149-158.

Baker, C. F. (1995). "Good practice for refining the types of fiber found in modern paper furnishes," *Tappi J*. 78(2), 147-153.

Ball, B., and Fuerstenau, D. W. (1973). "A review of the measurement of streaming potentials," *Miner. Sci. Eng.* 5(4), 267-277.

Balodis, V. (1967). "Electrokinetic properties of fiber surfaces," *Appita* 21(3), 96-103.

Barron, W., Murray, B. S., Scales, P. J., Healy, T. W., Dixon, D. R., and Pascoe, M. (1994). "The streaming current detector: A comparison with conventional electrokinetic techniques," *Colloids Surf. A*. 88(2-3), 129-139.

Barzyk, D., Page, D. H., and Ragauskas, A. (1997). "Acidic group topochemistry and fiber-to-fiber specific bond strength," *J. Pulp Paper Sci.* 23(2), J59-J61.

Bauer, D., Killmann, E., and Jaeger, W. (1998). "Flocculation and stabilization of colloidal silica by the adsorption of poly-diallyl-dimethyl-ammonium chlroide (PDADMAC) and of copolymers of DADMAC with *N*-methyl-*N*-vinyl-acetamide (NMVA)," *Colloid Polym. Sci.* 276(8), 698-708.

Baumgartner, H., and Bley, L. (1994). "Practical experiences with online charge titration on a paper machine," *Wochbl. Papierfabr.* 122(22), 894-898.

Beck, M. W. (1998). "Wet-end chemistry and paper machine stability," *Paper Technol.* 39(3), 33-38.

Berger, R., Renaud, S., and Waters, K. (2002). "Applying automatic chemical control from stock prep to the machine," *Appita J.* 55(3), 197-201.

Bernabé, Y. (1998). "Streaming potential in heterogeneous networks," *J. Geophys Res*. 103(B9), 20,827-20,841.

Berthold, J., and Salmén, L. (1997). "Effects of mechanical and chemical treatments on the pore-size distribution in wood pulps examined by inverse size-exclusion chromatography (ISEC)," *J. Pulp Paper Sci.* 23(6), J245-253.

Bley, L. (1992). "Measuring the concentration of anionic trash – the PCD," *Paper Technol.* 33(4), 32-37.

Bley, L., and Bischof, P. (1994). "Practical experiences with on-line charge titration in paper mills," *Wochenbl. Papierfabr.* 122(22), 894-898.

Bley, L., and Kästner, M. (1992). "On-line process charge control in papermaking," in *The Chemistry of Papermaking*, *Proc. PIRA Conf.,* Jan. 28-29, 1992, paper 17.

Bley, L., and Winter, E. (1997). "On-line charge monitoring – a wet-end strategy," *Proc. 1997 Engineering & Papermakers Conf.*, 297-301.

Briggs, D. R. (1928). "The determination of the zeta potential on cellulose – A Method," *J. Phys. Chem.* 33(5), 641-675.

Brouwer, P. H. (1991). "The relationship between zeta potential and ionic demand and how it affects wet-end retention," *Tappi J.* 74(1), 170-179.

Bull, H. B. (1934). "Electrokinetics. 14. A critical comparison of electrophoresis, streaming potential, and electrosmosis," *J. Phys. Chem.* 30(5), 577-583.

Buschle-Diller, G., Inglesby, M. K., and Wu, Y. (2005). "Physicochemical properties of chemically and enzymatically modified cellulosic surfaces," *Colloids Surf. A.* 260(1-3), 63-70.

Bygrave, G. and Englezos, P. (1998). "Fiber charge from potentiometric titration of kraft pulp and Donnan equilibrium theory," *Nordic Pulp Paper Res. J.* 13(3), 220-224.

Cardwell, P. H. (1966). "Adsorption studies using a streaming current detector," *J. Colloid Interface Sci.* 22(5), 430-437.

Carrasco, T., Mutjé, P., and Pelach, M. A. (1998). "Control of retention in paper-making by colloid titration and zeta potential techniques," *Wood Sci. Technol.* 32(2), 145-155.

Chang, M. Y. and Robertson, A. A. (1967a). "Zeta potential measurements of fibers. D-C. streaming current method," *Can. J. Chem. Eng*. 45(2), 66-71.

Chang, M. Y., and Robertson, A. A. (1967b). "Flocculation studies of fiber suspensions: Influence of zeta potential," *Pulp Paper Mag. Can.* 68(9), T438-T444.

Chen, J., Hubbe, M. A., and Heitmann, J. A. (2001). "Measurement of colloidal charge in the paper mill by streaming current," *TAPPI 2001 Papermakers Conf.,* electronic document, TAPPI Press, Atlanta.

Chen, J., Heitmann, J. A., and Hubbe, M. A. (2003). "Dependency of polyelectrolyte complex stoichiometry on the order of addition. 1. Effect of salt concentration during streaming current titrations with strong poly-acid and poly-base," *Colloids Surf. A* 223(1-3), 215-230.

Chen, S.-L., Wang, S., and Lucia, L. A. (2004). "New insights into the fundamental nature of lignocellulosic fiber surface charge," *J. Colloid Interface Sci*. 275(2), 392-397.

Chowdiah, P., Wasan, D. T., and Gidaspow, D. (1983). "On the interpretation of streaming potential data in nonaqueous media," *Colloids Surf. A* 7(4), 291-299.

Crill, M. (1991). "Fiber charge analyzers monitor furnish to optimize production," *Pulp Paper* 65(11), 92-95.

Davison, R. W., and Cates, R. E. (1975). "Electrokinetic effects in papermaking systems: Theory and practice," *Paper Technol. Ind.* 16(4), 107-144.

Denbrok, C., and Peacock, B. (1999). "Wet-end charge measurement using streaming current online titrators," *Tappi J.* 82(10), 57-59.

Dentel S. K., and Kingery, K. M. (1989). "Theoretical principles of streaming current detection," *Water Sci. Tech.* 21, 443-453.

Didwania, H. P. (1969). "The mechanism of strength increases in paper on hydroxyehtylation of fibers," *Tappi* 52(8), 1511-1517.

Eagland, D., and Allen, A. P. (1977). "The influence of hydration upon the potential at the shear plane (zeta potential) of a hydrophobic surface in the presence of various electrolytes," *J. Colloid Interface Sci* 58(2), 230-241.

Ehrnrooth, E., Htun, M., and de Ruvo, A. (1977). "Esterification as a means of improving the properties of once-dried fibers," in *Fiber-Water Interactions in Papermaking*, Trans. Symp. Oxford, 899-911.

Eriksson, R. (1987). "Method and apparatus for measuring the streaming potential of a particle suspension," *U.S. Pat. 4,687,986*.

Erickson, D., and Li, D. Q. (2001). "Streaming potential and streaming current methods for characterizing heterogeneous solid surfaces," *J. Colloid Interface Sci.* 237(2), 283-289.

Espy, H. H. (1994). "Alkaline-curing polymeric amine-epichlorohydrin resins," in *Wet-Strength Resins and their Application*, L. L. Chan, ed., TAPPI Press, Atlanta, Ch. 2, 13-44.

Espy, H. H. (1995). "The mechanism of wet-strength development in paper. A review," *Tappi J.* 78(4), 90-99.

Evans, B. E., Hemm, S. R., and Russell, R. A. V. (1985). "Apparatus for determining an electrical characteristic of a fibrous dispersion," *U.S. Pat 4,535,285*.

Fält, S., and Wågberg, L. (2003). "Influence of electrolytes on the swelling and strength of kraft-liner pulps," *Nordic Pulp Paper Res. J.* 18(1), 69-73.

Fardim, P., and Holmbom, B. (2003). "Fast determination of anionic groups in different pulp fibers by methylene blue sorption," *Tappi J.* 2(10), 28-32.

Fardim, P., and Holmbom, B. (2005). "Origin and surface distribution of anionic groups in different papermaking fibers," *Colloids Surf. A* 252, 237-242.

Farley, C. E. (1992). "Microelectrophoresis as a wet end chemistry teaching tool," *Proc. TAPPI 1992 Papermakers Conf.*, TAPPI Press, Atlanta, 509-512.

Farley, C. E. (1997). "Factors influencing the rate of charge decay," *Tappi J. 80* (10), 177-183.

Fleer, G. J., Cohen Stuart, M. A., Scheutjens, J. M. H. M., Cosgrove, T., and Vincent, B., eds. (1993). *Polymers at Interfaces,* Chapman and Hall, London, ISBN 0412581604, 343-375.

Forsström, J., Andreasson, B., and Wågberg, L. (2005). "Influence of pore structure and water retaining ability of fibers on the strength of papers from unbleached kraft fibers," *Nordic Pulp Paper Res. J.* 20(2), 176-185.

Fuerstenau, D. W. (1956). "Measuring zeta potentials by streaming potential techniques," *Trans. AIME* 205, 834-835.

Gerdes, W. F. (1966). "A new instrument – The streaming current detector," *12th Natl. ISA Analysis Inst. Symp.*, Houston, TX, 181-198.

Ghosh, B. N., and Pal, P.K. (1961). "Evaluation of zeta potential with correction of cell constant and surface conductance," *Trans. Faraday Soc.* 57, 116-122.

Goring, D. A. I., and Mason, S. G. (1950a). "Electrokinetic properties of cellulose fibers. 1. Stream potential and electro-osmosis," *Can. J. Res. B* 28(6), 307-322.

Goring, D. A. I., and Mason, S. G. (1950b). "Electrokinetic properties of cellulose fibers. 2. Zeta-potential measurements by the stream-compression method," *Can. J. Res. B* 28(6), 323-338.

Goulet, M. T., and Stratton, R. A. (1990). "The effect of pulping, bleaching, and refining operations on the electrokinetic properties of wood fines," *Nordic Pulp Paper Res. J.* 5(3), 118-125.

Gratton, R., and Pruszynski, P. (1995). "Cationic demand survey of paper machines: how can we measure and use it," *Pulp Paper Can.* 93(3), T103-T108.

Grignon, J., and Scallan, A. M. (1980). "Effect of pH and neutral salts upon the swelling of cellulose gels." *J. Appl Polymer Sci.* 25(12), 2829-2843.

Gruber, E., Grossmann, K., and Schempp, W. (1996). "Interactions of synthetic cationic polymers with fibers and fillers; influence on adsorption," *Wochenbl. Papierfabr.* 124(1), 4-11.

Halabisky, D. D. (1977). "Wet end control for effective use of cationic starch," *Tappi* 60(12), 125-127.

Hand, V., Koethe, J., Kuchibhotla, S., and Scott, W. (1993). "An evaluation of two pad-forming paper stock charge analyzers," *Papermakers Conf. Proc.*, TAPPI Press, Atlanta, 591-598.

Helmholtz, H. (1879). "Study Concerning Electrical Boundary Layers," *Weidemann Annalen der Physik u. Chemie*, 3rd Ser., 7, 337-382.

Herrington, T. M., and Petzold, J. C. (1992). "An investigation into the nature of charge on the surface of papermaking woodpulps. 1. Charge/pH isotherms," *Colloids Surf.* 64(2), 97-108.

Hidalgo-Alverez, R. (1991). "On the conversion of experimental electrokinetic data into double-layer characteristics in solid-liquid interfaces," *Adv. Colloid Interface Sci.* 34, 217-341.

Hiemenz, P.C., and Rajagopalan, R. (1997). *Principles of Colloid and Surface Science*, 3rd Ed., Dekker, New York.

Hoffman, F., Müller, F., Rohloff, E., and Tretter, H. (1975). "Experiences with the streaming potential method for retention and wet strength problems," *Papier* 29(12), 529-534.

Hoogeveen, N. G., Cohen Stuart, M. A., Fleer, G. J., and Böhmer, M. R. (1996). "Formation and stability of multilayers of polyelectrolytes," *Langmuir* 12(15), 3675-3681.

Horn, D., and Linhart, F. (1991). "Retention Aids," in *Paper Chemistry*, J. Roberts (ed.), Blackie, Glasgow, Chapter 4, 44-62.

Horn, D., and Melzer, J. (1975). "The effect of high molecular cationic dewatering aids on the electrokinetic properties of pulp," *Papier* 29(12), 534-541.

Horvath, A. E., Lindström, T., and Laine, J. (2006). "On the indirect polyelectrolyte titration of cellulosic fibers. Conditions for charge stoichiometry and comparison with ESCA," *Langmuir* 22(2), 824-830.

Hostetler, R. E., and Swanson, J. W. (1974). "Diffusion into and adsorption of polyethylenimine on porous silica gel," *J. Poly. Sci.* 12(1), 29-43.

Hubbe, M. A. (1979). "A modified reporting procedure for polyelectrolyte titrations," *Tappi* 62(8),120-121.

Hubbe, M. A. (1999). "Method and apparatus for measuring an electrical property of papermaking furnish," *U.S. Patent* 5,936,151.

Hubbe, M. A. (2000). "Selecting and interpreting colloidal charge measurements," in *Proc. Scientific & Technical Advances in Wet End Chemistry,* Barcelona, Spain, PIRA Intl., Leatherhead, UK.

Hubbe, M. A., and Chen, J. (2004). "Charge-related measurements – A reappraisal. Part 1. Streaming current," *Paper Technol.* 45(8), 17-23.

Hubbe, M. A., Rojas, O. J., Lee, S. Y., Park, S., and Wang, Y. (2006b). "Distinctive electrokinetic behavior of nanoporous silica particles treated with cationic polyelectrolyte," *Colloids Surf. A.*, accepted.

Hubbe, M. A., Rojas, O. J., Lucian, L. A., and Jung, T. M. (2006a). "Consequences of the nanoporosity of cellulosic fibers on their streaming potential and their interactions with cationic polyelectrolytes," *Cellulose*, submitted.

Hubbe, M. A., Wagle, D. G., and Ruckel, E. R. (1999). "Method for increasing the strength of a paper or paperboard product," *U. S. Pat.* 5,958,180.

Hubbe, M. A. and Wang, F. (2004). "Charge-related measurements – A reappraisal. Part 2: Fiber-pad streaming potential," *Paper Technol*. 45(9), 27-34.

Hunter, R. J. (1981). *Zeta Potential in Colloid Science*, Academic Press, London.

Hunter, R. J. (1987). *Foundations of Colloid Science*, Clarendon Press, Oxford, UK.

Isogai, A., Kitaoka, C., and Onabe, F. (1997). "Effects of carboxyl groups in pulp on retention of alkylketene dimer," *J. Pulp Paper Res. J.* 23(5), J215-J219.

Jackson, L. S., Heitmann, J. A., and Joyce, T. W. (1993). "Enzymatic modifications of secondary fiber," *Tappi J.* 76(3), 147-154.

Jacquelin, G., and Bourlas, H. (1964). "Measurement of the zeta potential of papermaking fibers. Effects of preliminary treatments," *Techniques et Recherches Papeteries* 3, 49-58.

Jain, L., Bharati, S., and Bharati, R. (1993). "Electrochemical potential in papermaking: significance and an instrument for measurement," *IPPTA* 5(3), 39-47.

Jaycock, M. J. (1995). "Assumptions made in the measurement of zeta-potential by streaming current/potential detectors," *Paper Technol.* 36(3), 35-38.

Jaycock, M. J., and Pearson, J. L. (1976). "Study of the retention of pigment during paper formation," *J. Colloid Interface Sci.* 55(1), 181-190.

Jones, I. S. (1979). "A theory of electrophoresis of large colloid particles with adsorbed polyelectrolyte," *J. Colloid Interface Sci.* 68(3), 451-461.

Kaunonen, A. (1989). "Online retention and cationic demand measurements and their utilization on a paper machine," *Paperi Puu* 71(1), 46-52.

Kaunonen, A., and Springer, A. M. (1988). "Evaluation of piston type streaming current detector for pulp and paper applications," *TAPPI Papermakers Conf. Proc.*, TAPPI Press, Atlanta, 225-230.

Kenaga, D. L., Kindler, W. A., and Meyer, F. J. (1967). "Studies of adsorption of cationic polyelectrolytes on pulp using streaming current detection," *Tappi* 50(7), 381-387.

Koethe, J. L., and Scott, W. E. (1993). "Polyelectrolyte interactions with papermaking fibers: The mechanism of surface-charge decay," *Tappi J.* 76(12), 123-133.

Koljonen, K., Mustaranta, A., and Stenius, P. (2004). "Surface characterization of mechanical pulps by polyelectrolyte adsorption," *Nordic Pulp Paper Res. J.* 19(4), 495-505.

Korpi, G. K., and De Bruyn, P. L. (1972). "Measurement of streaming potentials," *J. Colloid Interface Sci.* 40(2), 263-266.

Kumar, V., Jain, L., Bharati, R., Bharati, S., and Agrawal, S. K. (1991). "Application of zeta potential for retention optimization of bagasse pulp," *IPPTA* 3(3), 46-49.

Laine, J. (1997). "Effect of ECF and TCF bleaching on the charge properties of kraft pulp," *Paperi Puu* 79(8), 551-559.

Laine, J., Buchert, J., Viikari, L., and Stenius, P. (1996). "Characterization of unbleached kraft pulps by enzymatic treatment, potentiometric titration and polyelectrolyte adsorption," *Holzforschung* 50(3), 208-214.

Laine, J., and Stenius, P. (1997). "Effect of charge on the fiber and paper properties of bleached industrial kraft pulps," *Paperi Puu* 79(4), 257-266.

Leiviskä, T., Rämö, J., Sillanpää, M., and Vuorenpalo, V.-M. (2005). "Multimodal zeta potential distributions in chemical pulp bleaching filtrates," *J. Pulp Paper Sci.* 31(4), 167-174.

Li, H. C., and De Bruyn, P. L. (1966). "Electrokinetic and adsorption studies on quartz," *Surf. Sci.* 5(2), 203-220.

Lindgren, H., Öhman, L.-O., Gunnars, S., and Wågberg, L. (2002.) "Charge determinations of cellulose fibers of different origin – Comparison between different methods," *Nordic Pulp Paper Res. J.* 17(1), 89-96.

Lindström, T. (1992). "Chemical factors affecting the behavior of fibers during papermaking." *Nordic Pulp Paper Res. J.* 7(4), 181-192.

Lindström, T. (1996). "Electrokinetics of the papermaking industry," in J. C. Roberts, ed., *Paper Chemistry*, 2nd Ed., Blackie Academic, New York, Ch. 3, 25-43.

Lindström, T., and Carlsson, G. (1982). "The effect of carboxyl groups and their ionic form during drying on the hornification of cellulose fibers," *Svensk Papperstidn.* 85(11), R146-R151.

Lindström, T., and Söremark, C. (1975). "Zeta potential measurement during paper manufacture," *Papier* 29(12), 519-525.

Lindström, T., and Söremark, C. (1976). "Adsorption of cationic polyacrylamides on cellulose," *J. Colloid Interface Sci.* 55(2), 305-312.

Lindström, T., Söremark, C., Heinegård, C., and Martin-Löf, S. (1974). "The importance of electrokinetic properties of wood fiber for papermaking," *Tappi* 57(12), 94-96.

Lloyd, J. A., and Horne, C. W. (1993). "The determination of fiber charge and acidic groups of radiata pine pulps," *Nordic Pulp Paper Res. J.* 8(1), 48-67.

Marlow, B. J., Fairhurst, D., and Pendse, H. P. (1988). "Colloid vibration potential and the electrokinetic characterization of concentrated colloids," *Langmuir* 4(3), 611-626.

Marton, J. (1980). "Role of surface chemistry in fines-alum interactions," *Tappi J.* 63(2), 121-125.
Marton, J., and Marton, T. (1976). "Wet end starch: Adsorption of starch on cellulosic fibers," *Tappi* 59(12), 121-124.
Mathews, J., Gustafson, R. and Hodgson, K. (2004). "A method to determine the charge demand of single pulp fibers," *Nordic Pulp Paper Res. J.* 19(4), 453-459.
McKague, J. F., Etter, D. O., Pilgrim, J. O., and Griggs, W. H. (1974). "Practical applications of the electrokinetics of papermaking," *Tappi* 57(12), 101-103.
Melzer, J. (1972). "Zeta potential and its importance in the manufacture of paper," *Papier* 26(7), 305-332.
Miller, N. P., Berg, J. C., and O'Brien, R. W. (1992). "The electrophoretic mobility of a porous aggregate," *J. Colloid Interface Sci*. 153(1), 237-243.
Miyanishi, T. (1995). "On-line zeta potential analyses of a fine paper machine and a newsprint paper machine," *Tappi J.* 78(3), 85-91.
Miyanishi, T. (1995). "Effects of zeta potential on flocculation measurement in microparticle systems," *Tappi J.* 78(11), 135-150.
Miyanishi, T. (1999). "Wet end optimization for a neutral PCC filled newsprint machine," *Tappi J.* 82(1), 220-225.
Miyanishi, T., and Montegi, S. (1996). "Optimization of flocculation and drainage for microparticle systems by zeta potential control," *Proc. 1996 Papermakers Conf.*, TAPPI Press, Atlanta, 73-82.
Miyanishi, T., and Shigeru, M. (1997). "Optimizing flocculation and drainage for microparticle systems by controlling zeta potential," *Tappi J.* 80(1), 262-270.
Mocchiutti, P. and Zanuttini, M. A. (2005). "A useful equation for estimating the surface charge of pulp fibers," *Tappi J.* 4(5), 18-22.
Müller, R. H. (1996). *Zetapotential und Partikelladung in der Laborpraxis,* Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, see pp. 104-108.
Nazir, B. A. (1994). "An on-line streaming potential meter (SPM) – Wet-end applications," *Paper Technol. Ind.* 35(3), 28-35.
Neale, S. M. (1946). "The electrical double layer, the electrokinetic potential, and the streaming current," *Trans. Faraday Soc.* 42, 473-487.
O'Brien, R. W., and White, L. R. (1978). "Electrophoretic mobility of a spherical colloidal particle," *J. Chem. Soc. Faraday Trans II* 74(9), 1607-1626.
Öhman, L.-O., Wågberg, L., Malmgren, K., and Tjernström, A. (1997). "Adsorption of aluminum (III) on cellulosic fibers in neutral to alkaline solutions – Influence of charge and size of the particles formed," *J. Pulp Paper Sci.* 23(10), J467-J474.
Ohshima, H. (1997). "Electrophoretic mobility of a polyelectrolyte-adsorbed particle: Effect of segment density distribution," *J. Colloid Interface Sci.* 185(1), 269-273.
Ojala, T. (1993). "Charge measurements of different furnishes using polyelectrolyte titration with a streaming current detector," *TAPPI Papermakers Conf.*, 613-626.
Özdil, N., Özdoğan, E., and Öktoem, T. (2003). "Effects of enzymatic treatment on various spun yarn fabrics," *Fibres Textiles in Eastern Europe* 11(4), 58-61.
Padovani, E., and Colasurdo, A. R. (1995). "Online real-time measurement reduces wet-end variability," *Pulp Paper* 69(4), 57-62.

Pang, L., and Gray, D. G. "Heterogeneous fibrillation of kraft pulp fiber surfaces observed by atomic force microscopy," *J. Pulp Paper Sci.* 24(11), 369-372 (1998).

Pelton, R. (1993). "A model of the external surface of wood pulp fibers," *Nordic Pulp Paper Res. J.* 8(1), 113-119.

Pelton, R., Miller, P., McPhee, W., and Rajaram, S. (1993). "Strategies for improving electrophoresis data from the Coulter DELSA," *Colloids Surf. A*. 80(2/3), 181-189.

Penniman, J. G. (1991). "A method and apparatus for measuring an electrical characteristic of a fibrous dispersion," *Eur. Pat. Appl. 91304556.3*.

Penniman, J. G. (1992). "Comparison of pulp pad streaming potential measurement and mobility measurement," *Tappi J.* 75(8), 111-115.

Penniman, J. G. (1994). "On-line monitoring of micro-particulate papermaking process chemistry," *Proc. TAPPI 1994 Papermakers Conf.*, 229-236.

Petzold, J. C., and Allen, R. A. (1996). "Streaming potential measuring cell," *U.S. Patent* 5,495,751.

Phipps, J. S. (1999). "Some mechanistic insights for using the streaming current detector to measure wet-end charge," *Tappi J.* 82(8), 157-165.

Poppel, E. (1992). "The meaning of zeta potential in the wet end of paper manufacture and the possibilities for optimization by means of electrokinetic control," *Wochenbl. Papierfabr.* 120(14), 565-571.

Pursiheimo, P., and Thomas, S. (1998). "Controlling wet end chemistry based on the WIC system," *Proc. Measurement and Control of Papermaking*, Pira International, Leatherhead, Surrey, UK, Paper 15.

Quincke, G. (1859). "Concerning a new type of electrical current," *Annalen der Physic und Chemie (Poggendorff's Annal., Ser. 2)* 107, 1-47.

Räsänen, E., and Stenius, P. (1997). "The sorption of $Na^{+}$, $Ca^{++}$, $Mg^{++}$, and $Mn^{++}$ on cellulose fibers: Prediction and analysis of sorption equilibrium with electrostatic models," *Proc. ISWPC,* Paper 94, 1-5.

Räsänen, E., Stenius, P., and Tervola, P. (2001). "Model describing Donnan equilibrium, pH and complexation equilibria in fiber suspensions," *Nordic Pulp Paper Res. J.* 16(2), 130-139.

Revil, A., Pezard, P. A., and Glover, P. W. J. (1999). "Streaming Potential in Porous Media. 1. Theory of the Zeta Potential," *J. Geophys. Res.* 104(B9), 20021-20031.

Richter, R., Rohloff, E., Tretter, H., Müller, F., and Penniman, J. G. (1989). "A new instrument for automatic streaming potential measurements on the paper machine," *Wochbl. Papierfabr.* 117(15), 682-686.

Ricq, L., Pierre, A., Reggiani, J.-C., Pagetti, J., and Foissy, A. (1998). "Use of electrophoretic mobility and streaming potential measurements to characterize electrokinetic properties of ultrafiltration and microfiltration membranes," *Colloids Surf. A* 138(2-3), 301-308.

Roberts, J. C., and Tatham, J. (1992). "Succinylation of pulps," in *The Chemistry of Papermaking*, paper 08, Pira Int'l., Leatherhead, Surrey, UK.

Rojas, O. J., Claesson, P. M., Muller, D., and Neuman, R. D. (1998). "The effect of salt concentration on adsorption of low-charge-density polyelectrolytes and interactions between polyelectrolyte-coated surfaces," *J. Colloid Interface Sci*. 205(1), 77-88.

Rojas, O. J., Ernstsson, M., Neuman, R. D., and Claesson, P. M. (2000). "X-ray photoelectron spectroscopy in the study of polyelectrolyte adsorption on mica and cellulose," *J. Phys. Chem. B* 104(43), 10032-10042.

Rojas, O. J., and Neuman, R. D. (1999). "Adsorption of polysaccharide wet-end additives in papermaking systems," *Colloids Surf. A* 155(2-3), 419-432.

Rohloff, E., and Höschle, O. (1993). "Zeta potential experiences with laboratory and on-line measurements," *Wochenbl. Papierfabr.* 121(23-24), 990-994.

Rundlöf, M., Htun, M., Höglund, H., and Wågberg, L. (2000). "Mechanical pulp fines of poor quality – Characteristics and influence of white water," *J. Pulp Paper Sci.* 26(9), 308-316.

Sack, W. (1976). "Continuous measurement of streaming potential on a paper machine," *Papier* 30(10A), V42-V46.

Sack, W., Storbeck, W., and Winiker, R. (1993). "Ten years of research experience with continuous zeta potential measurements for paper machine process control," *Wochbl. Papierfabr.* 121(19), 803-805.

Saito, T., and Isogai, A. (2005). "A novel method to improve wet strength of paper," *Tappi J.* 4(3), 3-8.

Sanders, N. D. (1994). "The occurrence and importance of polymodal electrophoretic mobility distributions," in B. M. Moudgil and P. Somasundaran, eds., *Dispersion Aggregation, Proc. Eng. Found. Conf.* 1992, 183-190.

Scales, P. J., Grieser, F., Healy, T. W., White, L. R., and Chan, D. Y. C. (1992). "Electrokinetics of the silica-solution interface: A flat plate streaming potential study," *Langmuir* 8(3), 965-974.

Scallan, A. M. (1983). "The effect of acidic groups on the swelling of pulps: A review," *Tappi J.* 66(11), 73-75.

Scallan, A. M. (1989). "The electrical conductance of pulp suspensions," *Tappi J.* 72(11), 157-162.

Schurz, J., Ribitsch, V., and Jacobasch, H.-J. (1989). "Zeta potential as a means of characterizing surfaces," *Wochenbl. Papierfabr.* 117(11/12), 525-529.

Schwarz, S., Buschhammer, H.-M., Lunkwitz, K., and Jacobasch, H.-J. (1998). "Polyelectrolyte adsorption on charged surfaces: Study by electrokinetic measurements," *Colloids Surf. A* 140(1-3), 377-384.

Sennett, P., and Olivier, J. P. (1965). "Colloidal dispersion, electrokinetic effects and the concept of zeta potential," *Indus. Eng. Chem.* 57(8), 33-50.

Sezaki, T., Hubbe, M. A., Heitmann, J. A., Argyropoulos, D. S. (2006). "Colloidal effects of acrylamide polyampholytes. Part 2. Adsorption onto cellulosic fibers," *Colloids and Surfaces A*, in print.

Smith, D. C. (1992). "Chemical additives for improved compression strength of unbleached board," *Proc. TAPPI 1992 Papermakers Conf.*, TAPPI Press, Atlanta, 393-404.

Spence, G. G., Underwood, R. T., and Yarnell, J. R. (1997). "A titration procedure for determining the level of anionic impurities in a pulp furnish," *PaperAge* 113(7), 30-31.

Springer, A. M., and Taggart, T. E. (1986). "Quantitative evaluation of alternative methods of determining total charge," *Tappi J.* 69(5), 116-121.

St. John, M. R., and Gallagher, T. M. (1992). "Evaluation of the charge state of papermachine systems using the charge titration method," *Proc. 1992 TAPPI Papermakers Conf.*, TAPPI Press, Atlanta, 479-502.

Stark, H., and Eichinger, R. (1989). "Effect of zeta potential on the adsorption tendency and degree of organization of fiber suspensions," *Wochenbl. Papeirfabr.* 117(11/12), 521-524.

Stitt, J. B. (1998). "Charge control helps tissue producers achieve quality, productivity benefits," *Pulp Paper* 72(5), 109-114.

Stone, J. E., and Scallan, A. M. (1966). "Influence of drying on the pore structures of the cell wall," in R, Bolam, ed., *Consolidation of the Paper Web,* Vol. 1, British Paper & Board Makers Assoc., 145-174.

Stone, J. E., and Scallan, A. M. (1968). "A structural model of the cell wall of water-swollen wood pulp fibers based on their accessibility to macromolecules," *Cellulose Chem. Technol*. 2(3), 343-358.

Stratton, R. A., and Swanson, J. W. (1981). "Electrokinetics in papermaking. A position paper," *Tappi* 64(1), 79-83.

Strazdins, E. (1972). "Factors affecting the electrokinetic properties of cellulose fibers," *Tappi* 22(12), 1691-1695.

Strazdins, E. (1977). "Optimization of the papermaking process by electrophoresis," *Tappi* 60(7), 113-114.

Strazdins, E. (1980). "New approaches towards optimizing dry strength of waste paper containing stock furnishes," *Papier* 34(10A), V49-V55.

Strazdins, E. (1994). "Application of electrokinetics in optimization of wet-end chemistry," in L. L. Chan, ed., *Wet-Strength Resins and their Application*, TAPPI Press, Atlanta, 63-83.

Strazdins, E. (1995). "Critical issues in applying electrokinetics to papermaking," *Tappi J.* 78(8), 115-119.

Sukhorukov, G. B., Donath, E., Lichtenfeld, H., Knippel, E., Knippel, M., Budde, A., and Möhwald, H. (1998). "Layer-by-layer self assembly of polyelectrolytes on colloidal particles," *Colloids Surf. A* 137(1-3), 253-266.

Tanaka, H. (1984). "Studies on wet-end chemistry in papermaking. 2. Some considerations on physicochemical properties of mill white waters," *Japan Tappi* 38(8), 844-850.

Tanaka, H. (1984). "Studies on wet-end chemistry in papermaking. 3. Problems in measurement of zeta potential and characteristics of zeta potential of suspended solids in mill white waters," *Japan Tappi* 38(9), 965-972.

Tanaka, H., Ueda, I., Ichiura, H., and Kitaoka, T. (2000). "Determining cationic demand of suspensions using chromo-PDADMAC," *Proc. Intl. Symp. Environ. Friendly Emerging Technol. Sustainable Pulp Paper Ind.*, TFRI Extension Ser. 115, 68-71.

Tatsumi, D., and Yamauchi, T. (1997). "Depth profile of paper additives within a pulp fiber," in C. F. Baker, ed., *The Fundamentals of Papermaking Materials*, Pira Intl., Leatherhead, UK, 789-814.

Terayama, H. (1952). "Method of colloid titration (A new titration between polymer ions)," *J. Polym. Sci.* 8(2), 243-253.

Thiele, B., and Kopp, J. W. (1997). "Charge balances of paper machine systems - A method of process optimization," *Wochenbl. Papierfabr.* 125(11/12), 542-556.

Tripattharanan, T., Hubbe, M. A., Heitmann, J. A., and Venditti, R. A. (2004). "Effect of idealized flow conditions on retention and performance. Part 2. Polymer bridging, charged patches, and charge neutralization," *Appita J.* 57(6), 448-454.

van de Steeg, T. G. M., Cohen Stuart, M. A., de Keizer, A., and Bijsterbosch, B. H. (1992). "Polyelectrolyte adsorption: A subtle balance of forces," *Langmuir* 8, 2538-2546.

van de Ven, T. G. M. (1999). "Effect of fiber conductivity on zeta potential measurements of pulp fibers," *J. Pulp Paper Sci.* 25(7), 243-245.

Vanderhoek, N. (1994). "Optimizing paper machine performance through electrokinetic measurement," *Appita J.* 47(5), 397-401.

Van Wagenen, R. A., and Andrade, J. D. (1980). "Flat-plate streaming potential investigations - Hydrodynamics and electrokinetic equivalency," *J. Colloid Interface Sci.* 76(2), 305-314.

Varoqui, R. (1982). "Effect of polymer adsorption on the electrophoretic mobility of colloids," *Nouv. J. Chimie* 6(4), 187-189.

Veal, C. R. (1997). "On-line cationic demand measurement for wet end papermaking," *Proc. TAPPI 1997 Engineering Papermakers Conf.*, TAPPI Press, Atlanta, 287-296.

Vernhet, A., Bellon-Fontaine, M. N., and Doren, A. (1994). "Comparison of three electrokinetic methods to determine the zeta potential of solid surfaces," *J. Chim. Phys.* 91(11/12), 1728-1747.

Verwey, E. J. W., and Overbeek, J. Th. G. (1948). *Theory of the Stability of Lyophobic Colloids*, Elsevier, New York.

Wågberg, L., and Björklund, M. "On the mechanism behind wet strength development in papers containing wet strength resins," *Nordic Pulp Paper Res. J.* 8(1), 53-58.

Wågberg, L., and Ödberg, L. (2000). "Polymer adsorption onto cellulose fibers – A review," *Nordic Pulp Paper Res. J.* 15(5), 586-597.

Wågberg, L., Ödberg, L., and Glad-Nordmark, G. (1989). "Charge determination of porous substrates by polyelectrolyte adsorption," *Nordic Pulp Paper Res. J.* 4(2), 71-76.

Walecka, J. A. (1956). "An investigation of low degree of substitution carboxymethyl-celluloses," *Tappi* 39(7), 458-463.

Walker, C. A., Kirby, J. T. and Dentel, S. K. (1996). "The streaming current detector: A quantitative model," *J. Colloid Interface Sci.* 182(1), 71-81.

Wan, Q.-H. (1997). "Effect of electrical double-layer overlap on the electroosmotic flow in packed-capillary columns," *Anal. Chem*. 69(3), 361-363.

Wang, F., and Hubbe, M. A. (2001). "Development and evaluation of an automated streaming potential measurement device," *Colloids and Surfaces A* 194(1-3), 221-232.

Wang, F., and Hubbe, M. A. (2002). "Charge properties of fibers in the paper mill environment. 1. Effect of electrical conductivity," *J. Pulp Paper Science* 28(10), 347-353.

Winters, J. C. (1998). "Introduction to the electrokinetics of papermaking," in J. M. Gess, ed., *Retention of Fines and Fillers during Papermaking*, TAPPI Press, Atlanta, Ch. 3, 29-50.

Article submitted: May 8, 2006; Revision accepted: July 1, 2006; Published: July 26, 2006

# PROSPECTS FOR BIODIESEL AS A BYPRODUCT OF WOOD PULPING – A REVIEW

Sa Yong Lee,[a] Martin A. Hubbe,[a*] and Shiro Saka[b]

Effective utilization of byproducts can affect the profitability of kraft pulping to produce cellulosic fibers from wood. This review considers opportunities to use tall oil components, obtained from kraft pulping, as a source of raw material for biodiesel fuel, or as a source of additives for petrodiesel. Considerable progress has been achieved with respect to converting vegetable oils to diesel fuel, and some of what has been learned appears to have potential application for processing of wood-derived fatty acids and related compounds. Alkaline-catalyzed transesterification strategies, while seemingly well adapted for relatively pure vegetable oil source materials, may not be the best fit for the processing of tall oil fractions. The promising strategies to consider include acid-catalyzed esterification, enzymatic processes, hydrogenation, and the use of supercritical methanol.

*Keywords: Biodiesel, Diesel fuels, Tall oil, Kraft pulping, Transesterification, Esterification, Catalysis, Biorefinery, Extractives*

*Contact information: a: Department of Forest Biomaterials Science and Engineering, North Carolina State University, Box 8005, Raleigh, NC 27695-8005 USA; b: Department of Socio-Environmental Energy Science, Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku 606-8501, Japan; *Corresponding author: hubbe@ncsu.edu*

## INTRODUCTION

### Why Biodiesel from Wood?

Though biodiesel products are most often associated with chemical conversion of vegetable oils and seed oils, there is a powerful incentive to use lignocellulosic materials as an additional source for chemicals and fuels production. Because lignocellulosic materials constitute by far the largest proportion of biomass (Berndes et al. 2003; Kavalov and Peteves 2005; McKay 2006), such materials can be expected to help our society deal with the increasing scarcity of easily extractable petroleum (Berndes et al. 2003; Tester et al. 2005). The relatively low content of triglyceride oils and fatty acids in wood, *e.g.,* about 1-3% (Back and Allen 2000), must be viewed in the context of the huge volume of wood that is efficiently processed each year into pulp to be used in the manufacture of paper, *e.g.,* about 330 million tons worldwide (DeKing 2004). Though about half of the fiber needed for paper production comes from recycling, most of the remainder comes from the pulping of wood. In other words, the huge volumes of throughput can justify further work to maximize the value of various byproducts.

The pulping industry already has evolved a reasonably efficient "biorefinery" process, yielding cellulose in relatively pure form, while incinerating the lignin portion of

the wood to produce energy and recover most of the chemicals that were used to dissolve the lignin (Smook 1992). The burning of the lignin can supply a large proportion of the energy needed to run an integrated pulp and paper facility. In fact, technology is under development that holds promise to make such facilities net producers of electrical energy (Axegard et al. 2002).

Further potential for improving overall profitability often can be found in cases where certain chemical byproducts of pulping have a higher market value, compared to the value of the heat that can be recovered from them by incineration of pulping liquors. A conventional kraft pulp mill produces a byproduct called tall oil, which is a mixture of triglyceride oils, fatty acids, resin acids, other terpenoids, and other related materials (Johansson 1982). Though it is possible to incinerate these materials to recover their energy content, the market provides incentives to use or convert these materials for higher-margin applications. Diesel fuel is one such potential application. Though a cynic may point out that one is merely changing the location at which material is being incinerated, in a diesel engine rather than in a recovery boiler, diesel engines require fuel that has much higher purity, as well as more stringently specified properties. For instance, diesel fuel in the US must meet ASTM standards for acid number (test D 664), additive elements in lubricating oils (D 4951), total sulfur (D 5453), sulfate ash (D 874), and water and sediment (D 2709) (Ven Gerpen et al. 2004a). The market price of diesel fuel can be many times greater than the heat value of tall oil. During the period between January 2004 and June 2006, the price of diesel fuel approximately doubled from $1.57 to about $2.90 per gallon (DOE 2006a). To put this in context, the price of crude tall oil historically has been in the range of $90 to $160 per ton (Innov. Group 2006), a number which suggests a price per gallon of tall oil less than $1.

Petroleum-based diesel oil (PD) is an important automotive fuel (Song et al. 2000), with particular dominance in the heavy truck fuel market. As an auto fuel, diesel is popular in Europe, where high taxes have provided additional incentives to maximize fuel efficiency. Recent analysis indicates that the world supply of easily-extracted petroleum will soon reach and pass its "peak" if, in fact, it has not done so already (Hirsh et al. 2006). In light of the expected tightening of demand, one can expect that greater demand for energy-efficient vehicle technologies will pervade all regions of the world.

Combustion of petroleum-derived diesel fuel yields a net increase of carbon dioxide, which is considered to contribute to global warming (Drake 2000; OECD/IEA 2000; Tester et al. 2005). In addition, combustion of petroleum products often contributes to sulfuric pollution, a significant contribution to acid rain. Though there are those who doubt the certainty of the environmental effects (Lomborg 2001; Labohm et al. 2004), the weight of evidence appears to favor urgent action to limit net production of $CO_2$ and other greenhouses gases.

Accelerated by the oil crisis of the 1970s, there has been increasing research in alternative fuels (Hunt 1983; Lee 1996; da Rosa 2005; Tester et al. 2005). Research has focused on methanol, ethanol, compressed natural gas (CNG), liquid petroleum gas (LPG), liquefied natural gas (LNG), vegetable oils, reformulated gasoline, and reformulated diesel oil (Canakci and van Gerpen 2001a). Biomass fuels (Wyman and Goodman 1993; Vasudevan et al. 2005) offer particular promise, since the starting material can be highly

abundant, and it is obtained by photosynthesis, minimizing the net production of $CO_2$ during the fuel product lifecycle (Sheehan et al. 1998).

From the standpoint of conventional biodiesel production, the composition of tall oil is far from ideal. As will be reviewed in the next section, the most widely used procedures for biodiesel production begin with the conversion of triglyceride oils to the corresponding methyl esters, plus glycerol (Schuchardt et al. 1998). Although triglycerides can comprise a substantial proportion of the extractives component of wood (Back and Allen 2000), essentially all of that material becomes saponified under the hot, highly alkaline conditions of a kraft cook. The alkaline conditions most often employed during production of biodiesel products from vegetable oils would tend to leave the fatty acids in their soap form, and the physical behavior of soap tends to interfere with the desired conversion to biodiesel. Before considering these issues further, it can be instructive to review progress that has been achieved with respect to biodiesel production from high-grade vegetable oils.

## Lessons to be Learned from Existing Biodiesel Technology

To set the stage for consideration of tall oil as a biodiesel source, it makes sense to review various lessons that can be learned from present-day biodiesel technology, most of which is based on the use of vegetable oils. In the present discussion biodiesel will be defined as a composition in which fatty acids have been converted to alkyl esters (usually methyl esters). Unrefined vegetable oil itself is sometimes called "biodiesel," despite the fact that direct substitution of such oil in conventional diesel engines can cause problems in unmodified vehicles (Goering et al. 1982; Bagby 1987).

The relatively high viscosity and low volatility of vegetable oils can lead to engine-related deposits, injector coking, and piston ring sticking (Clark et al. 1984; Pestes and Stanislao 1984; Vellguth 1988). Such effects usually can be reduced or eliminated by converting vegetable triglyceride oils to their corresponding lower-viscosity methyl esters through a process of transesterification (Kusdiana and Saka 2001a, 2001b, 2004a, 2004b, 2004c; Saka and Kusdiana 2001a, 2001b; Shimada et al. 2002; Van Gerpen et al. 2004a; Warabi et al. 2004a, 2004b; Wang et al. 2005; Demirbas 2005; Imahara et al. 2006; Minami and Saka 2006). The resulting product is often called biodiesel. Transesterification of high-grade vegetable oil can produce a fuel having viscosity and many other characteristics that are similar to those of No. 2 diesel fuel. The transesterification can reduce viscosities from an initial 27-54 $mm^2/s$ down to about 4-5 $mm^2/s$ (Demirbas 2005). Another approach is to hydrolyze the triglycerides in sub-critical water to obtain fatty acids, and, in a second step, to esterify the freshly prepared fatty acids with supercritical methanol, together with the originally existing free fatty acids (Kusdiana and Saka 2004a, 2004c; Minami and Saka 2006).

Mittelbach (1996) describes the development of specifications for biodiesel composition and properties. Austria was the first to establish standards, which were in place by 1992, followed by others, including a German standard (DIN V 51606) in 1994. Strict limits are specified for density, viscosity, flash point, sulfur, carbon residue, sulfate ash, cetane number (a higher number indicating a shorter delay of auto-ignition under compression in a diesel engine), and neutralization number (related to the titratable acid

group content). Biodiesel derived from vegetable oils also needs to fall below prescribed maximum levels for methanol, free and total glycerol, as well as phosphorous. In the US, ASTM test number D 6751-02 describes the requirements for a product to qualify as biodiesel in the US (Van Gerpen et al. 2004b).

When the source material is a relatively pure mixture of triglycerides, transesterification can be carried out as illustrated by the reaction scheme in Fig. 1. The reaction is promoted by alkaline conditions and by the presence of solid catalysts, such as zeolites (Suppes et al. 2003), calcium compounds (Gryglewicz 1999), benzenesulfonic acid (Dittmar et al. 2003), as well as by lipase (Abigor et al. 2000; Arsan and Parken 2000). Glycerol, which is formed as a byproduct of the reaction, has applications in a worldwide production of 0.7-0.8 million ton for pharmaceutical, cosmetics, food, and plastics.

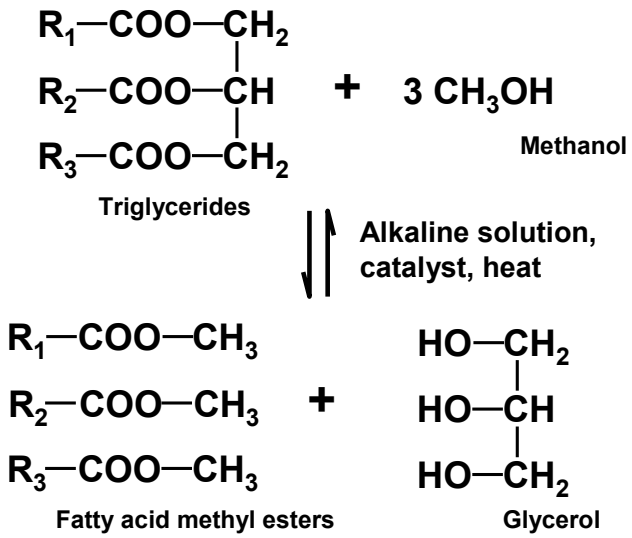



Fig. 1. Transesterification reaction suitable for relatively pure vegetable oils.

According to Fig. 1, the quality of biodiesel fuel prepared from relatively pure vegetable oils is often considered to be superior, in some respects, to that of conventional petrodiesel (Van Gerpen et al. 2004a). High-quality biodiesel can be added to petrodiesel to reduce the resulting emissions. Biodiesel produced from triglycerides is biodegradable and nontoxic. The exhaust emissions tend to be cleaner. No engine modification is needed. In addition, the Occupational Safety and Health Administration (OSHA) in the US considers biodiesel to be nonflammable, due to its high flash point of 160 $^{o}$C. It follows that biodiesel is safer to store and use, compared to its petroleum counterpart, which has a flash point of >62 $^{o}$C. For sake of comparison, gasoline is even more flammable, having a flash point of >45 $^{o}$C.

Figure 2 shows a typical process for the continuous production of biodiesel, using homogeneous catalysis (Bournay et al. 2005). Especially in the case of waste vegetable oil, when using alkali catalyst, the first step involves removal of water. Strong alkali is added as a catalyst to the methanol solution in a closed reaction vessel, vegetable oil is added, and

the vessel is then sealed to prevent loss of alcohol. The temperature is raised to about 55-70 $^{o}$C to promote the reaction that was shown in Fig. 1. Competing reactions can be minimized usually by adding an excess of the alcohol, beyond the stoichiometric amount needed to react with the triglycerides (Bournay et al. 2005). The usual reaction time varies between one to eight hours, partly dependent on the temperature. Based on the Arrhenius equation, the reaction rate is expected to double with each increase of 10 $^{o}$C. As shown, glycerol is separated from the mixture after the transesterification stage. The higher density of glycerol allows gravity separation, though some operations include centrifugation to speed up the process. Residual alcohol in the glycerol phase is removed by distillation and reused. The separated oil phase is washed with water to remove any of the undesired soap that may have formed. Advances in the separation of glycerol and alkyl ester phases have been patented by Noureddini (2000,2001). Fatty acids become a byproduct of the continuous alkali-catalyzed process, rather than being converted to biodiesel.

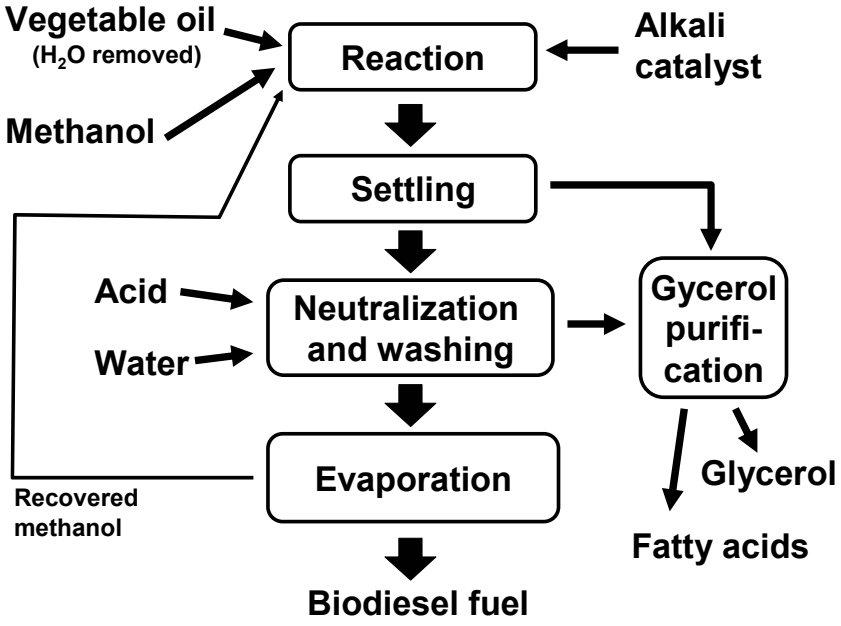



Fig. 2. Typical process for the continuous production of biodiesel using alkali catalyst

Work by Bookcock and coworkers showed that the rate of the initial reaction shown in Fig. 2 for alkali-catalyzed methylation can be increased considerably by use of a cosolvent, such as tetrahydrofuran (Bookcock et al. 1996a, 1998). The explanation is that the methanol and oil tend to exist as separate phases, thus limiting their ability to react with each other (Boocock et al. 1996b). The cosolvent allows the mixture to form a single phase. The molecular mechanism of alkali-catalyzed methylation, with an alumina catalyst, has been further elucidated by Vonghia et al. (1995).

## Low-Temperature Performance and Blends

Efforts to use tall oil as a source of diesel fuels need to take it into consideration some practical issues that are already experienced by users of biodiesel derived from vegetable oils. These issues include crystallization of saturated fatty acid alkyl esters in very cold weather and a higher likelihood of difficulty in getting a diesel motor to start (Dunn 1999; Tyson and McCormick 2006). In very cold weather, biodiesel may crystallize at higher temperatures than petrodiesel. The most common approach to avoid plugging of fuel filters and fuel lines in cold weather involves blending the biodiesel with petrodiesel. Various blends of biodiesel with substantial amounts of no. 2 low-sulfur diesel fuel and no. 1 kerosene have been used, depending on local costs of materials, seasonal temperatures, and engine characteristics (Dunn 1999; Tyson and McCormick 2006). Blends having ratios of 20% or less petrodiesel to biodiesel have become available in various locations (Sheehan et al. 1998). Though the results are highly dependent on the types of vegetable oils used to produce biodiesel, it has been found that the cloud point (an indication of crystallization) and the pour point of biodiesel-petrodiesel blends decreases almost linearly with the content of petrodiesel (DOE 2006b). Filter-plugging tests likewise showed substantial improvements in cold-weather performance, upon addition of petrodiesel to biodiesel, except that even B20 (20% biodiesel) blends made from yellow grease or tallow oils continued to plug filters at temperatures as high as 0 $^{o}$C. For temperatures higher than room temperature it has been clearly shown that addition of petrodiesel to biodiesel tends to reduce the viscosity in a predictable way, making the fuel more suitable for use as a direct substitute for petrodiesel (Yuan et al. 2005). In addition to blends, there has been some success in preparing isopropyl and other branched-chain esters, which are reported to reduce the crystallization temperature (Johnson and Hammond 1996; Wang et al. 2005).

Further practical difficulties that can be encountered with biodiesel products are associated with its relatively high level of unsaturated hydrocarbon chains, as compared to petrodiesel (Peterson et al. 1996). As noted in later discussion, the lower content of unsaturated fatty acids in tall oil (Zachary et al. 1965) can be considered to be an advantage in this respect. Heating of unsaturated hydrocarbons is expected to cause polymerization, resulting in the formation of gums. Though more study is needed to clarify this issue, it would make sense that differences in gumming effect ought to be found when comparing biodiesel fuels derived from oils having differing content of highly unsaturated fatty acid chains. To ensure the highest quality of biodiesel, it has been recommended to minimize the level of linolenic acid and other highly unsaturated components (Mittelbach 1966; Lang et al. 2001). As noted by Knothe (2004), although a high degree of unsaturation can help suppress crystallization of biodiesel at cold temperatures, the conjugated double bonds are susceptible to oxidation during storage, forming species that do not perform well in diesel engines.

Water in biodiesel can create additional problems. Condensation during storage can increase the amount of water, compared to freshly made biodiesel. Water not only reduces the heat of combustion, but it can cause higher emissions levels, harder starting, slower acceleration, and corrosion of vital fuel system components. If the temperature falls below 0 $^{o}$C, ice crystals can plug lines or nucleate crystallization within the organic phase. Water also tends to accelerate microbial growth, since biodiesel can be considered an excellent

food source for bacteria. Users of biofuels who employ heated tanks, for year-round use, can expect to face year-round problems with microbes (Van Gerpen et al. 2004b).

## BIODIESEL FROM HIGH FATTY ACID RAW MATERIALS

Tall oil consists of a mixture of triglycerides, free fatty acids, resin acids, and a variety of other materials (Johansson 1982; Back and Allen 2000). Because the extractives component of wood typically contains high levels of fatty acids, as well as substantial amounts of water, it can be instructive to consider various progresses that have been achieved with other source materials having similar characteristics. Waste frying oil can serve as an example.

### Waste Vegetable Oil

Waste vegetable oil comes mainly from industrial deep fryers in potato processing plans, snack food factories, and fast-food restaurants. Use of waste frying oils as a fuel source has been prompted by a desire to minimize costs, and also in order to avoid wasteful disposal of a resource (Zhang et al. 2003a, 2003b; Zaher et al. 2002; Felizardo et al. 2006; Kulkarni and Dalai 2006; Zheng et al. 2006). While the cost of waste frying oils is about half that of virgin vegetable oil (Supple et al. 2002), it is often possible to obtain relatively small amounts for free, picking it up from local fast-food restaurants. Such use reduces the burden of disposing of the oil as waste, maintaining public sewers, and treating oil-polluted water (Encinar et al. 2005). The amount of waste cooking oil produced has been estimated as up to one million tons per year in the EU countries (Supple et al. 2002), and about 120,000 tons per year, in the form of yellow grease, in Canada (Zhang et al. 2003a). According to a study done in 1993, the amount of yellow grease produced in the U.S. was approximately 1.5 billion pounds (0.7 million metric tons) (Hunter and Applewhite 1993). USDA yellow grease production estimates for the U.S. from 1995 to 2000 average 2.6 billion pounds (1.2 million metric tons), equivalent to 350 million gallons of biodiesel (Groschen 2002). In these studies yellow grease is defined as spent cooking oil and other fats and oils collected from commercial or industrial cooking operations.

From the standpoint of producing biodiesel, two of the biggest challenges in using waste frying oils are their water content and their free fatty acid content. Transesterification, as illustrated in Fig. 1, can carried out efficiently only in the case of sufficiently low levels of both water and free fatty acids in the feedstock (Ma et al. 1998; Saka 2004c). Transesterification can be defined as the conversion of one type of ester to another type of ester – in the present instance, from a triglyceride fat to a methyl or ethyl ester. Otherwise, the alkaline conditions used in this type of reaction cause saponification, converting an excessive amount of free fatty acids to their corresponding soap form, as illustrated in Fig. 3. The resulting soap tends to interfere with separation of the glycerol that is formed in the transesterification reaction.

The high temperatures of typical cooking processes and the water from the foods accelerate the hydrolysis of triglycerides and increase the free fatty acid content in the oil. Waste oil can have about 2 to 20% fatty acid content. Waste oil also has problems related to storage stability, partly due to polymerization of the unsaturated alkyl chains. Various

chemical differences can be distinguished when comparing typical waste vegetable oils *vs.* virgin oils (Tomasevic and Siler-Marinkovic 2003).

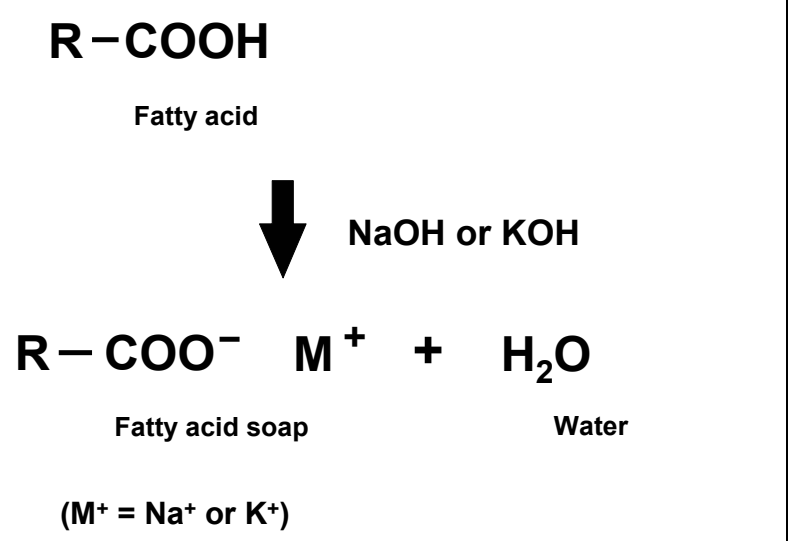



Fig. 3. Saponification reaction of fatty acids with alkali catalyst

Another critical point shown in Fig. 3 is the fact that the saponification of free fatty acids yields water. Water in the reaction mixture tends to hydrolyze triglycerides, lowering the yield and increasing the acid value. In the absence of water, hydrolysis of triglyceride esters cannot take place.

## Enzymatic Catalysis

Enzymes offer a promising route to biodiesel in the case of source materials having high content of free fatty acid. For example, immobilized Candida Antarctica lipase has been used for ethyl esterification of docosahexanoic acid (Shimada et al. 2001). This method later was adopted to produce fatty acid methyl ester. Through multi-stage transesterification of triglycerides (Fig. 1) and the esterification of the fatty acids, more than 98.5% by mass of fatty acid methyl ester was obtained. The latter reaction is illustrated in Fig. 4 (Hsu et al. 2002; Watanabe et al. 2005). The high conversion was achieved by a 24-hour reaction, involving six stages of transferring the enzyme to a fresh substrate mixture.

In comparison to the other transesterifications described in this article, the enzymatic process requires a substantially lower concentration of methanol, because methanol inhibits lipase activity. A problem that has been encountered in this type of enzymatic conversion is the generation of water during the methyl esterification of the fatty acid. The water also was found to inhibit the enzyme activity. Various means to overcome this problem have been suggested, based on use of immobilized enzymes and multiple stages (Bélafi-bakó et al. 2002; Hsu et al. 2002; Shimada et al. 2002; Watanabe et al. 2002).

While the enzymatic approach has been demonstrated as a route suitable for production of biodiesel, the reaction time required to achieve high rates of conversion has been considered to be a negative factor.

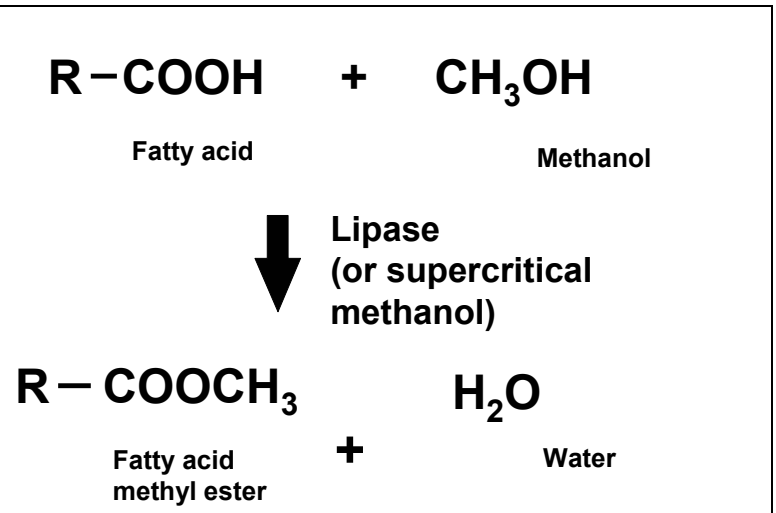



Fig. 4. Esterification catalyzed by enzyme or by supercritical methanol

## Acid Catalysis

Acid catalysis in methanol solution appears to be quite effective for converting free fatty acids (FFA) to esters, though the complete reaction, in samples that include triglycerides, may require 48 hours (Jeromin et al. 1987; Hammond 1998; Zhang et al. 2003a, Zheng et al. 2006). In order to apply such an approach to the case of low-grade vegetable oils, for instance, it would be necessary to employ a two-stage process in Figure 5. In the first stage the free fatty acids are converted to esters. In a second stage, alkali-catalyzed transesterification of the triglycerides present in the mixture is employed, as was illustrated in Fig. 1.

The latter reaction can be completed in much less time than would be possible with acid-catalyzed transesterification alone. Though acid catalysis of transesterification provides a way to avoid undesired saponification reactions, the reaction rates tend to be much lower, in comparison to the alkaline system. The ester conversion is strongly inhibited by the presence of water in the oil. If the water content is greater than 0.5%, the ester conversion rate drops below 90% efficiency (Canakci and van Gerpen 1999, 2001a; 2000b; 2000c).

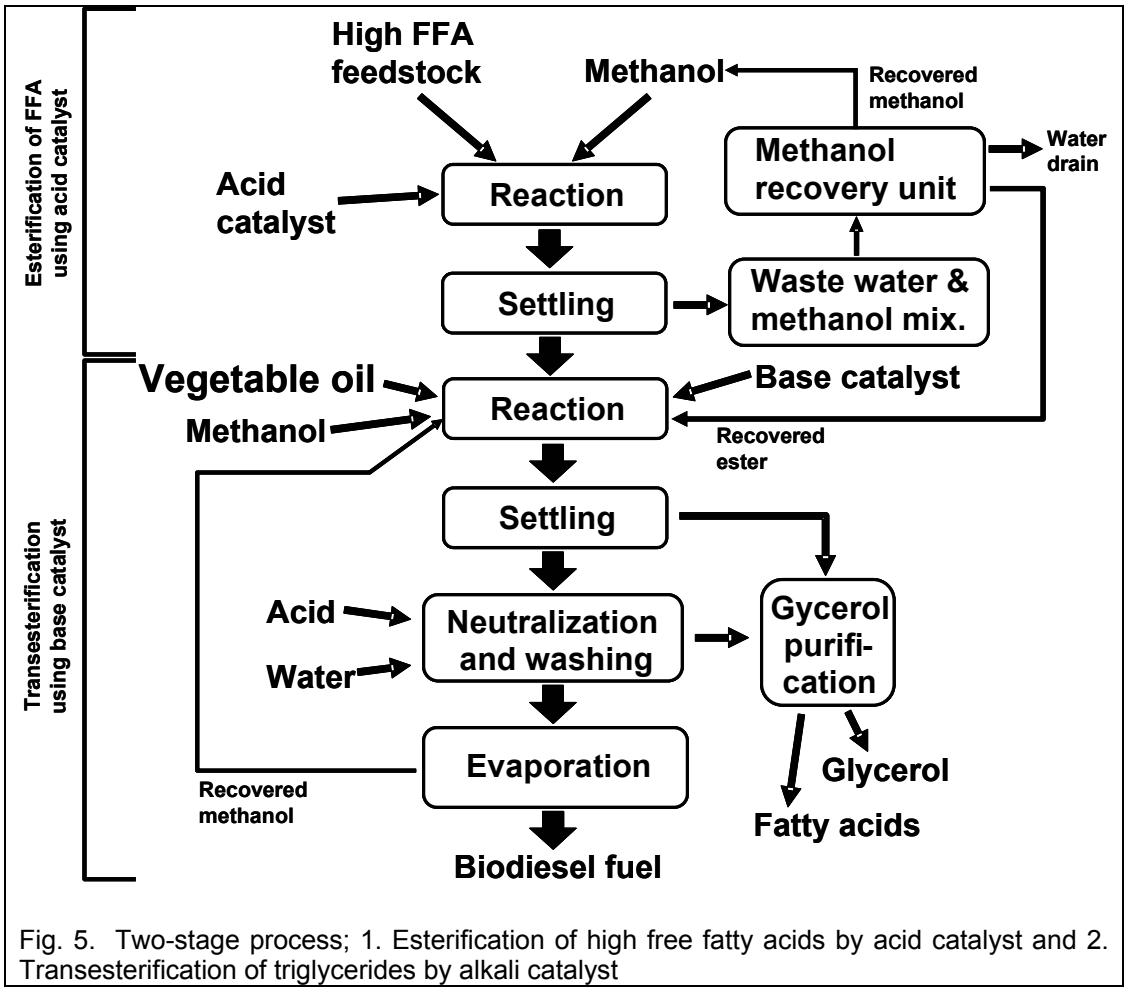



Fig. 5. Two-stage process; 1. Esterification of high free fatty acids by acid catalyst and 2. Transesterification of triglycerides by alkali catalyst

## Supercritical Methanol

Two intriguing strategies for producing biodiesel from a wider range of mixtures, which may include fatty acids, water, and oils/fats, involve the use of supercritical conditions of methanol. The term "supercritical" means that the temperature and pressure of the solvent in question are sufficiently high, above its critical point, so that the properties of the solvent are neither completely liquid-like nor completely gas-like (Saka 2000; Arai et al. 2002; Brunner 2004). Supercritical conditions of methanol have been found useful for transesterification and esterification (Kusdiana and Saka 2001a, 2001b, 2004a, 2004b, 2004c; Saka and Kusdiana 2001a, 2001b; Warabi et al. 2004a, 2004b; Demirbas 2005; Imahara et al. 2006; Minami and Saka 2006). The first such method (Saka method) involves a one-step transesterification and esterification with supercritical methanol (Saka and Kusdiana 2001b; Kusdiana and Saka 2001a, 2001b). The second method (Saka-Dadan method) involves two steps; first, a hydrolysis with subcritical water, then, after separation of glycerol, a supercritical methylesterification of the fatty acids (Kusdiana and Saka 2004a; Minami and Saka 2006). Such non-catalytic performance by supercritical methanol is due to its increased ionic product, which assists the methanolysis reaction without

catalyst. In addition, the decreased dielectric constant of methanol in the supercritical state results in better solubility of hydrophobic compounds in methanol such as triglycerides, diglycerides and monoglycerides, thus greatly promoting the reaction (Saka 2000; Kusdiana and Saka 2004a, 2004 c; Minami and Saka 2006).

Figure 6 illustrates the Saka-Dadan method. In the case of the initial hydrolysis reaction, a pressure of 10 MPa and a temperature of 270 $^{o}$C are sufficient to impart subcritical characteristics to water, making it possible to rapidly hydrolyze triglycerides to fatty acids. When the pressure is reduced, the mixture promptly separates into two phases, and the water phase can be separated to recover glycerol. Methanol becomes supercritical at a pressure of 10 MPa and temperature of 270 $^{o}$C, and the supercritical conditions favor rapid formation of methyl esters from the fatty acids.

Compared to the methods involving alkali or acid catalysis, the supercritical method for biodiesel production has advantages in terms of reaction time and yield. Instead of 1-6 hours, the reaction is substantially complete in about 240 seconds (Kusdiana and Saka 2001a). A reaction time of at least 9 minutes can achieve a total glycerol content less than 0.24 mass percent, as required in biodiesel standards (ASTM D6751-03). Any free fatty acids become converted to methyl esters. No catalysts need to be recovered or neutralized at the end of the process. An additional advantage is that not only methanol but also some other alcohols such as ethanol, 1-propanol, 1-butanol, and even 1-octanol can be available for fatty acid alkyl esters production. The fact that no catalyst is used makes it relatively easy to obtain glycerol as a byproduct.

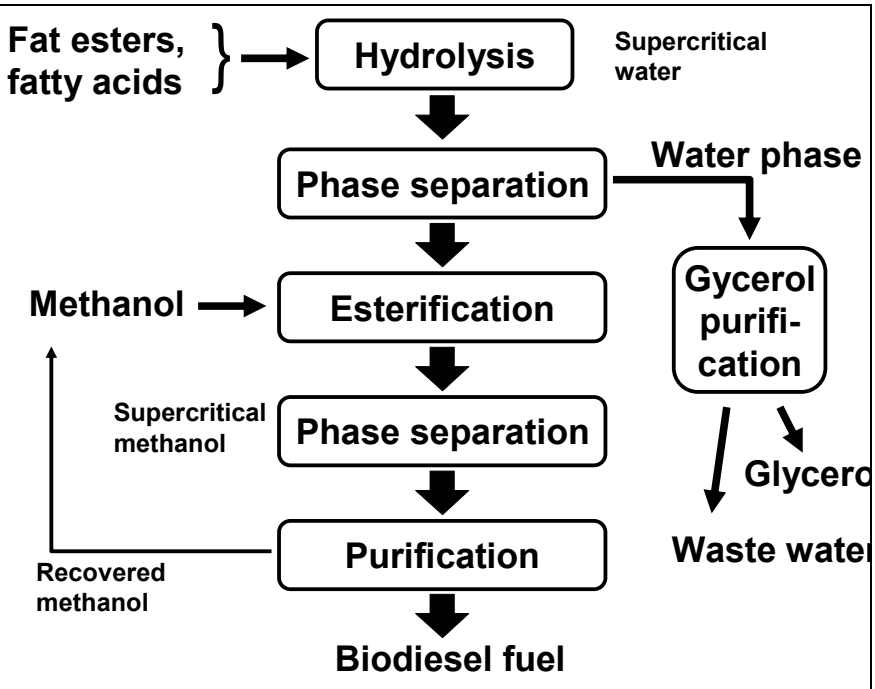



Fig. 6. Continuous process with supercritical water and supercritical methanol stages

Though it is beyond the scope of the present article to provide a detailed economic assessment, practical considerations suggest that supercritical technology may become a favored production route for biodiesel in the years ahead. The relatively short times required for reaction imply that the process can be carried out in considerably lower volumes compared to the other methods described in this review. Large processing tanks, which are required by the other technologies considered in this review, can be avoided. Though high pressures are required, the amount of thermodynamic work is moderate, due to the limited compressibility of the fluids involved. Further savings, relative to some of the other technologies considered, involve avoidance of catalysts. The cost of catalysts, including alkali, acid, or solid-phase, can involve later separation or disposal. However, as in any new technology, engineering studies and scale-up work will be needed in order to obtain estimates of capital costs, processing costs, and expected lifetimes and efficiencies of equipment.

## PROSPECTS FOR TALL OIL AS A SOURCE MATERIAL FOR BIODIESEL

Tall oil is a dark, viscous, and odorous liquid that phase-separates from the spent pulping liquor that remains after the kraft pulping of wood chips. The spent pulping liquor, commonly called black liquor, is a highly alkaline solution that contains the sodium soaps of rosin and fatty acids, byproducts of lignin, as well as inorganic ions, such as sulfate (Zachary 1965; Foran 1992). Crude tall oil forms a separate phase from the aqueous phase of black liquor following partial evaporation of the water. The majority of the soaps, together with other organic materials, can be removed at that point by skimming (Foran 1992). To promote separation, the mixture is acidified, converting the soaps into their respective free rosin acid and fatty acid forms (Zachary 1965). The aqueous phase is returned to the process, making it possible to reuse most of the sulfur and sodium for further pulping of wood. The composition of the extractives portion of typical tall oil is summarized in Table 1 (Holmlund and Parviainen 1999). As noted by Gullichsen and Lindeberg (1999), crude tall oil composition varies considerably, depending on the species of wood and the process conditions. Tall oil yields, per ton of kraft pulp, generally fall in a range between 18 and 60 kg, with some higher numbers reported (Gullichsen and Lindeberg 1999).

**Table 1.** Composition of Tall Oil Extractives from Kraft Black Liquor

| Component | N. Amer. Softwood | Scandinavian Pine | Scandinavian Spruce | N. Amer. Hardwood | N. Amer. Birch |
|---|---|---|---|---|---|
| Rosin acids (%) | 42 | 30-35 | 20-30 | - | - |
| Fatty acids (%) | 47 | 50-55 | 35-55 | 76 | 55-90 |
| Neutrals (%) | 11 | 5-10 | 18-25 | 24 | 5-35 |

As has been described in detail (Back and Allen 2000), some of the major components of crude tall oil are rosins, unsaponifiable sterols (5-10%), resin acids (mainly

abietic and its isomers), fatty acids (mainly palmitic, oleic, and linoleic acids), fatty alcohols, some sterols, and other alkyl hydrocarbon derivatives. Based on a total of 100%, the organic content of typical tall oil is comprised of about 5-15% volatiles ("tall oil heads" or "turpentine," *e.g.*, pinene), 25-35% fatty acids (or "soaps"), 5-15% of "distilled tall oil" (including unsaponifiable compounds), 25-35% rosins (as rosin soaps), and 15-25% higher-mass compounds ("pitch") (Zinkel and Russell 1989). According to another source, fractionation of crude tall oil is said to result in about 35% rosin acids, 30% fatty acids, and 35% of distllates, head, and pitch by mass (see Coll et al. 2001). Figure 7 shows the chemical structures of several components that tend to be relatively abundant in crude tall oil samples (Wang et al. 2001, 2002; Zinkel and Russell 1989; Back and Allen 2000).

Raw soap from tall oil typically contains significant residual water (as black liquor), lignin, fiber, sodium, calcium, and inorganic acids (Holmlund and Parviainen 1999). Of these, the lignin (from the aqueous black liquor phase), the calcium, and the fibers are considered to be the main contaminants (Gullichsen and Lindeberg 1999). As a first step in processing, tall oil is dehydrated to remove water, heated, and then flashed into a chamber where the lower pressure permits vaporization of most of the components (Zachary et al. 1965). The separation of the organic materials from the aqueous phase is facilitated by acidification, converting soaps into their respective free rosin acid and free fatty acid forms.

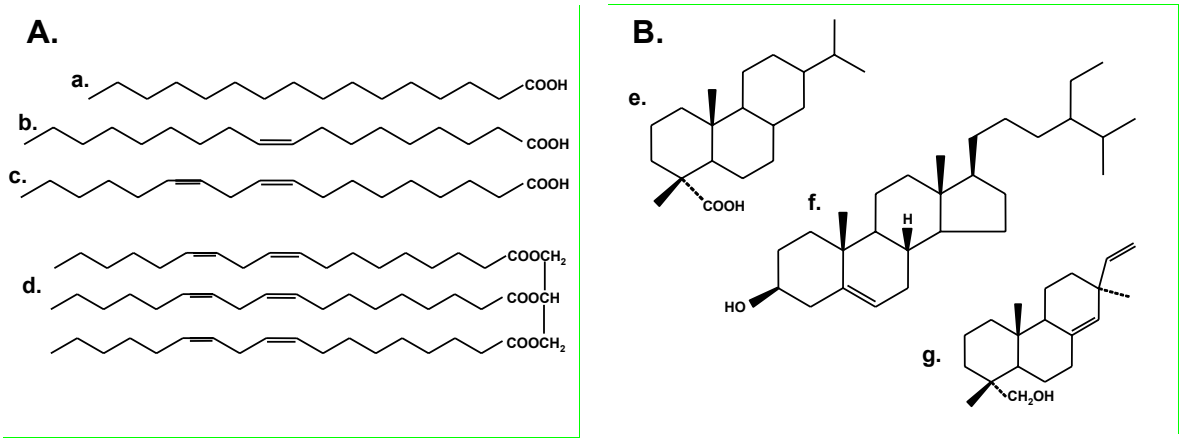



Fig. 7. Structures of some typical organic chemical species found in tall oil samples. Part A: Fatty acids and esters; a: palmitic acid; b: oleic acid; c: linoleic acid; d: triglyceride ester (fat) of linoleic acid. Part B: Resins and unsaponifiables; e: abietic acid (a rosin component); f: β-sitosterol (an unsaponifiable compound); g: pimarol (Wang et al. 2001, 2002; Zinkel and Russell 1989; Back and Allen 2000)

By distillation and other separation processes, crude tall oil can be separated it into a number of distinct fractions (Zinkel and Russell 1989). By further purification, reducing the rosin content to the range 1-10%, one obtains tall oil fatty acid (TOFA). Fatty acids derived from tall oil differ from typical vegetable oils by being richer in oleic acid (a C18 acid with one double bond), somewhat less linolenic acid (C18 acid with two unsaturated bonds), and almost no linolenic acid (C18 acid with three unsaturated bonds) (Zachary et al. 1965). Following from a point made earlier in this review, such differences can be

expected to reduce gum formation during combustion of tall oil biodiesel, in comparison to biodiesel obtain from vegetable oils (see Knothe 2005). Tall oil fatty acid is relatively inexpensive, and it can be used as an alternative to tallow fatty acids for production of soaps and lubricants. It also can be esterified with pentaerythritol and used in adhesives and oil-based varnishes. Tall oil also can be incinerated in a kraft liquor recovery boiler to recover its heat value, but the efficiency and relative safety of boiler operation tend to be reduced by such practices (Wong 1995).

Neutral fractions of tall oil typically are most prominent in the heads fraction of tall oil, the part of which distills first, before the rosin and fatty acid (Conner and Rowe 1975; Koebner 1983; Wang et al. 2001). Main species of the head fraction consist of resin alcohols (especially pimarol), steroids (especially β-sitosterol), fatty alcohols, diterpenes, sesquiterpenes, and resin aldehydes (Wang et al. 2001, 2002). Because neutral components of raw tall oil, as mentioned, can have other valuable uses, and due to their easy distillation, manufacturers would be expected to minimize the amounts carried over into streams intended for biodiesel production.

Table 2 summarizes some of the main hurdles, as well as some advantages of some of the conversion strategies already outlined in this article, with respect to their possible application in the case of tall oil as a source of biodiesel.

**Table 2.** Pros and Cons of Conversion Strategies for Biodiesel, Using Tall Oil from the Kraft Pulping of Wood

| Processing Strategies | Feasibility Issues |
|---|---|
| Alkaline catalysis | Saponification can cause problems; coking of reactors |
| Enzymatic catalysis | Slow; water removal required; multiple stages required |
| Acid catalysis | Promising; requires follow-up by alkaline transesterification |
| Supercritical methanol | Fast; high pressure equipment, development required |
| Hydrogenation alone | Suitable for use as cetane enhancer in petrodiesel blends |

As noted in Table 2, alkaline processing of tall oil fractions can be expected to suffer from saponification of the fatty acids, as well as from coking. Improved yields and reduced coke or pitch in the resulting biofuels have been achieved by use of methanol (Chantal et al. 1984), as well as different catalysts (Chantal et al. 1984; Baker and Elliot 1987), diluents (Sharma and Bakhshi 1991), or hydrogenation (Mathews et al. 1985; Baker and Elliot 1987) when producing the methyl esters of fatty acids obtained by wood. A relatively mild base, sodium carbonate, has been used in catalytic conversion of oils of poplar wood, obtained by high pressure liquefaction, using a zeolites catalyst (Sharma and Bakhshi 1991). The zeolite catalysts were found to be effective even in the absence of hydrogenation, but considerable coke formation was observed.

Progress has been achieved in studies of liquids obtained by pyrolysis or high-pressure liquefaction of wood (Sharma and Bakhshi 1991). Though the high inorganic content renders pyrolysis impractical in the case of black liquor, the process appears well suited to the processing of tall oil components to make other chemical products.

Coll et al. (2001) noted that the rosin fraction of tall oil tends to be available in excess quantities, relative to market demand. In effect, some of the rosin is an excess

byproduct of the separation and utilization of fatty acids and terpenoids from the pulping process. In order to convert such rosin into a useful biofuel, it is necessary to hydrogenate the double bonds within the ring structures of abietic acid and related isomers. Promising results were achieved by a catalytic hydrogenation and removal of carboxylic acids in a single-step hydrotreatment process.

Wong (1995) described catalytic hydrogenation of tall oil, yielding a product that can be used as an additive in petrodiesel fuel. It was not necessary to separate fatty acids from rosin components of the de-pitched tall oil mixture. Furthermore, no esterification was carried out.

Only limited work has been carried out using supercritical conditions to treat tall oil products. Taylor and King (2001) used supercritical carbon dioxide, containing a lesser amount of methanol, as a medium within which to evaluate the efficacy of various enzymes as catalysts for esterification. Though fatty acid methyl esters were produced, the aim of these researchers was tall oil analysis, rather than biodiesel production. The conversion rates were low, and the authors concluded that the supercritical conditions adversely affected the tertiary structure of the enzymes, relative to their intended activity. Other authors have employed supercritical $CO_2$ as a means of extracting oleophilic substances from wood (Harvala et al. 1987) or from tall oil itself (Ritter and Campbell 1991).

The foregoing results, while hardly demonstrating viable supercritical production of biodiesel from tall oil, at least provide some precedent for further research in that direction. The tolerance of supercritical conditions for relatively high levels of free carboxylic acids and water would appear to offer special advantages in the case of tall oil.

## Economic and Practical Motivations for Tall Oil Use

Though the discussion in the preceding paragraphs suggests various potentially feasible approaches to biodiesel production from tall oil fractions, it is not a forgone conclusion that the pulp and paper industry will rush to embrace any of these technologies. In qualitative terms, a venture into bio-fuels can be perceived as a risk, at least until another company has demonstrated the technological success. Even then, a large pulp and paper company may be reluctant to become involved in a technology that they do not think of as being part of their core business.

A key factor that has potential to help make tall-oil-to-diesel technology a reality is the price differential. The heat value of tall oil, as it is used to produce steam, is about 35-40 MJ/kg of solids (Wong 1995). The historical price of tall oil has been in the range of $90 to $160 per ton of solids from 1997 to 2002 (Innov. Group 2006). The price has risen recently by 50% (Anon. 2006) and the market is expected to tighten further as a general trend (Guzman 2002). If one uses $200 as a round-number estimate for the price per short ton of crude tall oil, the corresponding price per gallon is about $0.80. The wholesale price of diesel fuel is considerably higher. During the period between January 2004 and the present, the price of petrodiesel approximately doubled, reaching about $2.90 per gallon (DOE 2006a). In addition to the present price levels, investors also will be interested in longer-term forecasts of fuel prices. Engineering studies are needed to determine at what point the price difference is enough to trigger investments in the equipment changes needed to deal with specific issues related to the processing of tall oil as a biodiesel source.

Recent work by Uloth (2006) has helped to document an additional incentive for kraft pulp producers to convert tall oil for higher-value products, rather than just crude tall oil or direct combustion. More effective removal of tall oil soaps from black liquor was found to increase the capacity of recovery boilers by 2-8%. Because the kraft recovery boiler is often a process bottleneck, such changes can significantly increase the overall production capacity of a pulp mill. Advances in soap-skimming technology (Uloth and Wearing 1994; Propst 1995; Piirtinen et al. 2004) also can contribute to the feasibility of future tall-oil-to-biodiesel ventures.

### Summary

A variety of technological approaches may be used, including alkali- and acid-catalyzed esterification, transesterification, reactions in supercritical fluids, enzymatic conversions, and hydrogenation. Options involving supercritical methanol appear to be especially promising. Given the economic incentives, it appears probable that the kraft pulping process can become a significant source of biodiesel oil, helping to replace increasingly expensive petrodiesel products. Further research is needed in all of these fields as we continue to seek the most cost-effective, environmentally responsible, and energetically efficient schemes to power motor vehicles in the future.

## ACKNOWLEDGEMENTS

The present article grew out of activities related to the Ph.D. thesis candidacy of Sa Yong Lee. Support for the thesis was obtained from the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2004-35504-14655.

## REFERENCES CITED

Abigor, R. D., Uadia, P. O., Foglia, T. A., Haas, M. J., Jones, K. C., Okpefa, E., Obibuzor, J. U., and Bafor, M. E. (2000). "Lipase-catalysed production of biodiesel fuel from some Nigerian lauric oils," *Biochem. Soc.* 28, 979-981.

Anon. (2006). "Raw materials hot spots," *Raw Materials Facts* 3(1), 2, http://www.nationalstarch.com/pdf/06-0062_RMF_3-06_Final.pdf.

Arai, Y., Sako, T., and Takebayashi, Y. (2002). *Supercritical Fluids: Molecular Interactions, Physical Properties, and New Applications*, Springer, New York.

Arsan, J., and Parkin, K. L. (2000). "Selectivity of *Candida antarctica* B lipase toward fatty acid and (iso)propanol substrates in esterification reactions in organic media," *J. Agricultural Food Chem.* 48, 3738-3743.

Axegard, P., Backlund, B., and Warnqvist, B. (2002). "The eco-cyclic pulp mill. Focus on closure, energy-efficiency and chemical recovery development," *Pulp Paper Can.* 103(5), 26-29.

Back, E. L., and Allen, L. H., Eds. (2000). *Pitch Control, Wood Resin and Deresination*, TAPPI Press, Atlanta, GA.

Bagby, M. O., Freedman, B., and Schwab, A. W. (1987). "Preparation and properties of diesel fuels from vegetable-oils," *Fuel* 66(10), 1372-1378.

Baker, E. G., and Elliot, D. C. (1987). "Catalytic hydrotreating of biomass-derived oils," *ACD, Div. Fuel Chem.* 32(2), 257-263.

Bélafi-bakó, K., Kovács, F., Gubicza, L., and Hancsók, J. (2002). "Enzymatic biodiesel production from sunflower oil by *Candida antarctica* lipase in a solvent-free system," *Biocatalysis Biotrans.* 20(6): 437-439.

Berndes, B., Hoogwijk, M., and van den Broek, B. (2003). "The contribution of biomass in the future global energy supply: A review of 17 studies," *Biomass Bioenergy* 25(1), 1-28.

Bournay, L., Casanave, D., Delfort, B., Hillion, G., and Chodorge, J. A. (2005). "New heterogeneous process for biodiesel production: A way to improve the quality and the value of the crude glycerin produced by biodiesel plants," *Catalysis Today* 106, 190-192.

Brunner, G., ed. (2004). *Supercritical Fluids as Solvents and Reaction Media*, Elsevier, Boston.

Canakci, M., and Van Gerpen, J. (1999). "Biodiesel production via acid catalysis," *Trans. ASAE* 42(5), 1203-1210.

Canakci, M., and Van Gerpen, J. (2001a). "Biodiesel production from oils and fats with high free fatty acids," *Transaction of the ASAE* 44(6): 1429-1436.

Canakci, M., and Van Gerpen, J. (2001b). "Accelerated oxidation process in biodiesel," *Trans. ASAE* 42(6), 1565-1572.

Canakci, M., and Van Gerpen, J. (2001c). "A pilot plant to produce biodiesel from high free fatty acid feedstocks," *2001 ASAE Annual International Meeting* Paper No. 016049, Sacramento, California.

Chantal, P., Kaliaguine, S., Grandmaison, J. L., and Mahay, A. (1984). "Production of hydrocarbons from aspen poplar pyrolytic oils over HZSM-5," *Applied Catalysis* 10(3), 317-332.

Clark, S. J., Wagner, L., Schrock, M. D., and Piennaar, P. G. (1984). "Methyl and ethyl soybean esters as renewable fuels for diesel-engines," *J. Amer. Oil Chemists Soc.* 61(10), 1632-1638.

Coll, R., Udas, S., and Jacoby, W. A. (2001). "Conversion of the rosin acid fraction of crude tall oil into fuels and chemicals," *Energy Fuels* 15, 1166-1172.

Conner, A. H., and Rowe, J. W. (1975). "Neutrals in southern pine tall oil," *J. Amer. Oil Chemists Soc.* 52(9), 334-338.

Cook, C., Mies, W., Min, T., Rudder, G., and Smith, B. (2005). "Final, some good news," *Pulp Paper Intl.* 47(1), 26-28.

da Rosa, A. V. (2005). *Fundamentals of Renewable Energy Processes*, Elsevier, New York.

DeKing, N. (2004). *Pulp & Paper Global Fact & Price Book 2003-2004*, Paperloop, San Francisco, CA.

Demirbas, A. (2005). "Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol transesterification methods," *Prog. Energy Combustion Sci.* 31(5-6), 466-487.

Dittmar, T., Dimmig, T., Ondruschka, B., Heyn, B., Haupt, J., and Lauterbach, M. (2003). "Production of fatty acid methyl esters from rapeseed oil and spent fat in batch operation," *Chemie Ingenieur Technik* 75(5), 595-601.

DOE (2006a). "Weekly Retail On-Highway Diesel Prices," http://tonto.eia.doe.gov/oog/info/wohdp/diesel.asp.

DOE (2006b). "Biodiesel Handing and Use Guidelines," *DOE/GO* 102006-2288, 2nd Ed., March 2006, U.S. Dept. of Energy.

Drake, F. (2000). *Global Warming: The Science of Climate Change*, Arnold, London.

Dunn, R. O. (1999). "Thermal Analysis of Alternative Diesel Fuels from Vegetable Oils," *J. Amer. Oil Chemists Soc.* 76(1), 109-115.

Encinar, J. M., González, J. F., and Rodríquez-Reinares, A. (2005). "Biodiesel from used frying oil. Variables affecting the yields and characteristics of the biodiesel," *Industrial & Engineering Chemistry Research* 44(15), 5491-5499.

Felizardo, P., Neiva Correia, M. J., Raposo, I., Mendes, J. F., Beremeier, R., and Moura Bordado, J. (2006). "Production of biodiesel from waste frying oils," *Waste Management* 26(5), 4870494.

Foran, C. D. (1992). "Tall oil soap recovery," in *Chemical Recovery in the Alkaline Pulping Processes*, 3rd Ed., R. P. Green and G. Hough, eds., TAPPI Press, Atlanta, Ch. 4, 45-56.

Goering, C. E., Schwab, A. W., Dangherty, M .J., Pryde, E. H., and Heakin, A. J. (1982). "Fuel properties of eleven vegetable oils," *Transaction of the ASAE* 25(6), 1472-1477.

Groschen, R. (2002). "Overview of the feasibility of biodiesel from waste/recycled greases and animal fats," *Legislative Commission on Minnesota Resources "Evaluate Biodiesel from Waste Fats and Oils,"* Marketing Services Division, Minnesota Department of Agriculture.

Gryglewicz, S. (1999). "Rapeseed oil methyl esters preparation using heterogeneous catalysts," *Bioresour. Technol.* 70 (3), 249-253.

Gullichsen, J., and Lindeberg, H. (1999). "Byproducts of chemical pulping," in *Chemical Pulping*, J. Gullichsen and C.-J. Fogelholm, eds., Fapet Oy, Papermaking Sci. Technol. Ser. 6B, Ch. 18, B375-B389.

Guzman, D. de. (2002). "TOFA outlook improves on tightening vegetable oils and CTO consolidation," *Chem. Market Reporter*, Feb.

Hammond, E. G. (1998). Personal communication, Dept. of Food Science, Iowa Sate Univ., Ames, Iowa.

Harvala, T., Alkio, M., and Komppa, V. (1987). "Extraction of tall oil with supercritical carbon-dioxide," *Chem. Eng. Res. Design* 65(5), 386-389.

Hirsch, R. L., Bezdek, R., and Wendling, R. (2006). "Peaking of world oil production and its mitigation," *AIChE J.* 52(1), 2-8.

Holmlund, K., and Parviainen, K. (1999). "Evaporation of black liquor," in *Chemical Pulping,* J. Gullichsen and C.-J. Fogelholm, eds., Fapet Oy, Papermaking Sci. Technol. Ser. 6B, Ch. 12, B37-B93.

Hsu, A-F. Jones, K., Foglia, T. A., and Marmer, W. N. (2002). "Immobilized lipase-catalysed production of alkyl esters of restaurant grease as biodiesel," *Biotech. Appl. Biochem.* 36(3), 181-186.

Hunt, V. D. (1983). *Synfuels Handbook*, International Press, New York.

Hunter, J. E., and Applewhite, T. H. (1993). "Correction of dietary-fat availability estimates for wastage of food-service deep-frying fats," *J. Amer. Oil Chemists Soc.* 70(6), 613-617.

Imahara, H., Minami, E., and Saka, S. (2006). "Thermodynamic study on cloud point of biodiesel with its fatty acid composition," *Fuel* 85, 1666-1670.

Innov. Group. (2006).

Jeromin, L., Peukert, E., and Wollmann, G. (1987) "Process for the pre-esterification of free fatty acids in fats and oils, *U.S. Patent* No. 4698186.

Johansson, A. (1982). "By-product recovery and valorization in the kraft industry – A review of current trends in the recovery and use of turpentine and tall oil derivatives," *Biomass* 2(2), 103-113.

Johnson, L. A., and Hammond, E. G. (1996). "Soybean oil ester fuel blends," *U.S. Patent* 5,520,708.

Kavalov, B., and Peteves, S. D. (2005). *Status and perspectives of biomass-to-liquid fuels in the European Union*, European Union Directorate General, Joint Research Center, Inst. for Energy, The Netherlands, AUR 21745 EN.

Knothe, G. (2005). "Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters," *Fuel Proc. Technol.* 86(10), 1059-1070.

Koebner, A. (1983). "Separation of tall oil head fraction into fatty acids and unsaponifiables," *J. Wood Chem. Technol.* 3(4), 413-420.

Kreutzer, U. R. (1984). "Manufacture of fatty alcohols based on natural fats and oils," *J. Amer. Oil Chemists Soc.* 61(2), 343-348.

Kulkarni, M. G., and Dalai, A. K. (2006). "Waste cooking oil – An economical source for biodiesel: A review," *Ind. Eng. Chem. Res.* 45(9), 2901-2913.

Kusdiana, D., and Saka, S. (2001a). "Methyl esterification of free fatty acids of rapeseed oil as treated in supercritical methanol," *J. Chem. Eng. Japan* 34(3), 383-387.

Kusidana, D., and Saka, S. (2001b). "Kinetics of transesterification in rapeseed oil to biodiesel fuel as treated in supercritical methanol," *Fuel* 80, 693-698.

Kusdiana, D., and Saka, S. (2004a). "Two-step preparation for catalyst-free biodiesel fuel production," *Appl. Biochem. Biotechnol.* 115, 781-791.

Kusdiana, D., and Saka, S. (2004b). "Catalytic effect of metal reactor in transesterification of vegetable ," *JAOCS* 81, 103-104.

Kusdiana, D., and Saka, S. (2004c). "Effects of water on biodiesel fuel production by supercritical methanol treatment," *Bioresource Technol.* 91, 289-295.

Labohm, H., Rozendaal, S., and Thoenes, D. (2004). *Man-Made Global Warming: Unravelling a Dogma*, Multi-Science Publ., Brentwood, Essex, UK.

Lang, X., Dalai, A. K., Bakhshi, N. N., Reaney, M. J., and Herts, P. B. (2001). "Preparation and characterization of bio-diesels from various bio-oils, *Bioresource Technol.* 80(1), 53-62.

Lee, S. (1996). *Alternative Fuels*, Applied Energy Technol. Ser., Taylor and Francis, Washington, DC.

Lomborg, B. (2001). *The Skeptical Environmentalist. Measuring the Real State of the World*, Cambridge Univ. Press, Cambridge, UK.

Ma, F., Clements, L. D., and Hanna, M. A. (1998) "The effect of catalyst, free fatty acids, and water on transesterification of beef tallow," *Trans. ASAE*, 41, 1261-1264.

Mathews, J. F., Teplo, M. G., Eager, R. L., and Pepper, J. M. (1985). "Upgrading of poplar wood oil over HZSM-5 zeolite catalysis," *Can. J. Chem. Eng.* 63(4), 686-689.

McKay, H. (2006). "Environmental, economic, social, and political drivers for increasing use of woodfuel as a renewable resource in Britain," *Biomass Bioenergy* 30(4), 308-315.

Minami, E., and Saka, S. (2006). "Kinetics of hydrolysis and methyl esterification for biodiesel production in two-step supercritical methanol process," *Fuel*, in print.

Mittelbach, M. (1996). "Diesel fuel derived from vegetable oils. VI. Specification and quality control of biodiesel," *Bioresource Technology* 56(1), 7-11.

Noureddini, H. (2000). "Process for producing biodiesel with reduced viscosity and a cloud point below thirty-two (32) degrees fahrenheit," *U.S. Pat.* 6,015,440.

Noureddini, H. (2001). "System and process for producing biodiesel fuel with reduced viscosity and a cloud point below thirty-two (32) degrees fahrenheit," *U.S. Pat.* 6,174,510.

OECD/IEA. (2000). *Energy Technology and Climate Change: A Call to Action*, International Energy Agency, Organization for Economic Co-operation and Development, Paris.

Pestes, M. N. and Stanislao, J. (1984). "Piston ring deposits when using vegetable oil as a fuel," *J. Testing Eval.* 12(2), 61-68.

Peterson, C. L., Reece, D. L., Thompson, J. C., Beck, S. M., and Chase, C. (1996). "Ethyl ester of rapeseed used as a biodiesel fuel. A case study," *Biomass & Bioenergy* 10(5-6), 331-336.

Piirtinen, E., Stenius, P. Vuorinen, T., Kovasin, K., and Ala-Kaila, K. (2004). "A new method to improve soap separation in black liquor by adding extracted neutrals of tall oil," *2004 TAPPI Fall Tech. Conf.: Engineering and Pulping Proc.*, TAPPI Press, Atlanta, 505-515.

Propst, M. (1995). "Pine notes: Skimming off the top," *Naval Stores Rev.* 105(6), 14.

Ritter, D. C., and Campbell, A. G. (1991). "Supercritical carbon-dioxide extraction of southern pine and ponderosa pine," *Wood Fiber Sci.* 23(1), 98-113.

Saka, S. (2000). "Chemical conversion of wood by supercritical water, "*ASAST* 35, 5-10.

Saka, S., and Kusdiana, D. (2001). "Biodiesel fuel from rapeseed oil as prepared in supercritical methanol," *Fuel* 80, 225-231.

Schuchardt, U., Sercheli, R., and Vargas, R. M. (1998). "Transesterification of vegetable oils: A review," *J. Braz. Chem. Soc.* 9(1), 199-210.

Sharma, R. K., and Bakhshi, N. N., "Upgrading of wood-derived bio-oil over HZSM-5," *Bioresource Technol.* 35, 57-66.

Sheehan, J., Camobreco, V., Duffield, J., Graboski, M, and Shapouri, H. (1998). *An Overview of Biodiesel and Petroleum Diesel Life Cycles*, National Renewable Energy Laboratory (NREL), Golden, CO, TP-580-24772.

Shimada, Y., Watanabe, Y., Sugihara, A., and Tominaga, Y. (2002). "Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing," *Journal of Molecular Catalysis B: Enzymatic* 17, 133-142.

Shimada, Y., Watanabe, Y., Sugihara, A., Baba, T., Ooguri, T., Moriyama, S., Terai, T., and Tominaga, Y. (2001). "Ethyl esterification of docosahexaenoic acid in an organic solvent-free system with immobilized *Candida antarctica* lipase," *J. Biosci. Bioeng.* 92(1), 19-23.

Smook, G. A. (1992). *Handbook for Pulp and Paper Technologists.* 2nd Ed., Angus Wilde Publ., Vancouver.

Song, C., Hsu, C. S., and Mochida, I. (2000). *Chemistry of Diesel Fuels.* Applied Energy Technol. Ser., Taylor and Francis, London.

Suppes, G. J., Basari, M. A., Doskocil, E. J., Mankidy, P. J., and Goff, M. J. (2003). "Transesterification of soybean oil with zeolite and metal catalysts," *Appl. Catal., A* 257, 213-223.

Supple, B., Howard-Hildige, R., González-Gómez, E., and Leahy, J. J. (2002). "The effect of steam treating waste cooking oil on the yield of methyl ester," *J. Amer. Oil Chemists Soc.* 79(2), 175-178.

Taylor, S. L., and King, J. W. (2001). "Fatty and resin acid analysis in tall oil products via supercritical fluid extraction – supercritical fluid reaction using enzymatic catalysis," *J. Chromatographic Sci.* 39(7), 269-272.

Tester, J. W., Drake, E. M., Driscoll, M. J., Golay, M. W., and Peters, W. A. (2005). *Sustainable Energy: Choosing Among Options.* MIT Press, Cambridge, MA.

Tomasevic A. V., and Siler_Marinkovic S. S. (2003). "Methanolysis of used frying oil," *Fuel Processing Technol.* 81(1), 1-6.

Tyson, K. S., and McCormick, R. L. (2006) "Biodiesel Handling and Use Guidelines Second Edition," *Energy Efficiency and Renewable Energy, U.S. Department of Energy*, Technical Report, DOE/GO-102006-2288.

Uloth, V. C. (2006), "Tall oil and tall oil soap recovery," http://wcm.paprican.ca/wcmpaprican/publishing.nsf/AttachmentsByTitle/BO_Tall_Oil_Soap_PDF_Eng/$FILE/0225-E-TallOilSoapRecovery.pdf.

Uloth, V. C., and Wearing, J. T. (1994). "Use of lignosulfonate solutions to increase both soap recovery and tall-oil production efficiency," *Naval Stores Rev.* 104(3), 8-12.

Van Gerpen, J., Shanks, B., Pruszko, R., Clements, D., and Knothe, G. (2004a). "Biodiesel analytical methods," NREL/SR-510-36240, National Renewable Energy Laboratory, Colorado, USA.

Van Gerpen, J., Shanks, B., Pruszko, R., Clements, D., and Knothe, G. (2004b). "Biodiesel production technology," NREL/SR-510-36244, National Renewable Energy Laboratory, Colorado, USA.

Vasudevan, P., Sharma, S., and Kumar, A. (2005). "Liquid fuel from biomass: An overview," *J. Sci. Indust. Res.* 64(11), 822-831.

Vellguth, G. (1988). "Vegetable oil as diesel fuel substitute," *Landbauforschung Völkenrode* 38(1), 12-16.

Wang, S.-F., Furuno, T., and Cheng, Z. (2002). "Study of the extraction of phytosterol from mason pine raw tall oil," *J. Wood Sci.* 48(6), 505-511.

Wang, S.-F., Furuno, T., Cheng, Z., and Katoh, S. (2001). "Composition of neutral fraction in Chinese raw tall oil," *J. Wood Sci.* 47(5), 400-405.

Wang, P. S., Tat, M. E., and van Gerpen, J. (2005). "The production of fatty acid isopropyl esters and their use as a diesel engine fuel," *JAOCS* 82(11), 845-849.

Warabi, Y., Kusdiana D., and Saka, S. (2004a). "Reactivity of triglycerides and fatty acids of rapeseed oil in supercritical alcohols," *Bioresource Technol.* 91, 283-287.

Warabi, Y., Kusdiana D., and Saka, S. (2004b). "Biodiesel fuel from vegetable oil by various supercritical alcohols," *Appl. Biochem. Biotechnol.* Executive Editor: Ashok Mulchandani, Humana Press, vol. 115, 793-791.

Watanabe, Y., Pinsirodom, P., Nagao, T., Kobayashi, T., Nishida, Y., Takagi, Y., and Shimada, Y. (2005). "Production of FAME from acid oil model using immobilized *Candida antarctica* lipase," *J. Amer. Oil Chemists Soc.* 82(11), 825-831.

Watanabe, Y., Shimada, Y., Sugihara, A., and Tominaga, Y. (2002). "Conversion of degummed soybean oil to biodiesel fuel with immobilized *Candida antarctica* lipase," *J. Molecular Catalysis B: Enzymatic* 17, 151-155.

Wong, A. (1995). "Tall oil-based cetane enhancer for diesel fuel," *Pulp Paper Can.* 96(11), 37-40.

Wyman, C. E., and Goodman, B. J. (1993). "Biotechnology for production of fuels, chemicals, and materials from biomass," *Appl. Biochem. Biotechnol.* 39/40, 41-59.

Yuan, W., Hansen, A. C., Zhang, Q, and Tan, Z. (2005). "Temperature-dependent kinematic viscosity of selected biodiesel fuels and blends with diesel fuel," *J. Amer. Oil Chem. Soc.* 82(3), 195-199.

Zachary, L. G., Bajak, H. W., and Eveline, F. J. (1965). *Tall Oil and its Uses*, Dodge Co., Div. McGraw-Hill, New York.

Zaher, F. A., Megahed, O. A., and El Kenawy, O. S. (2003). "Utilization of used frying oil as diesel engine fuel," *Energy Sources* 25(8), 818-826.

Zhang, Y., Dubé, M. A., McLean, D. D., and Kates, M. (2003a). "Biodiesel production from waste cooking oil: 1. Process design and technological assessment," *Bioresource Technol.* 89(1), 1-16.

Zhang, Y., Dubé, M. A., McLean, D. D., and Kates, M. (2003b). "Biodiesel Production from Waste Cooking Oil: 2. Process Design and Technological Assessment," *Bioresource Technology 90* (3), 229-240.

Zheng, S., Kates, M., Dubé, M. A., and McLean, D. D. (2006). "Acid-catalyzed production of biodiesel from waste frying oil," *Biomass Bioenergy* 30(3), 267-272.

Zinkel, D. F., and Russell, J., eds. (1989). *Naval Stores, Production, Chemistry and Utilization,* Pulp Chemicals Assoc.

Article submitted: June 16, 2006; Revision accepted: Aug. 3, 2006; Published Aug. 4, 2006