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BioResources

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

In this Issue …

And much more …

Cover image: Pine trees at North Carolina State University's research farm

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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)

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FROM HERE TO SUSTAINABILITY

Martin A. Hubbe

Many readers and contributors to *BioResources* are working to develop sustainable technology. Such research attempts to use products of photosynthesis to meet long-term human needs with a minimum of environmental impact. Archeological and historical studies have concluded that the long-term success or failure of various past civilizations has depended, at least in part, on people's ability to maintain the quality of the resources upon which they depended. Though it is possible for modern societies to learn from such examples, modern societies are interconnected to an unprecedented degree. It is no longer realistic to expect one region to be immune from the effects of environmental mistakes that may happen elsewhere in the world. Research related to renewable, lignocellulosic resources is urgently needed. But in addition to the research, there also needs to be discussion of hard-hitting questions, helping to minimize the chances of technological failure. The next failed civilization may be our own.

Keywords: Sustainability, Depletion, Civilizations, Renewable resources

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

Future Generations

"Sustainability" means that all of us live and work in such a way that our children's children can enjoy lives filled with health, nourishment, and prospects for a bright future. Briefly stated, our society must find ways to support human welfare without depleting natural resources or polluting the environment.

Our present society has not yet earned the right to be called "sustainable." We are depending on nonrenewable resources such as oil, coal, and natural gas. Many readers of *BioResources* are personally involved with research that seeks to use renewable resources as a much larger part of our lives and our economy. Some parts of this journey "from here to sustainability" lie beyond the bounds of what scientists can do by themselves. Rather, prudent political decisions will be required – even a change in the mindset of people throughout the world. We are starting to see that change in attitude, as people awaken to the impacts of global warming and the coming end of cheap oil.

Humanity's Grand Experiments

Consider what can happen when groups of humans fail to live in harmony with the Earth. The book *Guns, Germs, and Steel* by Jared Diamond provides stunning examples of the fate of such groups. Humans have been experimenting for roughly 10.5 thousand years with substantial alterations to the natural environment. Some agricultural practices have been sustained for many centuries, providing a relatively stable symbiosis between people and the land. In other cases, the land became depleted of nutrients, and

societies disappeared. With advances in science we are coming closer to explaining why some ancient societies were able to live compatibly with the environment, whereas others tended to spoil their surroundings.

The experiment continues, but our human society has become global. It is no longer practical or morally acceptable just to move to new land. Rather, there are some hard questions that scientists and engineers need to ask. In theory, efforts to achieve sustainable lifestyles and sustainable manufacturing practices will contribute to a bright future for the world's citizens and their children's children. But if we get things wrong, as technologists, our mistakes will be at a global scale.

Hard Questions

Part of the role of journal such as *BioResources* is to provide a forum where scientists and engineers can exchange ideas in an effort to "get it right." Each of us probably can think of ideas that seemed great on paper, but which failed miserably upon execution. Let's ask each other some hard questions regarding ways in which efforts to build a truly sustainable economic infrastructure might fail:

- Is my research leading in the direction of a sustainable technological infrastructure?
- Is my work leading in the direction of products that are long-lasting, or at least can be recycled, composted, or safely incinerated to recover their energy value?
- Are the results of my research likely to be politically acceptable to large majorities of people, such that they stand a good chance of being implemented? Am I able to justify the technology to people having widely different political standpoints?
- Can the results of my research be implemented on a suitably small scale, so that their success does not require wasteful transportation of materials over long distances?
- Is it likely that the results of my work will lead to technology that does not depend on toxic herbicides, solvents, or increased demands for fresh water?
- Have I considered environmental implications of the "lifecycle" of materials, assuming that the technology that I am working on were to become implemented? Is the technology close to being carbon-neutral? Can it be energy-frugal?
- Am I being sufficiently bold in my research, helping to bring about changes that are sufficiently rapid that they can have a positive impact on such issues as global warming, human need, and the depletion of non-renewable resources?
- Are we designing a technological infrastructure that will tend to remove the motivations and opportunities for future wars?

Some of these questions are admittedly huge in scope, and we do not presume to provide answers to all of them in our journal. As is made abundantly clear in the pages of *Guns, Germs, and Steel*, humans can be very clever in figuring out ways to make civilizations survive. They also can be very stupid. In looking over published work, I have been struck by the inventiveness and global insight of authors and members of the Editorial Board of *BioResources*. I am convinced that we humans have what it takes to come up with ideas. My concern, though, is that we apply sufficient discussion of those ideas. We need to discuss which of them ought to be included as part of what may be humanity's final experiment in taking care of our earthly environment. We are honored here at *BioResources* to do our small part in this grand experiment.

IMPROVED PULP EVALUATIONS WITH RESTRAINED DRYING

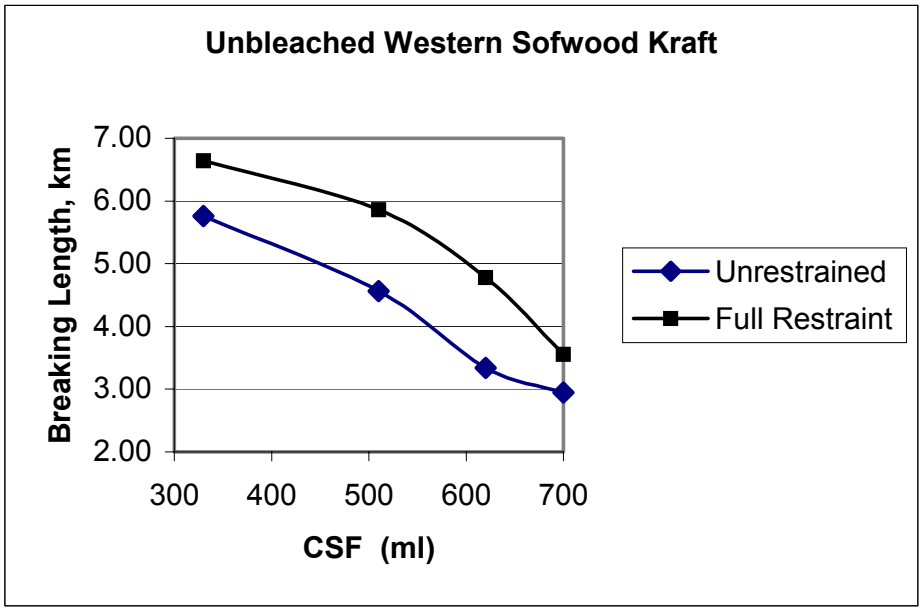
Charles Green[a]

Pulp evaluations traditionally use plate-dried handsheets. The evaluation of pulp could be improved significantly by using side-by-side comparison of handsheets that freely shrink when dried, in addition to handsheets dried in the usual way.

Keywords: Pulp evaluations, Paper properties, Predictions, Drying restraint

Contact information: a: 23 Maryvale Drive, Webster NY 14580, techman@papercurl.com

Pulp properties traditionally have been evaluated with plate-dried handsheets. Changes (increases) in tensile strength have been reported often for a pulp or process. The question is, are these increases actually seen in paper? Quoting Setterholm and Chilson (1965), "It is apparent that a correlation between properties handsheets and machine made sheets will never be satisfactory if allowances are not made for effects due to variations in restraint during drying." The data in the figure illustrate the effect of restraint and free shrinkage on breaking length.



Recently a model that makes these allowances has been proposed. (Wahlström and Mäkelä 2005). The isotropic strength values of plate and freely dried handsheets are measured along with the amount of shrinkage. These are transformed into machine- and cross-directional properties in terms of sheet shrinkage using appropriate equations and data.

In recent times several researchers have been including the effect of drying shrinkage in their research on paper properties. On the other hand, literature articles on pulp evaluations continue to use only standard TAPPI handsheets. To improve the value of pulp evaluation data significantly the following is proposed for research and production:

1. Prepare and additional set of handsheets that are dried without restraint, measuring the shrinkage.
2. Measure the properties of both sets of sheets normally measured, plus modulus of elasticity.
3. Using methods such as those of Wahlström and Mäkelä (2005), and a nominal 2:1 property ratio, report machine-directional properties with no shrinkage and cross-directional properties with 2 and 4 percent shrinkage.

This procedure should significantly increase the value of pulp evaluations and allow better application of information. The additional expense would well be justified.

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Wahlström, T., and Mäkelä, P. (2005). "Predictions of anisotropic multiply board properties based on isotropic ply properties and drying restraints," 13th Fund. Res. Symp., Cambridge.

ISOLATION OF CELLULOSE MICROFIBRILS – AN ENZYMATIC APPROACH

Sreekumar Janardhnan* and Mohini M. Sain

Isolation methods and applications of cellulose microfibrils are expanding rapidly due to environmental benefits and specific strength properties, especially in bio-composite science. In this research, we have successfully developed and explored a novel bio-pretreatment for wood fibre that can substantially improve the microfibril yield, in comparison to current techniques used to isolate cellulose microfibrils. Microfibrils currently are isolated in the laboratory through a combination of high shear refining and cryocrushing. A high energy requirement of these procedures is hampering momentum in the direction of microfibril isolation on a sufficiently large scale to suit potential applications. Any attempt to loosen up the microfibrils by either complete or partial destruction of the hydrogen bonds before the mechanical process would be a step forward in the quest for economical isolation of cellulose microfibrils. Bleached kraft pulp was treated with OS1, a fungus isolated from Dutch Elm trees infected with Dutch elm disease, under different treatment conditions. The percentage yield of cellulose microfibrils, based on their diameter, showed a significant shift towards a lower diameter range after the high shear refining, compared to the yield of cellulose microfibrils from untreated fibres. The overall yield of cellulose microfibrils from the treated fibres did not show any sizeable decrease.

Keywords: Cellulose, Cellulose microfibrils, Fungal / Enzyme pretreatment, Cellulose microfibrils isolation, Hydrogen bonds

*Contact information: Department of Chemical Engineering and Applied Chemistry, 200 College Street University of Toronto, Toronto, ON – M5S 3E5, Canada. *Corresponding author: s.janardhnan@utoronto.ca*

INTRODUCTION

Cellulose, the most abundant biopolymer on earth, is poly(β-1,4, D anhydroglucopyranose), which through a regular network of inter and intramolecular hydrogen bonds is organized into perfect sterioregular configurations called microfibrils. Each chain is stabilized by intrachain hydrogen bonds formed between the pyranose ring oxygen in one residue and the hydrogen of the OH group on C3 in the next residue (O5...H-O3') and between the hydroxyls on C2 and C6 in the next residue (O2-H...O6') (Liang and Marchessault 1959).

During biosynthesis, cellulose microfibrils are synthesized by the plasma membrane using an enzyme called cellulose synthase and are deposited onto the cell wall. In higher plants, despite its chemical simplicity, the physical and morphological structure of native cellulose is complex and heterogeneous, and in cell walls cellulose molecules are intimately associated with other polysaccharide moieties, resulting in even more

complex morphologies. Breakdown of these close physical and chemical associations between cellulose and other polysaccharides in a plant cell wall is vital for any economical utilization of these polymers. Researchers have achieved significant progress in converting lignocellulosic materials to materials of engineering importance such as reinforcing fibres, bioplastics and even biofuels.

The elementarization of natural fibres into their elementary cellulosic constituents such as nano- and microfibrils is gaining wider attention due to their (1) high strength and stiffness (Tashiro 1991), (2) high reinforcing potential, and (3) their biodegradability and renewability. Depending on the degree of elementarization, the defects and dimensions of the partly crystalline fibres of wood decrease, thereby improving their strength properties. The literature differentiates between Microfibrillated Cellulose (MFC) obtained through a mechanical homogenization (Herrick et al., 1983) and Microcrystalline Cellulose (MCC) that is generated by chemical treatment of various plant fibres. MFC has an aspect ratio around 50 to 100 and is extensively investigated for its reinforcing potential, while MCC with an aspect ration of about 3 is widely used as rheology control agents and as binders in the pharmaceutical industry.

Preparation and application of nanocomposites using cellulose nano- and microfibrils are expanding rapidly in biocomposite science. Numerous other high-end potential applications for cellulose microfibrils are currently being explored. Poor economics due to a high energy requirement in the isolation of cellulose microfibrils is a key challenge that could hamper the current momentum in the direction of commercialization. Microfibrils have been generated in the laboratory through a combination of high energy refining in a PFI mill, and subsequent cryocrushing under the presence of liquid nitrogen (Chakraborty and Sain 2005).

Isolation of Cellulose Microfibrils

Microfibrils are joined laterally by means of hydrogen bonding (Brown et al. 1976). In the cited study, as the microfibrils were generated, they were found to coalesce laterally through interfibrillar hydrogen bonding to form bundles. As stated by the authors, "the bundles associate with neighboring bundles to produce a composite ribbon of cellulose microfibrils".

The glucose and cellobiose structures show the presence of several hydroxyl radicals in the cellulose chain, and all these hydroxyl groups participate in hydrogen bonding. The interfibrillar hydrogen bonding energy has to be overcome in order to separate the microfibrils into individual entities. More than one type of H-bond is present in cellulose - intermolecular and intramolecular, so only a range of values can be used to quantify the hydrogen bond strength. This energy (U) for cellulose ranges between 19 and 21 MJ/kg mol (Nissan et al., 1985).

Young's modulus (E) of a hydrogen bond-dominated solid such as paper has been quantified (Nissan et al. 1985) as follows:

$$E = <k_R> n^{1/3} \quad (1)$$

where R is the total H-bond length, $\langle k_R \rangle$ is the average value of the force constant for stretching R by a unit distance, and n is the effective number of H-bonds per unit volume involved in taking up strain under uniaxial stress conditions.

Microfibrils are more flexible and agglomerate less in the presence of water. Fengel (1974) indicated that intensive disintegration in a homogenizer could split even the elementary fibrils and microfibrils down to molecular diameters.

Any attempt to loosen up the microfibrils by either complete or partial destruction of the hydrogen bonds before the mechanical process would be a step forward in the quest for energy-efficient generation of cellulose microfibrils. The focus of this research is to investigate and establish an enzymatic chemistry that would partially or completely nullify the hydrogen bonds between the microfibrils, making their isolation energy-efficient.

Enzyme Technology in Fibre Processing

The application of enzymes in fibre processing has been mainly directed towards the degradation or modification of hemicelluloses and lignin, while retaining the cellulosic portion. The enzymatic approach in the fibre processing sector has been based the idea of selected hydrolysis of certain components or limited hydrolysis of several components in the fibre. Some of the important areas of applications are (1) Fibrillation, inter-fibre bonding and strength enhancement (Bolaski et al. 1959; Yerkes 1968; Nomura 1985), (2) Drainage (Fuentes, 1988), (3) Modification of pulp properties (Uchimoto 1988; Paice 1984; Senior 1988; Jurasek 1988), (4) Enzymatic pulping (Nazareth 1987; Sharma and 1987, Morvan, C., 1990), and (5) Enzymatic pretreatments for bleaching (Tolan 1992; Viikari 1990).

Although enzymes have been widely used to modify cellulosic fibres for various applications, there hasn't been any research effort to understand and utilize the enzyme – fibre interaction at microfibrilar level. An understanding of the chemistry at this level and its exploitation to isolate high strength micro- and nanofibrils from plant cell walls in an economical manner would be a huge step towards isolation of cellulose microfibrils and their commercial scale utilization in various applications.

EXPERIMENTAL

Materials

Wood Fibre: Bleached kraft pulp – northern black spruce was used as the starting material for the isolation of microfibrils. Typical composition is described in Table 1.

Table.1. Composition of Bleached Kraft Pulp

Composition	%
Cellulose	86
Hemicellulose	14

Fungus: The fungus OS1, isolated in our laboratory from Elm tree infected with Dutch elm disease was used as the source of enzyme for the fibre treatment.

Methods

Bio-treatment: Twenty-four grams of oven-dry bleached kraft fibre was soaked overnight, disintegrated in 2 liters of water, and autoclaved for 20 minutes. A 24 gram sample size of fibre was chosen, as it was the optimum fibre charge to the high shear refiner that was used for further mechanical defibrillation for cellulose microfibrils isolation. OS1 fungal culture was added to this fibre suspension in a sterile flask with appropriate amount of sucrose and yeast extract to support the fungal growth. The fungus was left to act on the fibres at room temperature for different time duration with slow agitation. The fibres were autoclaved after their respective treatment time, washed and made into sheets of 10% fibre consistency ready for the mechanical refining and cryocrushing.

High shear refining: The fibres at 10% consistency were then sheared in a refiner for 125000 revolutions.

Cryocrushing: The refined fibres were then subjected to cryocrushing in which the fibres were frozen, using liquid nitrogen, and high shear was applied, using a mortar in a pestle. This step is critical in librating the microfibrils from the cell wall. The cryocrushed fibres were then dispersed in to water suspension using a disintegrator and filtered through a 60-mesh filter. The filtrate, a dilute water suspension of microfibrils, was used for further investigation.

Characterization of Ophiostoma Ulmi treated fibres

Weight loss: The weight loss of the bio-treated fibres was determined by simple difference between the weight of fibres before and after treatment.

Fibre composition: The cellulose and hemicellulose contents of the fibre after the bio-treatment were determined using the procedure adapted from Zobel and McElwee 1966).

Cellulose Microfibril Characterization

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to understand the surface morphology and diameter distribution of the treated fibres and cellulose microfibrils isolated.

RESULTS AND DISCUSSION

The results presented here focus on the effect of OS1 fungal pretreatment of bleached kraft softwood fibres on yield and diameter distribution of cellulose microfibrils obtained through subsequent defibrillation techniques such as high shear refining and cryocrushing. The action of fungal treatment on the morphology and the capacity of the bio-treatment to facilitate the internal defibrillation are extensively detailed here through Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The impact of bio-treatment and its extent on the physical and chemical characteristics of the fibres were studied by determining weight loss and cellulose content of the fibres.

Effect of OS1 Fungal Pretreatment of Fibres on Cellulose Microfibril Yield and Fibre Diameter Distribution

One of the major challenges impeding the isolation of cellulose microfibrils on a sizable scale for any intended application is the predominating hydrogen bonding between the cellulose microfibrils and also between microfibrils and hemicellulose. Cellulose microfibrils are generated and isolated through a combination of high energy refining, and subsequent cryocrushing under the presence of liquid nitrogen. A key reason for high shear refining of the fibres is to cause internal defibrillation, a process where only a minor portion of the total energy supplied to the refiner is utilized for internal defibrillation.

The interfibrillar hydrogen bonding energy has to be overcome in order to separate the microfibres into individual entities. This association energy for cellulose ranges between 19 and 21 MJ/kg.mol, with 20 MJ/kg.mol being used as an average value in most cases. If this value is taken to be the intermolecular H-bond energy binding the fibres together, then this much energy should be supplied to separate the microfibres into separate entities.

One of the main reasons to choose OS1 as the first fungal candidate for fibre treatment is our prior knowledge (Modification of interface in natural fibre reinforced composites, MASc Thesis, Deepak Gulati, 2006) of their effect on hemp fibres – its capacity to degrade and probably hydrolyze the cellulose. In this work, bleached kraft pulp was pretreated with OS1 fungus to study its effect on (a) overall yield of microfibrils, (b) number averaged fibre diameter distribution.

Yield of Cellulose Microfibrils

The yield of cellulose microfibrils is determined as the percent by weight of microfibrils that pass through a 60-mesh screen after refining and cryocrushing. The yield comparison is detailed in the Fig. 1. The overall yield of cellulose microfibrils from OS1 treated fibre is seen to decrease by an average of 5%. The decrease in yield of microfibrils seems to be noticeable only after a minimum of 4 days of treatment, which indicates that the fungus needs a minimum of 4 days to establish an active community and produce enzymes in an effective quantity.

The yield of microfibrils is seen to stabilize after 5 days of treatment, and there is no noticeable decrease with any further extent of treatment. This observation contradicts results of the earlier study of the effect of OS1 fungus on hemp fibres, which showed a significant activity of the fungus towards cellulose accompanied by a significant loss in the fibre strength after 4 days of treatment (Modification of interface in natural fibre reinforced composites, MASc Thesis, Deepak Gulati, Department of Chemical Engineering and Applied Chemistry, University of Toronto, 2006).

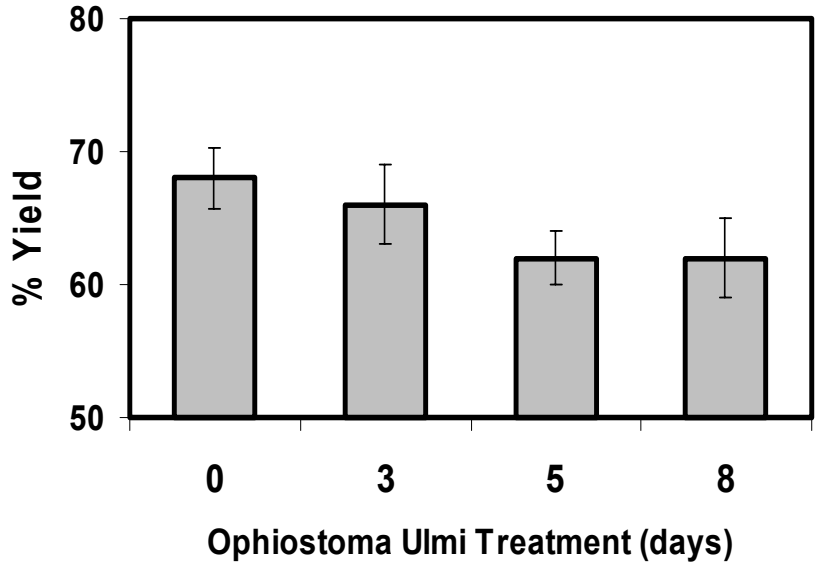


Fig.1. Yield of cellulose microfibrils with different Ophiostoma Ulmi treatment conditions

The low cellulolytic activity of OS1 fungus was further confirmed by a study of the weight loss and cellulose content of the treated fibres. The loss in fibre weight, as depicted in Fig. 2, showed a gradual drop up to a maximum 7.5 % of original fibre weight for 4 days treatment and tended to be insignificant thereafter. A similar trend is seen with respect to the cellulose content of the treated fibres. This is evident in Fig. 3, where the cellulose content is seen to decrease with the extent of treatment, and the loss of cellulose is proportionate with the weight loss of the treated fibres.

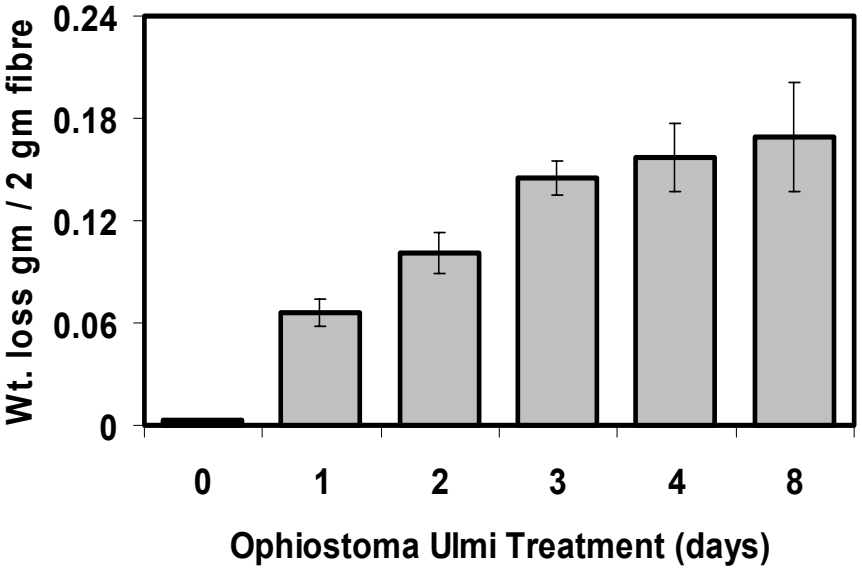


Fig. 2. Weight loss of fibres with different OS1 fungus treatment conditions

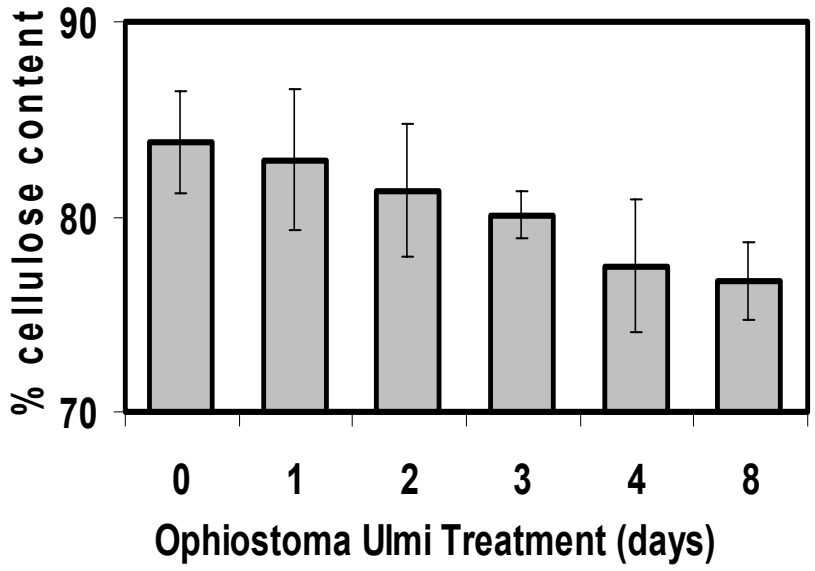


Fig. 3. Cellulose content of fibres with different OS1 treatment conditions

The weight loss and a proportionate decrease in cellulose content of the treated fibres imply that the action of fungal enzymes on the fibres is mostly limited to cellulose and not the hemicellulose. Now, the reason for this low level of activity against cellulose can be explained only once the specific enzymes are isolated and identified. This is the next phase of this project.

Microfibril Diameter Distribution

Having understood the level of OS1 activity against cellulose, it is vital to understand the effect of OS1 fungal treatment on the internal defibrillation tendency of the treated fibres during subsequent mechanical defibrillation techniques such as high shear refining or high-pressure homogenization. This is the first step towards testing the hypothesis that enzymes can help in internal defibrillation through either weakening the hydrogen bonds that exist between microfibrils or loosening up the fibrils through controlled hydrolytic activity.

Fibres treated with OS1 fungus were refined and the number-average diameter distributions of these refined fibres for a 4 days treatment are detailed in Fig. 4. A very significant shift in the diameter distribution of the fibres occurred towards the lower diameter range, with the maximum yield of fibres below 100 nm range for the 4 days treated fibres, while that for the untreated fibre were between 100 – 250 nm range. The fibre diameter distribution did not change in an appreciable manner with increase in treatment time longer than 4 days treatment (results not shown here). This shift in fibre diameter distribution curve towards the lower diameter range for a treated fibre after the refining is of importance in this work as this observed phenomenon can happen only if the treatment had an effect of facilitating the internal defibrillation in the fibre during refining. The mechanism is not apparent yet, but a good supposition is that the enzymes might have worked to reduce the hydrogen bonding between the fibrils, thus improving

the internal defibrillation during refining. This concept is more visible in the TEM images of an unrefined treated fibre as shown in Fig. 5 and the refined fibres treated with the fungus as detailed in Fig. 6.

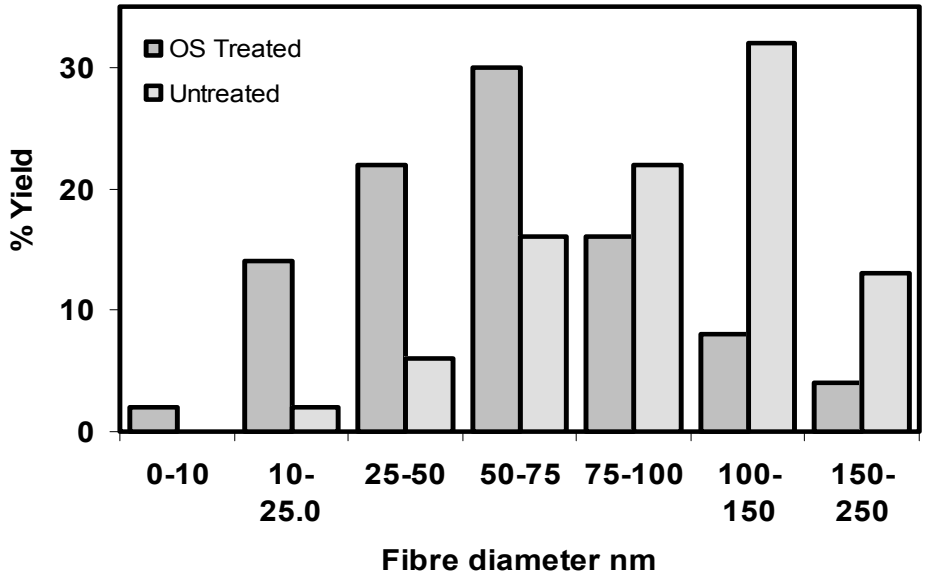
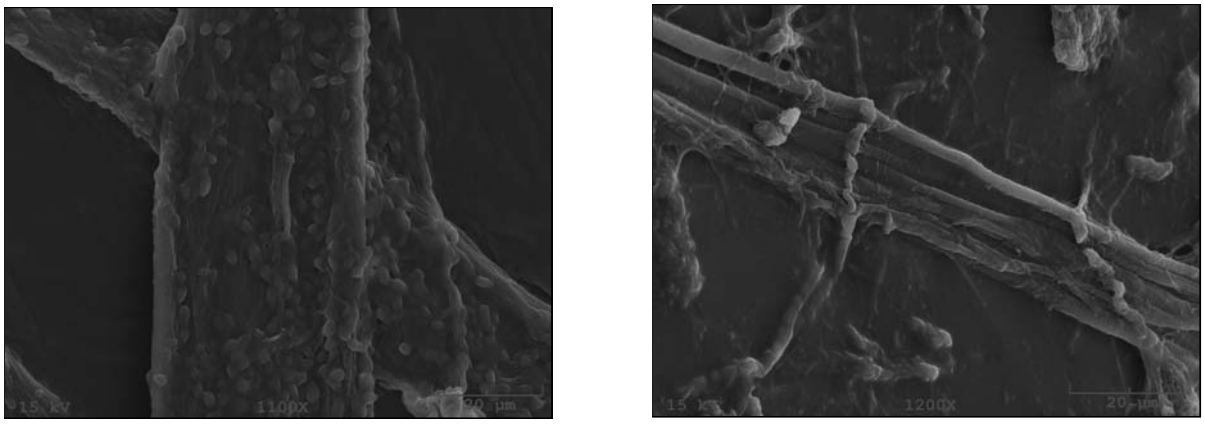


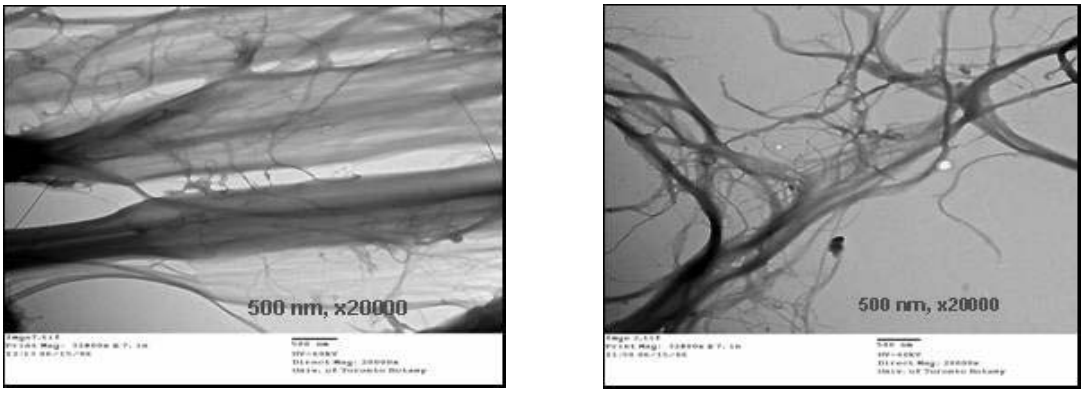
Fig. 4. Effect of OS1 fungal treatments on number averaged diameter distribution of fibres after refining for a 4 days treatment



(a) (b)

Fig. 5. OS1 fungal treatment of fibres, (a) fungus growing on the fibre, (b) treated fibre before high shear refining

The fibrillation of the treated fibres, as seen in Fig.6 (b), is more pronounced after refining, compared to the untreated fibres. The actual separation of elementary fibres takes place to a good extent with treated fibres, while high shear refining seems to have a reduced fibril separation effect on untreated fibres. This observation can explain the difference in fibre diameter distribution associated with treated and untreated fibres.



(a) (b)

Fig. 6. Effect of OS1 fibre treatments on internal defibrillation - (a) TEM of *untreated* fibre after high shear refining, (b) TEM of *treated* fibre after high shear refining

Cryocrushing is the final step that helps in the isolation of cellulose microfibrils into individual entities from the fibrillated fibres. An interesting point to note here is that a significant difference in fibre diameter distribution observed between treated and untreated fibres after refining, as was detailed in Fig. 4, no longer seems to demonstrate their significance in Fig. 7, which depicts the fibre diameter distribution of treated and untreated fibres after cryocrushing. The reason for such a distribution may be explained by a strong and positive effect of cryocrushing on microfibril isolation from fibres, such that the better defibrillation attained by treated fibres after refining is subdued.

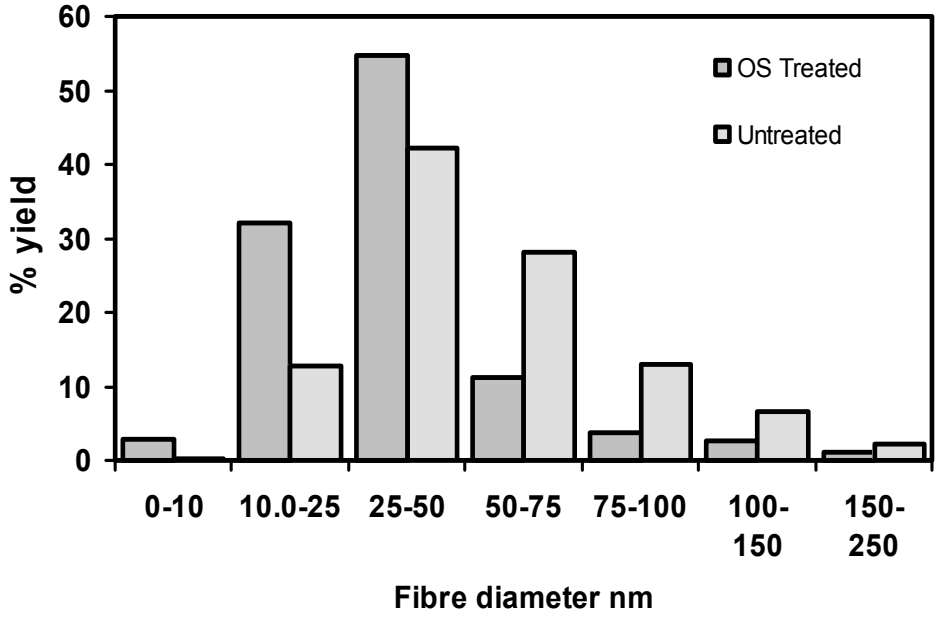


Fig.7. Effect of OS1 treatments of fibres on the yield and distribution of cellulose microfibrils after refining and cryocrushing

The fibre diameter distribution trend is similar for both treated and untreated fibres, with microfibrils from treated fibres showing a slight shift towards lower diameter range, and with the major fraction of fibres in the 0 – 50 nm range. The cellulose microfibrils isolated from treated fibres after cryocrushing showed very clear and distinct separation, as compared to cellulose microfibrils isolated from untreated fibre, as seen in Fig. 8 and Fig. 9.

This narrow shift in the fibre diameter distribution, as shown in Fig. 8, stems from the fact that isolation of cellulose microfibrils into distinct entities is not as good with untreated fibres as with treated fibres. This effect is evident from a closer look at TEM pictures, as shown in Fig. 8 and Fig. 9.

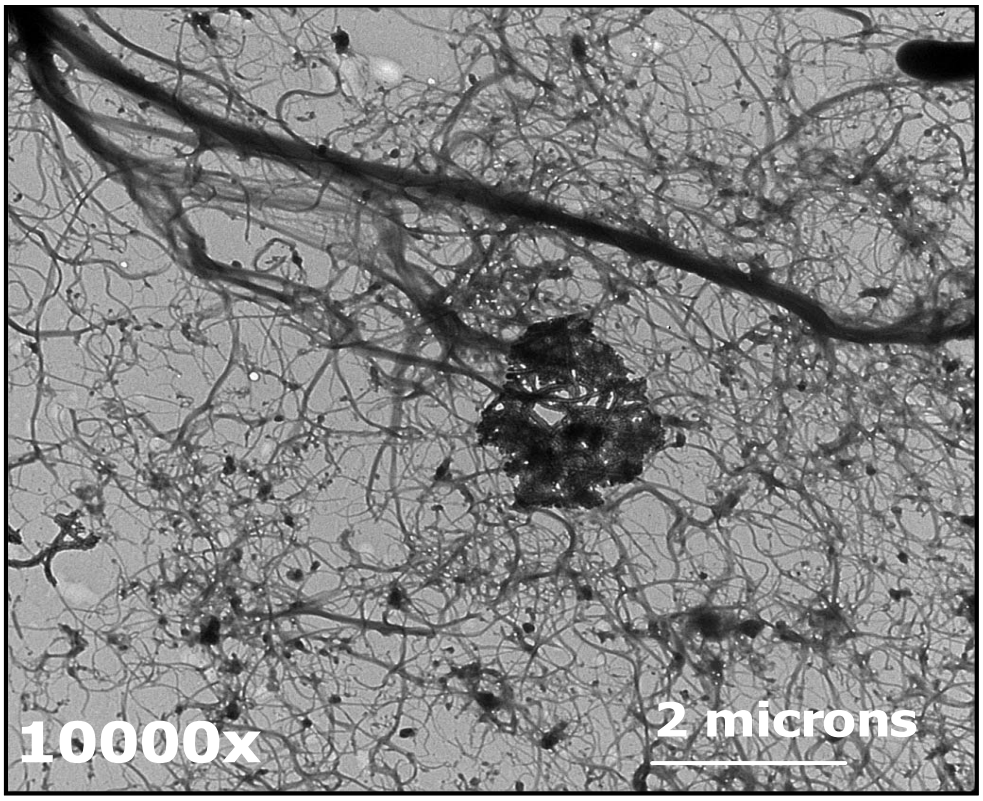


Fig. 8. TEM of cellulose microfibrils isolated from OS1 *treated* fibres through refining and cryocrushing

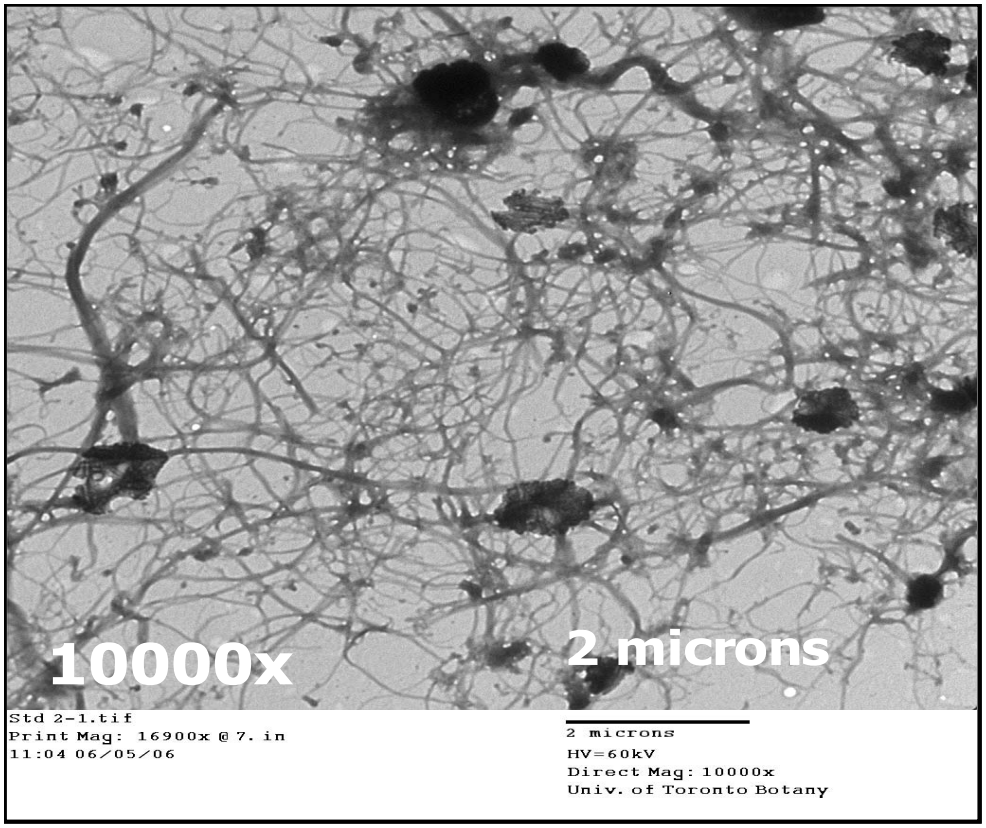


Fig. 9. TEM of cellulose microfibrils isolated from *untreated* fibres through refining and cryocrushing

The observations detailed above steer our thinking in two directions.

(a). The effect of fungal treatment has been shown to have a significant impact on the defibrillation characteristics of the fibres during defibrillation techniques such as the PFI refining we have used here. However, the impact seems to lose its significance once these refined fibres are cryocrushed. Therefore one may ask whether this enzymatic fibre treatment really benefits the isolation of cellulose microfibrils in a two-step process that includes PFI refining and cryocrushing.
(b). Having demonstrated the encouraging effect of OS1 treatment on the defibrillation of fibres during subsequent refining, it is worthwhile adopting a one-step process such as homogenization in a microfluidizer to authenticate the effect of fibre treatment and see if fewer passes through the microfluidizer are enough to isolate cellulose microfibrils, as compared to the number of passes required for untreated fibres.

In addition to isolating and identifying the extracellular enzymes involved in the treatment, the next phase of this work will include the isolation of cellulose microfibrils from Ophiostoma-treated fibre, using single-step / multipass high-pressure homogeniz-

ation. Fewer passes for treated fibres through a homogenizer for comparable microfibril yield will undeniably suggest a lower energy scenario in cellulose microfibril isolation.

CONCLUSIONS

1. The fungus OS1 treatment was shown to have a significant impact on the defibrillation characteristics of the fibres – a major step in the isolation of cellulose microfibrils.
2. Cellulose microfibrils isolated by refining and cryocrushing of treated fibres yielded very distinct microfibrils and a narrower microfibril diameter distribution, compared to that obtained for untreated fibres.
3. The fungus OS1 treatment of bleached kraft fibres seems to have only a mild activity against cellulose, which is of interest to this work, as this minimizes the loss of cellulose.

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A NEW APPROACH TO MEASUREMENT OF SACCHARIFYING CAPACITIES OF CRUDE CELLULASE

Bin Wu[a], Yue Zhao[a], Pei Ji Gao[a*]

A practical, quantitative approach has been designed, which makes it possible to accurately estimate the saccharifying activities of crude cellulase preparations for insoluble cellulosics. The challenge in activity determination imposed by changes in hydrolysis time and concentration of cellulase and cellulosics on the assay could be overcome by selection of the specific conversion percentage of cellulose as a function of cellulase concentration, that is, the hydrolysis percentage of filter paper by unit cellulase per minute, as the objective function with respect to different concentrations of crude cellulase. A rational and governing equation for crude cellulase assay was derived, and reliable results for quantitatively estimating the saccharifying activities of crude cellulases during the progress of hydrolysis of several cellulosic substrates were obtained.

Keywords: Cellulase; Cellulose; Conversion; Hydrolysis

*Contact information: a: The State Key Laboratory of Microbial Technology, Shandong University, Jinan, 250100, China; *Corresponding author: Gaopj@sdu.edu.cn*

INTRODUCTION

Crude cellulases are composed of multi-component enzyme mixtures, which can be classified into three types: endoglucanase (1,4-β-D-glucan glucohydrolase EC 3.2.1.4), exoglucanase (1,4-β-D-glucan cellobiohydrolase, EC 3.3.1.19), and β-glucosidase (β-D-glucoside glucohydrolase EC 3.2.1.21). A synergistic action of these enzymes is necessary for the complete hydrolysis of crystalline cellulose (Beguin and Aubert 1994). The hydrolysis rate is a compound function with reaction conditions that mainly depend on the ratio of the endo- to exo-glucanase, the concentrations of cellulase and cellulose, and the hydrolysis time. The reaction rate continuously declines as the conversion percentage of cellulose increases, and the specific rate at a given percentage of conversion also decreases with increasing cellulose concentration (Sharrock 1988). Because of the complexity of the cellulase-cellulose system, it is difficult to quantitatively describe the reaction rate and mechanism; thus, the kinetic characteristics in the initial reaction period and in the extended stages are significantly different, compared with the homogeneous enzyme catalytic reaction system, so that a Michaelis-Menten type rate expression cannot be applied to those enzymatic hydrolysis reactions. As Sharrock suggested, "the measurement of cellulase activity must be approached using somewhat uncertain techniques" (Sharrock 1988).

A variety of assays and substrates have been used for measurements of cellulase activities. These can be grouped into two basic approaches with respect to activity: One is based on the determination of the individual activity of the cellulase component;

whereas the other is based on the evaluation of the total saccharifying capacity of a crude cellulase system for the hydrolysis of certain cellulosics (Sharrock 1988). Until now, accurately determining cellulase activities, however, has been limited to cases with a single cellulase component with a soluble substrate (Wood and Bhat 1998; Day and Workman 1984; Deshpande et al. 1984; Manning 1981; Hagerman et al.1985; Wu et al. 2006).

A series of assay procedures has been used for estimating crude cellulase activities on insoluble cellulose. The activities of cellulase are commonly evaluated by endpoint analysis of the products, using a reducing sugars assay, and therefore, results are expressed as the saccharifying capacity. However, most of this work is not readily expressed in a quantitative manner, lacks a theoretical basis, and does not take into account all the effective factors, such as the concentration of cellulose and cellulase, the hydrolysis time, the ratio of crystalline and amorphous cellulose, and the proportion between different components in the enzyme preparation. These methods are neither rapid nor readily adaptable to kinetic measurements (Sharrock 1988). Thus, the applicability of those methods is sometimes limited to certain hydrolysis conditions; in fact, there is a considerable confusion inherent in the enzyme names, units and activities (Sharrock 1988; Deshpande et al. 1984; Manning 1981). Mandels and Webber proposed a filter paper assay (FPA) method for identifying the saccharifying activity of a cellulase preparation from cellulolytic fungi (Mandels and Webber 1969). Although this method has been recommended by the International Union of Pure and Applied Chemists (IUPAC) for evaluation of potential saccharifying capacity of a cellulase system (Ghose 1987), it has been long recognized for its complexity and susceptibility to operator error, and has a reputation for not being reproducible. Since then, some improved FPA (filter paper assay) methods have been proposed to improve the reproducibility of the filter paper assay, most of which are limited to improvements in sugar estimation (Griffin 1973; Kelly et al. 2003; Gao 1987); thus, the problem of an accurate and reproducible saccharifying assay for cellulase still remains.

In this study, a new approach to accurately estimating the potential capacity of a cellulase system for the hydrolysis of insoluble cellulosics is described.

EXPERIMENTAL

Materials and Methods

Cellulolytic substrates

Filter paper (Whatman No.1), which was adopted for the present work, is composed of crystalline and amorphous cellulose. De-waxed cotton fibers, composed of 95% cellulose, were used to represent highly crystalline native cellulose (DP about 1000). Crystalline cellulose (Avicel) PH101 was obtained from SERVA (DP about 200). Phosphate swelling cellulose, prepared by 85% phosphate acid swelling method (Wood and Bhat 1998), represented amorphous cellulose.

Production and purification of cellulase

A cellulolytic fungus, Trichoderma pseudokoningii S-38, was isolated previously by our laboratory and used for production of cellulase (Ma 1990). Enzyme production, purification and determination of its activity were performed as described in previous reports (Yan and Sun 1997; Yan et al. 1997).

Hydrolysis of cellulose samples by crude cellulase

The hydrolysis assay procedure was performed in 25 mL flasks that contained different concentrations of cellulose substrate (w/v) suspended in 5 mL (final volume) pH 4.8, 50 mM acetate buffer, to which was added the crude cellulase solutions. Hydrolysis was performed at 45ºC in a shaking bath at 15 rpm (adding 0.001% NaN3 w/v to prevent contamination). Every two hours, the mixture was centrifuged at 5,000 g for 10 min to separate the hydrolyzate. These samples were analyzed for reducing sugars content in the supernatant by the dinitrosalicylic acid (DNS) method (Ghose 1987).

Effects of cellulase and cellulose concentration on the hydrolysis kinetics

In general, the rate at which cellulase hydrolyzes cellulose can be defined in two ways. One is based on the number of reducing end groups, usually expressed as an equivalent weight of glucose, produced per unit volume of cellulose suspension after application of a defined amount of cellulase after a prescribed incubation time. This will be called the apparent hydrolysis rate. The second way is as the equivalent weight of glucose reducing end groups produced in the cellulose suspension per unit of time and per unit of cellulose (weight or volume) at a selected enzyme level.

RESULTS AND DISCUSSION

Results of the Hydrolysis Process

Filter paper was chosen as a typical substrate for cellulase catalysis assays, because it has both crystalline and amorphous fractions, moderate susceptibility to hydrolysis, and availability as a convenient and reproducible substrate.

Fig. 1 shows a typical time course for a fixed amount of filter paper (0.2 mg/mL) hydrolyzed by different concentrations of cellulase (0.25 mL to 1.25 mL added), while Fig. 2 shows a similar time course for different concentrations of filter paper (0.08 to 0.4 mg/mL) hydrolyzed by a fixed amount of cellulase (0.25 mL).

The results in Figs. 1 and 2 appear to be in good agreement with many published sets of data in this field (Beguin and Aubert 1994; Eriksson et al. 2002; Zhang et al. 1999; Valjamae et al. 1998; Ortega et al. 2001). All of the results led to the conclusion that as the more digestible material becomes consumed, the cellulose remaining was more and more difficult to be hydrolyzed. The hydrolysis rate depended on the concentration of cellulase and cellulose. Both the condition of the cellulose and the concentration of cellulase change with time during the course of an experiment. This was indicated, since the objective variable (reducing sugars formed) was a compound function, which increased with the hydrolysis time and the concentration of cellulase and cellulose.

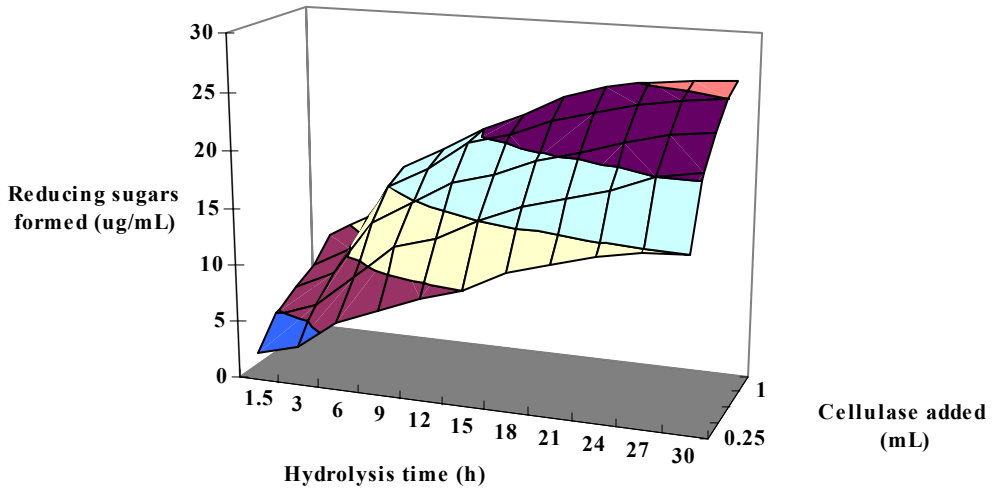

Fig. 1. Time course of hydrolysis curves for filter paper (0.2 mg/mL) by different concentrations of crude cellulase from *Trichoderma pseudokoningii* S-38

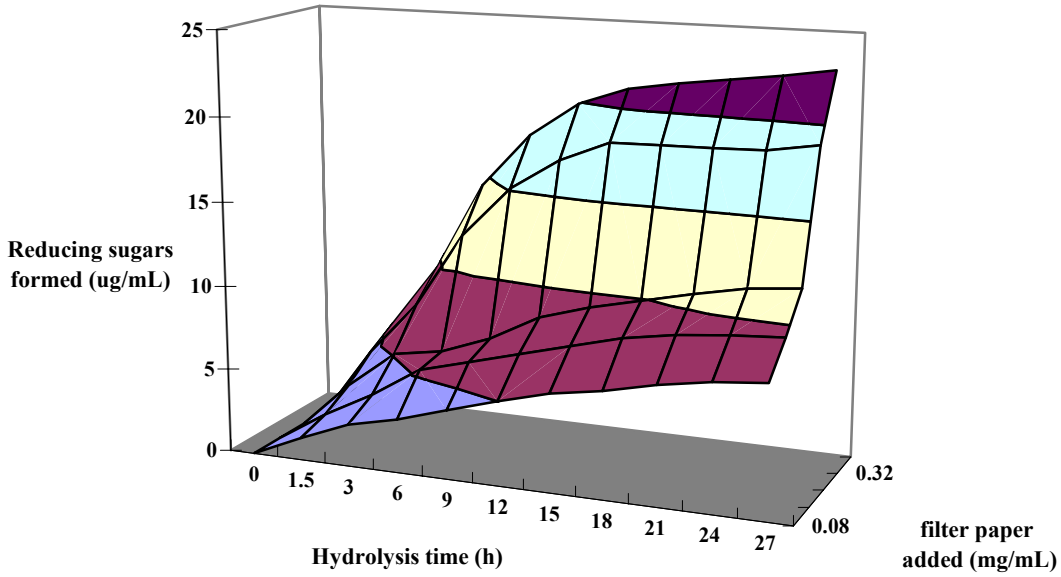

Fig. 2. Time courses of reducing sugars produced during hydrolysis of different concentration of filter paper by crude cellulase (0.25mL)

Under the conditions used, linear relationships between activities of enzyme and the cellulase added only appeared within a narrow range of the curves. Only the data in such ranges could be fitted to the linear equations. Significant effects on specific activity were observed only when considering relatively low concentrations of cellulase and relatively short hydrolysis times (Figs. 3 and 4). The specific activity was calculated as follows:

$$\text{Specific activity} = \frac{\text{Reducing sugar formed}}{t \times [\text{cellulase}][\text{cellulose}]} \quad (1)$$

where, t is the hydrolysis time, [cellulase] is the concentration of cellulase added, and [cellulose] is the concentration of cellulose added. Under these conditions, the majority of hydrolyzed cellulose is the amorphous fraction; thus, the intrinsic saccharifying capacity for cellulase cannot be accurately estimated based on those shorter hydrolysis times.

In Figs. 3 and 4, the plotted points represent the observed data, and the lines are the fitting results obtained by application of the one-phase exponential decay equation. The parameter R^2 is the coefficient of determination in the goodness of fit test.

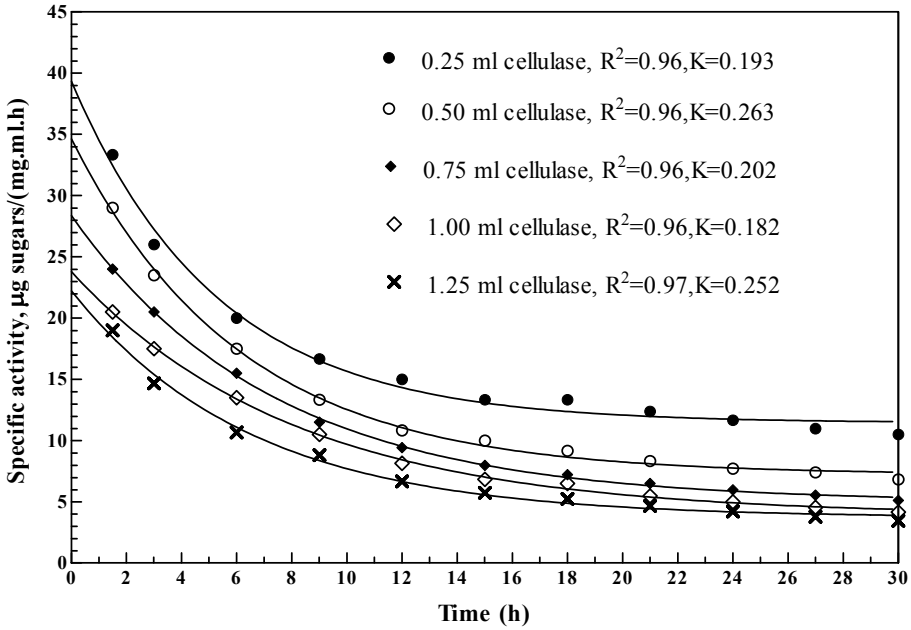

Fig. 3. Changes of the specific activities of cellulase during hydrolysis of 0.2 mg/mL cellulose at different concentrations of crude cellulase (0.25-1.25 mL) (data derived from Fig. 1).

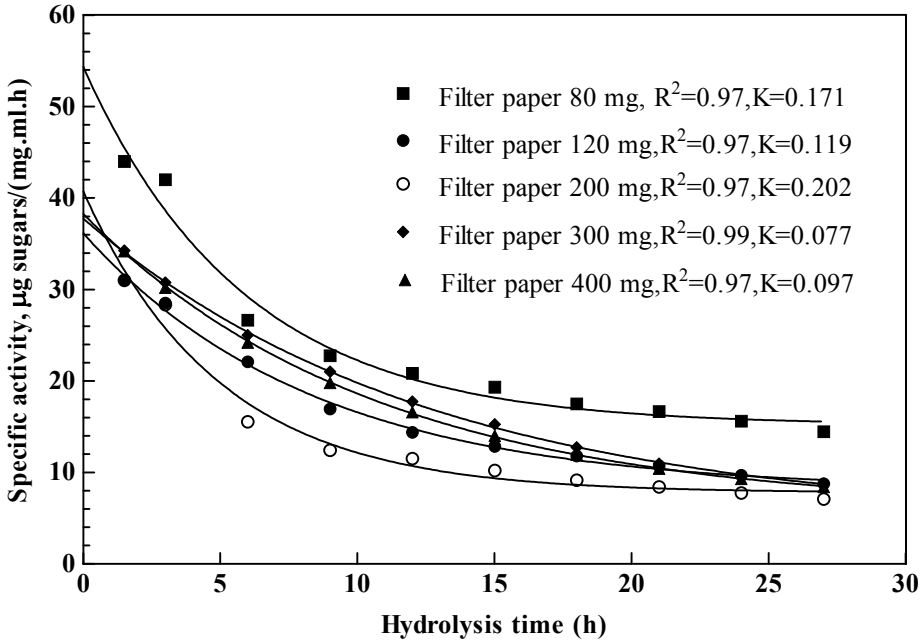

Fig. 4. Changes of the specific activities of cellulase during hydrolysis at different concentrations of filter paper (0.08-0.4 mg/mL) by 0.25mL of crude cellulase (data derived from Fig. 2).

Relationship of Hydrolysis Rate and Conversion Percent of Cellulosics during Hydrolysis Process

As mentioned above, in order to accurately estimate the potential capacity of a crude cellulase sample in the hydrolysis of insoluble cellulosics, the substrates must be hydrolyzed to a certain extent to show the synergism of all the cellulase components. As reported in our previous study, quantitatively estimating the activity behavior of cellulase during cellulose hydrolysis can be elucidated by multiple regression analysis (Wang et al. 2004). But this method is generally regarded as being applicable only for pure cellulase components, and is inadequate for crude cellulases.

For the present estimation, a new parameter, substrate conversion percentage (SC), was introduced. It was defined as the ratio between the cellulosics hydrolyzed (S_0-S, where "S" is the quantity of cellulosics remaining) and the original cellulosics (S_0):

$$SC = \frac{S_0 - S}{S_0} \times 100\% \qquad (2)$$

When results of the present study are viewed in combination with other accumulated reports (Mandels and Webber 1969; Ghose 1987; Lynd 2002), the combined data suggest that SC always increases during the progress of hydrolysis (Fig. 5), and the experimental data also produced a hyperbolic shape curve.

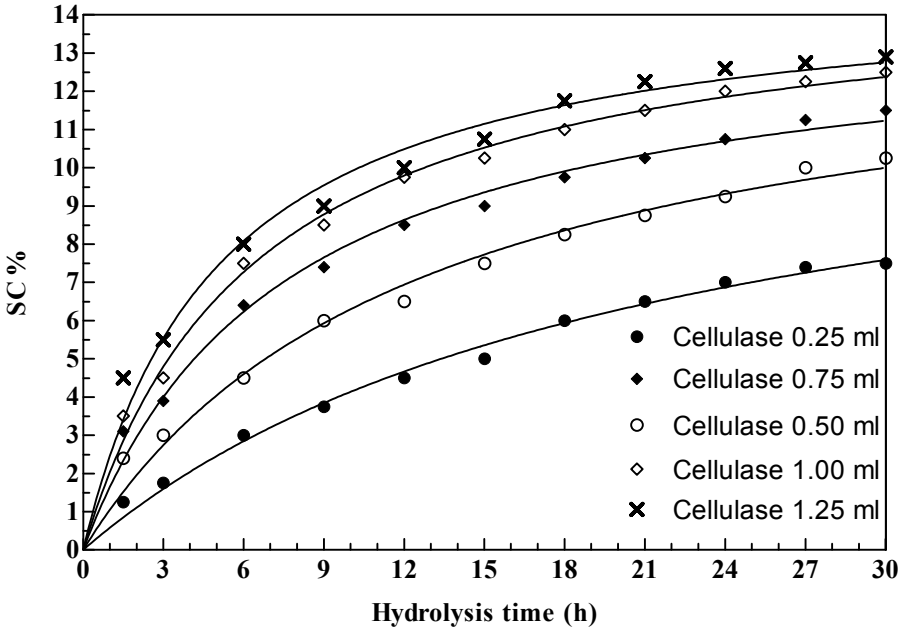

Fig. 5. Time courses of SC in the hydrolysis by different concentrations of cellulase (Data derived from Fig. 1)

The reaction rates all decreased continuously with hydrolysis time and increasing cellulase concentration. Based on these results, estimation of the cellulase activity is complex, because the accurate estimation of reactive value at each point required the combination of calculating the partial derivative of SC (Y) with respect to time (t) and cellulose concentration (C), respectively, as

$$dY = \frac{\partial Y}{\partial C} dC + \frac{\partial Y}{\partial t} dt \quad (3)$$

However, the calculation of the partial derivatives of Y with respect to C and t is very intricate.

We considered that two factors simultaneously caused the changes of SC during the progress of hydrolysis: first, the heterogeneous structure of cellulose, second, the cellulase reacting may be quantitatively responsible for the changes of Specific Substrate Conversion percentage (SSC). This parameter is calculated similarly to specific activity, and the only difference is that the SC replaces the value of reducing sugar formed.

$$SSC = \frac{SC}{t \times [cellulase][cellulose]} \quad (4)$$

where t is the hydrolysis time, [cellulase] is the concentration of cellulase added, and [cellulose] is the concentration of cellulose added. Time courses of SSC are shown in Fig.6.

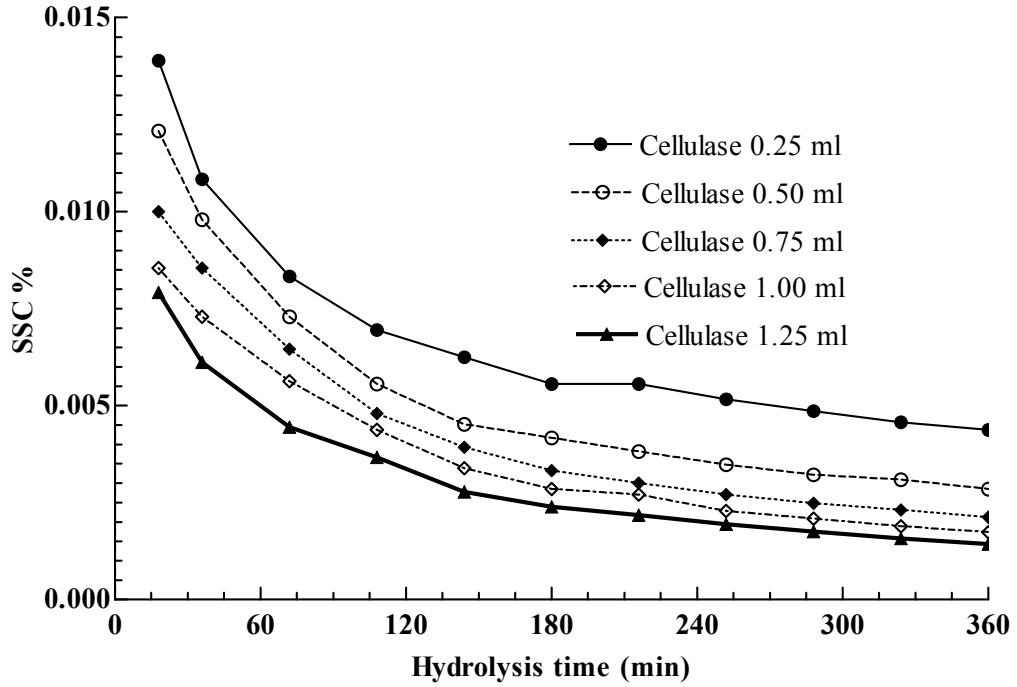

Fig. 6. Time courses of SSC (data derived from Fig. 5)

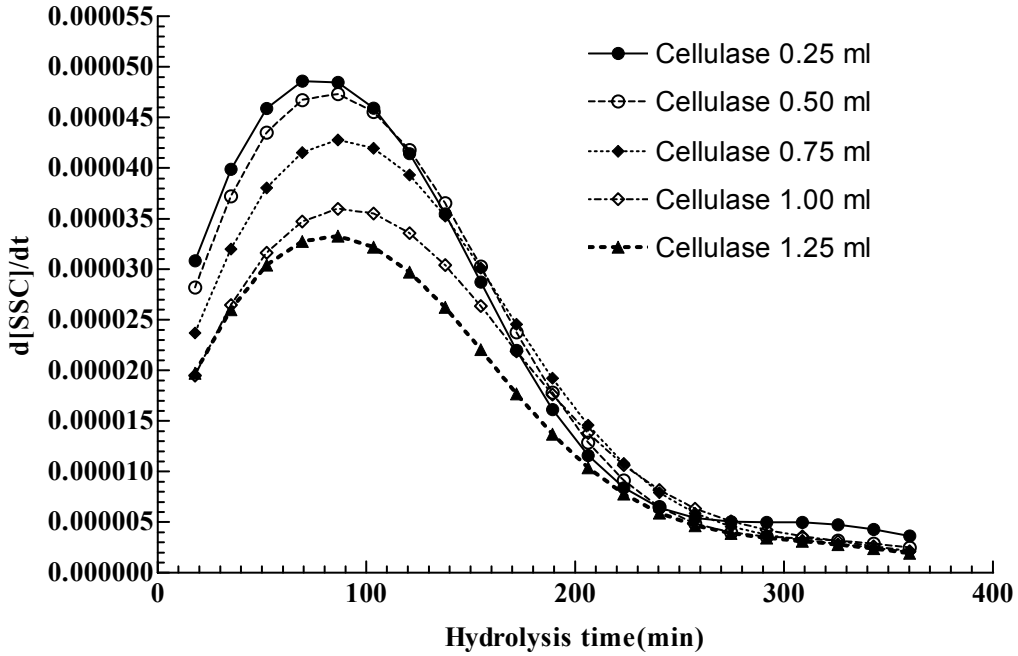

Fig. 7. Instantaneous rate curves of d[SSC]/dt for different cellulase concentrations and hydrolysis times

The critical index for evaluation in enzymatic capacity, according to the traditional approach, is its initial velocity; this can be solved by the instantaneous rates (Wu 2006). Figure 7 shows the instantaneous rates plot of d[SSC]/dt versus time under different cellulase concentrations. It appears as a bell-shaped curve, but with different kinetic characteristics and cannot be directly fit by a linear or non-linear equation that applies to the entire progress of reaction.

As mentioned above, for an insoluble and heterogeneous substrate – the cellulose – to expresses intrinsic catalytic capacity, the entire hydrolysis information is necessary. So we used the AUC method (the total area under the curve) for evaluation of cellulase capacity (Fig. 8). This is a common nonlinear method used in pharmacokinetics (Perrier 1982).

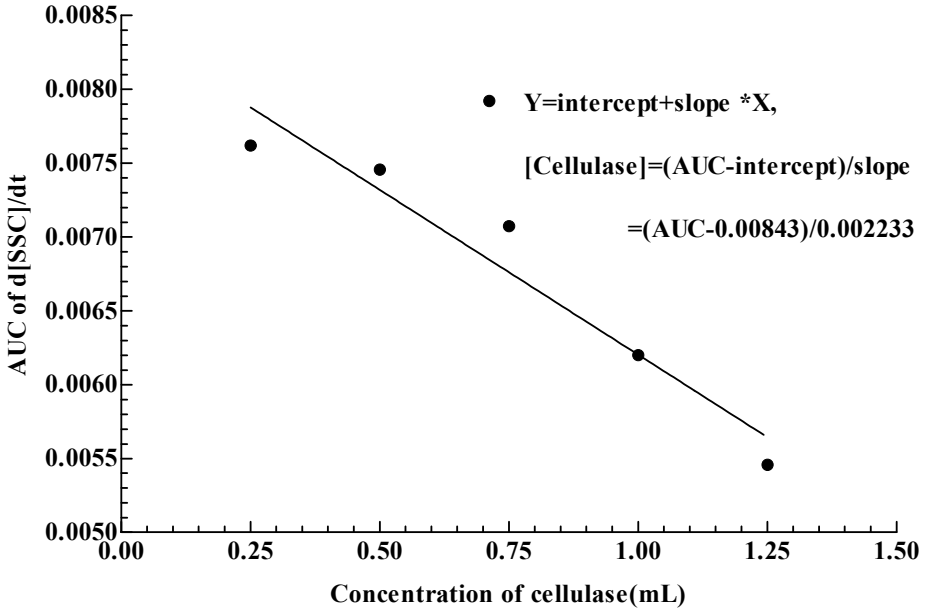

Fig. 8. The relationship of cellulase concentration and its AUC calculated as d[SSC]/dt

Reliable results were obtained, as the Specific·SC (SSC) varied in a wide range. The cellulose hydrolysis ranged from 1.5 to 12.5 % (W/V) for different concentrations of cellulase, according to which a rational and simple equation for crude cellulase assay was obtained:

$$Y = \text{intercept} + \text{slope} * X \quad (5)$$

in which Y is the AUC of d[SSC]/dt, and X is the concentration of cellulase.

As might be expected, the intrinsic saccharifying capacity for a crude cellulase can be obtained by this approach, because the effects of three factors – concentration of cellulase, cellulose, and hydrolysis time – are all considered in this equation. The slope of the linear equation could be represented as the total saccharifying capacity of a crude

cellulase sample under experimental conditions corresponding to the design of a practical hydrolysis process for insoluble cellulosics.

Similar results were obtained when choosing other cellulosics such as cotton fibers, microcrystalline cellulose, and phosphate swelling cellulose as substrates (data not shown).

Summary of Assay Procedure

The overall assay procedure, in summary, consists of the following steps:

1. Hydrolysis of cellulose samples by crude cellulase

Different concentrations of cellulose substrate are hydrolyzed by different concentrations of crude cellulase solutions. In general, five combinations for cellulose and cellulase with different concentrations would be enough, and the reducing sugars are determined every two hours. The total hydrolysis time is varied based on the structure of cellulosics; for example, for filter paper 12 hours is enough and for cotton fibers it may be 24 hours.

2. Calculation of the evaluation of cellulase capacity

Calculate the value of SC (substrate conversion percentage) and SSC (specific substrate conversion percentage), and convert the data to obtain AUC (the total area under the curve) for evaluation of cellulase capacity. Data analysis can be easily performed by Microsoft Excel and the graphical software Prism 6.0 or other public software.

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PROPERTY COMPARISONS AND BONDING EFFICIENCY OF UF AND PMDI BONDED PARTICLEBOARDS AS AFFECTED BY KEY PROCESS VARIABLES

Antonios N. Papadopoulos

The purpose of this paper was to compare physical properties of conventional particleboard bonded with amounts of UF and PMDI resin and to examine the effect of mat moisture content (MC), wax content and platen temperature on their bonding efficiency, as determined by internal bond strength. It was found that PMDI not only gave superior board properties compared with the UF, but the amount required was reduced considerably as well. The MC of the mat and the platen temperature did not significantly affect the bonding efficiency of PMDI bonded boards, but the bonding efficiency of UF bonded boards. The inclusion of 1% wax significantly affected the bonding efficiency of both resins, however the loss in strength was higher in UF than in PMDI bonded boards.

Keywords: Urea formaldehyde resin, Isocyanate resin, PMDI, Particleboard, Process variables, Wax, Mat moisture content, Platen temperature.

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. E-mail: antonios1974@hotmail.com, antpap@teikav.edu.gr

INTRODUCTION

Isocyanate resins, unlike urea formaldehyde (UF) resins which are normally soluble in water, are used either as a 100 percent solids content or in an emulsive form to which water and, or other additives may be added. Since they were first introduced to the German particleboard market in the early 1970s, the use of MDI (4,4'-methylenediphenyl isocyanate) binders in composite panels has grown significantly. When shipped to engineered wood products plants, MDI is a complex mixture of the isomers of di-, tri-isocyanates, and higher polymeric aromatic species derived from side reactions and generally sold as PMDI (polymeric MDI).

The purpose of this paper was two-fold:

i. To compare physical properties of conventional particleboard bonded with varying amounts of UF and PMDI resin,
ii. To examine the effect of mat moisture content (MC), wax content, and platen temperature on their bonding efficiency, as determined by internal bond strength.

This is the first study, as far as the author is aware, where these issues are addressed under identical manufactured conditions.

EXPERIMENTAL

Industrially produced wood chip furnish, comprising predominantly mixed softwoods (pine and fir) was used in this study. After screening (through a mesh with 5 mm apertures to remove oversize particles and then through a mesh with 1 mm apertures to remove undersize particles), the chips for the manufacture of UF bonded boards were dried to 2.5-3% moisture content (MC), while the chips for the isocyanate bonded boards were dried to 6.5-7% MC. It is known, in the latter case, that a higher mat MC can be tolerated (Deppe, 1977). The resins used for the manufacture of boards were UF (62.4% solids) and PMDI 100% solids. The water-repelling agent was an emulsifiable slack wax (E538) having 60% solids content. The amount of resin and wax used was based on the oven-dry weight of wood. A 2% aqueous solution of ammonium chloride (20% solids content), based on resin solids, was added to the UF as a hardener before spraying. All resins were applied to the furnish with a pneumatic atomising nozzle in a rotary drum blender. The order of spraying was wax, resin, followed by water to bring the mat to the correct moisture level.

Circular mats were randomly hand-formed on a circular aluminum caul. Mats were pressed without stops (10 mm/min), in a 30cm diameter electrically heated hot press for 3 min. Target board density was 650 Kg/m^3 and target board thickness 12.15 mm for all boards. Three replicates of each board were made, giving a total of 66 boards. Complete process information of the experimental design is in Table 1.

Boards were conditioned at 20^{o}C and 65% relative humidity prior to testing of internal bond strength (IB) (EN 319), modulus of rupture (MOR), modulus of elasticity (MOE) (EN310), and thickness swelling after 24hours immersion in water (TS) (EN317).

Table 1: Experimental design.

Variables	Constants
UF 7, 10, 13% PMDI 2, 4, 6%	Mat MC 10% Press temperature 180 ^{0}C Press time 3 min
Mat M.C 7, 10, 13 % UF 10% PMDI 4%	Press temperature 180 ^{0}C Press time 3 min Wax 1%
Press temperature 170, 180, 190^{0}C UF 10% PMDI 4%	Mat M.C 10% Press time 3 min Wax 1%
Wax 0 ,0.5 ,1 , 1.5% UF 10% PMDI 4%	Mat M.C 10% Press temperature 180 ^{0}C Press time 3 min

RESULTS AND DISCUSSION

Effect of Resin Type

Mechanical properties

The effect of resin type on bonding (IB) and bending properties (MOR, MOE) is shown in figures 1,2 and 3 respectively. From these, it can be seen that in order to

provide equivalent board mechanical properties using UF and PMDI, it is possible to use PMDI resin at a considerable lower dosage. For example, it can be seen from figure 1 that UF bonded boards required 7% resin to achieve an IB of 0.68 N/mm^2, while 2.8% PMDI was needed. This represents reductions in dosage of 60%. However, this tendency was reduced to 37.5% at higher resin levels, since UF bonded boards required 9.6% to achieve an IB of 1.01 N/mm^2, while 6% PMDI was needed. With regard to MOR, UF bonded boards required 7% resin to achieve a value of 13.1 N/mm^2, while 2.5% PMDI was needed (see fig.2). This represents a reduction in dosage of 64.2%. This tendency remained the same at higher resin levels, since UF bonded boards required 13% to achieve a MOR of 1.01 N/mm^2, while 4.5% PMDI was needed (reductions in dosage of 65.3%). Modulus of elasticity showed similar trends to MOR. The results are broadly in line with those reported by Adams (1980) and Frink and Layten (1985). According to the former study the isocyanate dosage can be reduced to about one quarter of the requisite UF dosage to achieve equivalent board properties, whereas in the later study an approximate binder reduction by a factor of about 60 is suggested.

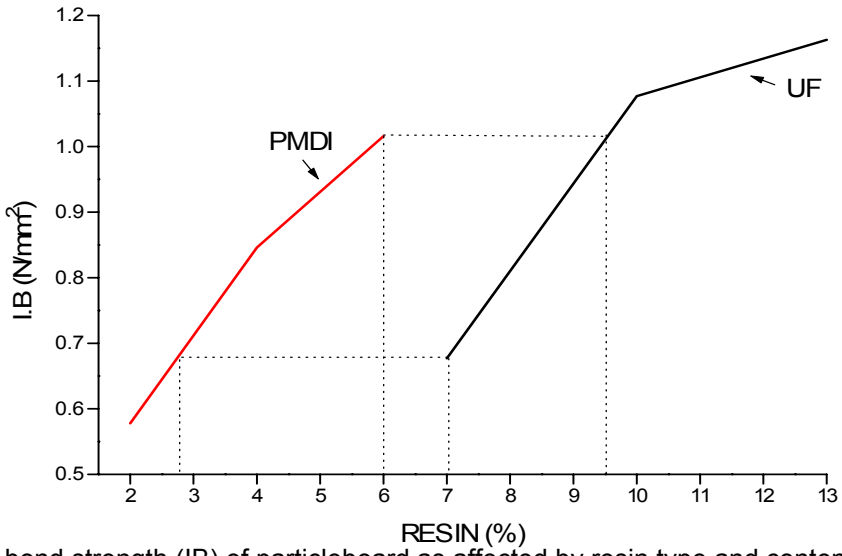

Fig. 1. Internal bond strength (IB) of particleboard as affected by resin type and content. Dashed lines indicate equivalent board property levels for comparisons.

Dimensional stability

The effect of resin type on 24 hours thickness swelling (TS) is shown in figure 4. In order to provide equivalent board dimensional stability using UF and isocyanate resins, it is possible to use isocyanate resin at a lower dosage. For example, an amount of 7.5% UF resin resulted in a thickness swelling of 28.6%, whereas the same level of thickness swelling was obtained at a much lower amount of PMDI (2%). This tendency remained the same at higher resin levels, since UF bonded boards required 13% resin to achieve a 20.95% TS, while 3% PMDI was needed (reductions in dosage of 76.2%).

From the above discussion, is clear that that in order to provide equivalent board properties using UF and PMDI resin, it is possible to use PMDI at a considerable lower dosage. This can be attributed to the way that the resins form bonds with wood surfaces.

Unlike formaldehyde resins, which bond by mechanical means (H-bond), isocyanate resins form covalent bonds with wood surfaces. A covalent bond has an energy in the range of 70-100 kcal per mole, while an H-bond, the type most normally associated with adhesion, is in the range of 5-10 kcal per mole. Technically, the reaction is characterized as an addition reaction, which yields a cross-linked, substituted urea and carbon dioxide (Deppe, 1977).

Another possible explanation for the superior performance of isocyanate resins is related to their high mobility on the wood surface. One reason for this, is their self-activated flow; when isocyanate droplets are placed on the wood surface they spread out spontaneously without the need of any external forces. This high mobility, according to Roll (1997), causes penetration to considerable depth into compressed particles, which can result in their total impregnation. This penetration may repair weak zones (chips in particleboard usually damaged by cracks and fissures) by sticking them together.

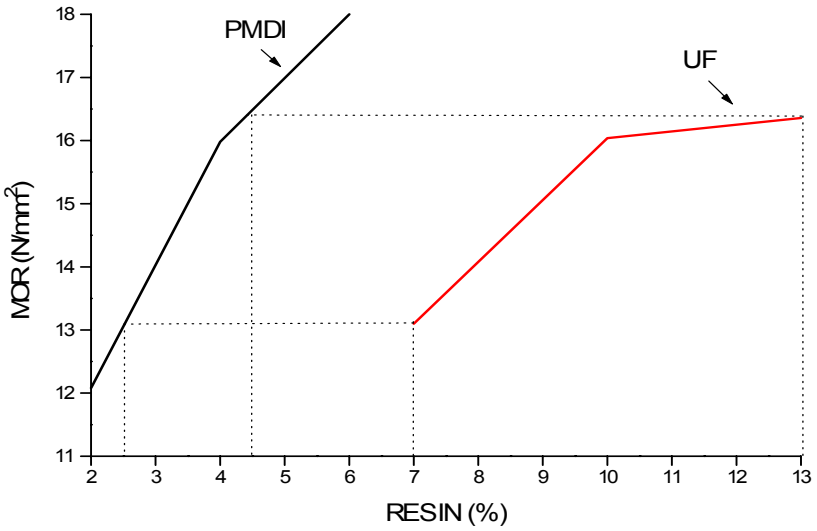

Fig. 2. Modulus of rupture (MOR) of particleboard as affected by resin type and content. Dashed lines indicate equivalent board property levels for comparisons.

Effect of Mat Moisture Content on Bonding Efficiency

The IB data at various mat moisture contents are shown in Table 2. From this, it can be seen that the bonding efficiency of UF resin was strongly influenced by the mat MC. Thus, the values at 10% MC were significantly higher than those developed at 7 or 13%, however between 7 and 13% there was no significant difference. The significant difference between 7 and 10% MC can be attributed to the fact that at 7% MC, the wood-glue interface was adequate for good glue bond formation (enough water to obtain sufficient hardening of the UF molecule), but the wood was not plasticised sufficiently for the maximum number of bonds to be formed, as evidenced by the failure of IB samples in the surface layers. Plastization of wood occurs when wood is heated at about 180^{o} (as in this study), however the low pressing time was a limited factor. The explanation for the significant difference between MC's of 10 and 13%, was due to the

excess amount of moisture at the 13% level, particularly in the centre of board as the press cycle proceeded, which reduced optimum conditions for glue bond formation, since water molecules act as a competitor in the formation of hydrogen bonds between the UF resin and the wood chips.

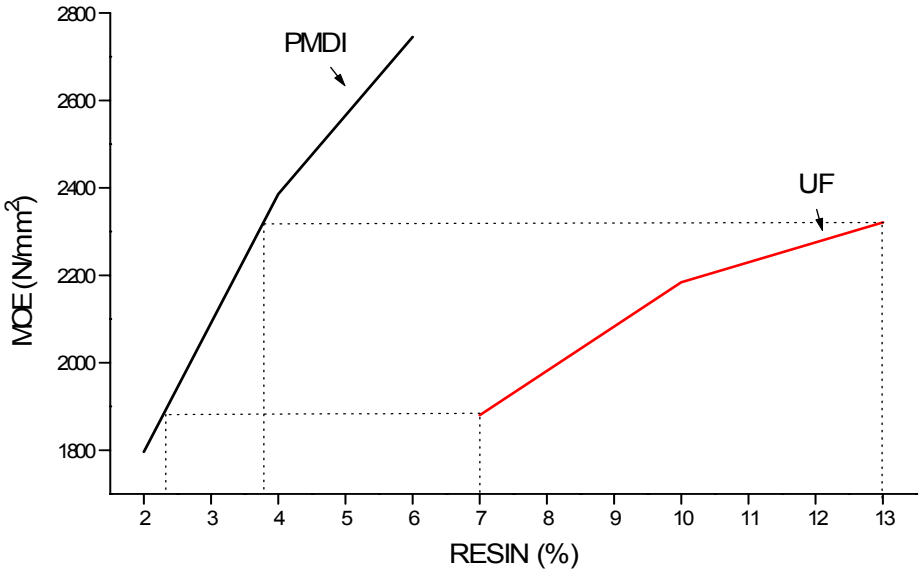

Fig. 3. Modulus of elasticity (MOE) of particleboard as affected by resin type and content. Dashed lines indicate equivalent board property levels for comparisons.

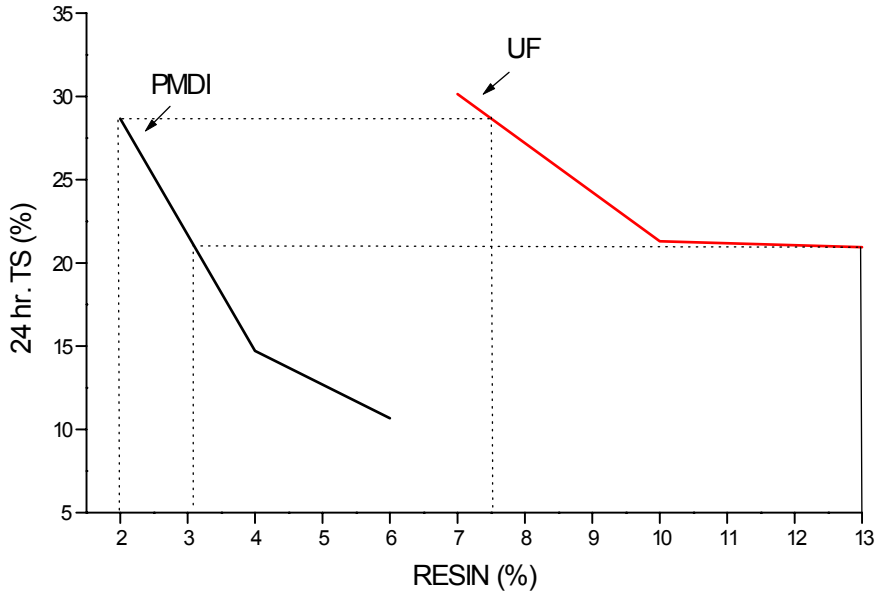

Fig. 4. Thickness swelling (TS) of particleboard as affected by resin type and content. Dashed lines indicate equivalent board property levels for comparisons.

The MC of the mat did not significantly affect the bonding efficiency of PMDI resin, indicating that isocyanate resins can be used at MC higher than those permitted for the aldehyde-based resins. This is due to the direct bonding between the resin and the wood surfaces. The results presented in this paper are in line with those reported by Deppe (1977) and Udvardy (1979), but they were not consistent with those reported by Johns et al. (1982). In the latter case a significant reduction of IB was observed when the MC of the mat was greater than 12%.

Effect of Platen Temperature on Bonding Efficiency

The IB data at various platen temperatures are shown in Table 2. From this, it can be seen that higher platen temperatures resulted in improved IB. This positive influence can be explained in terms of the amount of heat in the mattress, and in particular the core, when exposed to elevated temperatures. Because the core of the mattress is always at the lowest temperature compared to the surfaces, the platen temperature should be such to ensure that the core reaches a sufficiently high temperature to allow the resin to cure. Increased temperature will promote increased cross-linking and curing of the resin, in addition to improved plastization of wood, and hence improved those properties such as IB, which are particularly sensitive to bond quality.

From the Table 2, it can be also seen that the bonding efficiency of PMDI resin was not significantly affected by the platen temperature, whereas the bonding efficiency of UF bonded boards was significantly improved when the temperature was raised to 190^{o}C.

Table 2: Internal bond strength (IB) of particleboard specimens. Values in parentheses represent the coefficient of variation (%). Different letters show which values are statistically different at the 5% level. (For process variables involved see Table 1).

Resin Type	Mat MC (%)	IB (N/mm^2)	Platen Temperature (^{o}C)	IB (N/mm^2)	Wax (%)	IB (N/mm^2)
UF	7	0.689 A (8.8)	170	0.799 A (5.4)	0	1.077 A (8.7)
UF	10	0.828 B (7.8)	180	0.828 A (7.8)	0.5	0.929 AB (9.1)
UF	13	0.713 A (10)	190	0.912 B (9.8)	1	0.828 B (7.8)
PMDI	7	0.692 A (10.1)	170	0.681 A (11.1)	0	0.846 A (9.9)
PMDI	10	0.717 A (6.5)	180	0.717 A (6.5)	0.5	0.815 AB (6.7)
PMDI	13	0.731 A (8.4)	190	0.729 A (7.9)	1	0.717 B (6.5)

Effect of Wax Content on Bonding Efficiency

The IB data at various wax contents are shown in Table 2. From this, it can be seen that higher wax contents resulted in decreased IB. This suggests that the wax interfered with both UF and PMDI resin bonding during pressing, resulting in reduced bond quality. This reduction was significant above 0.5% wax, for boards bonded with either type of resin.

Although the performance of both resins was the same towards the wax content, the presence of wax was more detrimental in the boards bonded with UF, since an increase in wax content from 0 to 1% resulted in 30% reduction in IB, whilst the reduction in PMDI bonded boards was 18%. This might be due to differences in chemical bonding between UF and PMDI resins. The wax apparently interferes with UF resin when hydrogen bonds are formed, at least to a greater degree than in the case of covalent bonds of PMDI.

CONCLUSIONS

1. Compared with UF resin, PMDI resin had better bond strength for particleboard as determined by bending properties and internal bond.
2. Dimensional stability properties were also better for PMDI bonded board.
3. The MC of the mat and the platen temperature did not significantly affect the bonding efficiency of PMDI bonded boards, but the bonding efficiency of UF bonded boards were influenced.
4. The inclusion of 1% wax significantly affected the bonding efficiency of both resins, however the loss in strength was higher in UF than in PMDI bonded boards.

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MORPHOLOGY AND MECHANICAL PROPERTIES OF POLYPROPYLENE-WOOD FLOUR COMPOSITES

D. Dobreva,* S. Nenkova, St. Vasileva

The microstructure and mechanical properties of polymer composites based on polypropylene and wood flour modified with monochloroacetic acid were investigated. Scanning electron microscopy and wide-angle X-ray diffraction were used as methods to probe the composite microstructures, while the tensile test was used to measure the physical strength. The wood flour modification was performed at different levels of monochloroacetic acid, ranging from 0.01 to 1 mol, while the modified wood flour was used as filler for polypropylene at 10, 20 and 30 wt.-%. It was found that increasing the monochloroacetic acid fraction influences the microstructure of the composites and leads to more homogeneous products. The introduction of non-modified wood flour decreases the polypropylene crystallization degree, but it improves after introduction of monochloroacetic acid. Physical-mechanical tests showed positive effects on tensile tests and Charpy notched impact strength. The new composites appear to be promising materials for construction purposes.

Keywords: Morphology; Polypropylene-wood flour composites; Monochloroacetic acid; Mechanical Properties; Modification

*Contact information: Department of Polymer Engineering, University of Chemical Technology and Metallurgy, 8 Kl. Ohridski Blvd., 1756 Sofia, Bulgaria; *Corresponding author: Tel.: +359 2 8685493; fax: +359 2 8685488. E-mail address: dobromira.dobreva@gmail.com*

INTRODUCTION

Over the last few years, wood-fiber thermoplastic composites have received considerable attention from the wood and plastic industries. Although wood flour has been used as filler for thermosetting polymers for a long time, wood fibers or cellulose fillers are being increasingly used nowadays to reinforce thermoplastics for development of new applications. Wood flour is an attractive filler for thermoplastic polymers mainly because of its low cost and large availability (Nunez et al. 2002; Coutinho et al. 2004; Michler 1999; Marcovich et al. 1998a; Park and Balatinecz 1997a; Hristov et al. 2004). Several research groups reported on the use of wood flour as potential fillers for synthetic polymers (Marcovich et al. 1998b; Wu et al. 2000; Park and Balatinecz 1997b; Toriz et al 2002; Qiu et al. 2005; Shibata et al. 2005). Park and Balatinecz have investigated the effect of flour-surface treatment on the microstructure of wood flour filled polyolefins. In those studies, the composites were predominantly processed by extrusion and compression molding. To obtain a composite based on non polar thermoplastics with competitive final properties, it is necessary to achieve a good interface between filler and matrix, generally realized by the modification of the filler surface (Thielemans and Wool 2005; Toriz et al. 2001; Toriz et al. 2004; Albano et al. 2004; Tacterajvidi and Ebrahimi 2003; Oksman and Clemons 1998).

For improving the rheological properties it is necessary to optimize the interface between the filler and the polypropylene matrix using monochloroacetic acid for modify-cation of wood flour (Dobreva et al 2005). In earlier investigations, carboxymethylation of wood flour by an etherification reaction at room temperature with monochloroacetic acid was demonstrated (Dobreva et al 2004; Kishi and Shiraiski 1986; Tan and Yu 1997; Bazarnova et al. 2004). The carboxymethylated material enhances the polymer-phil-ic character of the filler so that such composites materials are obtained much more easily.

The main purpose of the current work was to investigate the structural evolution and morphological changes of polymer composite materials from polypropylene and wood flour, modified with different molar contents of monochloroacetic acid. In addition we have determined specific mechanical properties of the new materials for a more detailed characterization.

EXPERIMENTAL

Materials

The experiments were carried out with wood flour obtained from coniferous wood with particle size of about 140 μm, specific volume of 8 dm^3/kg and moisture content of 5 wt.%. Monochloroacetic acid, used as the modifying reagent, was purchased from MERCK (purity > 99 %) with a melting interval from 60 to 63ºC. Wood flour modify-cation was realized with monochloroacetic acid content ranging from 0.01 to 1 mole.

Isotactic polypropylene with trade mark BUPLEN 7523 - LUKOIL - Burgas with flow index of 4g/10 min was used for obtaining polymer composites. Wood flour is added to polypropylene in quantities of 10, 20 and 30 wt.-%. Some polymer composites included 5% compatibilizer Fusabond PMD353D Dupont (CA, USA).

Techniques

The chemical modification of wood flour is conducted with different contents of monochloroacetic acid ranging from 0.01 up to 1 mole by a suspensional method. The alkylation mixture consists of monochloroacetic acid and sodium base. Modified wood flour is chemically modified completely after approximately 48 hours. Before using the wood flour samples, they were flushed thoroughly with methyl alcohol. By this we obtained carboxymethylated wood flour (CMWF) without any contamination from sodium chloride or other chloride-containing materials.

The polypropylene, wood flour and the coupling agent MAPP (maleated polypropylene) were mixed in a Leistritz MICRO18 co-rotating twin–screw extruder (screw diameter 35 mm). The barrel temperatures were: 190°C for zone 1, 180°C for zone 2 and 185°C for zones 3-6 and 180°C at the last zone. The frequency of the screw rotation was 300 min^{-1} and the material output was 0.9 kg/h. The product temperature was 186°C. The extruder strands were then cooled in a water slide system, pelletized, and dried at 105°C. The compounded pellets were injection molded using a conventional BOY 22A reciprocating screw injection molder into standard DIN test specimens.

The mechanical behavior of composites was examined by tensile and fracture tests. Tensile tests were performed according to DIN 53834 (samples thickness 4 mm)

by using a Zwick machine (model 1445) at a crosshead speed of 50 mm/min. From the stress-strain curves percent elongation at break (ε_b) and E-modulus were determined.

Fracture tests were carried out on a Charpy instrument at an impact speed of 1 mm/s. For this test samples (in the form of small bars with dimensions 80 x 10 x 4 mm) are notched as follows: first, a blunt notch was produced by using a machine with – shaped tool, and then a sharp notch of ~ 2 mm depth was made by a razor blade fixed to a micrometric apparatus.

Scanning electron microscopy (SEM) observations of polypropylene and polymer composites were made with a JEOL electron microscope (Model JSM 6400). The samples were fractured after cooling in liquid nitrogen and the freshly fractured surfaces were coated with thin Au layers for elimination of charge effects in the microscope.

The samples for wide-angle X-ray diffraction were carefully homogenized by grinding the samples in an analytical mill cooled with liquid nitrogen. X-ray scattering curves of the powder samples were measured by means of a two-circle-X-ray diffractometer (Bruker-AXS) in symmetrical transmission technique using monochromized (Ge(111)-monochromator) CuK_α-radiation (40 kV, 30 mA). The angular range was 3-16° (θ) and the step width was 0.2° (θ).

The scattering curves were corrected with regard to background scattering, absorption, incoherent scattering and were normalized to electron units. The corrected and normalized Wide Angle X-ray Scattering (WAXS) curves were used to determine a degree of crystallinity, x_c, and a lattice disorder parameter k according to the Ruland/Vonk method (Ruland 1961).

RESULTS AND DISCUSSION

Figure 1 illustrates the impact strength by Charpy analysis for each composition. The poorer mechanical properties of the unmodified polypropylene wood flour composite are due to poor adhesion between the polypropylene matrix and wood flour.

The concentration of monochloroacetic acid in the wood flour was found to have a significant effect on the microstructure; more specifically, on the deformation mechanism and mechanical properties of the composites. Improvements in impact strength were determined in all samples including 10 and 20 wt.-% modified filler. An exception to this is the material modified with 1 mole NaOH and 1 mole $ClCH_2COOH$, and 5% compatibilizer. The impact energy was remarkably enhanced with the addition of 10 and 20 wt.-% wood flour to which was added 1 mol of NaOH and 1 mol of $ClCH_2COOH$. In fact, the impact energy increased from 2.6 to 4.8 kJ/m^2, leading to an almost two-fold increase versus composites having unmodified wood flour. Generally, impact values of the unmodified wood flour exhibit voids formation and brittle fracture of the matrix, wood particles debonding, and pulling out of the polypropylene matrix. A higher concentration of monochloroacetic acid and sodium hydroxide in the wood flour leads to a weak interaction between the polypropylene matrix and wood filler, which supports the formation of a plastic deformation zone around the wood particles and an improved ductility of the polypropylene matrix.

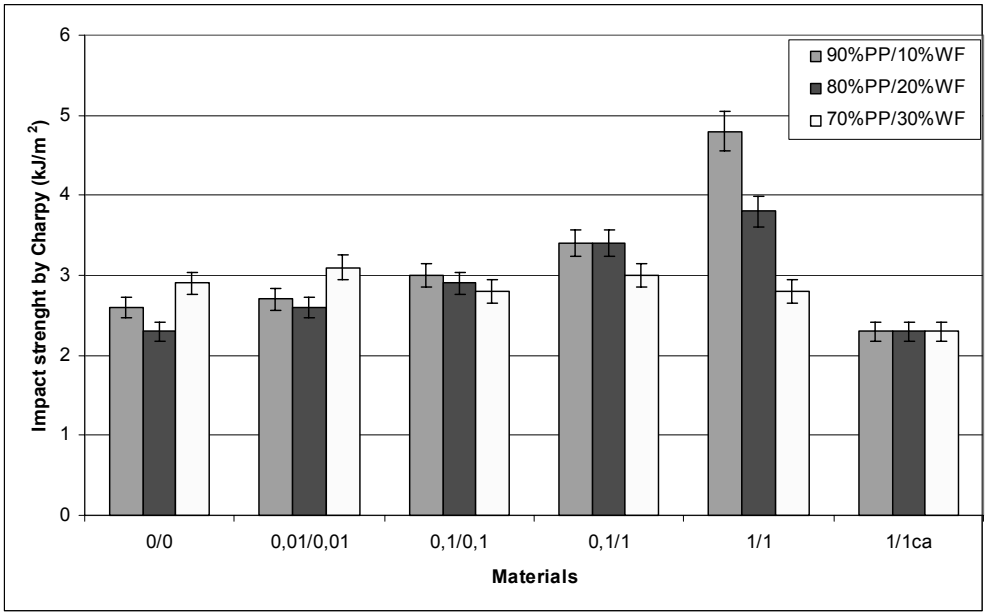

Fig. 1. Charpy notched impact strength of materials modified with different mole quantity $ClCH_2COOH$ and NaOH. (0/0: 0 mol NaOH / 0 mol $ClCH_2COOH$, 0,01/0,01: 0,01 mol NaOH / 0,01 mol $ClCH_2COOH$, 0,1/0,1: 0,1 mol NaOH / 0,1 mol $ClCH_2COOH$, 0,1/1: 0,1 mol NaOH /1 mol $ClCH_2COOH$, 1/1: 1 mol NaOH /1 mol $ClCH_2COOH$, 1/1: 1 mol NaOH /1 mol $ClCH_2COOH$ and 5% coupling agent)

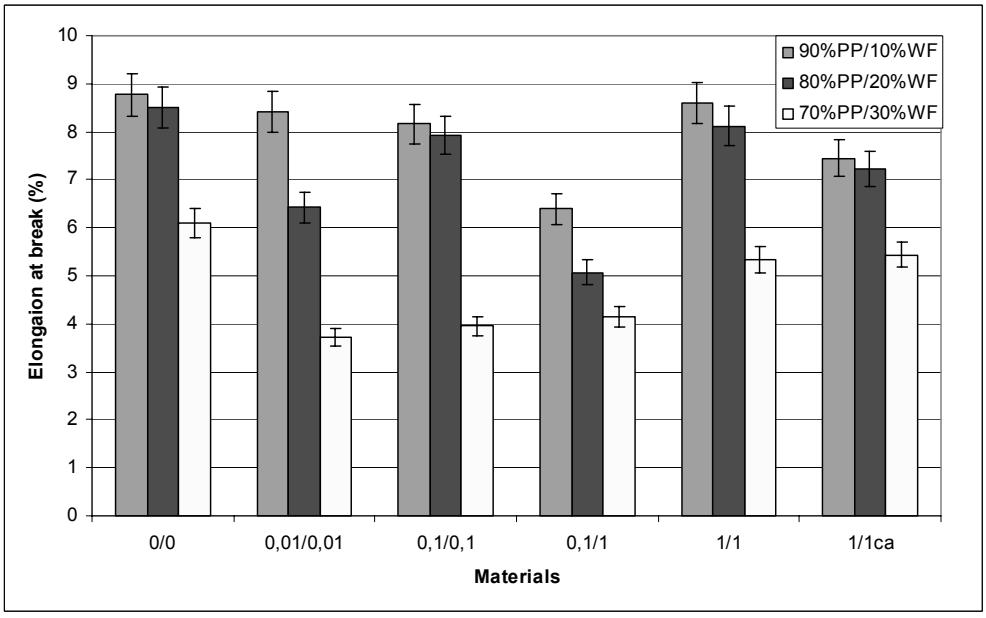

Fig. 2. The percent elongation at break of materials in percent modified with different mole quantities of $ClCH_2COOH$ and NaOH.

The tensile strength of the wood/filled composites decreased with increasing fraction of the wood flour mainly because of poor transfer of applied load through the interface. As a rule, fillers with higher stiffness than the matrix cause a dramatic decrease in the elongation at break (Oksman and Clemons 1998). The obtained results for the unmodified and modified polypropylene/wood flour composite are in accordance with the above mentioned rule (Fig. 2).

Fig. 3 shows the E-modulus (Elasticity modulus) of composites modified with different molar contents of modified agents. Flexural modulus is largely dependent on wood flour content in the composites. The E-modulus was improved with increase of wood flour concentration. The results suggest that wood flour can significantly enhance the stiffness of polypropylene composites.

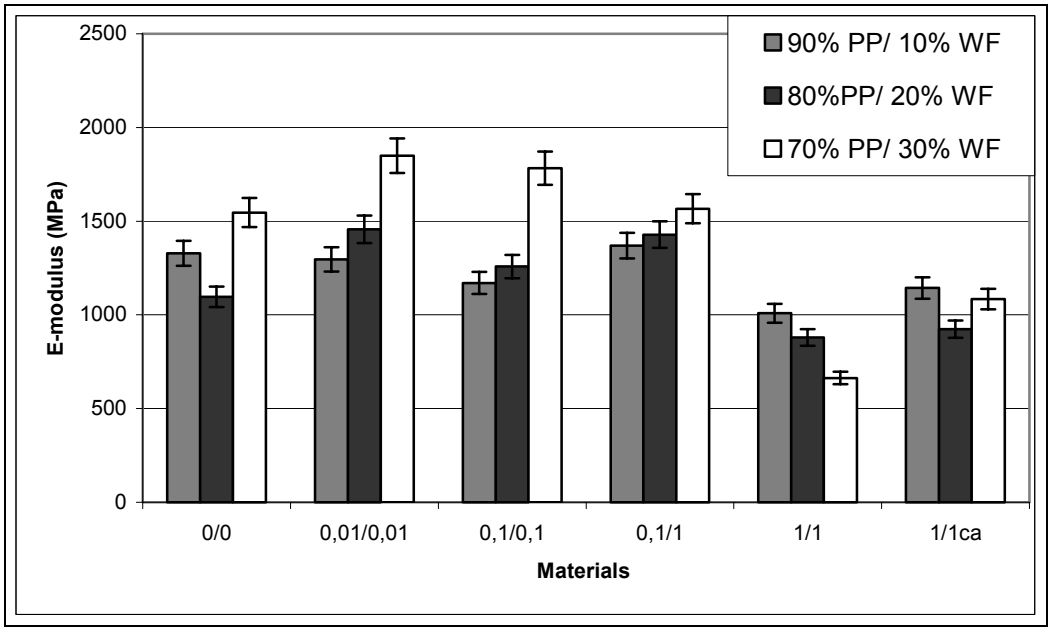

Fig. 3. E-Modulus of materials modified with different molar quantities of $ClCH_2COOH$ and NaOH.

When modified with the maximum molar quantities of monochloroacetic acid and sodium hydroxide, the wood flour composites showed a large decrease of the E-modulus. It is much higher in the composites with lower quantities of modified agents (0.01 mol $ClCH_2COOH$/0.01 mol NaOH and 0.1 mol $ClCH_2COOH$/0.1 mol NaOH), because the wood flour structure is not disordered and therefore the fiber character of the structure of the particle wood flour is retained.

Examination of the fracture surfaces of the composites by scanning electron microscopy gave information about how the modified agents influenced the morphology of the composite. Figures 4-6 illustrate how addition of wood flour to the polypropylene causes the formation of heterogeneous microstructures. The wood particle is not fractured and there are voids around the particle, indicating poor interaction between the wood surface and the polypropylene matrix. Lamellar structures, as they are typical for pure polypropylene, disappear (Fig. 5), and separated pieces of wood flour with size from 20 to 150 μm are observed. The structure of polypropylene composites containing 30

wt.-% wood flour modified with 1 mole $ClCH_2COOH$ and 1 mole NaOH is more homogeneous (Fig. 6) while homogenously dispersed wood flour particles in the size range of approximately 10 μm from the second phase are observed. Good adhesion between the filler and matrix was also observed. The improved impact strength obtained by Charpy compared with composites filled with unmodified wood flour can be explained by better toughness of the matrix itself.

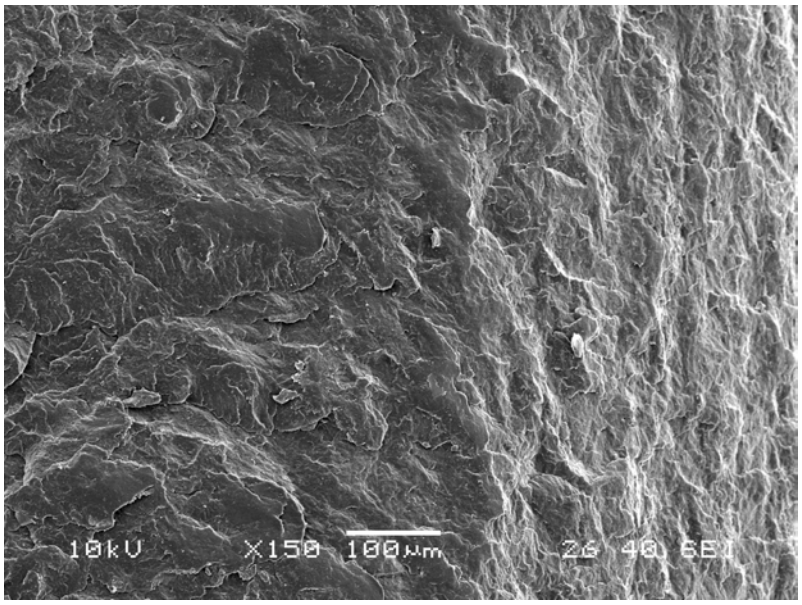

Fig. 4. Scanning electron micrograph of fracture surface of pure polypropylene.

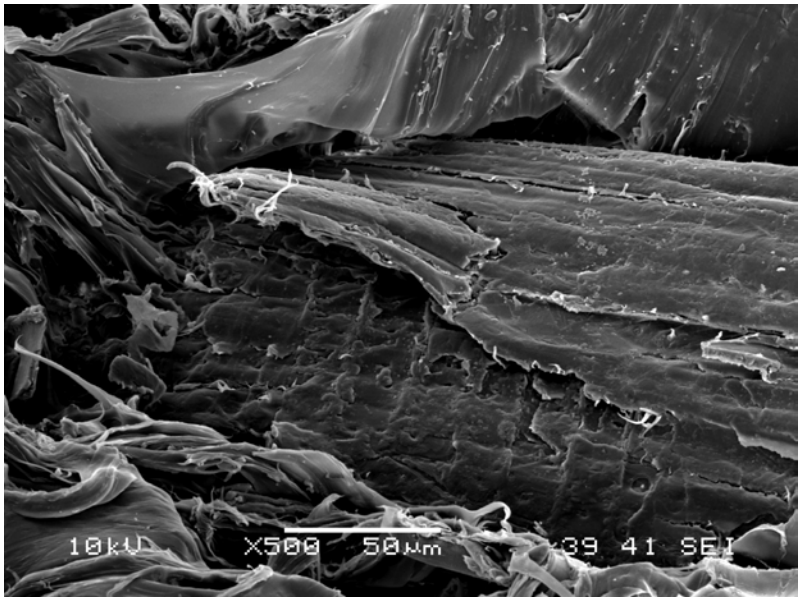

Fig. 5. Scanning electron micrograph of fracture surface of 70 wt.-% polypropylene/30 wt.-% unmodified wood flour.

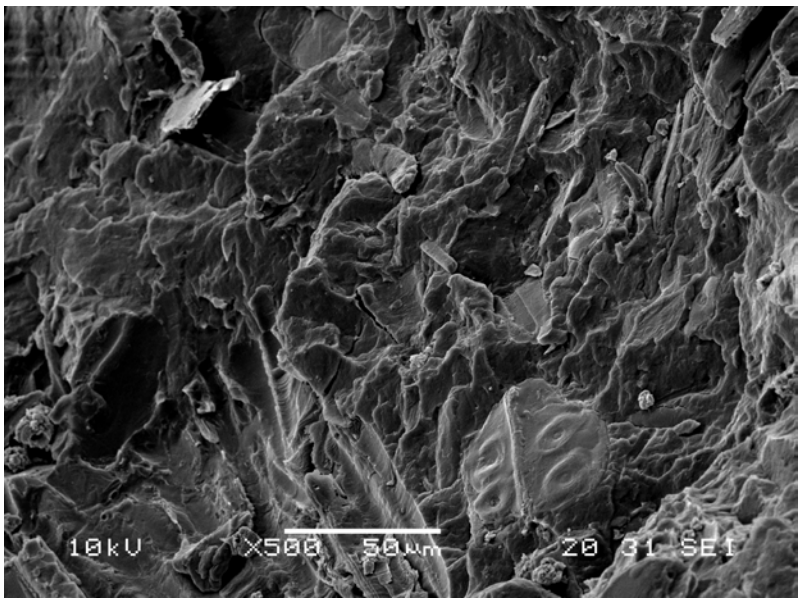

Fig. 6. Scanning electron micrograph of fracture surface of 70 wt.-% polypropylene/30 wt.-% modified wood flour with 1 mol $ClCH_2COOH$ and 1 mol NaOH.

For the polypropylene with unmodified wood flour composite, a decreased crystallinity degree is observed, as well as the presence of separate globular formations. These results are in good agreement with the WAXS data, which will be discussed shortly. The polypropylene composites' samples with modified wood flour (Fig. 6) show an increasing tendency to an amorphous state and good ability for inclusion of wood flour particles in the matrix.

The results from WAXS analysis of polypropylene (Fig. 7A) are typical for standard polymer material with high crystallization degree. In the WAXS data of composites containing modified wood flour (Fig. 7C) the X-ray diffraction patterns of polypropylene are presented, but with a lower degree of diffraction intensity in combination with signals of remaining products of the modification reactions. The degree of crystallization of polypropylene and polymer composites with non-modified and modified wood flour was determined by using the Ruland/Vonk method (Table 1).

The X-ray diffraction data show that the addition of 10 wt.-% wood flour in the polymer matrix simultaneously causes inversion in the intensity of polypropylene diffraction maxima and appearance of diffraction patterns of cellulose 11.3 Θ° (Fig. 7B). The diffraction maxima of cellulose for modified samples disappear from the diffraction pattern. The presence of modified wood flour leads to return of usual character of the diffraction patterns of polypropylene. This probably may be due to an improvement in the crystallization in the polypropylene or a more regular arrangement of macromolecules in polypropylene in the composite melts. This process could improve the homogeneous distribution of wood flour in the composite leading to significant improvement of some physical and mechanical parameters of the composites.

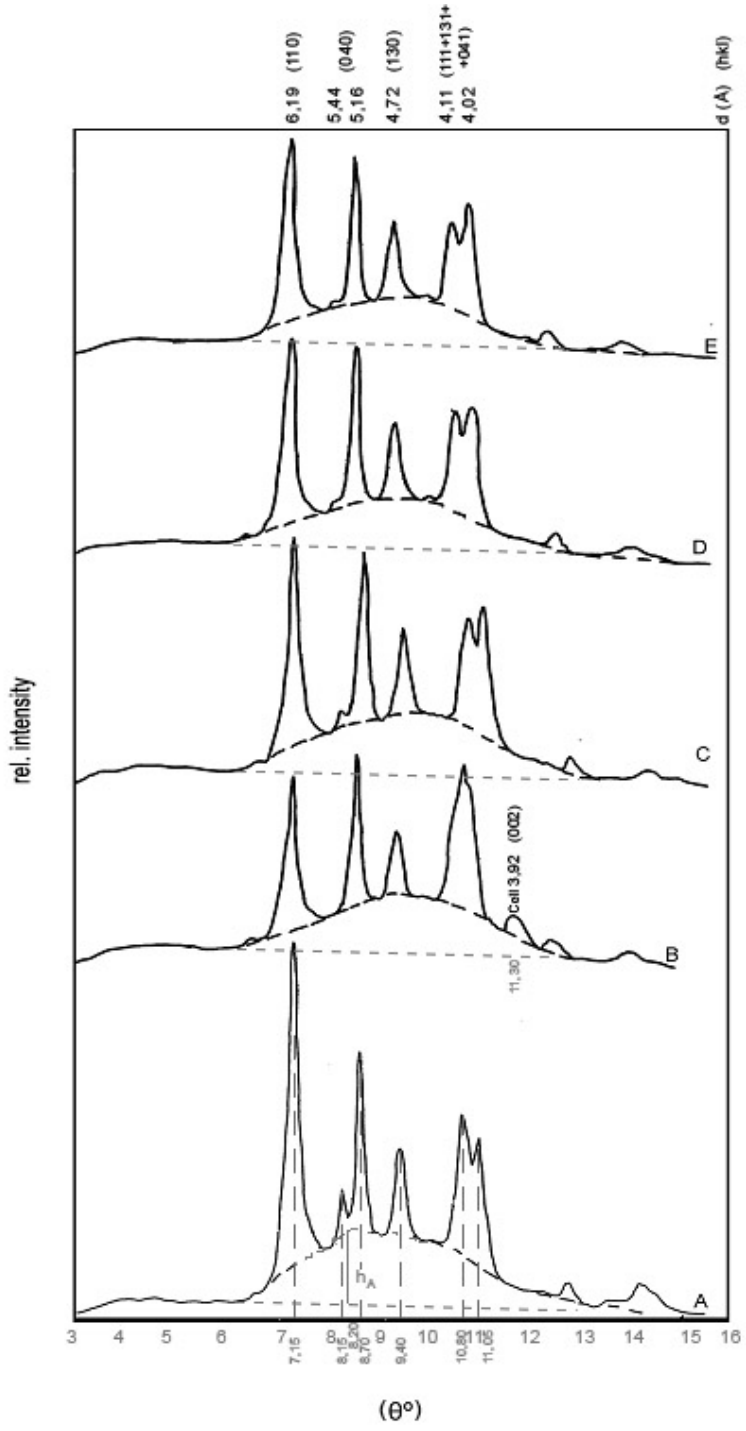

Fig. 7. WAXS of: A - polypropylene; B - polypropylene with 10 % unmodified wood flour; C - polypropylene with 10 % wood flour modified with 0.01 mol $ClCH_2COOH$ and 0.01 mol NaOH ; D - polypropylene with 10 % wood flour modified with 0,1 mol $ClCH_2COOH$ and 0.1 mol NaOH; E - polypropylene with 10 % wood flour modified with 1 mol $ClCH_2COOH$ and 1 mol NaOH.

Table 1. Crystallinity x_c and Disorder Parameter k

Sample	X_C [%]	k [10^{-2} nm^2]
polypropylene	39	1.8
wood flour	< 20	-
90% polypropylene/10% U wood flour	28	1.6
90% polypropylene/10%WF/0.01 mol $ClCH_2COOH$/0.01 mol NaOH	32	1.6
90%PP/10%WF/0.1 mol $ClCH_2COOH$/0.1 mol NaOH	35	1.7
90%PP/10%WF/1 mol $ClCH_2COOH$/1 mol NaOH	37	1.7

The absolute values for the crystallinity of polypropylene/wood flour-composites and polypropylene are not very reliable because the amorphous background scattering could not be measured due to the fact that no fully amorphous polypropylene sample was available. The amorphous background was calculated and not measured. The relative differences between the samples, however, are reliable while the pure wood flour phase has a low crystallinity of approximately less than 20%.

CONCLUSIONS

1. Composite materials based on polypropylene and carboxymethylated wood flour were generated.
2. SEM and WAXS analysis demonstrated that the addition of non-modified wood flour degrades the homogeneity of the samples and decreases the crystallization degree of polypropylene.
3. The preliminary modification of wood flour with monochloroacetic acid influences the microstructure of the composites which then display an increasing tendency towards an amorphous state with good inclusion of the uniformly dispersed wood flour particles.
4. The polypropylene composites filled with modified wood flour do have a more homogeneous surface compared to polypropylene-unmodified wood flour composites.
5. In the presence of modified wood flour, the crystallization degree of polypropylene depends on the added quantity of the modification agent.
6. The process of structural homogenization improves the rheological properties of polypropylene composites in such a way that allows conventional production methods to still be employed.

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CHEMICAL COMPOSITION, ANATOMY, LIGNIN DISTRIBUTION, AND CELL WALL STRUCTURE OF MALAYSIAN PLANT WASTE FIBERS

H. P. S. Abdul Khalil*, M. Siti Alwani, and A. K. Mohd Omar

The chemical composition, anatomical characteristics, lignin distribution, and cell wall structure of oil palm frond (OPF), coconut (COIR), pine-apple leaf (PALF), and banana stem (BS) fibers were analyzed. The chemical composition of fiber was analyzed according to TAPPI Methods. Light microscopy (LM) and transmission electron microscopy (TEM) were used to observe and determine the cell wall structure and lignin distribution of various agro-waste fibers. The results revealed differences in anatomical characteristics, lignin distributions, and cell wall structure of the different types of fibers investigated. Nevertheless, transmission electron microscopy (TEM) micrographs have confirmed that the well wall structure, in each case, could be described in terms of a classical cell wall structure, consisting of primary (P) and secondary (S_1, S_2, and S_3) layers.

Keywords: *Anatomy; Cell wall structure; Chemical composition; Lignin distribution; Plant fibers*

*Contact information: School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia; * Corresponding author : akhalil@usm.my*

INTRODUCTION

Plant waste fibers can be described as lignocellulosics, i.e. resources comprised primarily of cellulose, hemicellulose, and lignin. Lignocellulosics include wood, agricultural residues, water plants, grasses, and other plant substances (Rowell et al. 2000).

Plant waste fibers have the composition, properties, and structure that make them suitable for uses such as composite, textile, and pulp and paper manufacture. In addition, plant fibers can also be used to produce fuel, chemicals, enzymes, and food. Biomass, including agricultural crops and residues, forest resources, and residues, animal and municipal wastes, is the largest source for cellulose in the world. Approximately 2×10^{11} tons of lignocellulosics are produced every year, compared with 1.5×10^{8} tons of synthetic polymers. Organic plant wastes such as oil palm, pineapple, banana, and coconut fiber are annually renewable, available in abundance, and of limited value at present. These lignocellulosic byproducts could be a principal source for fibers, chemicals, and other industrial products (Reddy and Yang 2005).

In Malaysia, with such a large area of plantation of oil palm (3.87 million ha.), coir (147 thousands ha.), banana (34 thousands ha.), and pineapple (15 thousands ha), large quantities of cellulosic and non-cellulosic raw material are generated during

harvesting (MAO, 2006; MPOB, 2001). The explosive expansion of plantation in this country has generated enormous amounts of vegetable waste, creating problems in replanting operations and tremendous environmental concerns.

When left on the plantation floor, these waste materials create great environmental problems (Sreekala et al. 1997; Reddy and Yang 2005). Therefore, economic utilization of these fibers will be beneficial. In spite of the abundance of published work dealing with the technological aspects of the agro-fibers applications, the bibliography covering comprehensive fundamental aspects of specific agro-fibers is quite scare, disperse, and inadequate. In order to completely evaluate the potential of agro-wastes for new applications, a detailed and comprehensive study of fundamental properties is necessary.

The fundamental aspects considered in the previous literature, with other fibers, have been reported extensively (Balashov et al. 1956; Fengel and Shao 1984; McNeil et al. 1984; Bai et al. 1998; Donaldson 1996), except for oil palm, banana stem, and pineapple leaf fibers. Only the chemical composition and anatomy of these fibers have already been reported (Mansor and Ahmad 1991; Cordeiro et al. 2004; Mishra et al. 2004; John et al. 2005). However, no research has been reported to evaluate the cell-wall structure, lignin distribution, and physical properties of these major plant wastes fibers (oil palm, coir, banana stem, pineapple leaf) in Malaysia until now.

The main objective of the present study is, therefore, to make some observations on the fine structure of plant fibers, using light and electron microscopy. The research findings can lead to a better understanding and knowledge of chemical compositions, fiber dimensions, cell wall structure, and lignin distribution of the fibers. This information is very important to reduce the environmental and health hazards associated with the disposal of plant wastes such as oil palm, banana tree, and pineapple leaf fibers. Hence, this basic studies/knowledge can be used by the food technologist, material scientist, and polymer chemist for further applied research study.

EXPERIMENTAL

For chemical composition determination, agro-fibers were ground, and 40-mesh fractions were selected. The procedures were performed according to TAPPI Method T 264 om-88. The samples were first submitted to Soxhlet extraction with ethanol/benzene [1:2 (v/v)] for 6 hours. The determination of alpha-cellulose, lignin, and ash content were performed following the standard methods T 212 om-93, T 203 os-74, and T 211 om-93, respectively. Holocelluloses were determined according to a previous study (Wise et al. 1946).

For cell wall structure and lignin distribution determinations, agro fibers were chosen randomly and cut into 2 × 3 mm blocks. Samples were then dehydrated in an ethanol series and embedded in Epoxy resin (Epon), which was polymerized for 24 hours at 60°C. Transverse sections (1 μm) were cut from embedded material, using a Sorvall ultra microtome (MT 500) and stained with 1% Toluidine Blue for lignin distribution determination. The sections were viewed under polarized microscope (Olympus BX50). Ultra-thin sections (0.1μm) also were obtained from embedded samples, stained with 2%

uranyl acetate and lead citrate, and finally viewed under transmission electron microscopy (TEM) (Phillips CM12).

RESULTS AND DISCUSSION

Malaysia is the largest producer of oil palm (*Elaeis guineensis Jacq.*) (Fig. 1a) in the world. Total planted area of oil palm increased from 73,000, reaching 3.87 million hectares in 2004 (MPOB 2001). Oil palm can reach 18-24 m in height in nature, but is rarely more than 6 or 9 m in cultivation. Nowadays, oil palm frond (OPF) contributes 70% of the overall oil palm industry waste in Malaysia (Eng et al. 2004). It is reported that Malaysia alone produced, during the recent past years, about 30 million tonnes annually of oil-palm biomass, including trunks, fronds, and empty fruit bunches (Abdul Khalil and Rozman 2004).

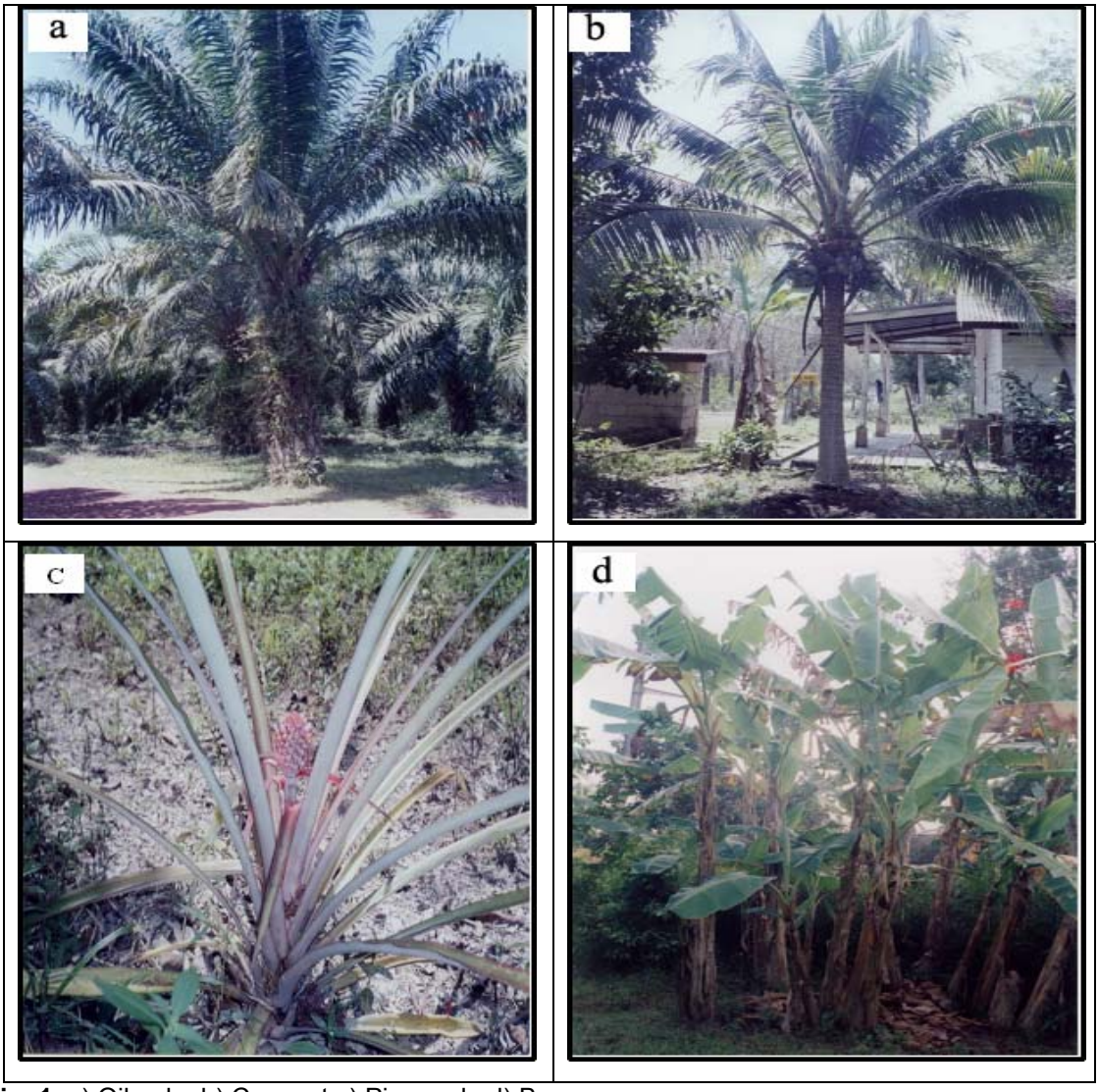

Fig. 1. a) Oil palm b) Coconut c) Pineapple d) Banana.

Coconut palm (*Cocos nucifera L.*) (Fig. 1b) is one of the most important crops in Malaysia. Statistics showed that total area planted of coconut palm increased from 117 000 (1998) to 147,000 hectares in 2004 (MAO 2006). Coir fiber is obtained from coconut husk. Coir fiber is one of the hardest natural fibers, because of its high content of lignin.

The pineapple (*Ananas comosus*) (Fig. 1c) is one of the important tropical fruits in Malaysia, which was produced in economic quantities on about 14,042.9 hectares in 2001 (MAO, 2006). Pineapples are rosette-forming, herbaceous monocots. The stems are short and inconspicuous in the center of the rosette of long and linear leaves.

Bananas (Musaceae) (Fig. 1d) are produced in large quantities in tropical and subtropical areas. The total planted area of banana in Malaysia (2001) was 33,704.2 hectares (MAO, 2006). Banana plants range in height from 0.8 m to more than 15 m. Each contains a flattened, modified stem, called a pseudostem, consisting of concentric layers of leaf sheath and crown of large leaves (Ennos et al., 2000). After harvesting fruit, the pseudostem is traditionally wasted, as it usually left in the soil plantation to be used as organic materials.

Chemical Composition

Table 1 shows the percentage of various chemical components present in OPF, COIR, PALF, and BS fiber. The data show that BS fibers exhibited the highest solubility in ethanol-benzene (10.6%), compared to other fibers. As for wood processing, higher ethanol-benzene extractive content in BS may be advantageous for decay resistance and will provide good strength in fiber processing, because of its higher specific gravity.

Table 1. Chemical composition of different lignocellulosic fibers

	Oil palm frond	Coconut	Pineapple leaf	Banana stem	Softwood*	Hardwood*
Extractive (%)	4.5	6.4	5.5	10.6	0.2-8.5	0.1-7.7
Holocellulose (%)	83.5	56.3	80.5	65.2	60-80	71-89
α-cellulose (%)	49.8	44.2	73.4	63.9	30-60	31-64
Lignin (%)	20.5	32.8	10.5	18.6	21-37	14-34
Ash (%)	2.4	2.2	2.0	1.5	<1	<1

* Tsoumis 1996

Generally, coir fibers contained the highest percentage of lignin (32.8%), but the lignin content of coir fiber was still lower than that of wood fiber (14-37%) (Tsoumis 1991). Generally, the high content of lignin in coir fiber made the fiber tougher and stiffer, compared to other fibers. This was because lignin provides plant tissue and individual fibers with compressive strength and stiffens the cell wall of the fibers, to protect the carbohydrates from chemical and physical damage (Saheb and Jog 1999). Lignin is an undesirable polymer, and its removal during pulping requires high amounts of energy and chemicals. PALF fibers had the lowest lignin content, which suggests that this material can undergo bleaching more easily and with the utilization of lower amounts of chemicals than coir fibers.

Paper strength depends on the cellulose content of raw plant materials. Plant materials with 34% and over of α-cellulose content were characterized as promising for pulp and paper manufacture, from chemical composition point of view (Nieschlag et al., 1960). Cellulose content was also at a satisfactory level (above 40%) for each type of fiber considered in the present study. PALF fibers contained the highest percentage of α-cellulose content (73.4%), which was higher than wood fiber (30-64%) (Tsoumis, 1991). The higher cellulose content in pineapple leaf fiber is probably due to the relatively higher weight of the fruit they support and the fact that they are less perishable (Reddy & Yang, 2005).

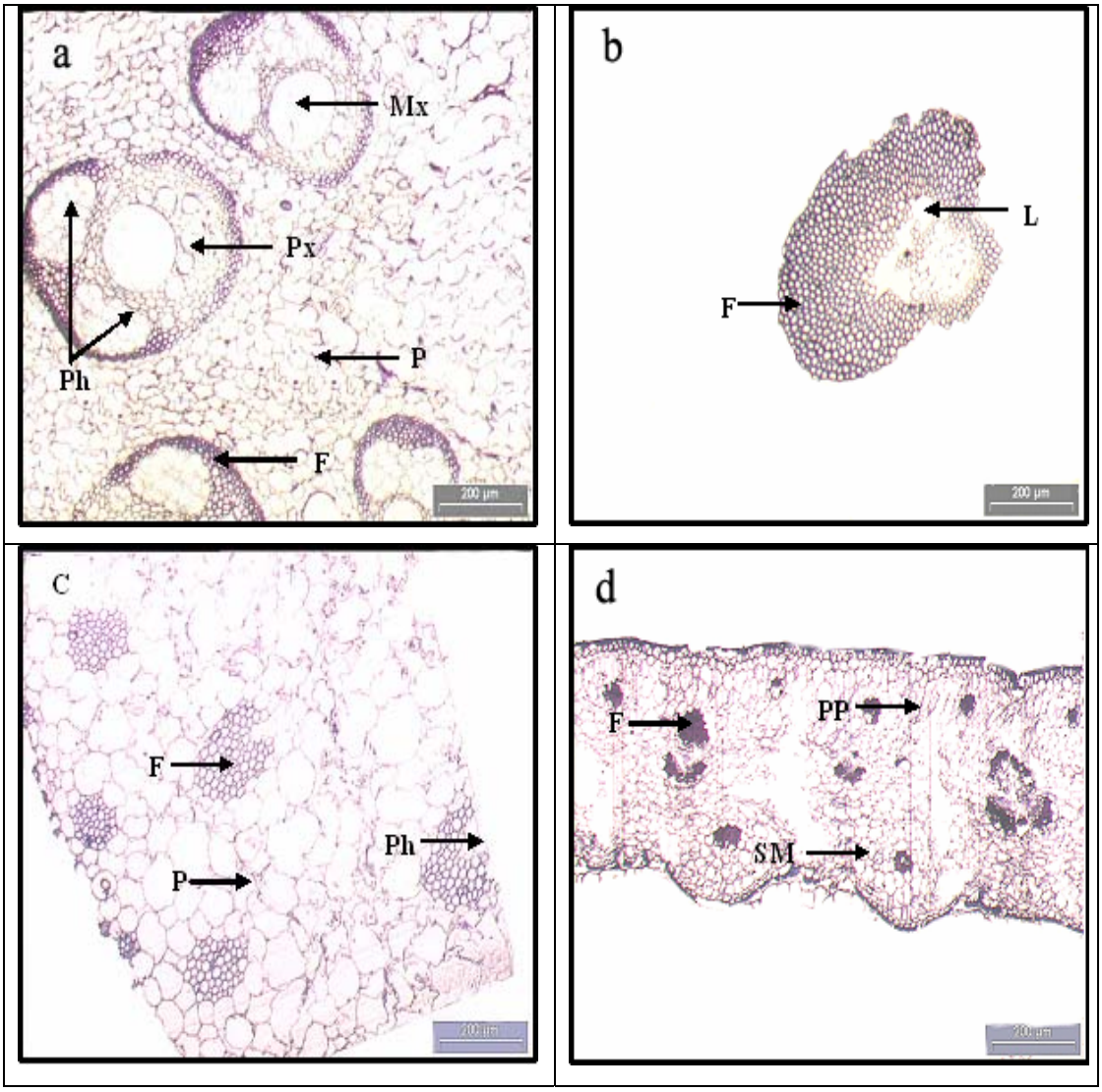

Fig. 2. Transverse section of different types of agro fibers at low magnification (4×). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF). F Fiber; P Parenchyma; Mx Metaxylem; Px Protoxylem; Ph Phloem. F Fiber; L Lacuna; PP Palisade Parenchyma; SM Spongy Mesophyll

Anatomical Characteristics

OPF fibers contained various sizes of vascular bundles. The vascular bundles were widely imbedded in thin-walled parenchymatous ground tissue. Each bundle was made up of a fibrous sheath, vessels, fibers, phloem, and parenchymatous tissues (Fig. 2a). Xylem and phloem tissues are clearly distinguishable. Phloem was divided into two separate areas in each bundle. Some vascular bundles also contained several well-defined protoxylem elements. Protoxylem and metaxylem vessels in the bundle were separated by a layer of parenchyma cells (Fig. 3a). According to previous study, within the stem and leaves, proto- and metaxylem vessels are separated by at least one layer of live parenchyma cells, which form a living barrier to possible transfer of gas bubbles between proto- and metaxylem vessels (Tomlinson et al. 2001).

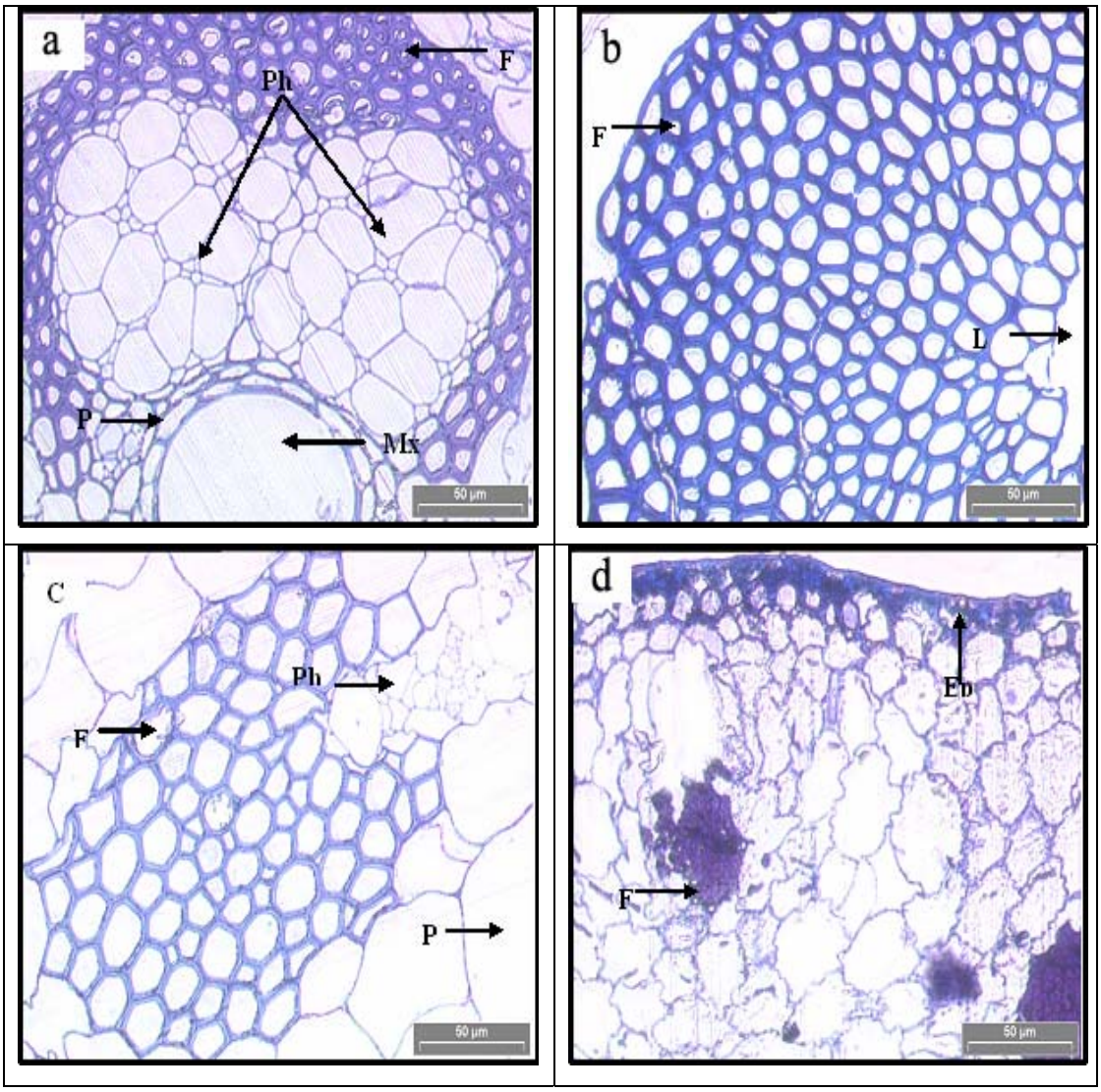

Fig. 3. Transverse section of different types of agro fibers at high magnification (20×). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF). F Fiber; P Parenchyma; Mx Metaxylem; Ph Phloem. F Fiber; L Lacuna; Ep Epidermis

COIR is a multicellular fiber that consists of fiber, phloem, and parenchyma cells. Unfortunately, from the cross section obtained from the fiber, only fiber and parenchyma fibers can be clearly seen (Fig. 2b). COIR fiber also consists of a central portion, called "lacuna" (Fig. 3b). This porous surface morphology is useful for better mechanical interlocking with the matrix resin for composite fabrication (Sreekala et al. 1997).

The vascular bundles of BS fiber also are widely distributed in parenchymatous ground tissue, which consists only of fiber and phloem, without any other vascular tissues (Fig. 2c and 3c). This situation may occur in certain types of monocot stem. In plant tissue, phloem plays an important role in organic nutrient transport, especially in the case of sugar that is produced during the photosynthesis process (Mauseth, 1988).

Leaf is a primary photosynthesis organ for a plant. Cross-sections of PALF showed that it consists of an epidermis, palisade parenchyma, spongy mesophyll, and fiber bundle (Fig. 2d). Figure 3d shows that the PALF vascular bundle consists only of thick-walled fibers, without other vascular tissues. This situation also may occur in certain species such as orchid and Bromeliad (pineapple). This was one of the adaptations for annual plants, to provide strength and stiffness to the leaf (Mauseth, 1988).

Lignin Distribution

Qualitative and quantitative determination of lignin distribution has been carried out in several species of agricultural fibers, such as wheat straw, sugarcane, rice plant, poplar and linseed flax (Donaldson 1996; Zhai and Lee 1989; He and Terashima 1991). Unfortunately, there appears to be little information on lignin distribution in monocotyledonous species, especially oil palm, pineapple leaf, and banana stem fibers.

An observation that has been made using an image analyzer illustrated that thick-walled fiber in OPF, COIR, BS, and PALF were strongly lignified after being stained with toluidine blue. The middle lamella showed a high level of lignification for all types of plant fibers (Fig. 4a-4d). Phloem and parenchyma cells in OPF (Fig. 4a) and BS (Fig. 4c) fiber, which consists only of a primary wall, were unlignified, as shown by a weak positive reaction with toluidine blue. Donaldson (1991) also found that lignification in linseed flax was greater in xylem fibers, compared to phloem fibers. In the OPF vascular bundle, fibers were more lignified than metaxylem vessels. Palisade and mesophyll cells, which were thin-walled parenchyma cells, also stained weakly (Fig. 4d). These thin walls are important to absorb sunlight in an optimum manner to be used in the photosynthesis process.

Cell Wall Structure

The electron microscope provides a unique tool for investigating cell wall ultrastructure. Electron microscopy observations showed that wood cell walls are composed of an intercellular layer, a primary, and a secondary wall. The primary cell wall is a thin layer produced by cell division and the subsequent growth of xylem mother cells, while the secondary wall is a thick layer deposited inside the primary wall. It consists of an outer layer (S_1), a middle layer (S_2), and an inner layer (S_3), each with a different orientation of cellulose microfibrils (Harada & Cote, 1985).

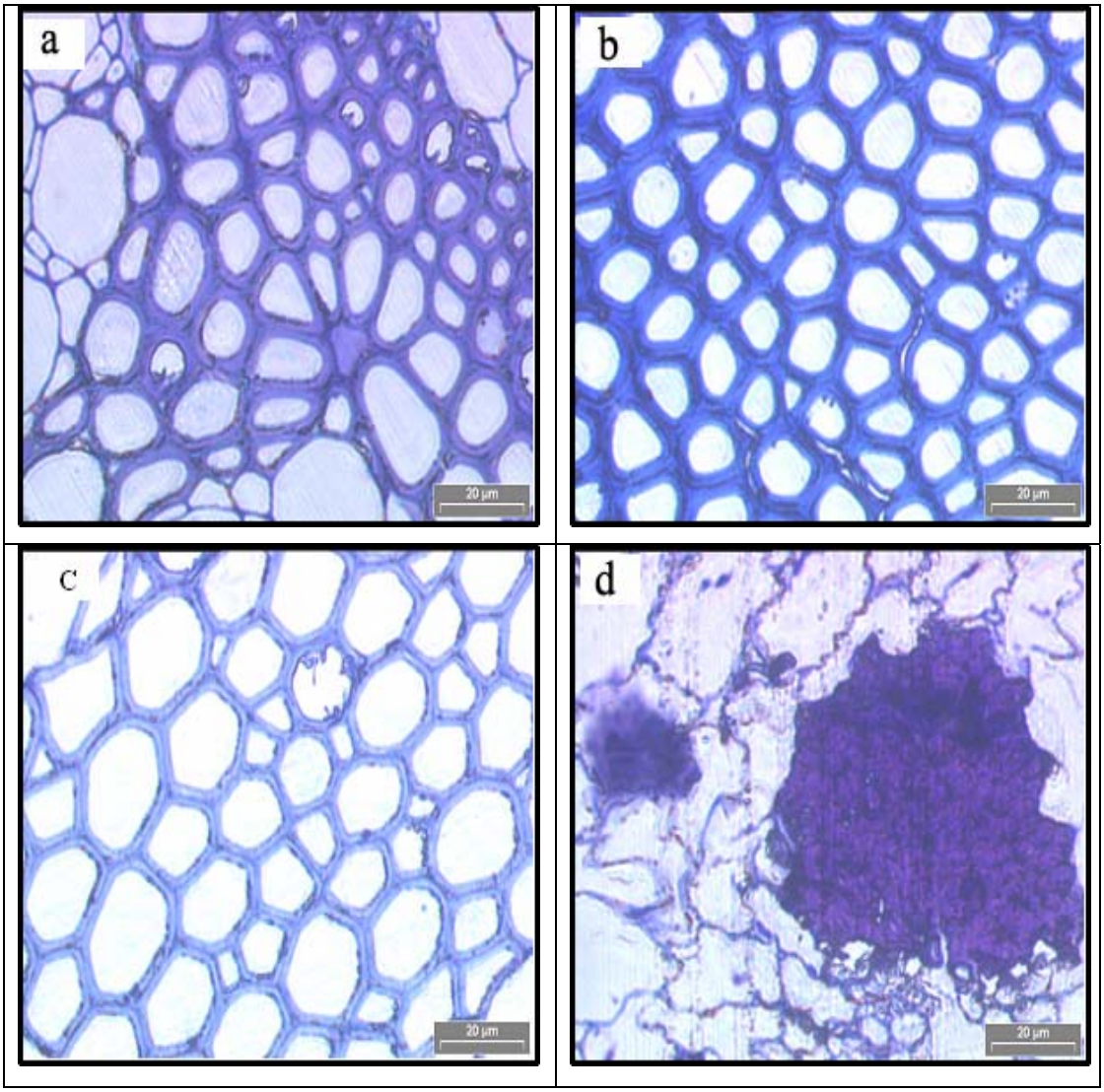

Fig. 4. Transverse section of agro fibers after stained with toluidine blue at high magnification (40×). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF).

Transmission electron microscopy (TEM) views of transverse sections of agro fibers are shown in Figs. 5-6. The electron microscopic observations were restricted mainly to the walls of fibers within the vascular bundles. Generally, all fibers showed great variability in size, shape, and structure of the cell wall (Fig. 5a-5d). The TEM electron micrographs have confirmed that the layered structure of OPF, COIR, PALF, and BS fiber wall contained primary (P) and secondary (S_1, S_2 and S_3) wall layers (Fig. 6a-6d). This structure was similar to the wood cell wall structure that has been proposed by Harada and Cote (1985) and other lignocellulosic fibers such as flax, jute, *etc*. However, according to Liese (1985), bamboo showed different structure of the cell wall layer. Bamboo culm fiber has a polylamellated wall structure [middle lamella, primary and secondary wall (S_0, $S_{1\text{-}l}$, $S_{2\text{-}t}$, $S_{n\text{-}l}$, $S_{n\text{-}t}$)]. A typical tertiary wall is not present, but warts cover the innermost layer of the cell wall.

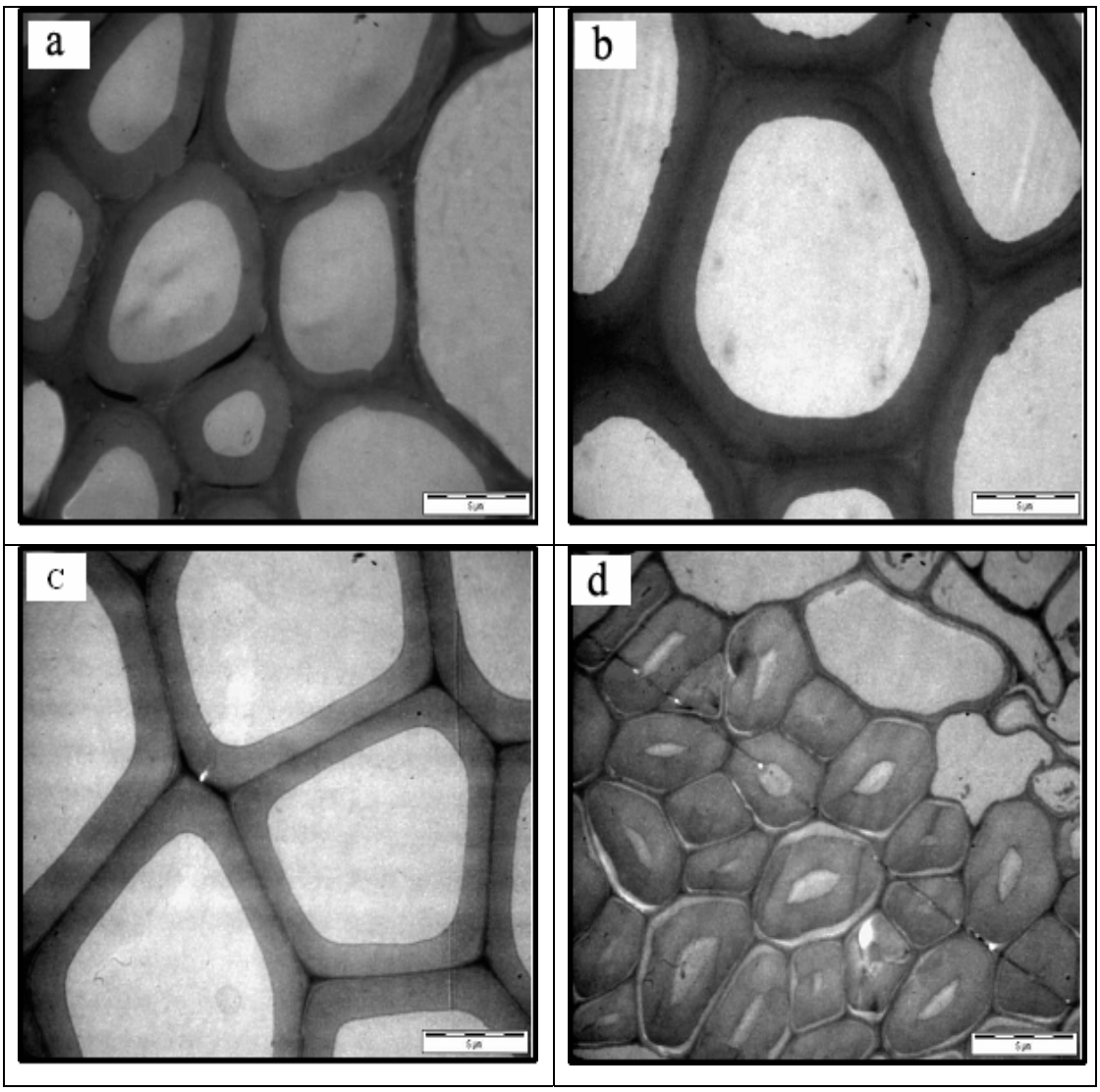

Fig. 5. Transmission electron micrograph of ultrathin section of agro fibers after stained with uranyl acetate and lead citrate at low magnification (3400×). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF).

The various wall layers (primary and secondary wall) clearly appeared in ultra-thin transverse sections of fibers. The primary wall appeared as a solid boundary of the cell (Fig. 6a and 6d). The middle lamella, which glues the cells together, showed a clear transition to the adjacent primary wall layers. The dark staining of the middle lamella indicated that it was strongly lignified. The S_1 layer of all fibers was well-defined and could be distinguished from the adjoining S_2 layer, as it was the brightest layer compared to other layers. Random measurements were taken on high magnification TEM micrographs, and the thickness of the S_1 layer in agro fibers was found to be in the range of 0.10 – 0.84 μm. Total cell wall thickness is largely controlled by the S_2 layer. The S_2 layer is reinforced by microfibrils that usually lie from 5 to 30 degrees to the axis, and it

is about forty times thicker than any of the other layers (Booker and Sell, 1998). Cells with thick walls contain a large S_2 layer, whereas thin-wall cells have a small S_2 layer. The S_2 layer of agro fibers occupied about 43-78% of the whole wall in thickness, and BS fibers exhibited the thickest S_2 layer (1.57 μm) (Fig. 6c).

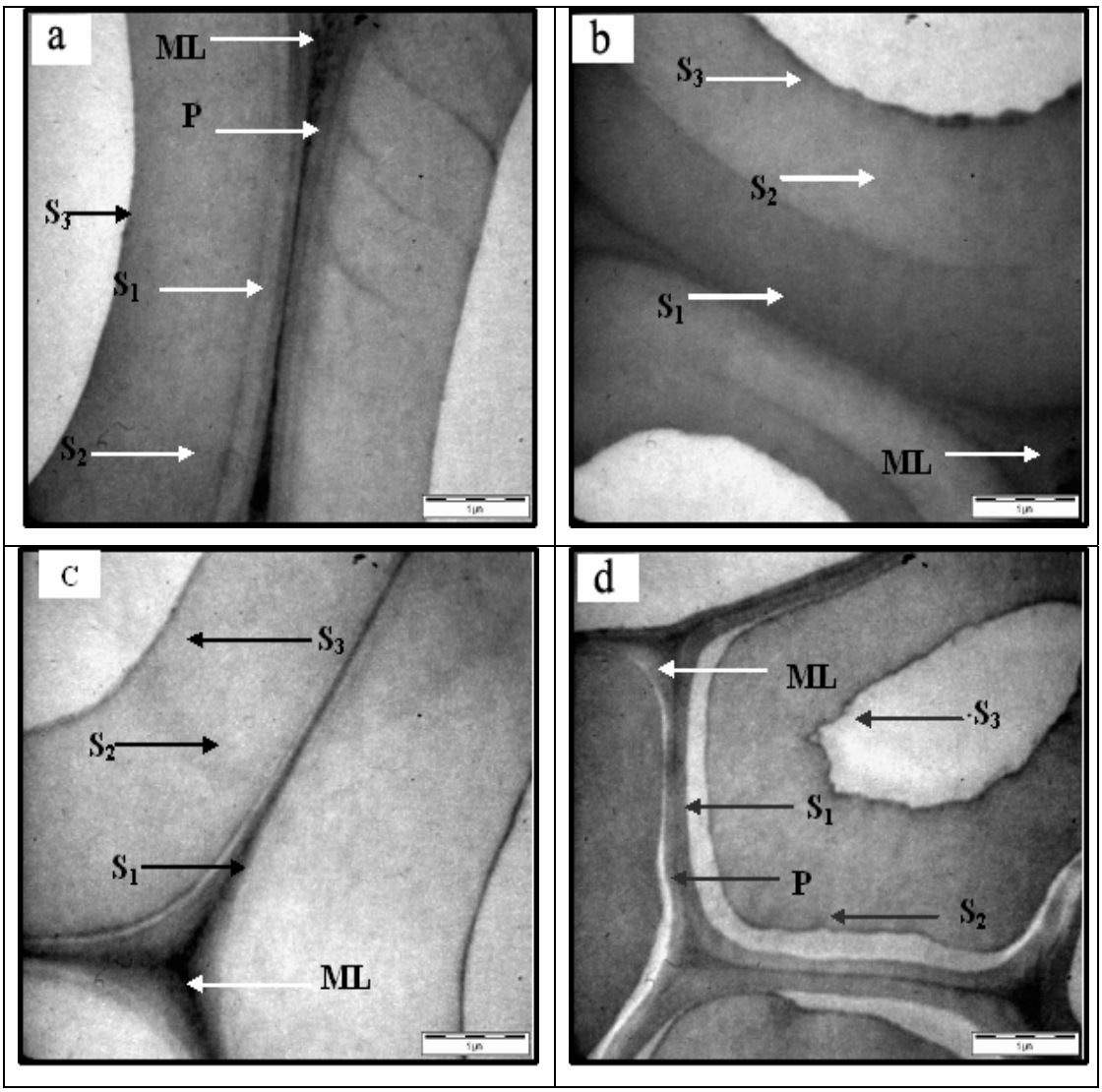

Fig. 6. Transverse section of a multi-layered structure of agro fibers at high magnification (17000×). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF). ML Middle Lamella; P Primary Wall; S_1 S_2 & S_3 Secondary wall sub-layers

The observations using TEM micrographs also provide evidence for the presence of a distinct S_3 layer in the cell wall of agro fibers. Agro fibers showed a great variability in the thickness of the S_3 layer. COIR fibers (Fig. 6b) contained a well developed S_3 layer, being the thickest S_3 layers (0.089 μm) among all fibers. COIR fibers are supposed to have more resistance against collapse due to water tension and buckling due to axial compression forces, as also proposed by Booker and Sell (1998). Previous studies have

reported that *P. radiata* earlywood tracheids also showed great deal of variability in the thickness of the S_3 layer, both within and between tracheids (0.06-0.3 μm). It has been suggested that the irregular thickness might be better suited to relieve the pressure of the axial compression force on the tracheid wall than one of uniform thickness (Singh et al., 2002).

CONCLUSIONS

The above studies can be summarized as follows:

i) In general, the chemical composition of oil palm fibers followed the order given below:
   Lignin: (highest) COIR > OPF > BS > PALF (lowest);
   α-cellulose: (highest) PALF > BS > OPF > COIR (lowest);
   Holocellulose: (highest) OPF > PALF > BS > COIR (lowest);
   Extractive: (highest) BS > COIR > PALF > OPF (lowest);
   Ash: (highest) OPF > COIR > PALF > BS (lowest).
ii) Determinations of lignin distribution in oil palm fibers revealed evidence of lignification in most of the fiber and vessel (meta- and protoxylem) material, except for the phloem. Most of the fibers showed positive staining with toluidine blue. The middle lamella showed a high level of lignification for all types of fibers.
iii) Transmission electron microscopy (TEM) micrographs have confirmed that cell wall structure of all types of agro fibers (OPF, COIR, PALF and BS) consist of primary (P) and secondary (S_1, S_2 and S_3) layers.

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PENETRATION AND PERFORMANCE OF ISOCYANATE WOOD BINDERS ON SELECTED WOOD SPECIES

T. Michael Gruver [a] and Nicole R. Brown [b *]

The penetration and performance of polymeric diphenylmethane diisocyanate (pMDI) wood binder was investigated according to three factors: substrate species (aspen, yellow-poplar, or southern yellow pine); anatomical bonding plane (radial or tangential); and moisture content (0%, 5%, or 12%). Compression shear block tests and fluorescence microscopy were used to examine bond performance and resin penetration. Statistically, each of the aforementioned factors impacted results. As moisture content increased, observed bond strengths and wood failure increased. Bond formation did not occur when the substrates were equilibrated to 0% moisture content, except for the radial bonding surfaces of pine, which did adhere. At 5 and 12% moisture contents, tangential bonding surfaces out-performed radial bonding surfaces. In terms of resin penetration, moisture content was clearly the most important variable. Little penetration was observed at 0% moisture content, while extensive resin penetration was observed at elevated moisture contents. Pine was the only wood species to exhibit resin flow through radial cells, possibly explaining the enhanced resin penetration depths observed in pine samples.

Keywords: pMDI, Isocyanate, Adhesion, Fluorescence microscopy, Compression shear block tests, Resin penetration

*Contact information: a: Hexion Specialty Chemicals Inc., 6210 Campground Road, Louisville, KY 40216 b: School of Forest Resources, Materials Research Institute, The Pennsylvania State University, 202 Forest Resources Building, University Park, PA 16802 USA; *Corresponding author: NRB10@PSU.EDU*

INTRODUCTION

The widespread use of polymeric diphenylmethane diisocyanate (pMDI) binders in structural wood composites, coupled with the resin's unique cure chemistry, led to a number of fundamental research investigations regarding wood-pMDI interactions. Differential scanning calorimetry, dynamic mechanical analysis, dielectric analysis, nuclear magnetic resonance spectroscopy, and Fourier transform infrared spectroscopy (Weaver and Owen, 1995; Wendler and Frazier 1996a; Wendler and Frazier 1996b; Marcinko et al. 1998; Schmidt and Frazier 2000; Harper et al. 2001; Bao et al. 2003, He and Yan 2005; Das et al. 2006) all have been used to probe the wood-pMDI interphase, largely with the aim of understanding either the scale of intermolecular interactions between the resin and wood polymers or resin cure chemistry. Urethane formation between the resin and wood hydroxyl moieties was widely debated in the literature, but now is generally regarded as insignificant under common industrial manufacturing conditions. The mechanism underlying species-dependent performance of pMDI resin, however, remains unclear and is the motivation for this research.

Johns et al. (1982; 1985) conducted studies on the effects of pMDI in binding species-exclusive composite panels and found distinct performance differences. Much later, Malmberg (2002) and Das et al. (2006) observed species effects while testing pMDI-bonded wood double cantilever beams via mode I opening fracture testing. Das et al. also conducted solid state NMR and dynamic mechanical analyses to investigate these differences (2006). He and Yan (2005) studied pMDI cure on wood via DSC. To date, little has been done to explore the relationships between species-dependent performance and pMDI penetration for selected species, moisture contents, and bonding planes; these factors will be investigated in this paper.

Fluorescence microscopy was used to observe resin penetration at the micron scale. This technique is well established in the literature as a means of probing resin penetration within the wood-adhesive interphase (Brady and Kamke 1988; Johnson and Kamke 1992; Sernek et al. 1999; Conrad et al. 2004). Lap shear, internal bond, and fracture mechanics tests are all common means of evaluating the performance of thermosetting wood adhesives. However, each of these methods has inherent flaws for evaluating adhesion on solid wood substrates when sample orientation (bonding plane) is varied. Here, bond line performance was measured, following a widely used standard test method (ASTM D 905). Bigtooth aspen (*Populus grandidenta*), yellow-poplar (*Liriodendron tulipifera*), and southern yellow pine (*Pinus spp.*) wood species were selected due to widespread commercial usage and their prevalence in past research. Moisture contents (MCs) of 0%, 5%, and 12% were utilized, also due to commercial relevance and correlations with prior work.

EXPERIMENTAL

Wood Sample Preparation

The experimental design was a three by three by two factorial with three levels of species (aspen, poplar, pine), three levels of moisture content (0%, 5%, 12%), and two levels of anatomical bonding plane (radial and tangential). Twenty compression shear blocks and five resin penetration samples (five measurements on each) were evaluated for each three-way factor combination, totaling 360 compression shear block tests and 450 resin penetration measurements. Compression shear block and fluorescence microscopy samples were taken from the same cured billet (Figure 1). Sapwood billets (15.2 cm x 1.27 cm x 5.08 cm) were prepared from kiln-dried boards equilibrated at 23°C and 40% ± 2% relative humidity (RH) such that either the radial or the tangential plane was exposed. Grain orientation was maintained such that "true" radial or tangential faces were obtained ± 15°; in rare cases variations in grain angle were observed up to ± 25°. Samples were equilibrated to moisture contents (MCs) of either 0% (0% RH), 5% (25% ± 2% RH), or 12% (67% ± 2% RH) prior to bonding. Bayer Mondur® 541 Light pMDI resin was applied with a dropper to freshly planed surfaces and then spread with a plastic applicator at an adhesive coverage of 80 g/m^2. Two adhesive-coated wood specimens (same species, and same planes exposed) were hot pressed at 175°C in a Carver Laboratory press for 25 minutes to form a cured billet (Figure 1). The hot press schedule was adapted from the work of Das et al. (2006), who prepared mode I opening fracture specimens; here the

cure time was extended to account for thicker samples. Cure of each wood species*moisture content interaction was probed with dielectric analysis, using a Micromet Dielectric Analyzer and Idex sensors (data not shown). Each billet was machined into two compression shear blocks and one microscopy sample as shown in Figure 1.

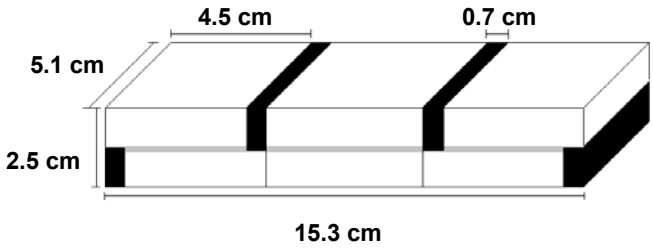

Fig. 1. Dimensions of a cured billet, where the bond line is the central horizontal gray line. Radial or tangential surfaces were bonded together to form the billet. Black portions were removed during machining, generating two compression shear blocks (ends) and one microscopy sample (middle).

Compression Shear Block Testing

Compression shear blocks were conditioned at 23°C and 40% ± 2% RH for one week prior to machining and testing. Samples were then loaded into a Tinius-Olsen testing machine fitted with an ASTM D 905 shear fixture. A continuous uniform loading rate of 0.51 cm/min was applied until complete sample failure. Percent wood failure and shear stress at failure were recorded.

Fluorescence Microscopy

Bond line cross-sections were prepared with an American Optical microtome. Sample thickness varied between 50 μm and 75 μm. Each section was placed in 0.5% (w/w in water) Toluidine-O-Blue (Harleco) solution for fifteen seconds, then sequentially rinsed in distilled water, then ethanol (Pharmco, 95%). Samples were placed on glass slides with two to three drops of glycerol (J.T. Baker, 99% pure), and a cover slip was placed on top. A total of five slides were prepared for each condition.

The fluorescence microscope was an Olympus BX-60 epi-fluorescent scope fitted with a Hamamatsu Orca-100 digital camera, a NIBA filter cube that allowed excitation wavelengths of 455 nm to 500 nm, and a dicroic lens, which reflects light with wavelengths less than 500 nm. Emission wavelengths of 505 nm to 560 nm were detected. Gain and exposure times were adjusted to minimize over-saturation. Measurements were taken with a 4x magnifying lens (40x magnification) and qualitative observations were made with either 10x or 20x magnifying lenses. Maximum resin penetration depth perpendicular to the bond line was measured (five times per sample at set interval spacing) with Image Pro v5.0 image analysis software.

Statistical Methodology

The statistical model for predicting the least squared means (Minitab, General Linear Model) was expressed as a function of the main effects (moisture content (α),

wood species (β), and bonding plane (γ)) plus the two- and three-way interactions of the main effects, as shown in Equation 1.

$$M_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} \quad [1]$$

Where: $i = 1, 2, 3$ $j = 1, 2, 3$ $k = 1, 2$

Analyses of variance (ANOVA) with Tukey multiple comparison tests were used to evaluate the data, where the criterion for significance was α=0.10. Data are illustrated via interaction plots where sample means are represented by a symbol and error bars represent the 90% confidence intervals about the means.

RESULTS AND DISCUSSION

Performance of Bonded Specimens

Statistical analysis of the compression shear block data revealed that all two- and three-way interactions of the factors were significant at α=0.10. Two-way interaction plots demonstrate the effects of the factors on bond shear strength and wood failure.

Out of the 120 billets bonded at 0% MC, 100 (~83%) failed to bond during the press cycle. Dielectric studies were conducted to monitor the extent of cure during hot pressing, however, at 0% MC no conclusive evidence for cure emerged due to extreme variability among the data. This raises the possibility that the samples were only partially cured—one potential explanation for the poor bonding. Alternatively, the dry conditions could have caused the isocyanate resin to act as a wood modification agent rather than a highly networked resin. Previous studies offer some support for this theory, as cure under dry conditions has been speculated to be due to urethane formation at accessible wood hydroxyl groups (Weaver and Owen 1995, He and Yan 2005). Under these dry conditions, the resin is not extensively networked, causing poor stress transfer and poor bonding. It should be noted that the 20 samples that did bond were all southern yellow pine samples with radial surfaces adhered. Of these 20 samples, five failed when the compression shear block samples were machined from the billet; the remaining 15 samples had 0% wood failure and failed at low average shear stress (8.34×10^5 N/m^2). The discovery that radial pine samples adhered at 0% moisture was unexpected. This finding may suggest that ray tissues have a unique interaction with pMDI. The surface chemistry in the rays may be different, or the morphology of the surface could be different, although these are both speculative comments. Little work has been conducted regarding the surface roughness or surface characterization of ray tissues.

Figures 2 and 3 reveal the effects of bonding the selected species at either 5% or 12% moisture content. Under these conditions, all samples bonded and corresponding dielectric data revealed complete cure. A networked topology based on polyurea dominates when moisture is present, thus providing resistance to applied loads (Weaver and Owen 1995, Zhou and Frazier 2001). Generally, wood failure data (Figure 3) paralleled the responses seen in the shear stress at failure data (Figure 2), with the exception that the variability was higher in the wood failure data. Wood failure is by

nature variable; nevertheless it remains an important component of the ASTM D 905 protocol. Data obtained here show surprisingly good correlations between wood failure and observed shear stress at failure; in Figure 7 the variability becomes more obvious. In terms of shear stress (Figure 2), results indicated that at 5% moisture content, southern pine and aspen samples behaved similarly, both performing better than yellow-poplar. When moisture content increased from 5 to 12%, ultimate shear stresses increased for each wood species, as did wood failure (Figure 3). Yellow-poplar showed the most pronounced sensitivity to moisture content, both in terms of wood failure and shear stress at failure.

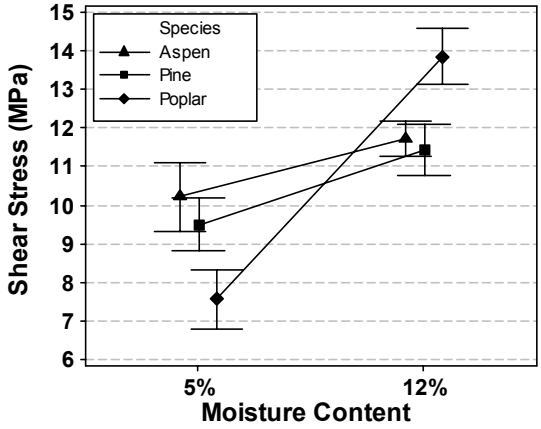

Fig. 2. Interaction plot of moisture content versus mean shear stress (A) as a function of wood species (all orientations pooled).

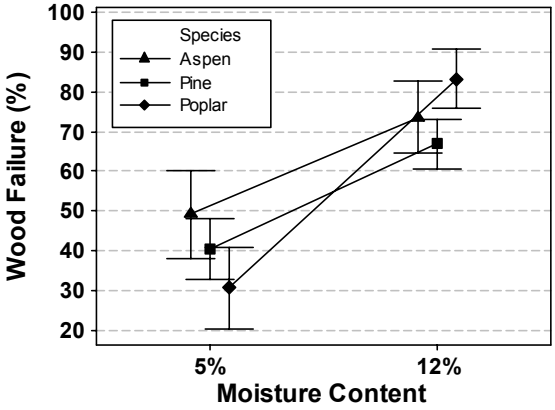

Fig. 3. Interaction plot of wood failure versus wood moisture content as a function of species (anatomical faces pooled).

Effects of Species and Anatomical Bonding Plane

At 5% moisture content, the bonding plane did not impact shear stress at failure, but at 12% MC, the tangentially bonded samples performed better than the radially bonded samples, as expected. Differences due to the anatomical planes were not evident

in the wood failure data; however, the trend of better performance at higher moisture contents is again supported (Figure 5).

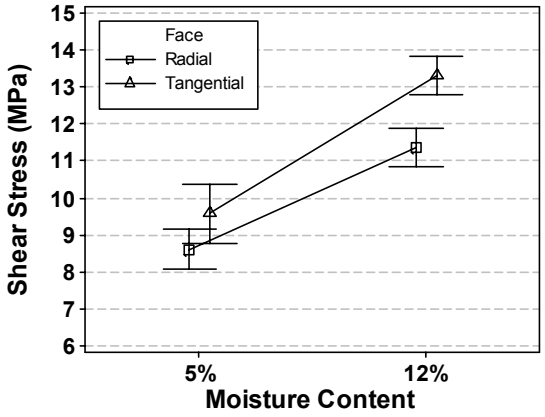

Fig. 4. Interaction plot of moisture content versus mean shear stress as a function of anatomical bonding surface (all species pooled).

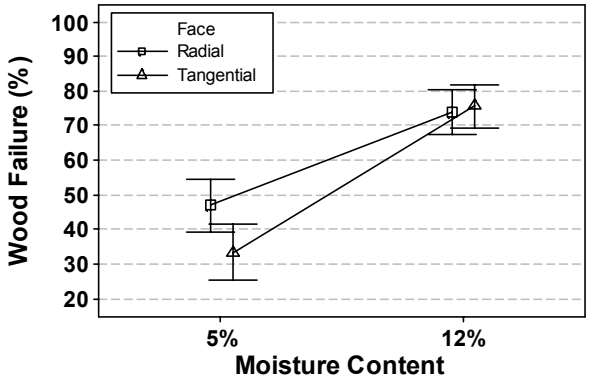

Fig.5. Interaction plot of wood failure versus wood moisture content as a function of face (species pooled).

Interactions between species and anatomical faces were only statistically significant for aspen samples (Figures 6), which had enhanced performance when tangential faces were bonded. The effect of substrate orientation and exposed resin penetration paths will be discussed further in the fluorescence microscopy section of this article. When tangential faces were bonded, there were no differences among the species; all radially bonded faces were also statistically identical, except the pine samples at 0% moisture content, which were previously described. Wood failure data (Figure 7) revealed no significant differences according to anatomical planes or species.

Very few research articles have investigated the strength of the pMDI-wood bond line in solid wood specimens. The fracture mechanics results obtained by Das et al. (2006) included southern yellow pine and yellow-poplar specimens bonded at 10% MC. Malmberg (2002) speculated that the higher initiation and arrest energies observed for pine were due in part to the higher surface free energy measured for pine. The 10% MC

level used by Malmberg (2002) is not replicated here. However, our results at 5% MC are similar to their findings (pine samples out-performed poplar in observed shear stress at failure). Our data indicates that this trend reverses at 12% MC (poplar out-performs pine), suggesting that the high surface free energy of pine is not the only factor influencing adhesion. Other important factors to consider regarding species-specific adhesion (also noted by Das et al. 2006) include: slight differences in cure chemistry, differences in interphase morphology, and differences in heat and mass transfer during hot pressing due to different wood anatomical features.

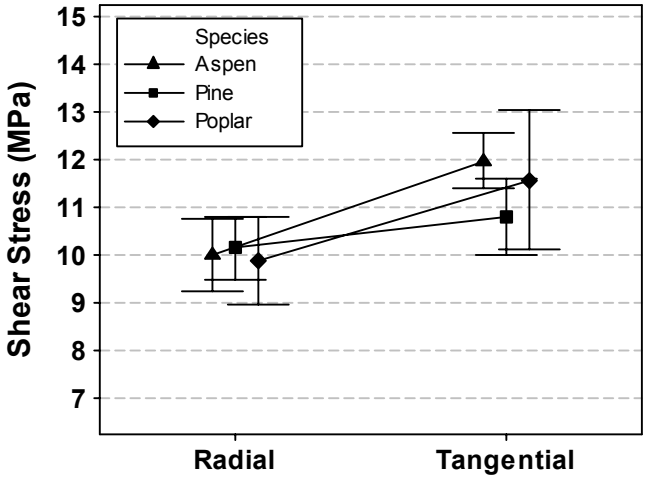

Fig. 6. Interaction plot of anatomical bonding plane versus mean shear stress as a function of wood species (all moisture contents pooled).

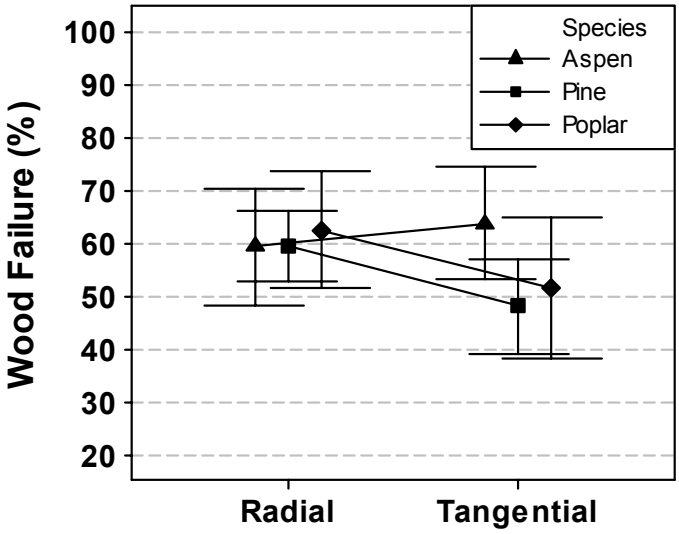

Fig. 7. Interaction plot of anatomical bonding plane versus mean wood failure as a function of wood species (all moisture contents pooled).

Resin Penetration

Factors Influencing Resin Penetration

One of the principle objectives here was to examine the effects of wood anatomy on resin penetration. Of the commercially important species used in OSB production, one softwood and two hardwood species were selected for study. Differences in hardwood and softwood structure are well documented, but the flow of adhesives and resins through specific anatomical tissues is not. Softwoods have a fairly homogeneous cell structure, constituted of more than 90% longitudinal tracheids, whereas hardwood structure is notably more heterogeneous. While vessels are the principle vehicle for bulk flow in hardwoods, softwood fluid flow occurs primarily through tracheid lumens, ray lumens, and the interconnecting pits (Siau, 1995). Radial permeability exceeds tangential permeability in softwoods, owing to the important contribution of rays (Banks, 1970). In hardwoods, this discrepancy is not as apparent. For unknown reasons, conduction through hardwood ray tissues is not nearly as important, despite the greater abundance of rays (Siau, 1995). Differences are also clear when comparing penetration in earlywood and latewood tissues. In softwoods, latewood tissues are generally more permeable than earlywood. This is presumed to be due to the more rigid pit membranes (tori) of latewood, which are less likely to be aspirated (Siau, 1995). Diffuse porous hardwood species show little difference in penetration between latewood and earlywood, as the pits in hardwoods lack tori, and thus cannot be aspirated (Siau, 1995).

The polarity of the resin is also expected to affect penetration. Independent research by Walters and Cote (1960), and Murmanis and Chudnoff (1979) suggested that nonpolar liquids travel via bulk flow through cell lumens and pitting. Polar compounds penetrate via both bulk flow and by diffusion through the wood cell wall. This raises an interesting question regarding the mechanism of pMDI penetration. While unreacted pMDI is non-polar, its hydrophilicity and polarity increase as it reacts with water present in wood. In addition to affecting the mechanism of pMDI flow, wood moisture content also impacts the ultimate penetration depth of pMDI. It is well known that the permeability of wood tissues is proportional to moisture content. Intercellular pitting becomes impermeable under dry conditions, suggesting that polar liquids must be present to facilitate penetration (Stamm 1953; Stone and Green 1959; Wardrop and Davies 1961; Murmanis and Chudnoff 1979). While increasing moisture contents favor tissue permeability, they also lead to pMDI cure, which increases resin viscosity and limits resin penetration. Thus, the pathway and extent of pMDI penetration in wood is difficult to predict.

Here, limited resin penetration was observed under dry conditions (Figure 8), especially for the two hardwood species, indicating that the effect of increased viscosity is not as important as the resin's accessibility to wood's ultrastructure. Note that above 0% moisture content, resin penetration remains constant for a given species. Pine has maximum penetration, and aspen minimum penetration. We observed that pine is the only species to show evidence of adhesive penetration through ray tissues, confirming Siau's observation that flow through rays is more important in softwoods than hardwoods (1995). Figure 9 further supports this observation, in that resin penetration is far greater when tangential surfaces are bonded. In this orientation, resin can flow through the open ends of rays and penetrate wood more deeply. In the two hardwoods, flow through the

exposed ends of rays did not contribute to enhanced resin penetration. In aspen, there is a notable decrease in penetration in this orientation. The reason for this decrease is not clear.

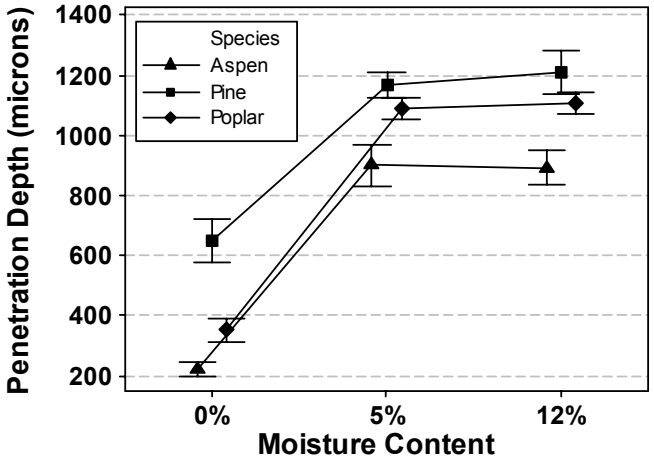

Fig. 8. An interaction plot of moisture content versus mean resin penetration depth as a function of wood species (all orientations pooled).

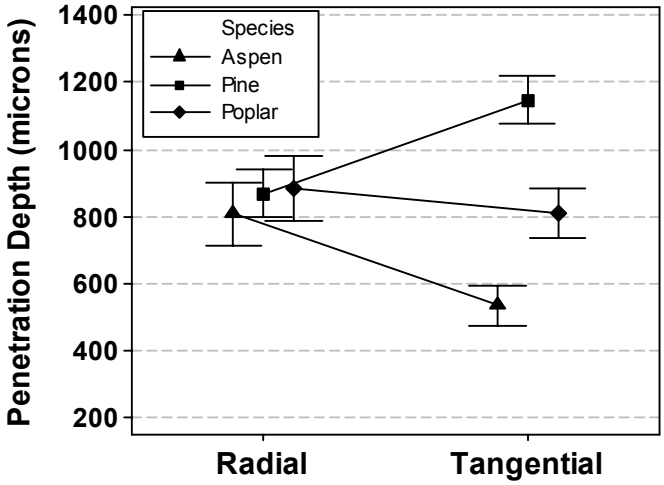

Fig. 9. An interaction plot of anatomical bonding plane versus mean resin penetration depth as a function of wood species (all moisture contents pooled).

Qualitative Features of Resin Penetration

Representative cross-sectional micrographs of resin penetration into radial and tangential bonding surfaces for aspen, poplar, and pine at 0% MC are shown in Figure 10, while micrographs at higher moisture contents are shown in Figure 11. There were no significant differences for resin penetration between 5% and 12% MC, therefore these micrographs are grouped in Figure 11. Fluorescing regions indicate the presence of pMDI resin.

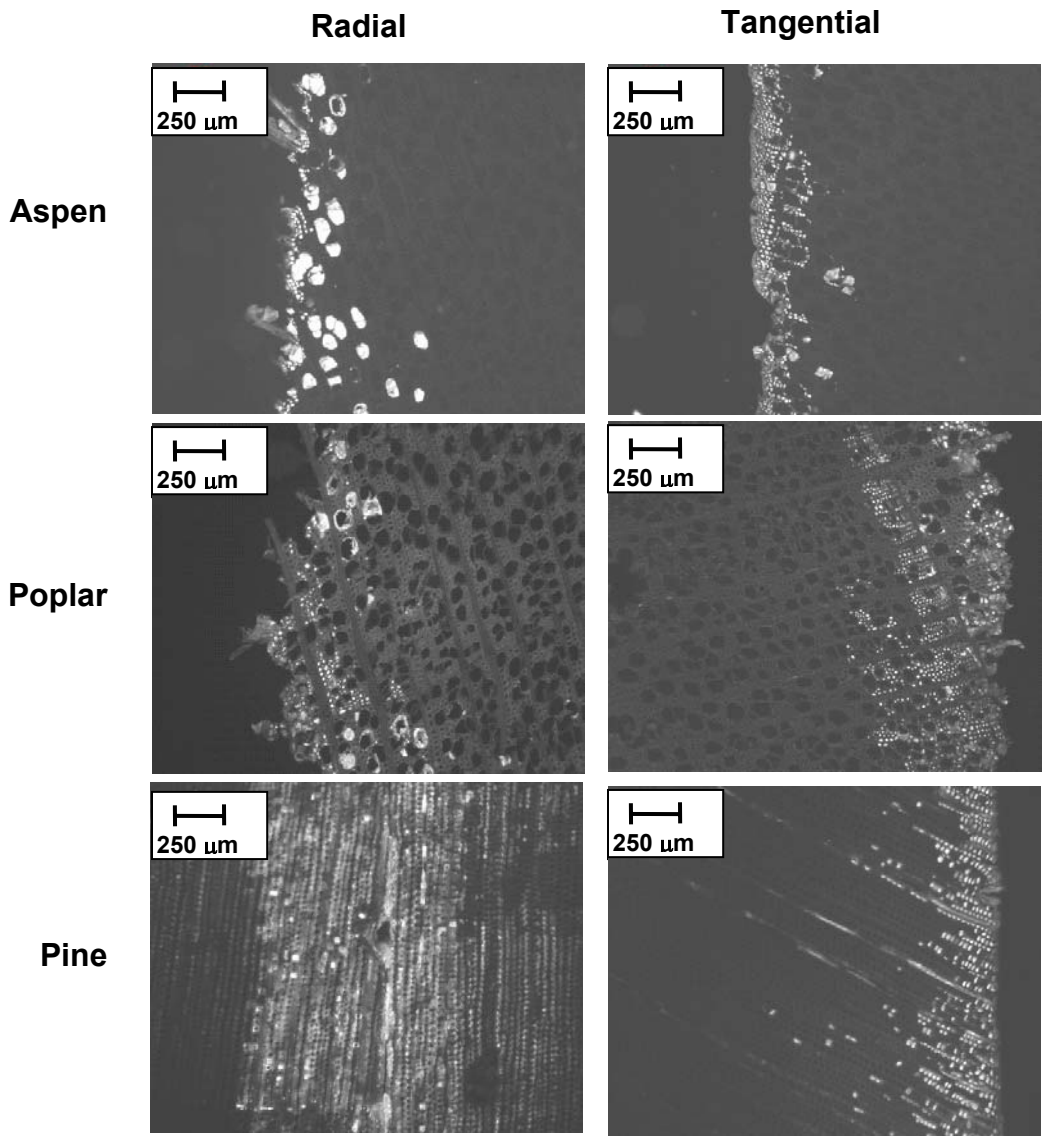

Fig. 10. Resin penetration into radial and tangential surfaces for aspen, poplar, and pine at 0% MC.

The figures reveal less resin penetration at 0% MC than at elevated MCs (confirming the results of Figure 8), and indicate the different distributions of resin according to the species and bonding plane exposed. Note that a high proportion of vessel elements (hardwoods) and lumens (all species) are completely filled with resin at 0% moisture content (Figure 10), but not at higher moisture contents (Figure 11). This lends support to the observation that resin penetration is highly dependent upon the accessibility of the resin to wood tissues. In cases where the wood is completely dry, the resin cannot penetrate far, thus it is limited to cell lumens. Note that pine ray tissues contain pMDI under all moisture conditions, but hardwoods did not show evidence of pMDI in ray tissues. This was referred to previously as support for increased penetration

when tangential surfaces of pine are bonded (Figure 9). Note that in hardwoods under dry conditions (Figure 10), the resin almost always appears in the cell lumens of fiber tracheids when applied to the tangential faces; further, it seems to prefer latewood lumens. This latewood preference was even more distinct in pine samples (Figure 11, lower right), a finding in support of Siau's claim that latewood tissues of softwoods are more permeable.

When moisture was present (Figure 11), the resin lightly coated the cell lumens and/or vessel walls instead of completely filling them. Also, the resin was mostly found in the vessel cells of the hardwood species, very rarely being evident in the longitudinal fibers. In pine, resin clearly preferred latewood tissues. Under these circumstances, the influence of steam generation on resin penetration, as mentioned by Brady and Kamke (1988) and Sernek et al. (1999), cannot be neglected. However, it is interesting to note that resin penetration differences between 5% MC to 12% MC are not evident, suggesting that either the effect due to steam generation at these MCs may be limited, or that some minimal level of steam is all that is required to promote penetration. Studying resin penetration at higher moisture content conditions would contribute to a greater understanding of the role of steam in the system. No studies to date have accounted for the carbon dioxide (CO_2) formed when isocyanate and water react, and this could similarly influence resin penetration.

Previous research quantitatively observing pMDI resin penetration is sparse. Buckley et al. (2002) studied pMDI penetration in aspen with chemical-state x-ray microscopy and found that resin travels via bulk flow through the cell lumens and pits. Zheng et al. (2004) determined pMDI penetration is greater in yellow-poplar than in southern yellow pine. These results conflict with results in this study. Zheng et al. (2004) also determined, via fluorescence microscopy, that pMDI had similar or less penetration than neat phenol formaldehyde (PF) resin, a surprising finding given the lower viscosity and lower surface free energy of pMDI. They attributed the limited extent of pMDI penetration to diffusion into the cell wall, which cannot be resolved via fluorescence microscopy. Evidence for cell wall penetration of pMDI was first noted by Marcinko et al. (1999) and later by Schmidt and Frazier (2000). The techniques used here cannot compare the extent of pMDI penetration into the cell wall; however, this issue will be explored by future dynamic mechanical analyses.

Correlations between Performance and Resin Distribution

Both shear stress at failure and resin penetration improved when the moisture content of the wood increased from 0% to either 5% or 12%. Overall, the correlation between shear stress at failure and maximum resin penetration depth was found to be quite weak ($r^2 = 0.59$). This is not surprising, as the influence of resin penetration on performance is difficult to resolve.

Further, correlations with performance may be limited by the inherent variability of the compression shear block test method. In cases where the substrate fails prior to the adhesive (true for virtually all cases for 5 and 12% MC), the true measure of "adhesion" is not obtained. This is a well-known limitation of the compression shear block test, yet it remains the industry standard method for probing wood-adhesive performance.

This study did not investigate the surface chemistry of the selected species. Resin wetting and contact angle studies could contribute to the overall understanding of species effects, resin distribution, and performance for pMDI resins.

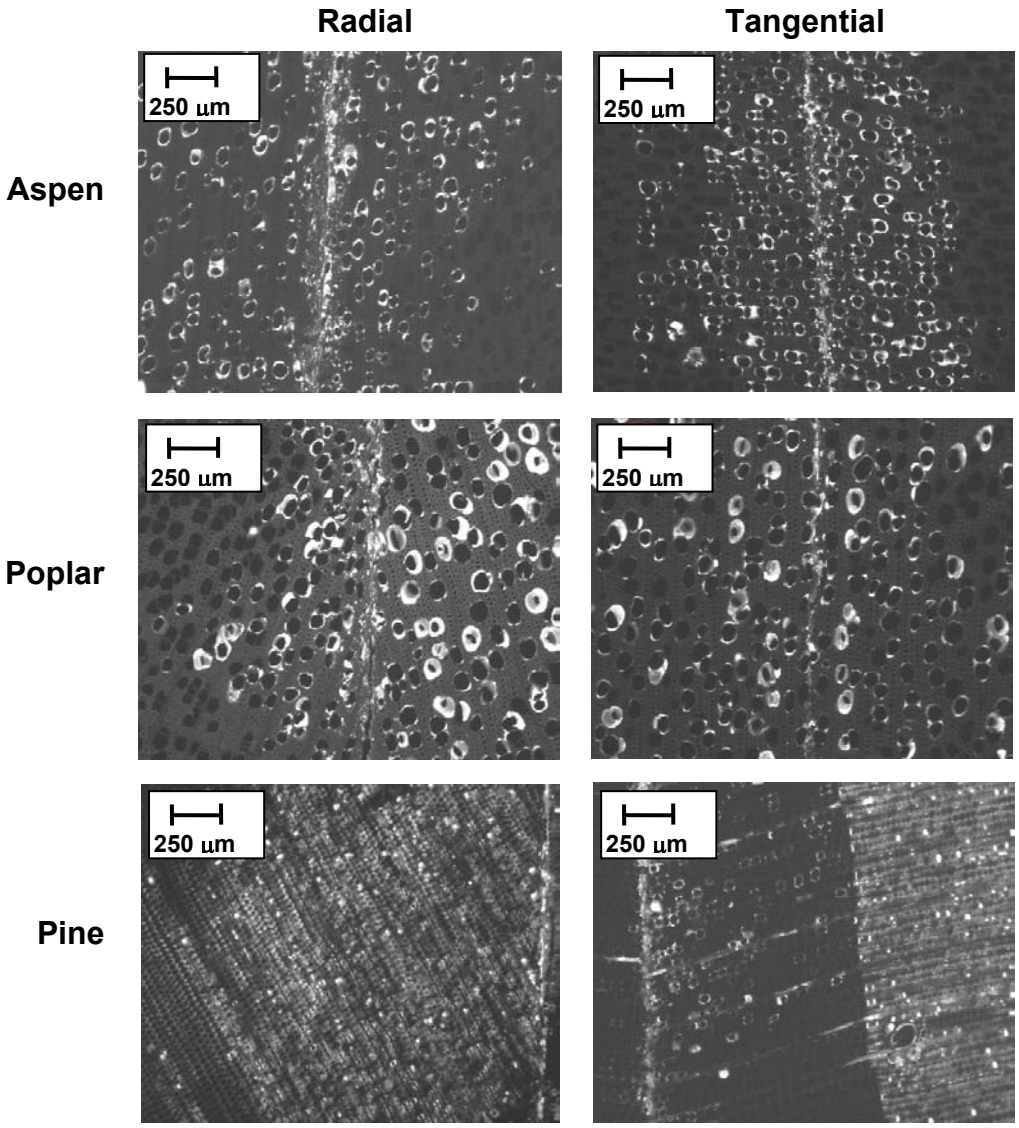

Fig. 11. Resin penetration into radial and tangential surfaces for aspen, poplar, and pine at 5% MC and 12% MC.

CONCLUSIONS

1. All factors investigated (wood species, moisture content, and bonding surface), and the interactions of these factors, had statistically significant effects on shear stress at failure and resin penetration.

2. Bond formation did not occur at 0% MC, except for the radial bonding surface of pine.
3. Performance increased with MC for all wood species; poplar displayed the greatest sensitivity to moisture.
4. Moisture content affected resin penetration in all wood species; little resin penetration was observed at 0% MC and extensive resin penetration was observed at elevated moisture contents. The differences at elevated moisture are attributed to intercellular structures and their ability to inhibit fluid flow in dry wood.
5. Resin filled lumens and vessels at 0% MC but only lightly coated them at elevated moisture levels.
6. Pine was the only wood species to exhibit resin flow through radial cells, possibly enhancing resin penetration.
7. Anatomical bonding surfaces affected resin penetration for pine (as mentioned previously) and for aspen.
8. Resin preferred latewood regions of wood, particularly in pine.
9. Bond performance was greater for tangential bonding surfaces than radial bonding surfaces.
10. Resin penetration and bond line performance show a weak correlation.

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LIQUEFACTION OF CROP RESIDUES FOR POLYOL PRODUCTION

Lingyun Liang,[a] Zhihuai Mao,[a*] Yebo Li,[b] Caixia Wan[b], Tipeng Wang,[a] Lianhui Zhang,[a] and Lingyan Zhang[a]

The liquefaction of crop residues in the presence of ethylene glycol, ethylene carbonate, or polyethylene glycol using sulfuric acid as a catalyst was studied. For all experiments, the liquefaction was conducted at 160°C and atmospheric pressure. The mass ratio of feedstock to liquefaction solvents used in all the experiments was 30:100. The results show that the acid catalyzed liquefaction process fit a pseudo-first-order kinetics model. Liquefaction yields of 80, 74, and 60% were obtained in 60 minutes of reaction when corn stover was liquefied with ethylene glycol, a mixture of polyethylene glycol and glycerol (9:1, w/w), and ethylene carbonate, respectively. When ethylene carbonate was used as solvent, the liquefaction yields of rice straw and wheat straw were 67% and 73%, respectively, which is lower than that of corn stover (80%). When a mixture of ethylene carbonate and ethylene glycol (8:2, w/w) was used as solvent, the liquefaction yields for corn stover, rice straw and wheat straw were 78, 68, and 70%, respectively.

Keywords: Corn stover, Wheat straw, Rice straw, Liquefaction, Polyol

*Contact information: a: College of Engineering, China Agricultural University 17 Qinghua Donglu, Beijing 100083, China, E-mail: maozhh@cau.edu.cn; b: Biological Engineering Program, North Carolina A&T State University, 1601 East Market Street, Greensboro, NC 27411, E-mail: yli@ncat.edu; *Corresponding author: maozhh@cau.edu.cn, yli@ncat.edu*

INTRODUCTION

Crop residues such as corn stover, wheat straw, and rice straw are abundant biomass resources in China. The amount of agricultural residues produced annually in China is estimated to be about 640 million tons (Anon. 2000). In order to increase the productivity of the land, crop rotation is a widely accepted practice in China. The second crop is immediately seeded after the harvesting of the previous crop. There is no time for the decay of the crop residues that should be removed from the land for better seedbed preparation. Open burning is widely used in the rural area to get rid of the crop residues, and this has aggravated the air pollution problem. On the other side, with the depletion of petroleum, bio-based products made of biomass are required to replace the petroleum-based products. Conversion of crop residues to bio-based products has the potential to be a win-win situation for the agriculture, processing industry, and the environment.

In past decades, biological, thermal, and chemical conversion technologies have been studied to convert lignocellulosic biomass to biofuel and bio-based products. Lignocellulosic biomass could be pretreated and fermented to produce ethanol and chemicals (Kaar and Holtzapple 2000; Kim et al. 2003; Ruan et al. 2004; Shahbazi et al. 2005), and gasified to produce synthesis gas, which is an alternative source of fuel for the

rural community (Dasappa et al. 2003). In the absence of oxygen, lignocellulosic biomass could be converted to liquids, gases and char with the fast pyrolysis process (Mohan et al. 2006). Lignocellulosic biomass could also be liquefied under acid conditions with liquefying reagents, such as ethylene glycol and ethylene carbonate, to produce polyol products (Yamada and Ono 1999; Yu et al. 2006a). Polyols are chemical compounds containing multiple hydroxyl groups. Some polyesters, polyurethanes, and fuels have been prepared from the liquefied polyol product (Montane et al. 1998; Yu et. al. 2006b).

The hydroxyl groups of the lignocellulosic biomass make it possible to carry out biopolymer production. Liquefaction is an effective method to convert lignocellulosic biomass into intermediates rich in hydroxyl groups. Wood has been successfully liquefied with polyethylene glycol and phenols in Japan to produce polyurethane foams and phenol-formaldehyde resin (Yao et al. 1996). Shiraishi et al. (1992) reported that liquefaction of lignocellulosic biomass can be done in the presence of these organic solvents using either acid catalysts at temperatures of 120-180°C or without catalysts at temperatures of 180-250°C. Phenol and phenol derivatives mixed with small amounts of sulfuric acid could promote the hydrolytic reactions of depolymerisation in the cellulosic chain (Yu 1982). Yu et al. (2006a) reported that corn stover could be converted to bio-polyols in the presence of ethylene glycol and ethylene carbonate, using sulfuric acid as a catalyst. However, we have not found any report on the comparison of the liquefaction yield of different types of crop residues.

The liquefied products could be used to produce biopolymers such as epoxy resins, which could be used as packaging and insulation materials and adhesives. As the liquefaction process could release some of the etherified hydroxyl groups and reduce the molecular weight of lignin, the reactivity of lignin can be improved (Xie and Chen, 2005). Yu et al. (2006b) reported that the liquefied corn stover could be directly used as feedstock for making polymers without further separation or purification.

In this present article, we designed the experiments to compare the performance of liquefaction process of different feedstock when different liquefaction agents were used. The liquefied products were also characterized with FTIR spectra. It is helpful for choosing the optimal liquefaction agents for different feedstock. The influence of organic solvents on the liquefaction yield of corn stover was also investigated. A first-order reaction model was then used to analyze the kinetics of the liquefaction process.

EXPERIMENTAL

Materials

Corn stover and wheat straw were collected from a local farm in a Beijing suburb and rice straw was collected from the Jiangxi province of China. All of the collected samples with moisture content of 8%-10% were stored in plastic bags at ambient temperature before use. The materials were milled, dried, and screened before tests. Only the fraction with particle size of 20-80 mesh was used for the liquefaction experiments. The compositions of the feedstock were analyzed by Van Soest measurements, using an Ankom Fiber Analyzer (ANKOM220, ANKOM Techno. Corp., NY). The compositions are shown in Table 1.

Sulfuric acid (97%) was used as a catalyst. Ethylene glycol, ethylene carbonate, polyethylene glycol +glycerol (9:1, w/w), or ethylene carbonate + ethylene glycol (8:2, w/w) were used as solvent in the liquefaction process. All chemicals used were reagent grade and obtained from commercial sources.

Table 1. Compositions of Feedstocks (dry basis)

Samples	Ash %	Cellulose %	Hemicellulose %	Acid-insoluble lignin %
Corn stover	5.92	34.51	23.86	6.61
Rice straw	12.47	38.48	20.51	6.42
Wheat straw	9.97	37.07	21.72	5.90

Biomass liquefaction

Liquefying chemicals (50g solvent and 1.5g catalyst) were added into a three-neck flask (250mL) equipped with a reflux condenser, a thermometer, and a motor-driven stirrer. The flask was then immersed in a silicon oil bath that had been preheated to 160°C. When the temperature of liquefying chemicals reached 150°C -160°C, 15 g crop residue samples were added. The mixture was continuously stirred during the liquefaction process to obtain homogeneously liquefied product. For all experiments, the liquefaction was conducted at 160°C and in the air at atmospheric pressure. After the preset time, the flask was immersed into cold water to quench the reaction and the liquefied products were collected for later use and analysis.

Analysis

The amount of residue in the biopolyols after aqueous dioxane extraction and rinsing was measured to evaluate the liquefaction yield. The liquefied biopolyols were extracted using an aqueous dioxane solution (dioxane: H_2O, 80:20). Unliquefied biomass residue was obtained by vacuum-filtration of the extracted solution, using quantitative filter paper (medium speed). The solid residue was then rinsed with the aqueous dioxane solution repeatedly until a colorless filtrate was obtained. The rinsed solid residue was then dried in an oven at 105°C for 24 hours to obtain the dry mass of the residue. The liquefaction yield was calculated by:

$$Liquefaction\ yield = \left(1 - \frac{dry\ mass\ of\ solid\ residue}{dry\ mass\ of\ feedstock}\right) \times 100 \quad (1)$$

Fourier transform infrared (FTIR) spectra of selected samples were obtained using a Perkin-Elmer Spectrum GX spectrometer equipped with DTGS detector. The liquefaction residue was analyzed with a KBr pelletization method.

RESULTS AND DISCUSSION

Effect of Organic Solvent

Liquefaction of corn stover with three different solvents (ethylene carbonate, ethylene glycol, and a mixture of polyethylene glycol and glycerol) was studied. The average liquefaction yields from three replicate tests with the above three organic solvents are shown in Fig. 1. Among the three liquefying solvents tested, ethylene carbonate was the most effective solvent for corn stover liquefaction. The liquefaction yield reached 80, 74, and 60% in 60 minutes of reaction, when ethylene carbonate, the mixture of polyethylene glycol and glycerol, and ethylene glycol were used as solvents, respectively. Liquefaction yield of 70% was obtained in 30 minutes when ethylene carbonate was used as solvent. The difference in liquefaction efficiency among different solvents might be caused by their dielectric constant values (Yamada and Ono 1999). The dielectric constants of ethylene carbonate and ethylene glycol at 40°C are 90.5 and 38.4, respectively (Sengwa, et al., 2000, Chernyak, 2006). The dielectric constant of ethylene carbonate is much higher than that of ethylene glycol. Higher liquefaction yield was obtained when solvent with higher dielectric constant was used. The results of the analysis of variance of liquefaction yield showed that liquefaction solvents have significant effect ($p<0.0001$) on the liquefaction yield.

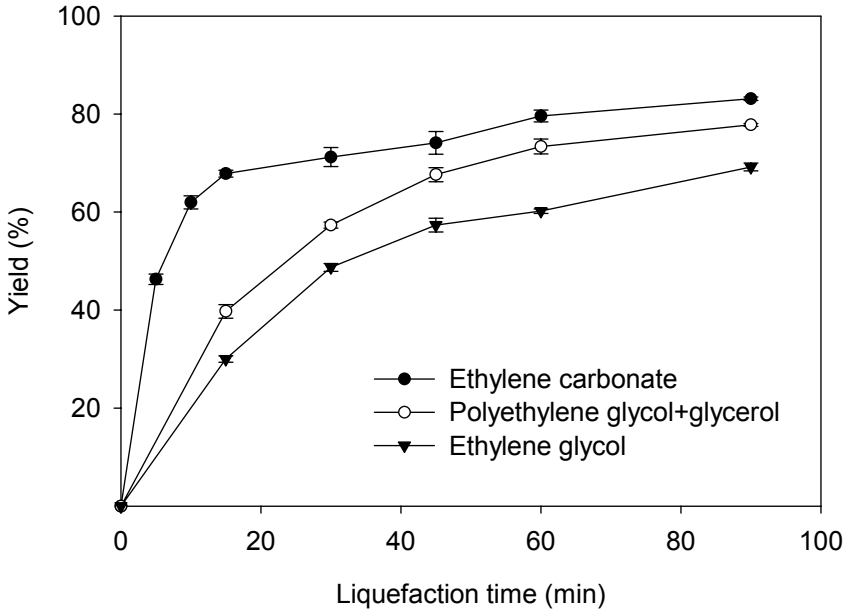

Fig. 1. Liquefaction yield of corm stover with different organic solvents

When the residue was plotted in log scale, there was a linear correlation between ln R and t, which demonstrated that the crop residue liquefaction process follows a pseudo-first-order reaction. The mass of unliquefied crop residue can be expressed as follows:

$$R = 1 - yield = A\exp(-kt) \quad (2)$$

where k is the rate constant, and R is the mass of unliquefied crop residue. The rate constant is an important parameter to evaluate the reaction rate of the liquefaction process. The values of the constant k for corn stover liquefaction with different liquefying solvents are shown in Table 2.

The highest reaction rate constant of 8.84×10^{-2}/min was obtained when ethylene carbonate was used as solvent. When ethylene glycol and a mixture of polyethylene glycol and glycerol were used as solvent, reaction rate constants of 1.91×10^{-2} and 2.49×10^{-2}/min were obtained, respectively, which were much lower than that of ethylene carbonate. The liquefaction rate constant of ethylene carbonate was 3.6 and 2.6 times higher than that of ethylene glycol and mixture of polyethylene glycol and glycerol, respectively.

Table 2. Rate Constants of Corn Stover Liquefaction with Different Liquefying Solvents

Liquefying solvents	Rate constant	Correlation coefficient (R^2)
Ethylene carbonate	0.0884	0.9702
Polyethylene glycol + glycerol	0.0249	0.9807
Ethylene glycol	0.0191	0.9815

Effect of Feedstock

In order to compare the liquefaction yield of different types of crop residues, wheat straw, rice straw, and corn stover were liquefied with ethylene carbonate and a mixture of ethylene carbonate and ethylene glycol (8:2, w/w). The liquefaction yield of corn stover, wheat straw, and rice straw with ethylene carbonate are shown in Fig.2. The liquefaction yield reached 80, 67, and 73% in 60 minutes for corn stover, rice straw, and wheat straw, respectively. The rate constants of rice straw and wheat straw were 7.02×10^{-2}/min and 5.47×10^{-2}/min, which is lower than that of corn stover (8.84×10^{-2}/min).

In the previous tests, the liquefaction yield of corn stover only reached 60% after 60 minutes of liquefaction with ethylene glycol. In order to improve the liquefaction yield, a mixture of ethylene carbonate and ethylene glycol (8:2, w/w) instead of ethylene glycol was used to evaluate the liquefaction yield of the studied crop residues. As shown in Fig 3, the liquefaction yield reached 78, 68, and 70% in 60 minutes of liquefaction for corn stover, rice straw, and wheat straw, respectively when mixture of ethylene carbonate and ethylene glycol was used as solvent. The rate constants of rice straw and wheat straw were 7.56×10^{-2}/min and 6.95×10^{-2}/min respectively. These constants were lower than that of corn stover (8.82×10^{-2}/min). A similar pattern was also obtained when ethylene carbonate was used as solvent, as shown in Table 3.

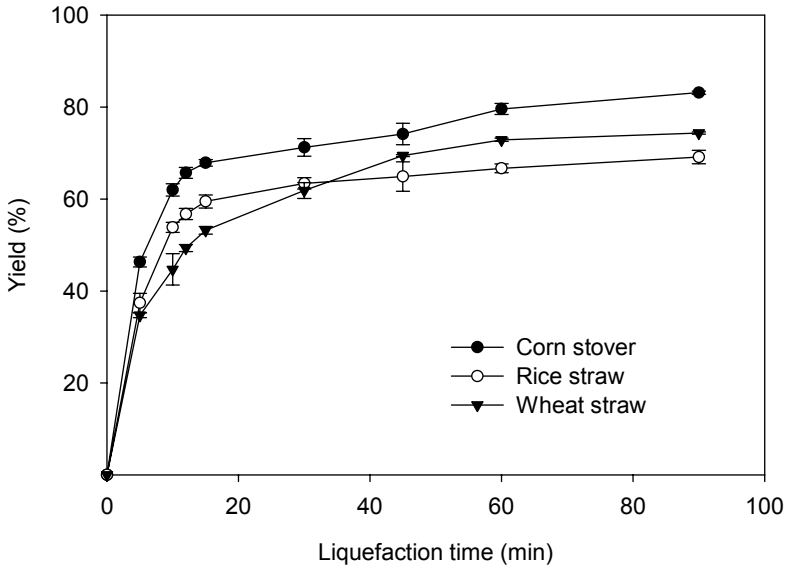

Fig. 2. Liquefaction yield of crop residues when liquefied with ethylene carbonate

For both of the solvents tested, highest liquefaction yield was obtained with corn stover, while the liquefaction yield of rice straw was very close to that of wheat straw. The analysis of variance performed on liquefaction yield showed feedstock had significant ($p < 0.0001$) effects on the liquefaction yield. There was significant interaction between solvent and feedstock on liquefaction yield ($p = 0.001$).

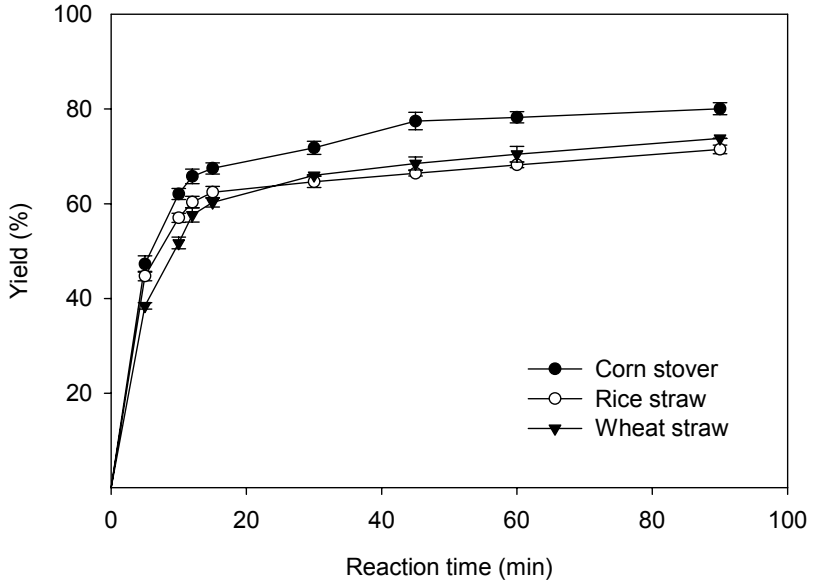

Fig. 3. Liquefaction yield of crop residues when liquefied with mixture of ethylene carbonate and ethylene glycol (8:2, w/w)

Different liquefaction yields among different feedstocks might be caused by the ash content of the biomass feedstock. The cell wall structure should be another factor which affects the liquefaction yield. Corn stover had the lowest ash content of 5.92%, as a result the highest liquefaction yield was obtained when corn stover was liquefied. Rice straw had the highest ash content of 12.47% and the lowest liquefaction yield was obtained when rice straw was liquefied. Increasing ash content caused lower reaction constants and liquefaction yield.

Table 3. Rate Constants of Different Feedstocks when Liquefied with Ethylene Carbonate and Mixture of Ethylene Carbonate and Ethylene Glycol (8:2, w/w)

Residue type	Ethylene carbonate	Correlation coefficient (R^2)	Ethylene carbonate + ethylene glycol	Correlation coefficient (R^2)
Corn stover	0.0884	0.9702	0.0882	0.9643
Rice straw	0.0702	0.9762	0.0756	0.9454
Wheat straw	0.0547	0.9499	0.0695	0.9742

Characterization of Liquefaction Products

The FTIR spectrum of the liquefied residue of corn stover with ethylene carbonate is shown in Fig 4.

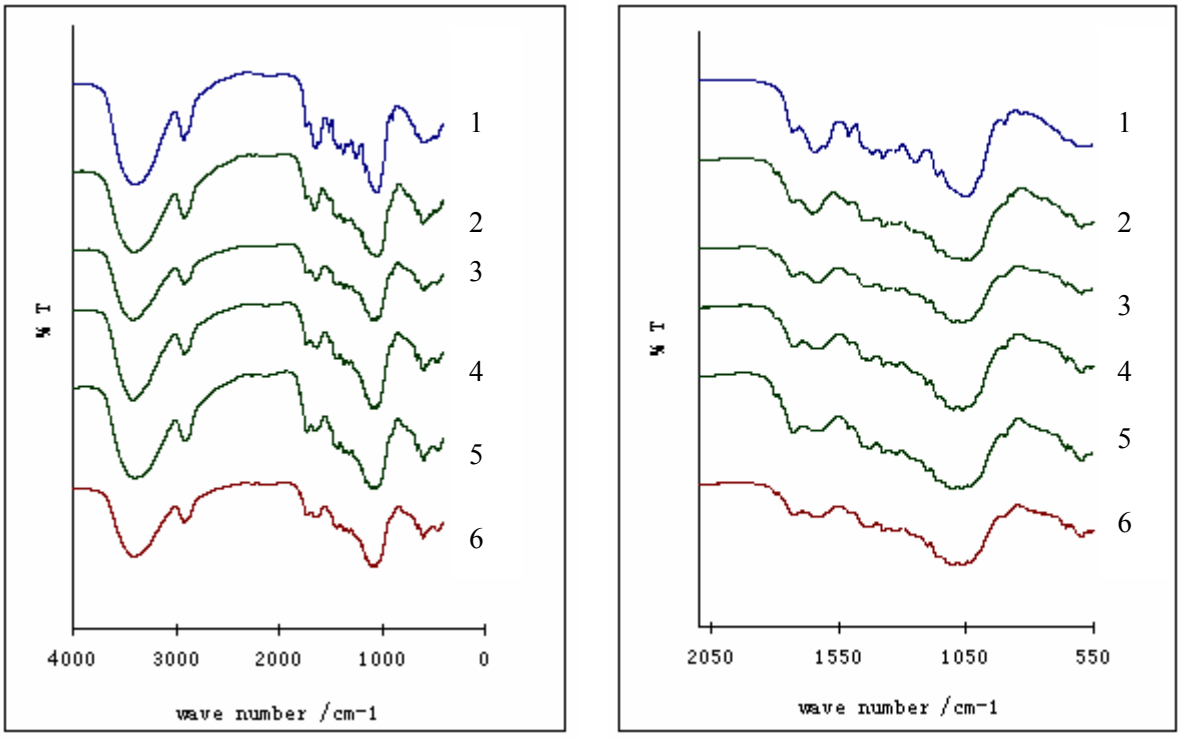

Fig. 4. FTIR spectra of residue liquefied corn stover at different reaction time (1- 0 min, 2- 5 min, 3- 15 min, 4- 30 min, 5- 60 min, 6- 90 min)

The absorption band at 1732 cm^{-1} is attributed to the stretching vibration of C=O in hemicellulose. The absorption bands at 1648 cm^{-1} and 1515 cm^{-1} are attributed to the stretching of the aromatic rings in lignin. The absorption band at 899 cm^{-1} is attributed to the stretching of β glycosidic bond in cellulose. It can be seen from Fig 4 that the absorption intensities of lignin and cellulose decreased substantially, while there was no obvious change for the absorption intensity of hemicellulose. It can be concluded that lignin and cellulose were liquefied prior to hemicellulose and the most significant conversion during liquefaction was observed in lignin. It was reported by Yadama and Ono (1999) that ethylene carbonate would promote the acid-catalyzed solvolysis of cellulose. Ethylene carbonate could be transformed into alcoholic compounds with the release of carbon dioxide during liquefaction. Further analysis, such as with GC/MS, will be helpful to understand the chemical reaction of the liquefaction process.

CONCLUSIONS

It is concluded that crop residues could be successfully liquefied by solvents of ethylene carbonate, ethylene glycol, and polyethylene glycol with sulfuric acid being used as catalyst. The kinetic studies of the liquefaction process demonstrated that the liquefaction process follows a pseudo-first-order reaction. The rate constant can be used to evaluate the liquefaction rate of the process. When corn stover was liquefied with ethylene carbonate, ethylene glycol, and a mixture of polyethylene glycol and glycerol, the highest liquefaction rate was obtained when ethylene carbonate was used as solvent. Among the three types of crop residues studied, the highest liquefaction yield was obtained with corn stover. The liquefaction yields of wheat straw and rice straw were very close. The physical, chemical and mechanical properties of the obtained liquefied crop residues will be further studied in order to be used as substrates for biomaterial production.

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PRODUCTION, CHARACTERIZATION, AND MECHANICAL PROPERTIES OF STARCH MODIFIED BY *OPHIOSTOMA* SPP.

Chun Bei Huang,[a] Robert Jeng,[b] Mohini Sain,[b] Bradley A. Saville[a*] and Martin Hubbes[b]

Microbial modification of starch with *Ophiostoma* spp. was investigated, with the purpose of developing a novel packaging material for the food or pharmaceutical industries. Various starch sources, such as tapioca, potato, corn, rice and amylopectin were tested as raw materials. The initial screening demonstrated that tapioca and potato starch had better performance for biopolymer production. The yield was about 85%. Preliminary characterization of the modified biopolymer was also conducted. Following microbial conversion, the percentage of molecules with molecular weight (abbreviated Mw) more than 10M (abbreviations of million) Daltons increased from 25% to 89% after 3 days, confirming that the modification increased the weight of the starch polymer. Fourier Transform Infrared (FT-IR) revealed changes in the chemical structure of the starch after the modification. Both pure starches and the modified biopolymers were cast into films and tested for mechanical properties. The tensile tests showed that after treatment with the fungus, the peak stress and modulus of the films increased about 10 and 40 times, respectively. Also, the water barrier property was improved. Therefore, microbial modification positively impacted proper-ties relevant to the proposed application. Although the role of the fungus in the modification and the function-property relationship of the biopolymer are not yet completely clear, the results of this study show promise for development of a novel biopolymer that competes with existing packaging materials.

Keywords: Ophiostoma spp.*, Microbial conversion, Polysaccharide, Starch films, Packaging material, GFC, FT-IR*

*Contact information: a: Department of Chemical Engineering, University of Toronto, Toronto, Ontario, M5S 3E5, Canada; b: Faculty of Forestry, University of Toronto, Toronto, Ontario, M5S 3B3, Canada; *Corresponding author:* : saville@chem-eng.utoronto.ca

INTRODUCTION

An increased emphasis on sustainability, eco-efficiency, and green chemistry has driven a search for renewable and environmentally friendly resources. Starch is a biodegradable polysaccharide, produced in abundance at low cost, and exhibits thermoplastic behavior. Therefore, it has become one of the most promising candidates as an alternative material to replace traditional plastics in certain market segments, such as the food packaging industry. Numerous studies have been conducted to optimize the performance of the starch-based plastics (Mali et al. 2004; Soest et al. 1997; Fama et al. 2005; Lawton 1996). Briefly, these studies have shown that important properties for evaluation of a packaging material include mechanical properties, gas and water vapor permeability, thermoforming properties, resistance, transparency, and availability (Weber

et al. 2001). However, the design and engineering of a starch-based packaging product that possesses all of these required properties is a significant challenge. Difficulties were encountered with cost, and technical hurdles such as brittleness associated with high loads, and poor water and gas barrier properties have to be overcome to finally commercialize the biomaterial (Lorcks 1997). Numerous studies have aimed to modify the functional properties of the starch to enhance the inherent bonding strength. Currently, most of the studies are focused on incorporating additives, such as plasticizers, to improve the performance of the material (Poutanen et al. 1996; Laohakunjit et al. 2003).

It has been reported that certain fungi have the ability to produce exopoly-saccharides that have great potential for use in cosmetic and food industries because of their possible bioactive characteristics, rheological behavior, and high stability at high temperature (Selbmann et al. 2003). *Ophiostoma* spp. was isolated from diseased Elm trees. This fungus is unique, as its natural habitat resides in xylem fluid. Preliminary laboratory studies demonstrated that *O.* spp. can produce exo-polysaccharides in the culture medium (Jeng et al. In press). When starch was used as the substrate in *O.* spp. medium, a biopolymer was produced that showed some characteristics well suited for a bio-packaging application. Therefore, a key objective of this study is to confirm that the fungus is capable of utilizing and modifying starch into a biopolymer with superior mechanical properties compared to starch, while identifying the impact of fermentation conditions.

The scope of the present work is to characterize the biopolymer derived from modification of different starch sources (corn, potato, tapioca and rice) by *O.* spp., and ultimately, develop a commercially viable process for large scale production of a biopolymer that can be used as packaging material for food or even medical applications. In these studies, the resulting biopolymers were characterized by gel-filtration chromatography (GFC) and Fourier Transform Infrared (FT-IR) to determine the changes in the biopolymer following fermentation. Films were also cast to test the mechanical properties of the polymer. Laboratory scale production was carried out in a 10 L carboy to investigate the compositional changes of the starch polymer during fermentation. Finally, it has not been previously reported that a fungus could be used to modify starch and improve its attributes as a bioplastic. Therefore, this research represents a paradigm shift in the realm of starch-derived plastics, and will provide valuable information regarding the starch modification process and its impact on the final product.

MATERIALS AND METHODS

Materials

The *Ophiostoma* spp. used in this study was supplied by the Great Lakes Forest Center, Canada Forest Service, Sault Ste. Marie, Ontario. Potato, rice, and tapioca starch were all normal commercial grade starch, purchased from the market. Corn starch was obtained by grinding dry kernels of food grade corn. Amylopectin was reagent grade, purchased from Sigma. Glycerol was reagent grade purchased from Fisher.

Starch Modification

Biopolymer production

25 g of starch was weighed into a 2-liter flask with 1000 ml of distilled water containing 2 g of yeast extract and 10 g of glucose. The medium was then sterilized at 121 ºC for 20 min. 100 ml of fungal spores (300 to 400 mg in dry weight) was inoculated into the starch suspension. The cultures were maintained as a shake culture (150 rpm) at 25 ºC for 4 days. Polymer was obtained by adding an equal volume of 95% ethanol to spore-free culture medium. The precipitated polymer was then washed with ethanol, lyophilized, and weighed. Substrates used in this study included starch from corn, potato, tapioca, rice, and amylopectin. The production rate of biopolymer from each feedstock was calculated.

The same procedure, but without the fungal inoculation, was also conducted using tapioca starch as a control. The precipitate, extracted by ethanol, was collected and lyophilized.

Time course of biopolymer production

Laboratory scale fermentation was also conducted in a 10 liter Nalgene carboy containing 7 liters of starch suspension. To determine the time course of biopolymer production, 1000 ml of spore-free culture filtrate was withdrawn daily from day 1 to day 4. Polymer was recovered by the methods described above, and then characterized.

Characterization of the biopolymer

Gel-filtration chromatography (GFC)

GFC was used to determine the molecular weight of the biopolymer and its molecular weight distribution. A TSK-GEL 4000 PWXL column from Tosoh Science was attached to the GFC system, which was equipped with Shimadzu-6A column oven, Perkin Elmer Series 200 pump, Perkin Elmer Advanced LC sample processor ISS200, and Shodex RI-71 refractive index detector. The Totalchrom software was used.

The HPLC operated at a flow rate of 1.0 ml/min and at ambient temperature. The run time for each sample was 15 minutes. Dextran standards from Sigma with Mw from 1270 to 24M were injected to obtain the standard curve.

Approximately 0.5 g of modified starch sample was put into a small flask and mixed in about 2 ml of water. The flask was shaken using an Orbit shaker, at the speed of 1500rpm for at least 3 hours. The supernatant was withdrawn and centrifuged before analysis through the GFC column.

Fourier transform infrared (FT-IR)

Infrared spectra of the samples were recorded on an ATR-FTIR (Bruker Tensor 27) equipped with an MCT detector. The sample pellets were prepared by mixing the fine sample powder with KBr (Sigma-Aldrich, FT-IR grade). Each spectrum obtained wavenumbers from 400cm-1 to 4000cm-1, representing the average of 64 scans. All optical measurements were performed at room temperature under ambient conditions.

Film Casting

The generic film formulation was constant for all the films produced, which contained 3.9 g of the "polymer" sample (biopolymer, control or pure starch) in water. Around 100 ml of water was added and heated for an hour. The solution was then poured into a 15 cm diameter Petri-dish. The dish was first left at room temperature for 4-5 hours, and then placed into a 35 ºC oven to dry. After 48 hrs, the translucent film was removed from the dish for further tests.

Tensile Test

The films were stored at 35ºC in the oven for at least two weeks before the tensile test. Five "dog bone" shaped specimens (ASTM D638, type I) were cut from each film. Each specimen had a width of 3.00 mm, and the thickness was measured at a minimum of 5 locations with a caliper gauge. The smallest number was recorded as the thickness of the specimen. Most of the specimens had thicknesses between 0.19 mm and 0.26 mm.

Tensile tests were completed using a Sintech Universal Tensile Test Machine Model #1. The gage length was 25.4 mm. The specimen was fixed into the slit and pulled apart by the machine at 2.5 mm/min, until failure occurred or the preset time was reached, usually 5 minutes. Each test was run in duplicate; the total number of impact tests ranged from two to ten for each type of starch/biopolymer, depending on the availability of the samples. The data for peak stress, elongation at break, and modulus of the films, which represent the strength, elasticity and elasticity in tension of the material, were recorded and analyzed. All of the tests were carried out in a climate controlled room at 23˚C and 50% RH.

Water Absorbance Test

The weight of each of the cast film sample was measured. The films were then soaked in water at ambient temperature. Every 60 minutes, the films were taken out, dried with paper towel, and weighed. The process was repeated until the weight of the films stopped increasing. The water absorbance was calculated from the final weight of the water-laden sample relative to the weight of the original film sample. These data are reported as water absorption per gram of the biopolymer film.

RESULTS AND DISCUSSION

Starch Modification/Biopolymer Production

Starch is a complex homo-polymer composed of α-D-glucose units that are linked together in two different forms, the linear form amylose and the highly branched amylopectin (Soest et al. 1997). The composition and structure of starch granules vary considerably between different plants, affecting the properties and functions of starches from different crops (Jobling 2004). Therefore, starches from various botanical sources were adopted as testing materials in the microbial modification, including corn kernels, potato starch, wheat flour, tapioca starch, rice starch, and millet starch. Among them, corn, potato, and tapioca were selected for further study after a pre-screening experiment, because they showed superior overall properties compared to the others. By increasing

the amount of starch in the medium, a polymer yield of more than 85% could be reached. Tapioca starch gave the highest conversion rate, and the polymer yield from tapioca was also generally the highest. (Fig. 1).

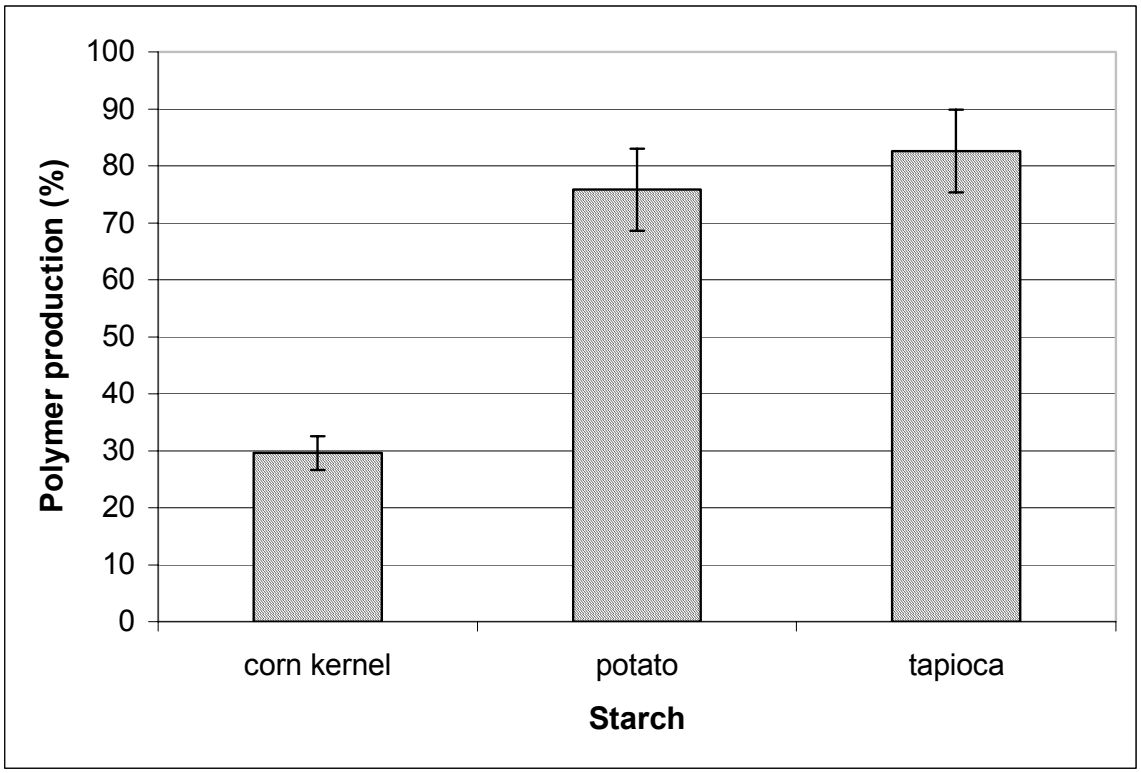

Fig. 1. Biopolymer production with different starches as raw materials after 4 days (values shown as mean +/- SD, N=3)

Characterization of the Biopolymer

Molecular weight and its distribution

Since the primary purpose of starch modification is to produce packaging material, the molecular weight and MW distribution of the biopolymer should be important indicators of its mechanical properties. Studies on the effect of molecular weight on biopolymer film characteristics have reported that the mechanical strength of certain films increased with increasing molecular weight of the biopolymer (Nunthanid et al. 2001). This finding might be attributable to an entanglement network forming during film formation from higher molecular weight material.

In this study, the molecular weight during the modification process, from day 1 to day 4, was analyzed using the GFC column, with the results shown in Fig. 2. The "not identified" portion in the figure is the difference between the total weight of the biopolymer injected into the column and the calculated result from the GFC standard curve. The disagreement is due to the difficulty in obtaining accurate response factors over such a wide range of molecular weights. However, the GFC assay has shown clearly that the majority of the bio-polymer has a molecular weight (abbreviated Mw)

more than 8M. It was also observed that the contribution of the high Mw fraction increased, from 25 wt. % on day 1, to 89 wt. % on day 3, then dropped slightly to 78% on day 4. This profile established that the modification by the fungus increased the size of the biopolymer. Based on the observations of J. Nunthanid et al. (2001), it could be speculated that the bio-polymer has a better inherent strength than that of pure starch, although the exact mechanism of the modification still remains to be determined.

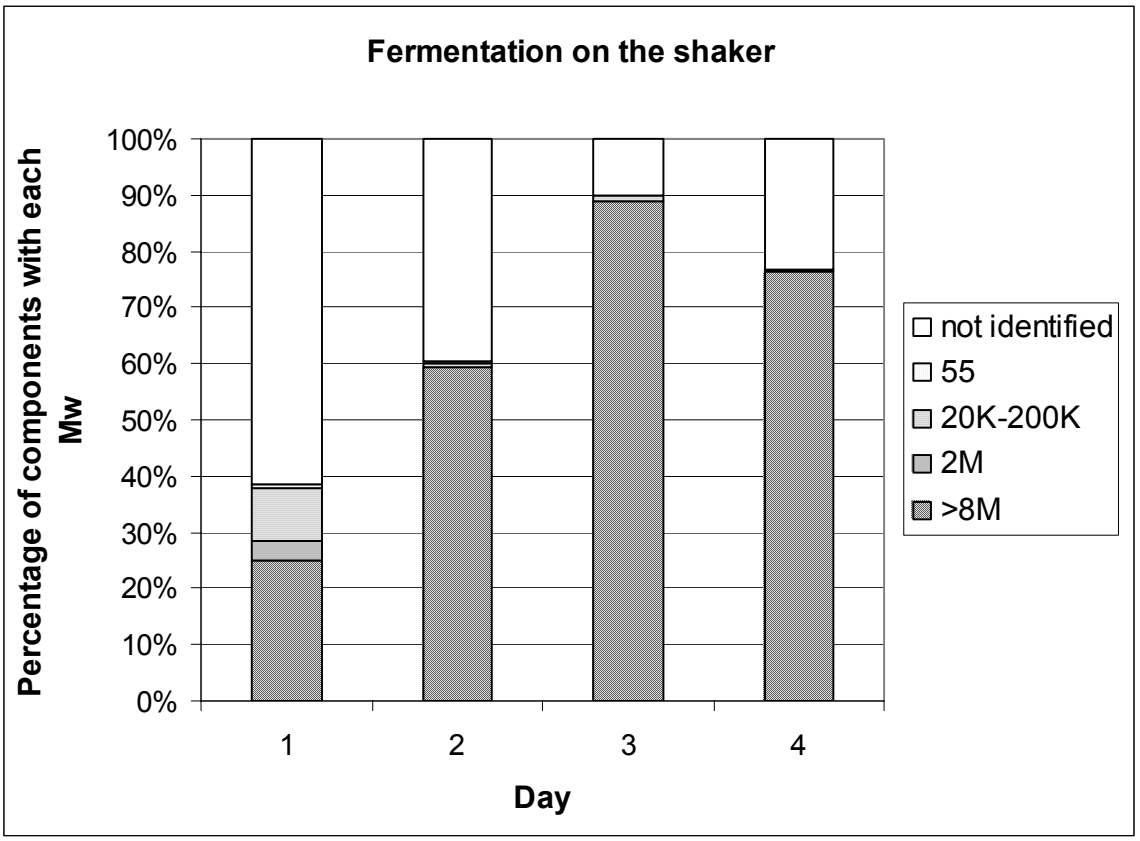

Fig. 2. Molecular weight analyses during the fermentation process with tapioca starch

The biopolymer produced by fungal fermentation was further characterized by acid digestion, followed with a Liquid Chromatography assay. Glucose was identified as the major degradation component. Very likely, there are one or more other components that contribute to the properties of the modification product.

The GFC profile of the pure starch is not measurable because of its extremely low solubility. But after modification, the biopolymer possesses greater water solubility than the original starch substrate, even though the solubility of the biopolymer varies, depending on the different starch sources.

FT-IR

The molecular level changes during the modification process were studied by FT-IR, as shown in Fig. 3. The spectra a and b represent the native potato starch and the biopolymer harvested from the fungal modification of starch, respectively.

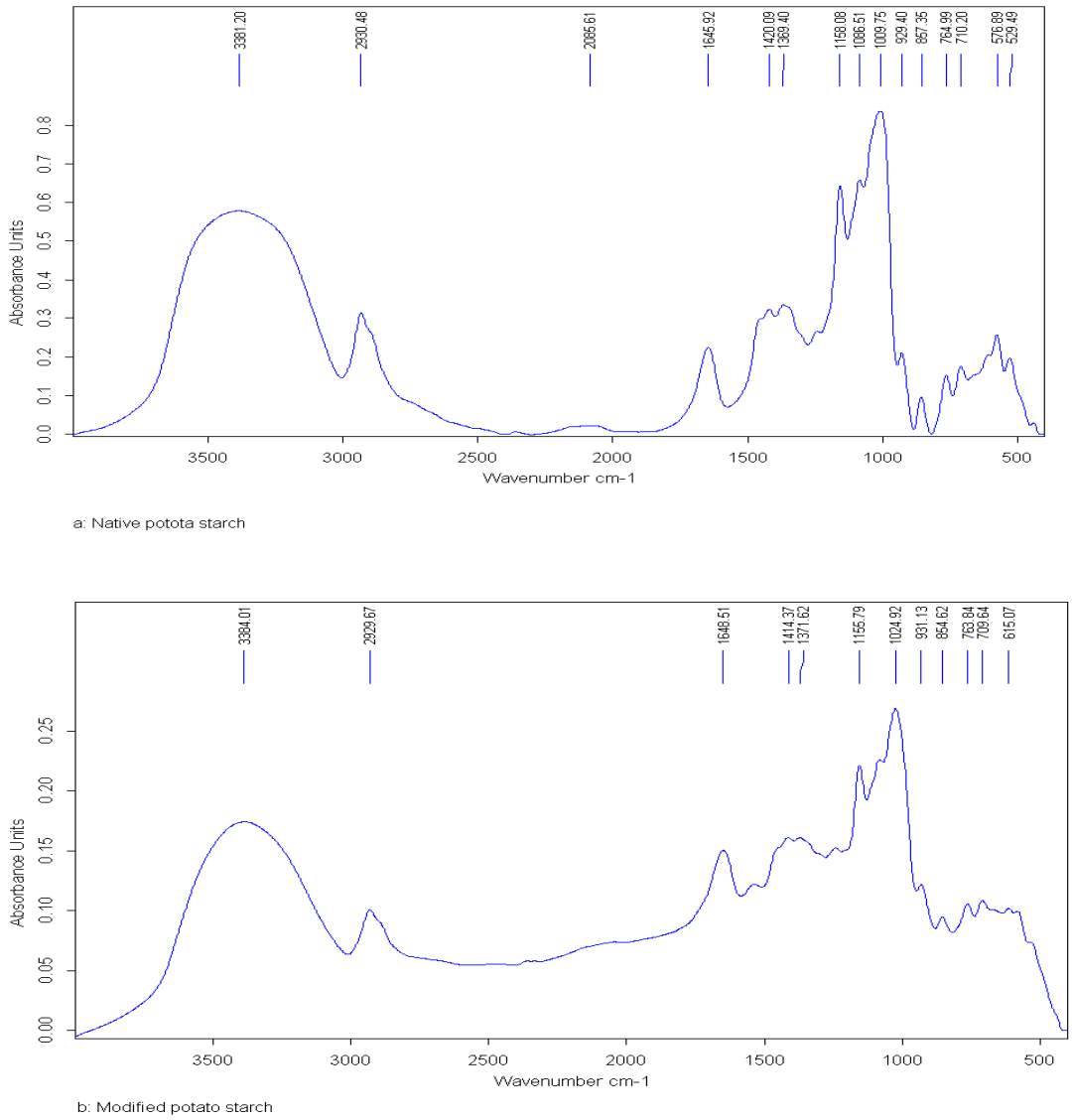

Figure 3. FT-IR spectra of starch and modified starch

Both spectra in the region below 800 cm^{-1} exhibited complex vibrational modes due to the skeletal mode vibrations of the pyranose ring in the glucose unit (Kizil et al. 2002). In the starch fingerprint region of 800 cm^{-1} to 1500 cm^{-1}, spectral characteristics of the starch and modified starch were similar. However, distinctive changes could also be found. Peaks at around 1080 cm^{-1} to 1158 cm^{-1} were characteristic of a C-O-H bond. Compared the peaks in native starch with wavenumber 1158 and 1081cm^{-1}, they were consistently observed shift to 1156 cm^{-1} and 1080 cm^{-1} after the modification. Also, the peaks between 990 and 1030 cm^{-1}, which represent the anhydroglucose ring O-C stretch (Fanga et al. 2002), were different after fungal modification. This phenomenon may be

related to the stability and intensity of hydrogen bonds. Pawlak et al. (2003) showed that the stability and strength of hydrogen bonds were indicative of correlative peak shifts and the peak style changes. Moreover, significant ratio changes were observed in the infrared absorption band around 1080 cm^{-1}, 930 cm^{-1}, and 861 cm^{-1}, consistent with changes in the glycosidic linkages in starches (Kizil et al. 2002). A characteristic peak occurred at 1644 cm^{-1} in native starch, which is presumably a feature of tightly bound water (Fanga et al. 2004). However, in the biopolymer, the peak shifted to 1652 cm^{-1} and also increased significantly in intensity. Irudayaraj et al. (2002) showed that the absorbances at around 3389 and 2930 cm^{-1} can be assigned to O–H and C–H bond stretching, respectively. The intensity and shape of the 2930 cm^{-1} peak in modified starch was substantially different than that of native starch. All the above observations support the conclusion that fungal fermentation has changed the structure and bonding of the starch substrates.

Mechanical Properties of the Bio-polymer Films

Both pure starch and the microbially modified biopolymer were cast into films and subjected to tensile testing. The experimental results confirmed the above prediction, i.e., that the modified starch has better strength properties, and is well suited for use as a packaging material (Fig. 4 and Table 1). The difference in the number of samples is mainly due to the availability of the sample material—some films, especially rice and amylopectin film cracked after drying, and thus, very few complete specimens could be cut from the films. The problem with film cracking was one of the reasons why films from these materials were not selected for further study. Also, if the films did not fail at the end of the test, no data could be recorded for "elongation at break". This was observed twice for potato starch, 4 times for tapioca starch, twice for rice polymer, and once for amylopectin polymer.

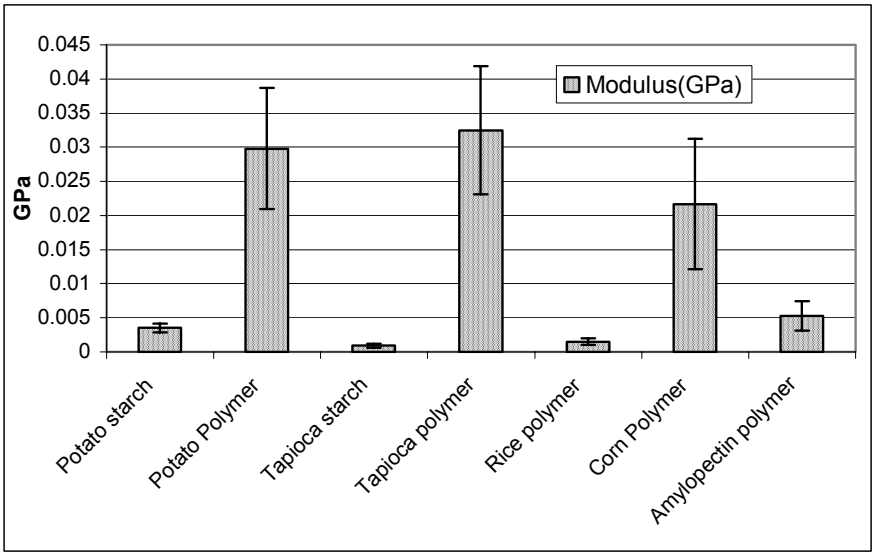

Fig. 4. Tensile modulus of the starch and the modified biopolymer films (values shown as mean +/- SD, N=5, 4, 6, 6, 10, 4, 3, respectively)

Table 1. Tensile Tests of Various Starches and the Fungal Modified Biopolymer Films

	Material		Mean	95% confidence limits of the mean		N (number of measurements)
Peak Stress (MPa)	Potato	Starch	1.60	1.18	2.01	5
		Polymer	3.58	3.22	3.92	7
	Tapioca	Starch	0.37	-0.01	0.75	6
		Polymer	3.60	3.30	3.89	10
	Rice	Polymer	0.43	-0.04	0.89	4
	Corn	Polymer	2.52	2.14	2.90	6
	Amylopectin	Polymer	0.97	0.44	1.51	3
Elongation at break (mm)	Potato	Starch	40.78	37.05	44.50	3
		Polymer	10.78	8.34	12.31	7
	Tapioca	Starch	48.33	43.76	52.89	2
		Polymer	10.77	8.73	12.81	10
	Rice	Polymer	34.79	30.22	39.35	2
	Corn	Polymer	13.36	10.73	16.00	6
	Amylopectin	Polymer	21.72	17.16	26.28	2

Statistical analysis was conducted using SAS software to generate 95% confidence limits for the mean values of peak stress and elongation at break for different materials before/after the modification. The results showed that the polymers produced by fungal modification of tapioca and potato starch possessed superior mechanical strength compared to rice and amylopectin. When compared to their native starch counterparts, a great improvement in the peak stress was obtained at a 99.9% confidence level for both potato and tapioca, with a reduction in elongation at the breakpoint. For instance, fungal modification of the tapioca starch increased its peak strength from 0.37 MPa to 3.50 MPa, demonstrating a huge impact of the microbial treatment. But at the same time, the elongation at break decreased from 48 mm to 12 mm. Therefore, after fungal modification, the polymer increased in strength at the expense of ductility. Aging of the films was also an important factor influencing polymer properties; this accounts for some of the relatively large error bars in the results, which simply aggregate data from films of similar composition, without accounting for their age.

After modification, biopolymers derived from potato and tapioca starches exhibited a much lower water absorption, which indicates higher moisture resistance, a

favorable property for packaging material applications. After soaking film samples in water, all the unmodified starch films broke into pieces within 30 minutes, and continued to absorb water. However, all films made from the modified biopolymer remained intact, even after 24 hours. Furthermore, their water uptake capacities reached a maximum in an hour, and then exhibited a plateau thereafter. The results from the film water absorption tests are shown in Fig. 5.

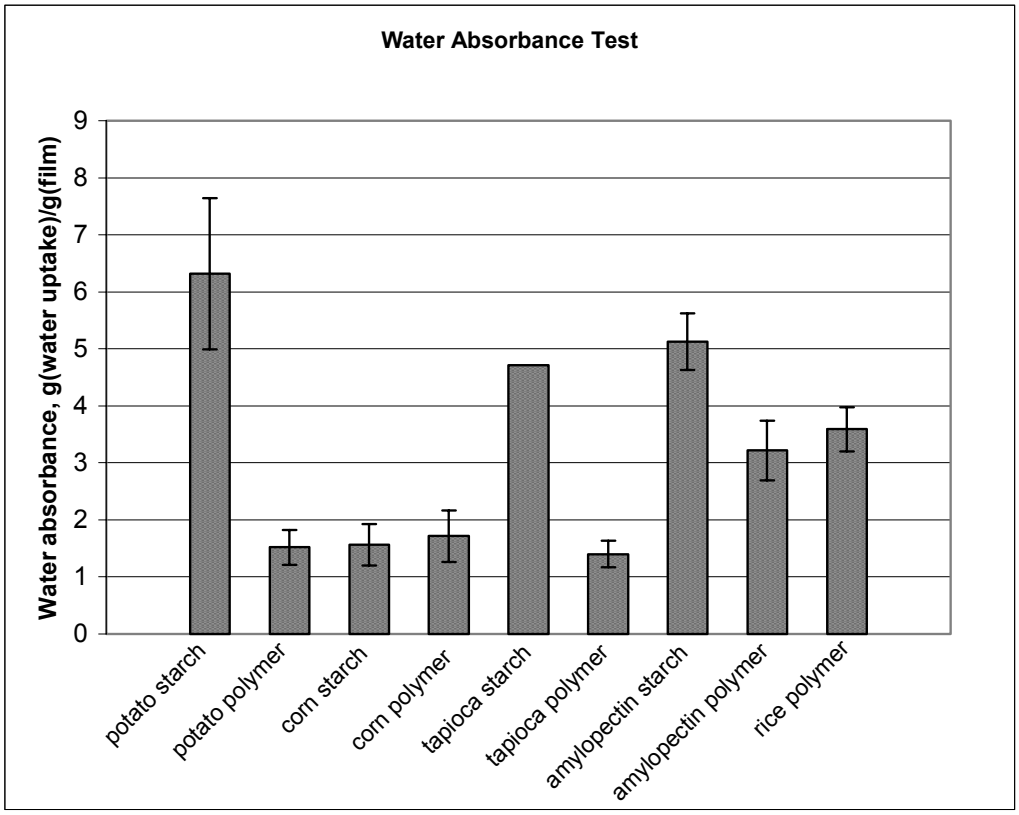

Fig. 5. Water absorbance tests for the starch and modified biopolymer films (values shown as mean +/- SD, N=1~3)

Tensile tests were also conducted with the potato starch from the carboy fermentation, shown in Table 2 and Fig. 6. Statistical analysis of the results showed that the biopolymer quality from the third and fourth day was superior to that obtained from the earlier days of large scale fermentation. On the third day, the properties of the films were very close to those of the potato biopolymer produced in a 2L lab-scale process, suggesting that the process can be readily scaled up, and supporting the concept of future mass production. The modified biopolymer has greater water solubility than the original starch, but the solubility varies, depending on the different starch sources.

Table 2. Tensile Tests with the Time Course Fermentation of Potato Starch

	Day	Mean	95% confidence limits of the mean		N (number of measurements)
Peak Stress (MPa)	1	0.58	0.38	0.77	5
	2	1.30	1.10	1.49	5
	3	2.36	2.17	2.55	5
	4	2.24	2.04	2.43	5
Elongation at break (mm)	1	8.75	7.74	9.75	4
	2	9.82	8.41	11.23	2
	3	6.95	6.06	7.85	5
	4	6.29	5.40	7.19	5

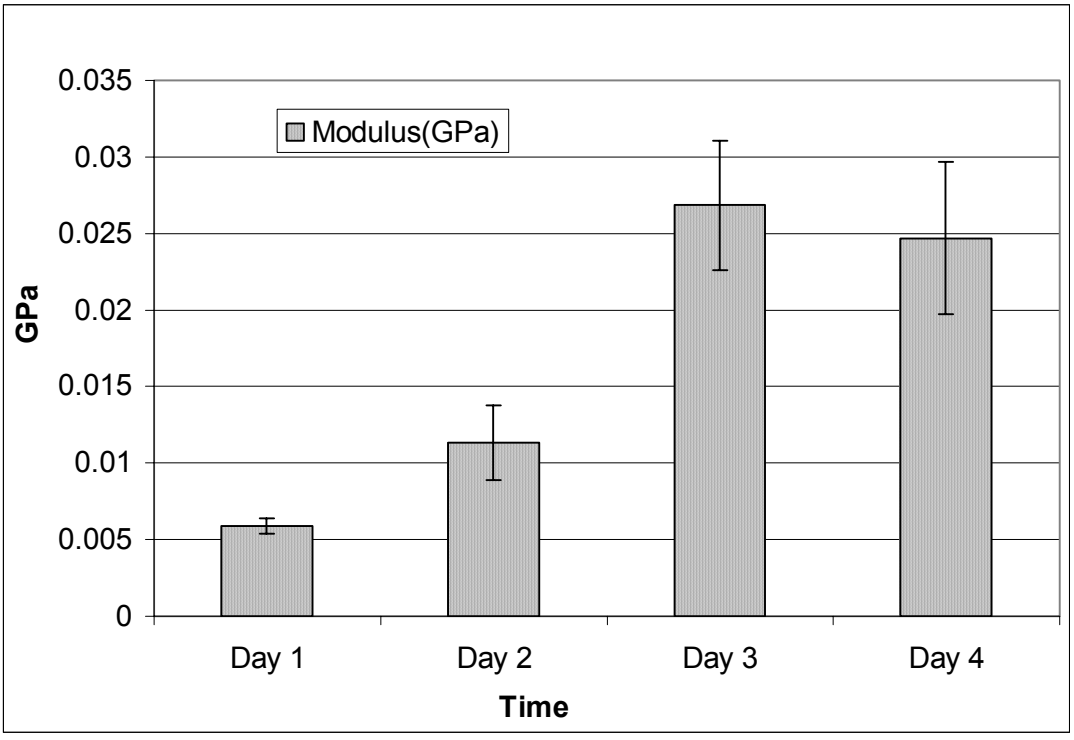

Fig. 6. Tensile modulus tests with the time course fermentation of potato starch (values shown as mean +/- SD, N = 5)

The overall results proved that over 72 hours, the fungus *O.* spp. is able to modify starch into a biopolymer with a higher molecular weight and greater mechanical strength. Changes in chemical bonds were also observed through FT-IR analysis. Observations of stronger hydrogen bonding indicated the enhancement of inter-molecular strength.

CONCLUSION

The overall results proved that *O.* spp. was able to successfully modify starch into a biopolymer with improved mechanical properties in about 72 hours. Results from GFC demonstrated a substantial increase in MW during the modification process. The increasing molecular weight also contributed to the improved mechanical properties of the starch films. Changes in the starch structure were studied through FT-IR. The pyranose ring was maintained after the modification, but the hydrogen bonds between molecules intensified. Peak shifts and ratio changes suggested the fixation of new chemical functional groups or new linkages between starch molecules. Based on previous (Jeng et al. In press) and current studies of the fungus, two possible pathways of the modification could therefore be proposed. One pathway involves the fungus producing a polymer that can bond starch molecules together and form new crosslinked structures. The second possible pathway involves the fungus attaching one or more functional groups that help to strengthen the starch polymer.

Work is under way to increase the understanding of the fungal modification process–a key step prior to commercial development. As more is learned about the structure-function relationships of the biopolymer, opportunities for commercial application will surely increase.

ACKNOWLEDGEMENTS

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CELLULOSE FOR MEDICAL APPLICATIONS: PAST, PRESENT, AND FUTURE

Nicholas Hoenich [a]

Films and tubes manufactured from cellulose have historically been used in the treatment of renal failure, but their use for this purpose has declined in recent years in favour of films manufactured from synthetic material blends. As the clinical application of cellulose for dialysis declines, new applications for its use are emerging, of which the most promising appears to be the use of microbial cellulose synthesized by Acetobacter xylinum as a novel wound healing system and as a scaffold for tissue regeneration.

Keywords: Cellulose, Modified cellulose, Cellulose acetate, Films, Tubes, Dialysis, Haemodyalysis, Tissue scaffolds, Wound care, Microcapsules, Sutures, Implants

Contact information: a: School of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH; nicholas.hoenich@ncl.ac.uk

HISTORICAL OVERVIEW

Cellulose is a naturally occurring material found in wood, cotton, hemp, and other plant-based materials, and consists of repeating anhydroglucose units joined by ß-(1,4) linkages, forming the basic repeating unit. It was first isolated from wood in 1885 by Charles F. Cross and Edward Bevan at the Jodrell Laboratory of the Royal Botanic Gardens, Kew, London. The process for manufacturing cellulose film from viscose was discovered by three English chemists, Charles Frederick Cross, Edward John Bevan and Clayton Beadle in 1898. However it was not until 1913 that Dr Jacques Brandenberger developed thin transparent cellulose film into commercial production at the 'La Cellophane SA factory in Bezons, France.

Although the primary use of cellulose films has been for wrapping purposes, it has also found an application in the treatment of renal failure, as well as in a variety of more recent and evolving clinical applications such as for scaffolds in tissue engineering, temporary skin substitute, a haemostatic agent, post operative adhesion barrier, and as a culture material for hepatocytes.

In this paper, the use of cellulose in medical applications are reviewed and discussed.

Cellulose for Use in the Treatment of Renal Failure

When the human kidney fails, either as a consequence of disease (chronic renal disease) or following traumatic injury, or the ingestion of poisons, the blood-borne metabolites of protein break down, water that would normally be handled by the kidney accumulates, and supportive therapy is required to sustain life. The most widely used treatment is haemodialysis, with over 1.1 million persons receiving such treatment

worldwide in 2002 (Lysaght 2002). Haemodialysis refers to the dialysis of blood, during which the patient's blood is passed through an artificial kidney or haemodialyser, containing a membrane. Blood flows on one side of the membrane, whilst the other side is bathed by an electrolyte or salt solution (dialysis fluid) continually produced by a proportionating system (the artificial kidney machine), which also monitors and controls treatment. Molecules small enough to pass through the membrane, such as salts and low molecular protein breakdown products, tend to move in the direction of decreasing concentration. Larger substances, such as proteins and cells having dimensions greater than the pore diameter, are retained. Haemodialysis is generally performed three times weekly for patients with irreversible renal or kidney failure, whilst the treatment regimens for acute or reversible renal failure are governed by clinical requirements and may include intermittent or continuous treatment over a period of several days.

Although diffusion is the primary driving force within the dialyser for solute transport, other mechanisms, such as convection, a consequence of fluid flux across the membrane, and adsorption to the membrane, also make small contributions, and both have been developed to provide therapies for specific groups of patients (Rabindrahath et al. 2006).

The term dialysis was coined by Thomas Graham, Professor of Chemistry at Anderson's University in Glasgow. In 1861 his experiments demonstrated that crystalloids were able to diffuse through vegetable parchment coated with albumin (which acted as a semi-permeable membrane; Gottschalk et al. 1997). Although Graham predicted that his findings might be applicable to medicine, he did not venture into this area. The first historical description of dialysis was published in 1913 when Abel, Rowntree and Turner "dialyzed" anesthetized animals by directing their blood outside the body and through tubes of semi-permeable membranes (Abel et al. 1990). These early membranes were made from a mixture of cotton and sulphuric and nitric acids, which were dissolved in alcohol. The tubes, which were connected at either end to a glass manifold to receive and return the blood, were approximately 8 cm in length and were encased in a glass cylinder through which the dialysis fluid passed. The tubes were in many respects similar to the hollow fibre designs in use today. Such membranes were difficult to produce and sterilise, and were subject to leakage and lacked consistency in pore size.

In 1925 Erwin O. Freund in Chicago discovered that he could make a sausage casing based on cellulose by the use of the viscose process. The cellulose was dissolved in alkali, producing a viscose solution, which when extruded into an acid bath converts the viscose into cellulose. This material became known as Visking tubing (Visking Corporation Chicago, Ill, USA). In the same year in Europe, Kalle Ag (Wisebaden, Germany) began the production of Cellophane, and this material was used in the early treatments of acute or reversible kidney failure by Kolff in the Netherlands in the 1940s (Jacobze et al. 2005). For such treatments a long cellophane tube spirally wrapped around a cylinder and rotated in a stationary dialysis fluid bath was used. The method was subsequently developed by Kolff and Berk, and became the first apparatus in widespread clinical use (Kolff et al. 1997).

The widespread application of haemodialysis for the treatment of renal failure became a possibility with the availability of robust methods to gain access to the patients' circulation (Konner 2005). In parallel with this, several new designs of artificial kidney,

including the coil, parallel flow and the hollow fibre dialysers became available. Early variants of such dialysers used membranes based on cellulose. Worldwide, the largest producer of membranes suitable for use in the coil and parallel plate designs was J. P. Bemberg AG (Wuppertal, Germany), who produced the membranes by the solubilisation of cellulose in an ammonia solution of cupric oxide (cuprammonium process). The original variant of the hollow fibre dialyser utilized cellulose acetate as the membrane, which was produced by the Dow Chemical company and originally used for desalination applications (Gotch et al. 1969). European production of hollow fibres based on cellulose began some years later in 1974.

Clinical use of the coil dialysers has declined, but parallel plate devices such as those shown in **Fig. 1** continue to be used. The majority of patients today receive their treatment using a hollow fibre dialyser (**Fig. 2**). In such devices, which are available in a range of surface areas to meet differing clinical requirements, the hollow fibres have internal diameters within the range 180-220 μm and wall thicknesses between 6 and 15 μm.

Fig. 1. Plate type dialyser utilizing sheet membranes supported on extruded polypropylene plates [Illustration courtesy of Gambro Ab, Lund, Sweden]

CONTEMPORARY ISSUES

Concerns began in the 1970s regarding the narrow range of molecules that could be removed by cellulose membranes. These concerns led to the development of membranes with an open-pored structure and new treatment techniques such as haemodiafiltration, a process in which whole blood is first diluted with physiologic electrolyte solution and then ultrafiltered across a membrane (Henderson et al. 1970). Since there were no cellulose-based membranes commercially available at that time that could meet the requirements of haemodiafiltration or haemofiltration, the procedure utilized membranes manufactured from synthetic polymer blends, such as the polysulfone membrane developed by the Amicon Corporation. These membranes were highly

permeable, yielding high filtration rates at low transmembrane pressures. Furthermore, they could be fabricated to provide good size selectivity, with a molecular cutoff of ca. 50,000 Da. Subsequent modifications of the manufacturing process of cellulose-based membranes have meant that today cellulose membranes are available for use in these therapies.

Fig. 2. Contemporary hollow fibre dialysers are available in a variety of sizes to meet differing clinical treatment requirements [Illustration courtesy of Fresenius Medical care, Bad Homburg, Germany.

In contrast to cellulose-based membranes, membranes manufactured from synthetic copolymers were associated with a reduced lecopaenia, or a transient loss of leucocytes from the circulation (Woffindin and Hoenich 1988). Subsequent research indicated that the leucopaenia was a consequence of pulmonary sequestration of the cells mediated by the contact of blood with the membrane surface (Craddock et al. 1977). It is now well accepted that C3a, C5a, and the terminal complement complex (TCC) stimulate the expression of receptors on leucocytes, leading to aggregation and sequestration in the pulmonary microvasculature (Dhondt et al. 1998). The desire to minimize these responses resulted in the modification of the classical cellobiose unit of the cellulose strands in which the OH groups are either replaced or the blood contacting surface is coated by materials such as poly-ethylene glycol (PEG) to minimize blood exposure to the hydroxyl groups on the surface (see **Fig. 3 and Table 1**).

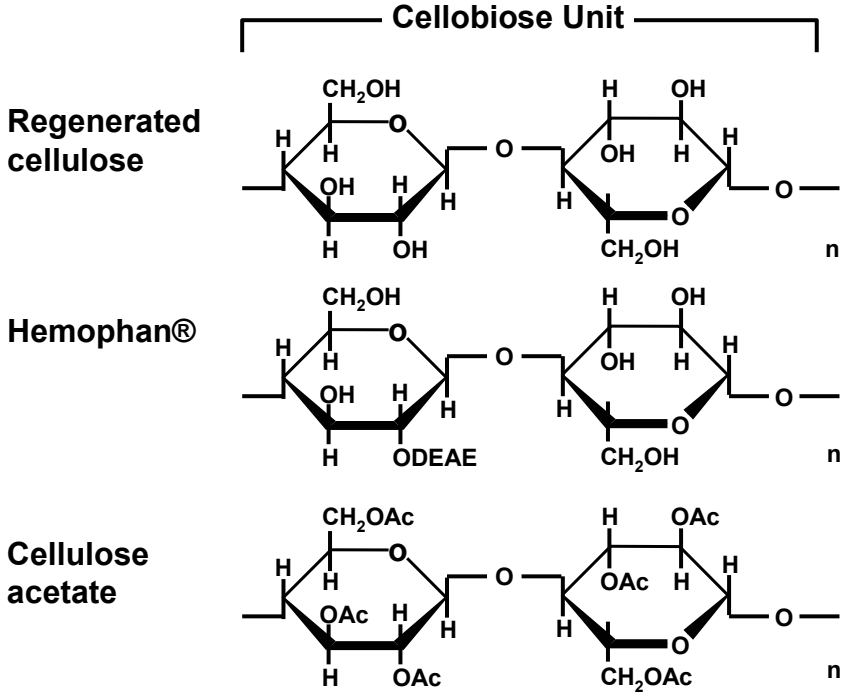

Fig. 3. The chemical structure of cellulose membranes showing partial modification of the OH groups. [DEAE = diethylaminoethyl; Ac = acetyl]

Table 1. Cellulose based Haemodialysis Membranes

Cuprammonium rayon	Regenerated cellulose (unmodified)
Cuprophan™	Regenerated cellulose (unmodified)
Hemophan™	Cellulose etherified with dimethylaminoethyl DEAE
Excerbane™	Regenerated cellulose coated with Vitamin E
PEG modified cellulose	Cuprammonium rayon coated with poly ethylene glycol (PEG)
SMC™	Synthetically modified cellulose in which there is benzyl group substitution of the hydroxyl groups
Cellulose acetate	Regenerated cellulose esterified with acetate
Cellulose tri-acetate	Regenerated cellulose esterified with 3.0 acetate
Cellulose 2.5 acetate	Regenerated cellulose esterified with 2.5 acetate

Membranes were historically considered as inert phase separators; however, repeated blood contact takes place with the dialysis membrane, which typically has an area of 1–1.5 m^2. This contact results not only in the activation of the body's humoral and cellular processes, but may also play a role in the outcomes associated with dialysis treatment. It is now recognised that patients receiving dialysis are subject to oxidative stress associated with the dialysis treatment, which contributes to the pathogenesis of

vascular injury and in the progression of atherosclerosis (Himmelfarb 2005). To minimize the activation of polymorphonuclear leukocytes on the surface of dialysis membranes, a vitamin E–coated cellulose membrane has recently been produced, which reduces oxidative stress induced by the treatment (Galli et al. 1998).

In the artificial kidney, the membrane acts as a semi-permeable barrier, separating the sterile blood side from the non-sterile dialysis fluid. Within the dialysis fluid, potential exists for bacterial contamination and the formation of biofilm in the dialysis machine or in the distribution systems delivering water to the dialysis machines (Tapia and Yee 2006). Bacteria, due to their size, cannot cross the membrane, but bacterial fragments or endotoxins are able to do so, and this has raised concerns that membranes with a large pore size may confer a risk to the patient of exposure to cytokine-inducing bacterial substances in the dialysate, contributing to the microinflammatory state of patients undergoing regular dialysis (Yao et al. 2004).

Membranes manufactured from cellulose are a homogenous gel, but membranes manufactured from polymer blends have a more complex structure. Several studies have reported higher transfer of cytokine-inducing bacterial substances through low-flux cellulosic compared to high-flux synthetic membranes (Weber et al. 2004a). This surprising paradox is explained by adsorption of cytokine-inducing bacterial substances to synthetic membranes (Hayamaa et al. 2003).

Other Medical Applications of Cellulose

Wound care

If a wound is to heal effectively, it must be maintained in a wet condition. The best dressing is the patient's own skin, which is permeable to vapour and protects the deeper layer tissue against mechanical injuries and infection. For many years biological dressings of pig skin or human cadaver skin have been used, but such products are expensive and may only be used for a short period.

Microbial cellulose synthesized by Acetobacter xylinum shows considerable potential as a novel wound healing system, resulting from its unique nanostructure. During the process of biosynthesis, various carbon compounds of the nutrition medium are utilized by the bacteria, then polymerized into single, linear β-1,4-glucan chains and finally secreted outside the cells through a linear row of pores located on their outer membrane. The subsequent assembly of the β-1,4-glucan chains outside of the cell is a precise, hierarchical process. Initially, they form sub-fibrils (consisting of 10–15 nascent β-1,4-glucan chains), then later micro-fibrils, and finally bundles of micro-fibrils consisting of a loosely wound ribbon. The thick, gelatinous membrane formed is characterized by a 3-D structure consisting of an ultrafine network of cellulose nanofibres (3–8nm) which are highly uniaxially oriented have a high cellulose crystallinity (60–80%) and mechanical strength.

The first efforts to commercialize microbial cellulose on a large scale were initiated by Johnson & Johnson in the early 1980s. The patents arising from this work were subsequently licensed to Xylos, a company responsible for the manufacture of The XCell® family of wound-care products.

A number of other commercial and academic institutions are developing this material. A Brazilian company, BioFill Produtos Bioetecnologicos (Curitiba, PR Brazil), produces a range of products based on this material, including Biofill® and Bioprocess® (used in the therapy of burns, ulcers as temporary artificial skin), and Gengiflex® (applied in treatment of periodontal diseases; Fontana et al. 1990).

Several Japanese companies and national governmental organizations have collaborated to set up interdisciplinary research programs, whilst the Technical University of Lodz in Poland has undertaken basic research studies on cellulose biosynthesis (Czaja et al. 2006).

A number of questions concerning this material remain unresolved. First, there is no clear indication as to its mechanisms of action, which is most likely to be a result of its unique nanostructure, creating optimal moist conditions for wound healing and skin regeneration. Production factors influencing its mode of action remain undefined, and the availability of large-scale, efficient fermentation for production is lacking.

Encapsulation

Activated charcoal is effective for the direct removal of various circulating toxic materials and waste metabolites from the blood (haemoperfusion). It may be used in the treatment of poisonings, as well as a bridge to liver transplantation, and occasionally in acute liver failure until the liver regenerates (in conjunction with other treatment modalities). The use of uncoated charcoal, however, is associated with adverse effects, such as hypotension and the appearance of platelet aggregates in the circulation, as well as particulate release. Coating of the material results in an improved biocompatibility, and a variety of coatings, including cellulose nitrate have been used (Chang 1984).

Materials other than charcoal, as well as cells may be encapsulated for a variety of clinical applications. Cellulose acetate butyrate microcapsules, as well as cellulose-based microspheres, have been used for the delivery of drugs (Lin and Wu 1999; Weber et al. 2004b; Fundueanu et al. 2005; Zhou et al. 2005). More recently, this approach has been extended to bovine spermatozoa (Weber et al. 2006).

Acute renal failure frequently occurs following sepsis. The removal of endotoxins by extracorporeal adsorption processes is a promising approach to the treatment of Gram-negative sepsis and endotoxin shock. Microspheres manufactured by coating a cellulose matrix with polyethyleneimine have demonstrated high adsorptive capacity for endotoxins (Weber et al. 1995; Fang et al. 2004; Taniguchi et al. 2006).

Cellulose as an implant material

Sutures are the largest group of devices implanted into humans. Silk is a traditional fiber used as sutures in medical applications. Although biodegradable, its major disadvantage is its lower tensile strength and reaction with tissues. The problem of strength retention in the case of bio-absorbable commercial sutures is being tackled by different surface modification techniques. Polypropylene and polyester filament coated with Teflon, for example, are employed for vascular surgery. Sutures by the modification of cellulose filaments also have been produced. Carbon fiber based sutures also have been produced by the modification of cellulose filaments (Narat et al. 1950).

Bacterial cellulose derived from Acetobacter xylinum has an ultrafine network architecture, high hydrophilicity, and mouldability during formation. In addition to the applications discussed, it is also suitable for use in micronerve surgery and as an artificial blood vessel suitable for microsurgery. Such products in the form of BActerisal Synthesized Cellulose (BASYC®) have been described in the literature (Klemm et al. 2001).

Haemorrhage continues to be a serious complication of surgical procedures. Surgicel® (oxidized regenerated cellulose) is used widely to control haemorrhage, as this material, when saturated with blood, swells rapidly (Sharma et al. 2003).

One of the goals of tissue engineering is to manufacture scaffolds, suitable for transplantation, which are infiltrated with cells. Both viscose cellulose sponges and non-woven cellulose have been used experimentally to fabricate cartilage transplants (Pulkkinen et al. 2006; Muller et al. 2006). Cellulose derived from Acetobacter xylinum, as discussed above in the context of wound healing, has also been explored as a potential scaffold material, due to its unusual material properties and degradability (Svensson et al. 2005).

THE FUTURE

The most widely used application of cellulose in medicine has been as a membrane in the treatment of renal failure. Worldwide trends in the use of membranes for the treatment of chronic renal failure indicate a move away from cellulose-based membranes in favour of synthetic membranes (Grassmann et al. 2005).

There are many reasons for this trend. But an important question is whether patients treated with cellulose membranes are at a disadvantage, compared to patients receiving treatment using synthetic membranes, in terms of outcomes and wellbeing. Historic studies suggested that this may have been the case, but a recent Cochrane review found no evidence of benefit when synthetic membranes were compared with cellulose membranes or modified cellulose membranes in terms of reduced mortality or the reduction in dialysis-related adverse symptoms (Macleod et al. 2005). Despite this review, the European Best practice guidelines suggest that dialyser membranes with the lowest degree of complement and leukocyte activation should be applied. Dialyser membranes that induce strong complement and leukocyte activation, inflammatory reactions, and/or a blunting of the response of leukocytes to stimuli should be avoided (Anon. 2002). This set of practices certainly precludes the continuing use of unmodified cellulose membranes. Discontinuation of the use of *modified* cellulose membranes in favour of synthetic membranes is, however, less compelling, since such membranes overlap in terms of their biocompatibility with those manufactured from synthetic materials (Hoenich et al. 1995). Furthermore, there may be other factors, such as cost, favouring the continuing use of such membranes in emerging economies.

As the clinical application of cellulose in the form of membranes for dialysis declines, new applications are emerging. Of these, the most promising appears to be the use of microbial cellulose synthesized by Acetobacter xylinum as a novel wound healing system.

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BONDING BETWEEN CELLULOSIC FIBERS IN THE ABSENCE AND PRESENCE OF DRY-STRENGTH AGENTS – A REVIEW

Martin A. Hubbe

Various hydrophilic polyelectrolytes, including cationic starch products, are used by papermakers to promote inter-fiber bonding and increase paper's dry-strength. Thus, papermakers can meet customer requirements with a lower net cost of materials, more recycled fibers, or higher mineral content. In the absence of polymeric additives, key mechanisms governing bond development between cellulosic fibers include capillary action, three-dimensional mixing of macromolecules on facing surfaces, conformability of the materials, and hydrogen bonding. Dry-strength additives need to adsorb efficiently onto fibers, have a hydrophilic character, and have a sufficiently high molecular mass. Though it is possible to achieve significant strength gains by optimal usage of individual polyelectrolytes, greater strength gains can be achieved by sequential addition of oppositely charged polyelectrolytes. Superior strength can be achieved by *in-situ* formation of polyelectrolyte complexes, followed by deposition of those complexes onto fiber surfaces. Polyampholytes also hold promise as efficient dry-strength additives. Opportunities for further increases in performance of dry-strength agents may involve fiber surface modification, self-assembled layers, and optimization of the dry film characteristics of dry-strength polymers or systems of polymers.

Keywords: Dry strength, Adhesion, Bonding, Polyelectrolytes, Polyampholytes, Cationic starch, Cellulosic fibers, Paper, Polyelectrolyte complexes, Surface modification

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

INTRODUCTION

Glue is not required in order to make a sheet of paper. This is one of the lessons that schoolchildren learn when they are fortunate enough to take part in a hands-on demonstration of papermaking. Usually the first step involves tearing sheets of paper into scraps and adding them to water in a blender. Subsequently, the suspension of fibers is formed into a new sheet of paper, pressed, and then dried by evaporation. The young papermaker leaves with a newly recycled sheet, taking advantage of a unique ability of cellulosic fibers to adhere to each other when they are dried while in contact.

This review considers a variety of chemical strategies that can be used in an effort to make a good thing better, by strengthening the inter-fiber bonds. The great majority of such strength-enhancing treatments are fully compatible with the recyclable nature of papermaking fibers (Zhang et al. 2002). The diversity of bonding agents, as well as the circumstances under which they are found to be effective, provide a viewpoint from which to better understand the surfaces of the fibers themselves. The goal of this review

is to highlight strength-enhancement strategies that tend to increase the value of paper products, while at the same time help to minimize the environmental impact of papermaking operations.

Why Enhance Bonding?

Expressed in terms of function, the three main categories of paper or paperboard product can be classified as "packaging," "printing and writing," and "absorbing and wiping." Though paper within each of these categories can benefit from enhanced inter-fiber bonding, the manner in which such bonding benefits the product can differ.

Packaging paper and paperboard grades require sufficient strength to contain and protect various contents. For example, a corrugated box must have sufficient edge crush resistance (Seth et al. 1979; Fellers 1983) to support high loads when the boxes are stacked. Theoretical studies suggest that edgewise compressive failure of corrugated containers often involves delamination at points of high shear stress within the linerboard (Sachs and Kuster 1980; Fellers 1983). Thus, it is not surprising that edgewise compression strength can be improved by the use of dry-strength agents (Smith 1992). Though inter-fiber bonding also can be increased by refining (Seth et al. 1979; Paulapuro and Thorp 1983), the resulting internal delamination of individual fibers makes them less able individually to bear compressive loads. Refining also tends to decrease the rate at which water can be removed from the wet web of paper, limiting the rate of production. Thus, a promising approach is to add a polyelectrolyte, such as cationic starch, to enhance bonding (Moeller 1966; Harvey et al. 1973; Greif and Gaspar 1980; Hofreiter 1981; Howard and Jowsey 1989; Ghosh 1994; Alince et al. 1990; Formento 1994). Compressive strength also can be increased by papermaking technologies involving high wet-press pressures or dwell times (Smith 1992; Worsick 1994; Slater 2003), or drying the sheet continuously while it is under constraint (Kunnas et al. 1993; Retulainen 2003).

There are several ways in which manufacturers of containerboard products can convert strength enhancement into cost savings. One way is by reducing the weight of the product. Changes in the ways that linerboard is specified for shipping containers have allowed papers to reduce the weight of their product, as long as it still meets strength requirements (Gutmann et al. 1993). In other cases, papermakers can use lower quality fibers, having a lower price. Chemical additives can play a role in keeping the strength high enough to meet product requirements (Reynolds and Wasser 1980; Smith 1992). In addition, there is always a trade-off between what one pays as energy input for refining *vs.* the expense of dry-strength additives. Increases in one of these expenses tend to be offset the by reductions in the other expense.

To cite another example, consumers often judge the quality of milk cartons by their resistance to bulging. A package that appears to be bloated makes the consumer suspicious that the contents of the package may have spoiled. If, indeed, there is pressure build-up due to fermentation within the container, then the problem is beyond salvaging by means of paperboard strength. In other cases a suitably high stiffness will ensure that a carton doesn't bulge excessively. Because stiffness, according to the simplest theory (Kajanto 1998), is proportional to the cube of thickness of a material, the papermakers have an incentive to maintain a low density of the paperboard material. Excessive refining should be avoided, minimizing the tendency for fibers to flatten into ribbons and

conform to each other in bonded areas. Again, dry-strength chemicals can help to compensate for the reduced inter-fiber bonding, when refining is reduced (Chan 1976).

Printing and writing papers can have different requirements, depending on the converting and printing operations for which they are designed. Offset lithography, one of the most important printing processes, places high demands on paper's surface strength and resistance to delamination. High levels of ink tack are required to achieve high fidelity of multi-color imaging. Though it would be technically possible to obtain the needed internal bonding strength by refining alone, while still maintaining the needed opacity, the most economical results usually can be achieved by a combination of refining and chemical additives (Tanaka et al. 2001). Use of cationic starch, for instance, can compensate for the lower bonding ability associated with recycled kraft fibers (Strazdins 1984; Hipple 1991; Nazhad and Pazner 1994; Zhang et al. 2002). Dry-strength additives also help to compensate for the debonding effect of mineral fillers, which are added in order to achieve brightness and opacity specifications (Lindström and Florén 1984; Alince et al. 1990). Strength gains can be achieved with little sacrifice of opacity, especially if acrylamide-type strength resins are used (Farley 1987). In addition to chemicals added to the fiber furnish, papermakers usually add relatively large amounts of starch solution to the paper surface, in a so-called size-press operation (Ecklund 1989).

Xerographic copy papers, though similar to uncoated offset papers in many ways, present some different requirements. To pass successfully through copiers at high speed, with a minimum of jamming, copy paper needs to be relatively stiff, with a low tendency to curl. Again, due to the dependency of stiffness on the third power of the paper's thickness (or caliper), papermakers avoid measures that would excessively densify the paper. Large amounts of cationic starch, often in the range of 0.5 to 1.5% of the total product mass are usually added to the papermaking furnish in order to meet various strength specifications (Harvey et al. 1973; Formento et al. 1994).

Absorbent paper products, such as tissue and towel, represent the opposite of typical packaging products in terms of density. In order to maximize the amount of fluid that can be held in the product during its use, there is a critical need to maintain relatively large air-filled spaces among the fibers in the structure. At the same time, the paper needs to be strong enough to run efficiently through various manufacturing and converting processes. In many cases the papermaker's preferred option is to minimize the amount of refining, maintaining the initial relatively stiff, tube-like nature of the fibers. When using recycled fibers obtained from production with bleached kraft pulp, the history of drying tends to stiffen the fibers, making them less conformable (Scallan and Tigerström 1992; Nazhad and Pazner 1994; Dulemba et al. 1999; Hubbe et al. 2003b). The bulky nature of the resulting paper tends to be desirable, from the standpoint of tissue production. On the other hand, many recycled kraft fibers already have been subjected to extensive refining, tending to produce a relatively dense sheet of paper. In an attempt to thread the needle among various competing goals, papermakers often use combinations of seemingly incompatible additives. Dry-strength agents can be used to meet the strength requirements of the product. Meanwhile, debonding agents can be used to maintain the desired bulky nature of the paper (Conte and Bender 1992; Poffenberger et al. 2000). The mechanisms by which these two contrasting types of additives interact are not well known, and this is likely to be an interesting area of research. It is possible

that debonding agents remain localized in patches on fiber surfaces, creating areas in which the fibers remain unbonded.

Trends in both packaging and printing grades point in the direction of increasing demands for more effective dry-strength treatments. Viewed over a span of years, almost every grade of paper or paperboard, for a given application, tends to decrease in basis weight. Lower weight yields savings not only with respect to the amount of materials needed for a given area of paper or paperboard product, but also due to lower costs of shipping and mailing. At the same time, filler levels tend to rise year by year. Filler particles interrupt inter-fiber bonding, and their surfaces are not conformable. Factors tending to favor increasing filler levels include their low cost, relative to fibers, and their contribution to opacity and brightness. In addition, there has been a trend towards greater use of fibers that are known to have less capability of inter-fiber bonding, *e.g.* high-yield mechanical pulps, and recycled office waste, and containerboard fibers. The trends just mentioned suggest that the main dry-strength strategies used by today's papermakers are likely to be sufficient to meet tomorrow's dry-strength goals. On the one hand, papermakers will continue to rely on refining and cationic starch to enhance dry strength. In addition, one can anticipate a need for innovative dry-strength additives, as well as a need to better understand how their effects can be optimized.

Each of the trends mentioned above has the potential to reduce the amount of newly pulped cellulosic fibers required to meet market demands for paper products. As will become clear in later sections of this report, commonly used dry-strength additives for addition to the papermaking furnish are generally non-toxic, biodegradable, and efficiently retained in paper during the manufacturing process. To set the context for discussing the effects of dry-strength additives, the following section describes some basic issues related to the development of adhesion between untreated cellulosic fibers that are dried in contact with one another.

BONDING BETWEEN UNTREATED CELLULOSIC FIBERS

Got Contact?

No contact, no bonding. If one assumes that hydrogen bonding between fibers plays a critical role in paper strength (Campbell 1959; Davison 1980; Fowkes 1983; Nissan et al. 1985; Tiberg et al. 2001), then it is necessary to assume that the at least parts of the facing surfaces can approach each other within about 0.27 nm (Linhart 2005). Distances associated with London dispersion forces are more forgiving (Hiemenz and Rajagopalan 1997; Lindström et al. 2005), but still, in order to explain development of significant inter-fiber bonding, one must assume contact within molecular dimensions.

Cellulosic fiber surfaces are rough on a scale of 0.01 to 10 μm. Studies of the refined kraft fibers indicate the presence of a wide range of fibrils and microfibrils extending outwards from the fiber surfaces (Clark 1985a,b; Neuman 1993; Pelton 1993, 2004). Dimensions can range from about 2-5 nm in the case of primary microfibrils (Fujita and Harada 2000) to much larger fibrils resulting from partial delamination of the outer layers of the cell wall. Roughness can severely reduce molecular contact between adjacent surfaces, depending on the scale and the material properties (Thomas 1999).

Figure 1 shows two ways to represent the effect of roughness between cellulosic fibers in the wet state. Part A of the figure might be considered as a conventional view, in which roughness involves a series of indentations and asperities relative to a planar surface. The illustration in part B of the figure assumes that fibrils and microfibrils, which are directed outwards from the fiber surfaces in the wet state, play a major role with respect to inter-fiber contacts between wet fibers.

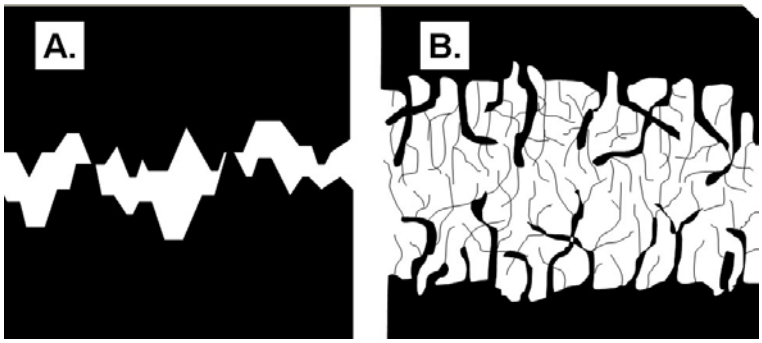

Fig. 1. Contrasting schematic concepts of contact between real solid surfaces. A: Conventional view. B: Fibrillated surfaces in the wet condition.

In light of fibers' roughness, one is forced to conclude either of two things with respect to the development of inter-fiber bonding. On the one hand, one might conclude that actual molecular contact between fibers within paper is highly inefficient on a molecular level, but somehow it is still sufficient to meet the needs of papermakers. Based on this view, it might be expected that there are tremendous opportunities to improve paper strength by suitable treatment or processing. On the other hand, one might assume that there is a mechanism by which fiber surfaces efficiently become drawn into molecular contact during the ordinary processes of papermaking. Based on this second view, it would make sense to explore ways in which dry-strength additives can further improve the efficiency of the process of bond formation as water is evaporated from paper. Two principles mechanisms appear to be mainly responsible for this "drawing together," and they will be discussed in the following two subsections.

Capillary Forces

Sandcastles provide a mundane example of capillary forces at work. Those who build sandcastles learn almost immediately that three phases – solid, liquid, and gas – all need to be present in order to build a successful castle. Attempts to build with dry sand are abandoned almost immediately in favor of damp sand, as a young builder settles into her or his activity. Attempts to build a castle under water can be even more futile, since the sand grains lack adhesion, and it is more difficult to view one's handiwork. By contrast, damp sand can be fashioned into a fantasy word. Such a castle can stand on its own, at least until the last films of water between the grains of sand are able to evaporate.

In addition to explaining the strength of sand castles, capillary forces also can account for the ability of wet paper to withstand significant tensile loads even before it has been dried. As noted by Lyne and Gallay (1954), the capillary forces within a damp sheet of paper tend to pull adjacent fibers towards one another. Depending on the

coefficient of friction between those fibers, in the wet state, the forces normal to the plane of the sheet becomes translated into considerable strength within the plane of the sheet. Such a mechanism has been demonstrated in cases of paper made from glass fibers. Sheets formed from untreated glass fibers have almost no strength after drying (Lyne and Gallay 1954; Hubbe 2005), due to the inefficient contact between the stiff, rounded surfaces. Maximum tensile strength of the damp glass sheets was observed at a solids content of 20-25% (Lyne and Gallay 1954).

Campbell (1947, 1959) was the first to employ similar reasoning to explain the development of paper strength during drying. An estimate of the magnitudes of capillary forces acting within a damp paper sheets, as it is being dried, can be obtained by imagining two smooth ribbon-like fibers of the same size that cross each other at a right angle. Now assume that there is a droplet of water positioned at the point of close contact, and that the water perfectly wets the fiber surfaces. In other words, one assumes a contact angle of zero degrees. In that case, Campbell (1959) showed that the negative pressure within the meniscus is given approximately by the following expression,

$$\Delta P \approx \gamma / r \approx 2\,\gamma / x\,, \qquad (1)$$

where γ is the water-vapor interfacial tension, r is the smaller radius of curvature at the edge of the film of water, and x is the distance of separation between the solids. This situation is illustrated in Fig. 2. Lyne and Gallay showed that capillary forces, including the type represented by Eq. (1), can result in a several-fold reduction of thickness of wet paper as water is removed from it. To paraphrase Ratliff (1949), one assumes that any parts of the adjacent fibers that are within the distances associated with optical contact will be drawn into molecular content during drying, as a result of the capillary forces.

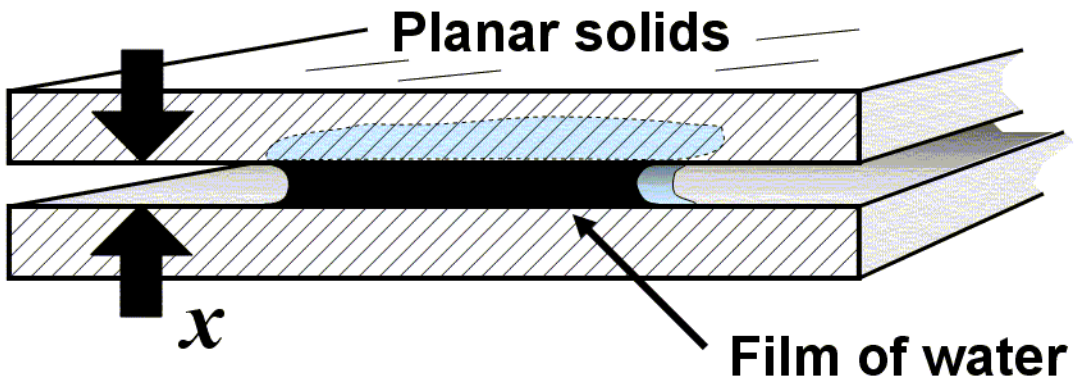

Fig. 2. Illustration of film of water between two flat, planar, perfectly-wetting surfaces, resulting in an attractive force proportional to the wetted area and inversely proportional to the separation distance

If one attempts, uncritically, to apply Eq. (1) to various situations, one quickly discovers that the negative pressure drawing two surfaces together is predicted to become infinite at zero separation. Since such a result is absurd, it is clear that the assumptions under which the equation was derived must cease to be accurate long before the last of

the water has evaporated. Nevertheless, it has been predicted that negative pressures within capillaries can reach about 2 metric tons per cm^2 during the drying of paper (Campbell 1959; Clark 1985a). Though these values are a factor of ten or more times lower than the pressures exerted within the nip of a wet press on a paper machine (Back 1987; Mather et al. 1987), the latter forces act only during the brief instant while the wet web of paper is passing through a nip. The capillary forces, on the other hand, can continue to act over longer periods of time. On this basis it is sometimes concluded that the capillary forces overcome the effects of roughness and force the fibers into molecular contact (Campbell 1959).

Some of the strongest evidence in support of the "drawing into molecular contact" theory just described consists of the dramatic decrease in fiber surface area when paper is dried (Davison 1980). The changes in kraft fiber properties associated with drying are generally attributed to partially irreversible closure of nanopores within the cell walls (Stone and Scallan 1966; Hubbe et al. 2003b), making the fibers less conformable and less able to swell with water (Nazhad and Pazner 1994). Capillary forces appear to be at least partly responsible for such effects.

Generally, it is not possible to discern spaces between fibrils at the surfaces of dried fibers, even by use of the most sensitive electron microscopic methods (Nanko et al. 1989; Nanko and Ohsawa 1989; Duchesne et al. 2003). Mayhood et al. (1962) observed nearly a constant strength of per unit area of apparent bonding between different kinds of papermaking fibers; on this basis they assumed that capillary forces are generally strong enough to overcome surface roughness and bring facing surfaces of fibers into molecular contact, regardless of the conditions of pulping and refining. Further evidence that molecular contact is achieved comes from the appearance of fiber surfaces after tensile failure. Areas that formerly were in optical contact generally show damage, such as raised and broken fibrils (Page 1960; Sachs and Kuster 1980; Clark 1985a; Nanko et al. 1989; Stratton and Colson 1993). Such damage was found to increase when a strength agent was used during formation of the paper (Stratton and Colson 1993). In many cases bonding between fibers is sufficiently strong, during the drying of paper, that lateral shrinkage of one fiber can cause lengthwise crimping of an adjacent fiber, resulting in an overall shrinkage of paper during unrestrained drying (Page and Tydeman 1962).

Though there would seem little reason to doubt the capillary mechanism of dry-strength development, as just described, some of the experimental evidence in its favor is difficult to interpret. For instance, it has been shown that the presence of surface-active materials often results in weaker paper (Brandal and Lindheim 1966). In principle, surface-active materials are expected to decrease the value of γ in Eq. (1), thus decreasing the capillary forces that draw surfaces together as paper is dried. However, there is a second explanation, which appears equally able to explain such results. That is, surface-active materials at fiber surfaces tend to block potential sites for hydrogen bonding (Brandal and Lindheim 1966). The latter mechanism is better able to account for the fact that cationic surface-active agents are much more harmful to paper's dry-strength, compared to anionic or nonionic surfactants, which have less tendency to adsorb onto anionic cellulosic surfaces (Touchette and Jenness 1960). Freeze-drying of paper, so that capillary forces are minimized, results in paper with no strength (Lyne and Gallay 1954); however such results also can be attributed to the non-conformability of frozen fibers.

Mutual Diffusion of Macromolecules at Facing Surfaces

A second explanation to account for the development of molecular contact between the facing surfaces of two fibers is based on the thermodynamics of mixing (Robinson 1980; McKenzie 1984; Pelton 1993). Water is envisioned as a solvent for segments of macromolecules such as hemicellulose and microfibrils of cellulose, *etc.*, that protrude from each fiber's surface (Clark 1985b). The idea is that the molecular segments already tend to mix with each other in the wet state, before the start of the drying process. A random process of molecular motions would be expected to result in interpenetration and tangling, *i.e.* a three-dimensional zone of contact (McKenzie 1984; Pelton 1993). As water is removed from such a swollen polymeric mass, the two surfaces become welded together. Consistent with this mechanism, it is generally found that inter-fiber bond strength increases as fibers become more highly swollen with water (Thode and Ingmanson 1959), either as a result of refining or chemical treatment.

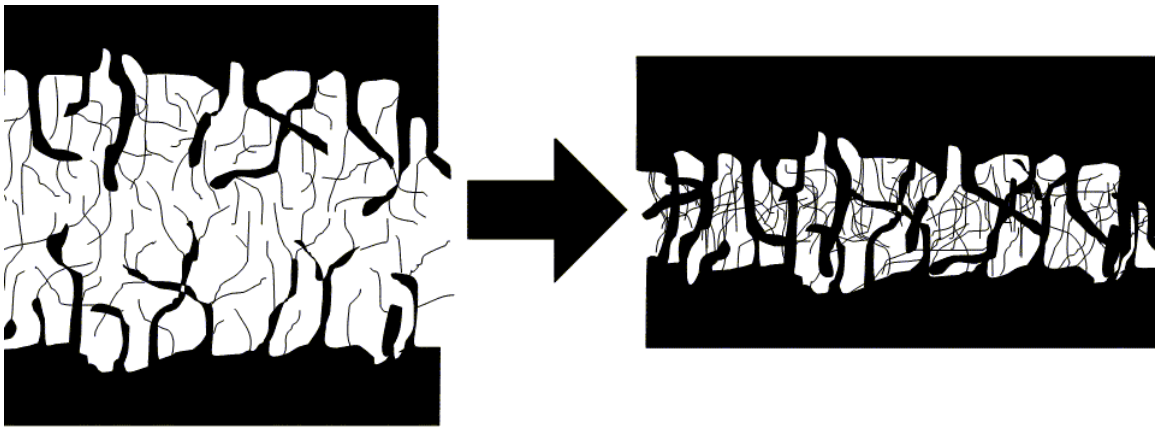

Fig. 3. Conceptual illustration suggesting that diffusional mixing of macromolecular segments extending from two wetted surfaces is likely to condense into a welded, 3-dimensional joint when the structure is dried

Cogent evidence supporting the inter-diffusion theory of bonding has been based on the principle that chemically different polymers do not tend to mix with one another, even if both are fully soluble in a surrounding liquid phase (Paul and Barlow 1982; Pelton et al. 2000). Such mixtures spontaneously remain as separate phases, each phase rich in one of the components. Pelton *et al.* (2000) tested such a mechanism by treating certain fibers with a water-loving cationic polyelectrolyte and other fibers with a polyelectrolyte having the same density of cationic charge, but a proportion of hydrophobic groups, such that the two polymer solutions did not mix. Highest strength was observed when fibers having the same surface treatment were dried. Lowest strength was obtained when incompatible fibers were blended together at an intermediate ratio. Earlier it had been shown that cellulose acetate and cellulose ether fibers, which are partly soluble in acetone, can be formed into very strong sheets, using acetone as a suspending medium (Bletzinger 1943). Ordinary cellulosic fibers, which are completely insoluble in acetone, did not develop any bonding when the paper was formed from acetone.

A different view of how cellulosic microfibrils behave during the drying of paper has been described by Nanko and Ohsawa (1989). Rather than observing a random

process of macromolecular arrangement, as might be assumed based on the diffusional theory of bonding, these authors observed a lining up of microfibrils, flat against the adjacent fiber surfaces. Many microfibrils appeared to arrange themselves as bridges between the adjacent surfaces. Thus it appears that the inherent nature of cellulosic materials, tending to form fibrillar structures, creates a bias towards regularity of structures within bonded regions between fibrillated surfaces.

Conformability

Unless the surfaces of the fibers are conformable under the conditions present during drying, there is little hope of forming a strong sheet of paper (Clark 1973). This fact can be amply demonstrated by forming paper from glass microfibers (Lyne and Gallay 1954; Hubbe 2005). Such fibers resemble cellulosic fibers with respect to being fully wettable by water and forming a relatively uniform suspension in water, as long as the solids content is kept quite low. Though it is possible to form nice-looking paper test sheets from such fibers suspensions, in the absence of polymeric treatment the resulting dry-strength is close to zero. Blowing on the glass "paper" causes the fibers to come apart and rise up into the air (Hubbe 2005).

It is well known that cellulosic fibers become more conformable when they experience multiple compressions and shearing action, as they pass through a refiner in the presence of water (Baker 1985; Scallan and Tigerström 1992; Paavilainen 1993). There are two lines of thought as to how this happens. On the one hand, the repeated "impacts" of pinch points within a refiner are known to delaminate the fibers, both internally and on the surface. One can use the analogy of a rope, in which the filaments can slide past one another, to explain greater flexibility following delamination; by contrast, a board, in which the filaments are fused together, is much more rigid. The other main explanation for increased flexibility is that refining increases the swelling of fibers (Scallan and Tigerström 1992; Dulemba et al. 1999). In other words they hold more water. In certain cases it is possible to find strong correlations between fibers' swelling ability, as indicated by water retention during centrifugation, and the strength of the resulting paper (Ingmanson and Thode 1959). In other cases the correlation between strength and water retention can be weak (Jayme and Büttel 1968; Fält and Wågberg 2003), showing that swelling is not the only important factor.

The conformability of cellulosic fibers also can be changed by chemical modification. By oxidizing or derivatizing the fibers, creating a higher proportion of carboxylate groups at pH values greater than about 4, one can increase the degree of swelling (Roberts 1992). Fibers modified in this way have been found to yield stronger paper (Walecka 1956; Minor et al. 1991; Fors 2000). This mechanism helps to explain why paper formed under alkaline conditions tends to have a strength advantage relative to sheets formed from acidic media (Lindström and Kolman 1982).

It is not certain whether the strength benefits due to increased acidity of the fiber are a mainly a bulk phenomenon or a surface phenomenon. On the one hand, an overall higher negative charge of the fibers would be expected to increase their interaction with water (Grignon and Scallan 1980; Fors 2000) and also make fibrils extend outwards from the fiber surface (Clark 1985a; Pelton et al. 2000). On the other hand, derivatization reactions can be directed towards the outer surfaces of fibers, either by use of non-

swelling solvents (Ehrnrooth et al. 1977) of by drying the fibers to close up pores and make the cell walls inaccessible to the reagent (Barzyk et al. 1997). By making the fiber surfaces more bondable, while leaving the interior of fibers in their original stiff, it is possible to achieve bulky, yet strong sheets of paper (Barzyk et al. 1997; Fors 2000).

Taking an opposite approach, acetylation at moderate to high levels tends to decrease the conformability of cellulosic fibers, when they are wetted by aqueous solution (McKenzie 1987). Thus, paper made from acetylated fibers tends to be bulkier and the strength can be reduced substantially (Nissan and Sternstein 1964). It would be easy to jump to the conclusion that even small degrees of acetylation would decrease the water-holding ability of cellulose or hemicellulose macromolecules at the fiber surface, thus interfering with the diffusional bonding mechanism described earlier. Tests of paper formed from slightly acetylated fibers showed, however, that the strength per unit of bonded area either remained constant or increased (Swanson 1956; McKenzie 1987). This effect can be attributed to the fact that low degrees of derivatization tend to disrupt the regularity of the macromolecules so that there tends to be less local crystallization. Greater mobility of the macromolecules at the fiber surfaces appears to enhance bonding.

There is a reasonable doubt that the preceding discussion of conformability makes sense in the case of mechanical pulp fibers, which are generally stiffer than kraft fibers. However, mechanical pulps tend to have relatively high levels of fiber fines. Moss and Retulainen (1997) showed that fines play a key role in the establishment of inter-fiber bonds in paper formed from thermomechanical pulp (TMP). Paper formed in the presence of fines was denser than paper formed from fractionated pulp with the fines removed (see also Seth 2003). Micrographs appeared to show tethers of fibrils from a layer of fines connected to each of two facing fibers. Such tethers tended to contract upon drying, drawing the fibers in the sheet closer to each other. It appeared that the presence of fines in the junctions between fibers helped to stabilize bonds, keeping the bonded areas from jumping apart after the paper became dry. Nanko et al. (1989) reported related observations of colloidal material efficiently filling up the space within bonded regions between fibers in dried paper. More recently, Lindström et al. (2006) proposed that dry-strength agents may help stabilize aqueous menisci as paper is dried.

Hydrogen and Other Non-Covalent Bonds

Due to the hydrophilic nature of fiber surfaces, it is to be expected that hydrogen bonds, in addition to London-van der Waals (dispersion) attractions, will hold the fibers together after paper is dried (Pierce 1930; Campbell 1947, 1959; Page 1969; Davison 1980; Fowkes 1983). The strong dependence of paper strength on relative humidity (Page 1969), as well as the ease with which paper can be recycled by addition to water, testify to hydrogen bonds' central importance. To keep things in perspective, however, there are many other kinds of materials that are quite strong even though they are unable to form hydrogen bonds. Polypropylene is a good example. The macromolecular chains within a polypropylene bottle are held together mainly by dispersion forces.

In the case of cellulose, individual macromolecules appear to have both internally and externally directed hydrogen bonds (Mann and Marrinan 1958; Gardner and Blackwell 1974; Kadla and Gilbert 2000). The latter, in principle, can contribute to bonding of hydroxyl groups on one macromolecular segment with adjacent chains in a

fiber or with other materials at the fiber surface. The formation of hydrogen bonds between cellulosic surfaces appears to be involved in the partial irreversibility of pore closure in the cell walls when kraft fibers are dried (Stone and Scallan 1966). Corte and Schaschek (1955) estimated that only about 0.5 to 2% of OH groups present at the fiber surfaces are involved in inter-fiber bonding. This observation is worth keeping in mind for later in this article, when different papermaking additives are considered. One might expect, correctly, that additives capable of forming hydrogen bonds would tend to be the most successful dry-strength agents. As described by Robinson (1980), the presence of highly flexibility, water-loving macromolecules, such as hemicellulose, makes it possible for a higher proportion of hydroxyl groups at the fiber surfaces in the zone of contact to take part in adhesion.

Interfiber Bonds as the Weak Link

Paper's ability to resist tensile failure can be modeled by considering the strengths of individual fibers and the bonds between those fibers (Helle 1965; Page 1969). For kraft fibers, reasonable success has been achieved by applying a concept of relative bonded area, *RBA*, a quantity that is based on light scattering experiments (Parsons 1942; Ingmanson and Thode 1959; Page 1969). To carry out this analysis, one assumes that the scattering of light, as it passes through paper, is directly proportional to the fiber surface area in contact with air. Bonded areas do not contribute to light scattering, since the light can pass from one fiber to the next with no change in refractive index.

To determine the value of *RBA* in a given case, the light scattering coefficient of a paper sample of interest is compared relative to a sheet of similar composition that is formed from a non-swelling solvent, such as butanol (Parsons 1942). Because the cellulosic fibers cannot conform to each other, when paper is prepared using the non-swelling solvent, the resulting sheets are extremely weak. To a reasonably good approximation, one can assume that each fiber's surface is surrounded by air, giving close to the maximum number of changes in refractive index as light passes through the non-bonded "paper." The relative bonded area is then defined as follows,

$$RBA = [s_{\text{non-bonded}} - s_{\text{test sample}}] / s_{\text{non-bonded}} \quad , \qquad (2)$$

where $s_{\text{non-bonded}}$ is the scattering coefficient of the paper sheet formed from butanol, and $s_{\text{test sample}}$ is the scattering coefficient of the test sample, which is assumed to have an identical composition. In the case of kraft pulps it has been shown that paper's tensile strength can be approximately modeled according to the following equation (Page 1969),

$$1/T = 9 / (8Z) + 12\, A\rho\, g / [\, b\, P\, L\, (RBA)] \qquad (3)$$

where T is the maximum tensile force before failure, usually expressed as a breaking length, Z is the zero-span breaking length, giving an indication of fiber strength, A is the average fiber cross-sectional area, ρ is the density of the fiber material, g is gravitational acceleration, b is the shear bond strength, P is the perimeter of a fiber, L is the length of a fiber, and *RBA* is the relative bonded area, expressed as a fraction. In more recent work

the value of *RBA* has been calculated based on procedures that do not require preparation of a non-bonded sheet (Görres et al. 1995; Batchelor and He 2005).

The first term in Eq. (3) involves breakage of individual fibers, whereas the second term involves separation of inter-fiber bonds. In many cases of practical interest it can be shown that bond strength tends to be the limiting factor (Page 1969; Robinson 1980). Thus, despite the various potential contributions to bonding strength, as outlined in the previous subsections, there is still plenty of motivation for papermakers to increase the strength or toughness of bonded regions, by addition of chemical agents. The latter help paper withstand various types of stresses and strains. Görres et al. (1995) found that the shear bond strength *b* was higher in the case of kraft fibers, in comparison to mechanically refined fibers, a result which is consistent with the less hydrophilic nature of lignin and the debonding effect of extractives present in the high-yield pulps.

POLYMERIC ENHANCEMENT OF INTER-FIBER BONDING

Two concepts continue to be important when water-soluble polymer additives are used to enhance Interfiber bonding, beyond what can be achieved by forming paper from the untreated fibers. The first concept, as already noted, is that there is reason to believe that inter-fiber contact is likely to be inefficient, especially if papermakers are under constraints that limit the degree of refining of the fibers. The second notion is that events taking place on a scale of nanometers during drying are likely to play a huge role in the development of paper strength, with or without polymeric additives.

Individual Polyelectrolytes

Substantial gains in paper's strength properties are often observed following addition of individual polyelectrolytes to the fiber slurry (Reynolds and Wasser 1980; Hofreiter 1981; Bhardwaj et al. 1997; Ketola and Andersson 1999; Linhart 2005; Lindström et al. 2005). Commonly used dry-strength additives include derivatives of biopolymers such as starch, guar gum (Leech 1954; Dugal and Swanson 1972), and carboxymethylcellulose (CMC) (Horsey 1947; Beghello et al. 1997; Zhang et al. 2002; Hubbe et al. 2003a; Ekevåg et al. 2004; Watanabe et al. 2004; Lofton et al. 2005). Among these, cationic starch is by far the most widely used (Moeller 1966; Harvey et al. 1973; Marton and Marton 1976; Greif and Gaspar 1980; Hofreiter 1981; Lindström and Florén 1984; Roberts et al. 1986; Howard and Jowsey 1989; Alince et al. 1990; Formento et al. 1994; Beaudoin et al. 1995). Copolymers of acrylamide are also widely used as dry-strength agents (Reynolds and Wasser 1980; Farley 1987; Spence 1999). Additives that perform effectively for strength enhancement tend to share some common features. These include a water-loving nature, a mechanism by which the material is retained efficiently at fiber surfaces, sufficiently high molecular mass so that the additive remains on the outer surfaces of the fibers, and an ability to form hydrogen bonds (Davison 1980; Farley 1987; Reynolds and Wasser 1980; Robinson 1980; Tiberg et al. 2001). The hemicellulose component of fibers shares many of these same attributes, so it is not surprising that addition of hemicellulose products to papermaking furnish tends to increase the strength (Obermanns 1936; Mobarak et al. 1973; Laine et al. 1997).

As a general rule, increasing amounts of adsorbed dry-strength polymer are expected to yield increasing strength benefits (Roberts et al. 1986). Efficient retention on the fiber surfaces usually is achieved by charge interactions (Fleer et al. 1993; Wågberg 2000). For example, retention of cationic starch at the wet end of a paper machine is substantially more efficient, in comparison to uncharged, native starch (Roberts et al. 1987). Best results usually can be achieved if the polymer has only a relatively low substitution with cationic groups (Harvey et al. 1979; Park and Tanaka 1998; Wågberg 2000). Exceptions to this rule are observed at high concentrations of electrolytes or when the fibrous slurry contains high levels of anionic colloids. At conductivity levels above about 2000 μS/cm it can be an advantage to use relatively high-charge density cationic dry strength products (Glittenberg et al. 1994; Beaudoin et al. 1995; Bobacka et al. 1998; Malton et al. 1998). In the case of wet-end starch products, levels of nitrogen between about 0.2 and 0.33%, *i.e.* between about 2.4% and 4% degree of substitution, are most common (Harvey 1979; Hofreiter 1981). Cationic acrylamide copolymers intended for use as dry-strength agents can have up to about 10% of cationic groups. As is depicted in Fig. 4, higher levels of cationic charge tend to reduce the amount of adsorbed additive (Durand-Piana et al. 1987; Zhang et al. 2000; Wågberg 2000). This effect has been attributed to two factors. First, higher positive charge provides a higher driving force for the polymer to lie down flat on the negative fiber surface. A flat adsorbed conformation is more efficient for the covering of a surface, relative to a three-dimensional conformation. Second, the charge on the adsorbing species can overwhelm the initial negative charge of the fiber surface, leading to a build-up of excess positive surface charge. Higher-charged cationic starches can maintain their dry-strength performance with increasing salt concentrations of the aqueous suspension (Beaudoin et al. 1995), but there is greater likelihood of overcharging the system.

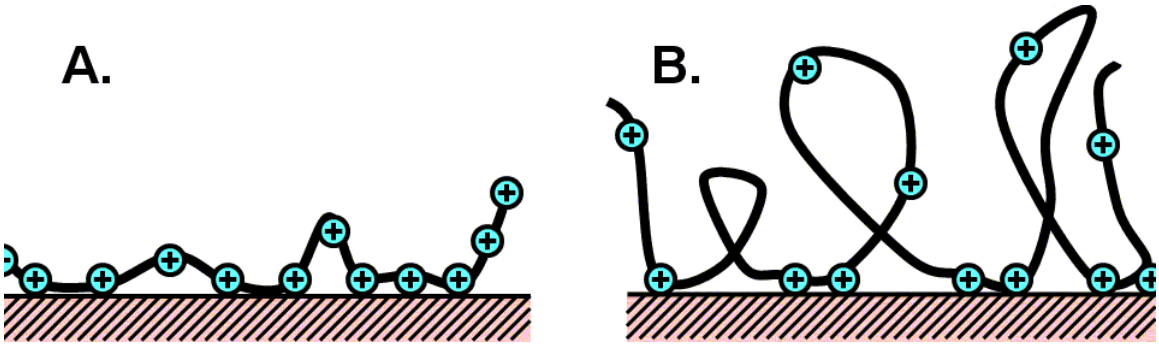

Fig. 4. Schematic illustration suggesting a greater degree of molecular extension from a surface in the case of a relatively low-charge cationic polyelectrolyte adsorbing onto a negatively charged substrate

The efficiency of cationic starch as a dry-strength agent usually is highest at addition rates up to about 1% on product mass (Roberts et al. 1986; Formento et al. 1994), or somewhat higher if the furnish contains relatively high amounts of filler and well-refined fibers, offering a relatively high surface area (McKenzie 1964). Beyond about 2% addition, one can expect that increasing proportions of the added cationic starch will remain in the solution phase rather than becoming adsorbed. As shown by Marton (1980), the available surface area is likely to become the limiting factor.

There does not appear to be a simple relationship between molecular mass and strength benefits (Hofreiter 1981; Zhang et al. 2001). In the case of acrylamide copolymers, some research has suggested an optimum molecular mass of about 100,000 to 500,000 grams per mole (Reynolds and Wasser 1980). Other work has suggested increasing effectiveness with increasing molecular mass (Carlsson et al. 1977; Pelton et al. 2003). Linear acrylamide copolymers of very high molecular mass tend to act as flocculants, hurting the uniformity and strength of the resulting paper. By contrast, the molecular mass of amylopectin molecules in commonly used starch products has been estimated to be in the range of hundreds of millions of grams per mole (Whistler et al. 1978; Swinkles 1985; Olsson et al. 2003; Modig et al. 2006). Higher dry-strength performance is sometimes reported for starch products that are rich in amylopectin (Beaudoin et al. 1995). The important distinction appears to be that the amylopectin molecules are highly branched, giving them a more compact conformation in solution and a reduced tendency to flocculate fibers.

The relative ineffectiveness of various low-mass analogues of dry-strength polymers provides an important clue as to how these materials function. By means of electrokinetic tests such as microelectrophoresis (Strazdins 1977; Koethe and Scott 1993; Farley 1997) and streaming potential tests (Penniman 1992; Koethe and Scott 1993; Wang and Hubbe 2002) it can be shown that cationic polyelectrolytes of decreasing molecular mass tend to penetrate more rapidly beneath the outer surfaces of kraft fibers. In addition, the amount of dry-strength resin adsorbed onto fibers often increases with increasing time of mixing (Abson and Brooks 1985). Though other explanations are possible, it appears that time may permit strength resins to migrate into hidden positions, either within the cell wall or within layers of fibrils at the fiber surfaces (Zhang et al. 2001). Such results can help to explain a decreased effectiveness of various strength resins with increasing time of exposure to the fiber slurry (Spence 1999).

As noted in a recent review (Hubbe et al. 2006a), it is not certain whether most of this migration of polyelectrolytes into fibers involves the 2-100nm pore spaces within the cell walls themselves, or just spaces within layers of fibrils at the fiber surfaces. Adsorption experiments with silica gel, having well-defined pore size, have shown relatively little penetration of high-charge, linear polymer into 15nm pores over time scales of several minutes (Hostetler and Swanson 1974; Hubbe et al. 2006b). Results of the more recent studies involving bleached kraft fibers (Hubbe et al. 2006a) suggest that the interior spaces within cell wall pores remain mostly uncovered by cationic polymers within the time periods usually associated with papermaking operations. Likewise, Tatsumi and Yamauchi (1997) observed only limited migration of cationic polymers into kraft fibers.

Gupta and Scott (1995) observed the most efficient contributions of cationic starch products to strength in cases exhibiting a strong decay of streaming potential with time. Such observations are consistent with non-equilibrium adsorbed conformations. In principle, a macromolecule that has not yet reached an energetically favorable adsorbed conformation would be expected to have a greater capability of associating with a second surface during the formation and drying of paper.

Effects of molecular mass hold the potential to shed light on the mechanism of bonding. In principle, a larger molecule, having a larger effective size in solution, would be expected to be more effective in filling void spaces, in areas where the facing surfaces

of rough materials are otherwise unable to make contact on a molecular case. Such "space-filling" mechanisms, to account for the action of dry-strength additives, have been proposed (Tiberg et al. 2001). Howard and Jowsey (1989) carried out critical experiments to address this question in the case of cationic starch. Independent variables in their study included whether or not cationic starch was added to the fiber suspension and the pressure applied to the sheets before they were dried. Increasing wet-pressing increased paper strength and decreased opacity, consistent with an increase in *RBA*, as was defined in eq. (2). Relative to the baseline, addition of starch increased the paper's tensile strength with only a minor decrease in opacity. The authors concluded that cationic starch mainly increased the bond strength per unit of bonded area. Related results were reported by others (Reynolds and Wasser 1980; Gaspar 1982; Retulainen and Nurminen 1993; Zhang et al. 2001; Yamauchi and Hatanaka 2002), some of whom observed significant increases in *RBA* with increasing cationic starch addition (Formento et al. 1994). Hofreiter (1981) suggested that water-loving polymers, such as cationic starch, can tend to draw paper together into a denser structure as paper is dried.

When interpreting results of the type just described, it is important to recognize that eq. (2), which defined the meaning of *RBA*, is based on optical measurements. Such measurements are relatively insensitive to the presence of air gaps thinner than about 200 nm. If cationic starch acts by "filling in void spaces," its effects must be associated with gaps much narrower than a wavelength of light. As noted by Pelton (2004), when one considers the amounts of various dry-strength additives that adsorb onto papermaking materials, one would expect the dried polymer layers to be about 1 nm thick.

As noted earlier in this issue of *BioResources* (Green 2006), paper's strength is affected by stresses within the plane of the sheet as it is being dried. Though details of this subject lie beyond the scope of this review, a study by Vainio et al. (2006) has revealed a connection between drying stresses and dry-strength additives. The authors observed, in general, that drying stresses tended to decrease the density of inter-fiber bonding, even in cases where the in-plane tensile strength was improved. However, it appeared that optimum dry-strength chemicals or polyelectrolyte complexes (see later) were able to counteract the negative effect of drying stress on bond strength, yielding an overall improvement in paper strength properties. The authors suggested that the dry-strength polymers can provide a flexible layer at the junction between fibers.

Anionic Polyelectrolytes

When attempting to achieve higher strength gains than could be achieved by starch products, one of the first kinds of wet-end additives considered by papermakers has been anionic copolymers of acrylamide (Chan 1976). To retain such polymers efficiently onto cellulosic fibers, which are tend to be anionic, it is necessary to add something cationic to the system. During the 1960s and 1970s, when most printing papers in the US were manufactured under acidic pH conditions, aluminum sulfate (papermaker's alum) was commonly used in such a role. The effectiveness of alum, in combination with anionic acrylamide copolymers, tended to be maximized at pH values between 4.2 and 4.5 (Reynolds 1961; Linke 1968; Reynolds 1980; Reynolds and Wasser 1980). Such a maximum, versus pH, is consistent with the expected formation of highly cationic oligomeric aluminum species that becomes prevalent within that range of pH,

depending on such factors as concentration, time, temperature, and the concentration of sulfate ions (Akitt et al. 1972; Bottero and Fiessinger 1989; Crawford and Flood 1989; Strazdins 1989). Results also suggest that best strength results are achieved when the amount of aluminum ions is sufficient to neutralize the negative charge of the polymer. The same mechanism appears to account for the effectiveness of certain anionic derivatives of natural products. These include carboxymethylcellulose (CMC), added sequentially with alum under acidic papermaking conditions (Horsey 1947). Anionic starches also show promise, when added sequentially with cationic polymers (Schneider and Huang 1993; Brouwer 1997; Wielema and Brouwer 2003).

To be fair, the explanation just given is not the only credible explanation of the cited results. For instance, one can suppose that the cationic materials in the system adsorb onto solids surfaces and they then can act as anchoring points for the anionic polyelectrolytes. Evidence for this anchoring mechanism was found in a study in which the dosages were carefully controlled, allowing a highly cationic polymer to become adsorbed almost quantitatively, prior to the addition of an anionic dry-strength polyelectrolyte (Hubbe et al. 2003a). Under certain conditions, the contribution of anionic CMC to the compression strength of linerboard could be maximized if the fiber surfaces had been exposed to just enough cationic polymer to saturate their adsorption capacity. Not only did those conditions achieve the highest compression strength of the paper made from never-dried unbleached kraft fibers, but the advantage continued to be clearly evident when the same fibers were reslurried and formed into recycled paper.

Recently a contrary approach has been advocated in which fibers are treated with anionic carboxymethylcellulose (CMC) in the absence of cationic additives. Even though CMC has a negative charge, enough of it appears to adsorb onto fiber surfaces so that substantial dry-strength gains can be achieved (Beghello et al. 1997). In addition to acting as a bonding agent, it seems likely that the adsorbed CMC acts as a kind of lubricant on the fiber surfaces (Mason 1950; Swanson 1950; Leech 1954; Zauscher and Klingenberg 2000), thereby reducing the flocculation tendency. More uniform paper is expected to be stronger (Linhart et al. 1987; Nazhad et al. 2000). More recently it was found that higher levels of CMC can be attached to fiber surfaces by prolonged heating under pressure at 120 ^{o}C in the presence of 0.05M calcium chloride (see Fors 2000). It appears that CMC is able to hydrogen bond efficiently to the cellulosic surfaces due to chemical similarity, and that such bonding is difficult to reverse completely.

Regardless of how the anionic polyelectrolyte is made to adsorb onto fiber surfaces, it is possible to draw an analogy to the fiber surface derivatization (Walecka 1956; Marton and Marton 1976; Wågberg et a. 1989; Minor et al. 1991; Roberts 1992; Fors 2000) and fiber surface oxidation work (Alince 1975; Saito and Isogai 2005), some of which was cited earlier. Recall that certain such treatments, on their own, had a very positive effect on the dry strength of the resulting paper, even without the addition of polyelectrolytes (Walecka 1956; Minor et al. 1991; Fors 2000). Contributing mechanisms, to explain those effects, included a greater swelling of the cellulosic material, *i.e.* a greater tendency to hold onto water. Treatment of fiber surfaces with anionic polyelectrolytes can be viewed as an alternative way to increase the water-holding ability of the fiber surfaces. The anionic charges are expected to induce an osmotic effect, causing water to swell the polymeric material. Seen in another way, repulsion between the ionic

double-layers surrounding the negatively charged polymer chains is expected to make them extend outwards from the fiber surface, enabling more efficient interdiffusion of macromolecules at the fiber surfaces. One of the problems, though, is that papermakers sometimes have to "use up" some of the negative polymer charge by addition of cationic materials in order to achieve efficient fine particle retention.

Polyelectrolyte Complexes Formed *In-Situ*

When solutions of oppositely charged polyelectrolytes are mixed, the resulting complexes can display a wide range of properties, including decreased solubility or an increased tendency to adsorb (Kötz 1993; Kekkonen et al. 2001; Mende et al. 2002; Gernandt et al. 2003). The charge and colloidal stability of polyelectrolyte complexes (PECs) can be controlled by adjusting the ratio of the two polymers (Gernandt et al. 2003). Results also can depend on salt concentration and mixing conditions. Substantial gains in dry strength can be achieved by addition of such pre-formed PECs to papermaking furnish (Heath et al. 1974; Nagata 1991; Gärdlund et al. 2003; Koljonen et al. 2003; Wu et al. 2004; Gärdlund et al. 2005). For example, it was found that combinations of cationic and anionic acrylamide copolymers were especially effective in increasing paper's dry-strength (Koljonen et al. 2003).

Several studies have shown evidence of paper strength increases due to *in-situ* formation and deposition of polyelectrolyte complexes within a fiber suspension (Carr et al. 1974, 1977; Smith 1992; Maximova et al. 2005). Related effects were observed during some recent work in which oppositely charged polyelectrolytes were added sequentially (Hubbe et al. 2003a). The working hypothesis was that the most promising gains in dry strength would be achieved if the fibers were treated first with just enough highly cationic polymer to saturate the fibers' adsorption capacity. Unexpectedly, however, even greater tensile strength of handsheets formed form repulped copy paper was observed if the first additive was greatly in excess of the saturation level. To explain the results, it was proposed that the excess of cationic polyelectrolyte in solution formed complexes with the subsequently added anionic polymer, and that the complexes adsorbed onto the fibers, contributing to dry strength (Hubbe et al. 2003a). The concept is illustrated in Fig. 5.

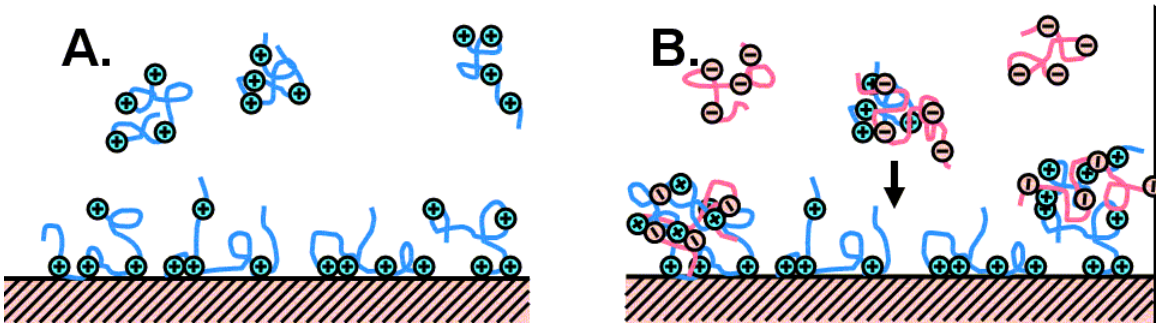

Fig. 5. Concept of a two-step treatment, starting with an excess of cationic polyelectrolyte, followed by anionic polyelectrolyte, creating polyelectrolyte complexes in the bulk phase, which subsequently deposit onto fiber surfaces, where they can contribute to paper strength

This mechanism was corroborated in subsequent work, based on a tendency of polyelectrolyte complexes to increase the light scattering of an aqueous suspension (Lofton et al. 2005; Hubbe et al. 2005; Hubbe 2005). The most promising results were obtained when the charge ratio of acidic and basic groups on the polyelectrolytes were within about a 2/3 to 3/2 ratio of each other (Hubbe et al. 2005; Hubbe 2005). Earlier work involving sequential addition of oppositely charged polyelectrolytes likewise showed that dry strength derived by this type of mechanism tends to be maximized when the ionic charges are approximately equal (Carr et al. 1977).

The manner in which polyelectrolytes are added to a fiber slurry can be critically important when one is attempting to increase strength by deposition of polyelectrolyte complexes. For instance, by pre-treating the fibers to make them cationic it was possible to shift the ratio of PEC charges corresponding to maximum retention of the polyelectrolyte complexes (Hubbe et al. 2005). PECs having a net negative charge were retained more effectively on the cationically pretreated fibers, yielding more efficient strength development. Follow-up tests with non-bonding glass fibers showed that sequential addition of the polyelectrolytes gave far superior results, compared to premixing the polyelectrolytes just before their addition to the fiber furnish (Hubbe 2005). A further decrease in strength contribution of the PECs was observed if there was a time delay before adding the polymer mixture to the fiber furnish. It appears that *in-situ* formation of PECs, in the presence of fibers, is able to take advantage of some non-equilibrium structures, which are somehow able to interact with fibers in a beneficial way before they become deactivated. A deactivation mechanism is consistent with an expected reconformation within freshly-formed PECs (Hubbe 2005).

A notable feature of PEC deposition, as a mechanism of dry-strength development, is the fact that the resulting paper strength continues to rise as the net polymer dosage increases to high levels. Thus, it was possible to form stiff, paper-like sheets from glass fibers (Hubbe 2005), skipping the mat impregnation step that is often used industrially for products made from non-bonding fibers (Williamson 1993). In one set of tests, strength increases continued to be very significant up to a level of about 40% dry weight of polyelectrolytes on kraft fibers (Heerman et al. 2006), above which point the fibers were so sticky that it was difficult to form laboratory sheets. In such cases it appears that strength is limited mainly by the high expense required to add so much polyelectrolyte and by the increased likelihood of sticky deposits.

Having demonstrated the mechanism, it is possible to cite other reported findings that appear to involve *in-situ* formation of PECs and their deposition onto fiber surfaces (Chan 1976; Lindström and Florén 1984; Nealey et al. 1989; Retulainen and Nurminen 1993; Schneider and Huang 1993; Zhang et al. 2002; Maximova et al. 2005). For instance, by sequential addition of anionic starch and polyaluminum chloride (PAC) it was possible to achieve paper strength gains usually associated with use of a size press (Brouwer 1997). A similar explanation might also account for the ability of polyvinylamine products to retain potato starch, which naturally has anionic phosphate groups (Lorenčak et al. 2000). In support of this mechanism, polyvinylamine was not effective for retaining unmodified tapioca starch, which lacks any anionic phosphate groups on its surface. Whereas conventional addition of cationic starch is limited to about 1 to 1.5% based on dry mass of fiber (Brouwer 1997; Glittenberg et al. 2004), it was possible to use

as much as 6% anionic starch, added in sequence with PAC (Brouwer 1997). A recent study showed that paper strength can be increased by *in-situ* formation of complexes with wood-derived colloidal materials, which are subsequently retained in the paper (Maximova et al. 2005).

Polyampholytes

Based on chemical similarity, one might guess that some of the advantages obtained by the use of PECs, as just described, might be achieved more reliably and simply by using single polymers having both positive and negative ionic groups. Thus, the papermaker would need to handle just one additive, not two. Concerns about controlling the charge ratio and mixing procedures could be avoided in the paper mill. In particular, because the additive can contain a defined ratio of both positive and negative ionic groups, there is much less concern that the system becomes over-charged with positive or negative species (McQueary 1990; Dalidowicz 2000). In principle, an over-dose of the system with a single-charged polymer can lead problems such as slow dewatering or inefficient retention of fine particles (Horn and Melzer 1975; Lindström and Söremark 1975; Goossens and Luner 1976; Strazdins 1994).

Evidence suggesting superior performance of amphoteric polymers in the role of dry-strength additives has been shown in a number of recent studies. For instance, a study by Yoshizawa et. al. (1998) showed that starch products prepared with both positive and negative ionic groups sometimes adsorb more efficiently on cellulosic fibers, in comparison with conventional cationic starches. Other studies have demonstrated increased rates of dewatering (McQueary 1990), and superior dry strength (McQueary 1990; Glittenberg 1993; Ghosh 1994) when using amphoteric starches. Related promising results also have been reported in the case of synthetic polyampholytes, based on acrylamide chemistry (Ye et al. 1990; Fukunaga 1999; Yoshimoto et al. 2004).

A series of studies was undertaken recently at North Carolina State University in order to better understand how polyampholyte composition and structure affect its behavior in aqueous solution, its adsorption characteristics, and its performance as a dry-strength additive. Polyampholytes were prepared by random, free-radical polymerization with differing charge ratio (Sezaki et al. 2006a) and with differing density of charged groups at a fixed charge ratio (Wang et al. 2006a). The results of potentiometric titrations, sensing the dissociation of acidic and basic groups as a function of pH, were found to be consistent with molecular compositions, as confirmed by NMR analysis.

Electrokinetic tests were used to characterize the polyampholytes. The electrophoretic mobility, as determined when the polyampholytes adsorbed onto microcrystalline cellulose indicator particles, depended strongly on pH, even near the apparent isoelectric point in each case (Sezaki et al. 2006a). By contrast, the results of polyelectrolyte titrations, using a streaming potential endpoint, showed relatively little interaction of the polyampholytes with strongly charged polyelectrolytes over a wide range of pH, especially in cases where the proportions of positive and negative charged groups were not too different (Sezaki et al. 2006a; Wang et al. 2006a). Streaming potential tests showed that polyampholyte-treated bleached kraft fibers or microcrystalline cellulose particles tended to be closer to neutral potential, compared to fibers treated with positive or negative polyelectrolytes of similar charge density (Wang et al. 2006a,b).

Consistent with predictions of polyampholyte molecular conformations in aqueous solution, both the specific viscosity and the turbidity of polyampholyte solutions were found to be strongly dependent on pH (Sezaki et al. 2006a; Hubbe et al. 2006c; Song et al. 2006). Lowest viscosity and highest turbidity were observed in the neighborhood of the isoelectric point, where the quantities of positive and negative dissociated groups were approximately equal. These results were attributed to macromolecular contraction, due to association of oppositely charged groups. Addition of salt, sufficient to raise the conductivity to 1000 μS/cm, resulted in a relative increase in viscosity and decrease in turbidity. Such observations provide examples of anti-polyelectrolyte behavior (Lowe et al. 1999). The usual explanation is that the salt ions tend to screen interactions among the charged groups of the polyampholyte macromolecules, decreasing the tendency for molecular contraction under pH conditions at which the amounts of oppositely charged groups are similar.

Follow-up tests showed that polyampholyte adsorption onto bleached kraft fibers was maximized not far from the same pH values where bulk viscosity of polyampholyte solutions was at a minimum and turbidity was maximized (Sezaki et al. 2006b; Wang et al. 2006b). In principle, one can expect there to be ionic association between charged segments in adjacent polyampholyte molecules, leading to higher levels of adsorption (Glittenberg 1993). However, as has been found in another study of polyampholyte adsorption (Yoshizawa et al. 1998), the pH of maximum adsorption was shifted relative to the isoelectric pH in such a way that the net charge was weak, but opposite to that of the substrate. Except at pH values above 9, polyampholyte adsorption was found to be more efficient in comparison to adsorption of a polybase of similar charge density and molecular mass. Various kinetic effects were observed, suggesting that the polyampholytes are able to readjust their molecular conformations gradually after adsorption in response to the electrical field (Dobrynin et al. 1997; Wang et al. 2006b). This concept is illustrated in Fig. 6.

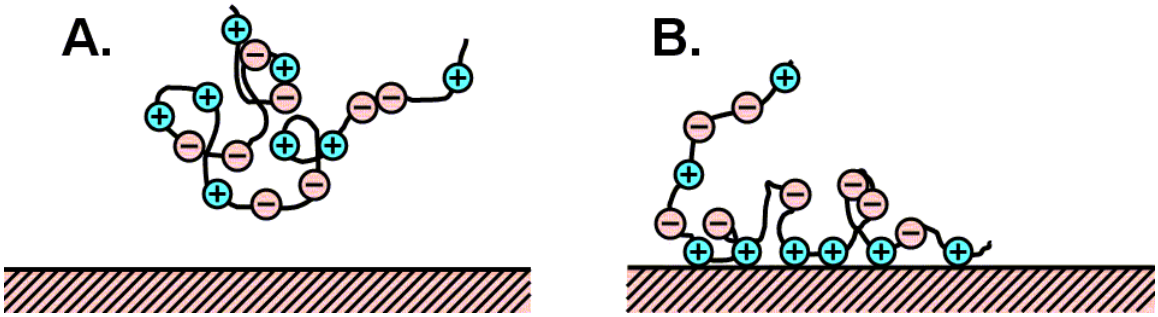

Fig. 6. Concept of macromolecular rearrangement of polyampholytes, making it possible for them to adsorb efficiently onto substrates having either sign of charge, assuming that the monomer charges are in moderate proportion with each other

Strength gains upon addition of polyampholytes to bleached kraft furnish were generally consistent with the results of the adsorption tests, and the polyampholytes were much more effective than ordinary polyelectrolytes of similar composition and mass (Hubbe et al. 2006c; Song et al. 2006). Effectiveness, in terms of dry strength, also increased with increasing density of ionizable groups. A further clue to the effectiveness

of the polyampholytes, in addition to their more efficient retention on fibers, was obtained by centrifugation tests; the water retention of polyampholyte-treated fibers was significantly increased. No significant increases were observed when the fibers were treated with solutions of single cationic or anionic polyelectrolytes. These results agree with other studies, suggesting that water-swollen polymeric layers at fiber surfaces can yield higher bonding strength (Jayne et al. 1950; Carlsson et al. 1977; Fors 2000).

Further gains in dry-strength performance could be achieved by addition of poly-aluminum chloride (PAC) before addition of polyampholytes to fiber suspensions (Song et al. 2006). The greatest relative boost in strength, due to PAC addition, was in the case of a polyampholyte having a relatively low level of charged groups, 2.5% base and 2% acid on a molecular substitution basis. The results appeared to be consistent with a complexation between positive aluminum ionic species and carboxylate groups on the polyampholytes, shifting the polymer change towards being more cationic.

While factors such as adsorption efficiency, a tendency to swell with water, and a tendency to achieve lower absolute values of streaming potential may be sufficient to account for the superior dry-strengthening effect of polyampholytes, under specified papermaking conditions, there might also be a contribution to bond strength arising due to ionic interactions. Unfortunately, there does not seem to be a completely satisfactory way to distinguish the contribution of ionic forces relative to other bonding contributions, such as London dispersion forces and hydrogen bonding. Also, the degree to which segments of a dry-strength polymer are charged can profoundly affect its ability to adsorb onto fibers, a key precondition of its effectiveness. Though some evidence has been presented in support of an ionic bonding mechanism (Reif 1972; Allan and Reif 1977; Allan et al. 1993), some of the results suggest that the contribution is not as important as other factors in terms of dry strength development (Akagane et al. 1979). Possibly the best evidence that ionic bonding plays a significant role in dry strength development comes from an early study of cationic starch (Moeller 1966). The strength gain due to the starch was approximately the same, regardless of whether the sheets were dried in the ordinary way or were freeze-dried. Since freeze-drying bypasses the effects of capillary forces, as well as the efficient development of hydrogen bonding, Moeller concluded that the bonding contribution of the starch must be mainly ionic.

PROSPECTS FOR FUTURE DEVELOPMENTS

Preparing the Substrate

In principle, additional anionic groups can be created on fiber surfaces and within the cell walls by oxidative bleaching, ozone, peroxide, or oxygen treatment (Barzyk et al. 1997). Carboxymethylation of fiber surfaces has shown advantages in both the ease of refining of kraft pulps and in the strength of paper made from those pulps (Walecka 1956; Minor et al. 1991; Fors 2000). In the vapor phase, negative charge can be induced by exposure to corona discharge (Goring and Suranyi 1969; Cramm and Bibee 1982; Clark 1985a) or to the vapors of maleic anhydride (Hubbe et al. 1999). A reaction between cellulosic or hemicellulosic hydroxyl groups with a halo-acetic acid compound will derivatize the cellulosic material with carboxymethyl groups (Barzyk et al. 1997).

By carrying out such derivatization on recycled kraft fibers, which have experienced a drying cycle, the negative groups can be mainly limited to the outer surfaces (Fors 2000). Substantial strength gains were achieved, even tough the reagent could not penetrate into the fibers to change their bulk characteristics, such as conformability or swelling ability. Rather, the effects were attributed to increased bondability of the surface.

Marton and Marton (1976) were apparently the first to show that increasing the negative charged character of fibers could be used as a way to enhance their ability to adsorb cationic starch. The fibers were carboxymethylated to various degrees. The authors also demonstrated that cationic starch, once adsorbed onto fiber, could not be removed even by rinsing in hot water. Retulainen et al. (1993) showed that higher-yield fibers, having a higher density of negative charge, were able to adsorb cationic starch more efficiently compared to low-yield, bleached kraft fibers. Watanabe et al. (2004) showed that CMC-treated fibers achieved larger gains in strength upon subsequent addition of strength additives, in the presence of alum.

Figure 7 illustrates a possible way to implement fiber modification in a paper machine system, while adding a cationic polyelectrolyte. As shown, the pulp first is treated, by some means, to create a higher level of acidic groups on its surface. Next it is proposed that excess reagent ought to be washed from the pulp. Otherwise, excess chemical and byproducts might result in problems for the papermaker or end-user. Cationic dry-strength additives added after the washing stage, as indicated, would be expected to adsorb with high efficiency, leading to higher dry-strength performance.

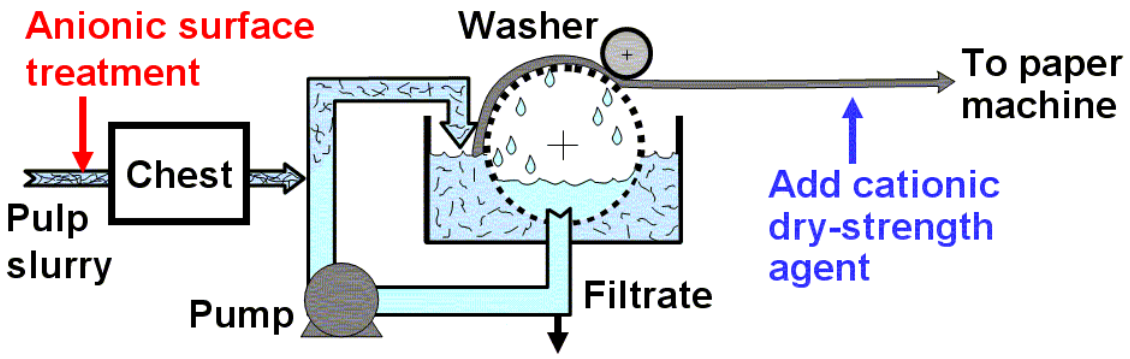

Fig. 7. Generalized concept of a two-step treatment, involving a surface derivatization, to make the fiber surfaces more negative, followed by treatment with positively charged dry-strength polymer

Controlled Assembly

The words "self-assembled" have become popular in recent studies that fall under the category of nanotechnology (Decher et al. 1992; Advincula et al. 1996; Decher 1997; Koetse et al. 2002). When they use this term, authors usually are referring to procedures by which individual macromolecules or colloidal particles align themselves in a regular order. In other words, the organization follows from the characteristics of the large molecules and the order of the mixing steps. The general principles of this approach recently were demonstrated in the case of papermaking materials (Wågberg et al. 2002). In the cited study self-assembled multi-layers (SAMs), composed of alternating layers of

oppositely charged polyelectrolytes were deposited onto silicon wafers and onto cellulosic fibers. The total thickness of deposited macromolecules increased in regular steps during the self-assembly process. In a corresponding manner, the dry-strength of paper prepared from SAM-treated cellulosic fibers increased markedly with increasing numbers of deposited layers (Wågberg et al. 2002; Eriksson et al. 2005a,b). In certain cases the strength was strongly influenced by the nature of the final deposited layer (Wågberg et al. 2002; Eriksson et al. 2005a).

Despite the intriguing and seemingly promising nature of the results just cited, a key technological hurdle needs to be overcome before SAM technology can be considered to be a practical means of improving paper's dry strength. SAM procedures, in their current state of development, depend on alternating treatment of fibers with solutions containing excess amounts of polyelectrolyte, followed by rinsing steps (Hoogeveen et al. 1996; Decher 1997; Caruso et al. 1999; Dubas and Schlenoff 1999). The expense to install and run multiple sets of washing steps would tend to make SAM treatment economically unattractive. Fortunately, there is reason to be optimistic. An inherent problem with existing scientific studies of SAMs is that investigators tend to concentrate on forming alternating layers of supremely high regularity and purity. It has not been demonstrated that such ideality of alternating polyelectrolyte layers is truly important relative to the practical goals of increasing paper's strength.

Figure 8 illustrates a concept that can be likened to what happens when a chef goes on a camping trip with only one pot. Just as in a conventional papermaking operation, the chef can add ingredients to the pot, but nothing can be removed until the mixture is "ready." The main decisions to be made involve the order of addition and the amounts of materials that are added in each step. Occasionally the chef may taste a morsel. In principle, one would want to add just enough polyelectrolyte, in each alternating step, so that the fiber surface would become saturated, leaving no significant excess of that charge of polyelectrolyte in solution (Hubbe et al. 2003a).

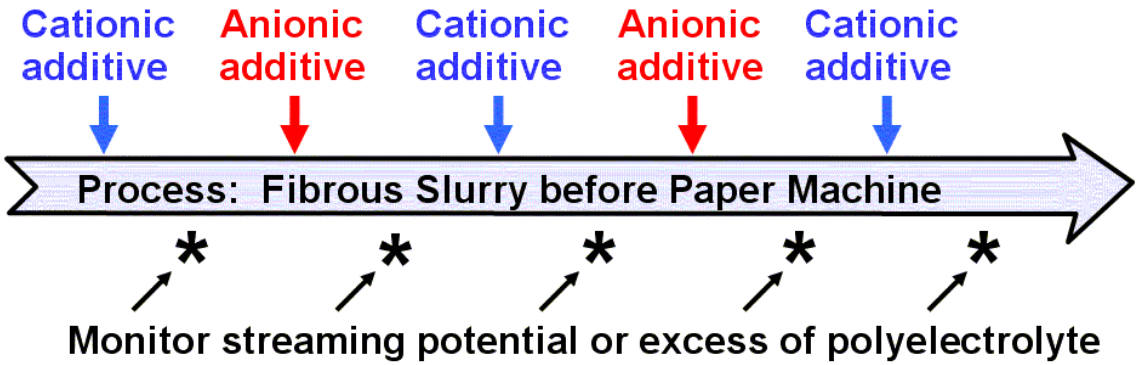

Fig. 8. Concept of how self-assembled multi-layers of dry-strength polymers might be applied to papermaking fibers in a continuous process, with online control of dosages and optimization of electrokinetic properties. This concept requires that adequate adsorption efficiency can still be achieved in each successful step, even though there cannot be any large excesses of polyelectrolytes used, in the absence of rinsing steps.

Let's assume, for the moment, that future studies show that it is indeed feasible to increase paper strength by "one pot" successive treatments with alternating polyelec-

trolytes, without the use of rinsing steps. A big challenge, then, will be to fine-tune the amounts of added polyelectrolyte in each step. Too little polyelectrolyte would be considered undesirable, since a strong reversal of net surface change is required at each step, in order to render the surface attractive to the subsequent, oppositely charged layer. Too much polyelectrolyte would be considered undesirable, since the excess of unadsorbed polyelectrolyte would be subject to uncontrolled complexation with the next oppositely charged polyelectrolyte to be added, before the latter has a chance to reach the fiber surfaces. Though polyelectrolyte complexes appear to have a promising role to play with respect to development of dry strength in paper (Lofton et al. 2005), such interactions would defeat the goal of achieving multilayer self-assembly.

Fortunately, there are two parallel electrokinetic methods that hold promise for monitoring and controlling levels of polyelectrolyte addition, in order to achieve a controlled one-pot multilayer assembly, as just described. One such method involves titration with a highly charged polyelectrolyte in order to determine the net colloidal charge of the bulk solution, after a given treatment step. Automated systems are available for such tests, using streaming current measurements as an indication of the titration endpoint (Bley 1998; Hubbe and Chen 2004). As envisioned in Fig. 8, one would want to control the added amount, in each step, so that a very small controlled excess of that polymer remains in solution after mixing with the fibers. Fiber-pad streaming potential measurements are the other kind of electrokinetic measurement to be considered for this kind of application (Nazir 1994; Miyanishi 1995; Hubbe and Wang 2004). Such measurements sense the electrical potential adjacent to the surfaces of the fibers. Recently it was discovered that the sign of charge of the outer-most layers of cellulosic fibers can be detected by carrying out streaming potential measurements in the presence of aqueous solution having a low electrical conductivity (Hubbe et al. 2006a). Online equipment has been developed for continuous measurements of streaming potential in paper mills (Nazir 1994; Miyanishi 1995). In order to use such equipment for the purposes just described it would be necessary to modify the equipment so that the fiber slurry is first dewatered, then resuspended in low conductivity solution, then subjected to the automated zeta potential test (Hubbe et al. 2006a).

Film Quality

As noted by Linhart (2005), wet-end additives that are most promising in terms of dry-strength development also tend to form strong films, in cases where dilute aqueous polymeric solutions are applied to smooth substrates, followed by evaporation. This correlation suggests that the cohesive strength within the adhesive layers provided by dry-strength additives may be an important factor. In support of this view, Lertsutthiwong et al. (2002) attributed the good dry-strength performance of chitosan to the fact that it forms a good film. The authors stressed that chitosan has a compatible structure with cellulose, tending to promote efficient interdiffusion, essentially welding the fibers together upon drying of the paper.

Though issues related to the cohesive strength of the "glue" used to hold papermaking fibers together has received relatively little attention until recently (Pelton 2004; Linhart 2005), there is reason for concern. The cohesion within a layer of polyelectrolytes and other substances at a fiber surface can be only as strong as its

weakest point. Processes associated with the flocculation interactions of polymers (Fleer et al. 1993; Kötz 1993; Mende et al. 2002) might be expected to produce non-uniform distributions of density, including areas of weakness in films. In addition, it is known that cracks that develop in an adhesive film as a result of the drying process can have a dominant effect on failure of glue joints (Veselovsky and Kestelman 2002).

As noted by Helle (1965), in considering the tensile strength of paper it is often more useful to think in terms of a rope, rather than thinking in the terms of an isotropic solid material. Rope's toughness, flexibility, and high tensile strength are due in part to the non-rigid association between adjacent strands. Likewise, it would appear important that inter-fiber contacts in paper be designed with an appropriate amount of compliance, depending on requirements for the paper grade. Leech (1954) proposed that gums can increase paper strength by increasing the flexibility of bonded areas between fibers. On the other hand, it would make sense that stiff paper products can benefit from the use of additives such as starch (Retulainen and Nieminen 1996), which tend to produce relatively stiff films upon drying. This issue is not settled, however, since more recent work by Yamauchi and Hatanaka (2002) suggested that wet-end starch increases the ability of paper to withstand tensile strains without breaking; this observation might be taken as evidence of increased compliance within the bonded areas. One can expect that there are many additional opportunities to match the cohesive and viscoelastic behavior of dry-strength additives, in their solid form, relative to paper performance.

Let us assume that high tensile strength that can be achieved in a flexible structure that allows some internal movement among the component members. As noted by Van den Akker (1969), most of the energy that is consumed during the tensile breakage of paper is expended in the flexing of fibers. Consistent with this idea it has been found that superior dry tensile strength can be achieved if the furnish is fractionated, the agent added to the fiber fraction, and then the furnish components are recombined (Strazdins 1980; Stratton 1989). By restricting the effects of the strength additives mainly to the crossing-points of fibers, at locations not covered by fines, a certain level of slippage is possible within the paper structure, helping to distribute loads. Despite the promising nature of such results, no published accounts were found describing industrial applications of fractionation, followed by selective addition of dry-strength agents. It appears likely that the lack of commercial interest is due to the relatively high costs associated with fiber fractionation. What is needed, it seems, is a chemical-based way to achieve similar objectives, a kind of "spot welding" within the paper sheet, rather than uniformly increasing bonding over all of the contacting surfaces. This, in addition to many other of the ideas presented in this review, imply that there will be plenty of interesting questions to occupy paper scientists for a long time to come.

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