DOCTOR OF PHILOSOPHY

The protection of wood against fungal decay by isocyanate chemical modification.

Cardias, Maria de Fatima Castro

Award date:
1992

Awarding institution:
Bangor University

THE PROTECTION OF WOOD AGAINST FUNGAL DECAY BY ISOCYANATE CHEMICAL MODIFICATION

BY

MARIA DE FATIMA CASTRO CARDIAS

DEPARTMENT OF FORESTRY AND WOOD SCIENCE

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR, U.K.

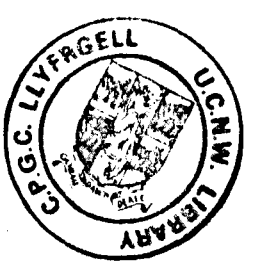
A thesis submitted in candidature for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF WALES

JUNE/1992



ACKNOWLEDGMENTS

I would like to express my high appreciation to my major supervisor Dr. M. D. Hale for his kind guidance, assistance and encouragement during the course of this research. Special recognition is given to Dr. W. B. Banks and Dr. J. M. Lawther for their valuable comments, suggestions, and attention during my studies. Thanks are extended to Mr. G. L. Jones, Mr. G. Mcleod and all members of the wood science division, Bangor University, for their kind technical assistance, helpful attention during laboratory work.

Sincere thanks are for the directors and staff of the Instituto Nacional de Pesquisas da Amazonia - INPA, and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico - CNPq. for their financial support.

I would like to thank my mother and family for their constant encouragement, support and sweetness in my whole academic life.

Special thanks goes to my friends and those who directly or indirectly allowed me to complete this study by being patient, supportive and understanding.

SUMMARY

The purpose of this study was to assess the bioprotectant performance of chemical modification with three different isocyanates (n-butyl, hexyl and 1,6-diisocyanatohexane, BuNCO, HeNCO and HDI respectively) in Corsican pine (Pinus nigra Schneid) sapwood. Wood-isocyanate bond formation was verified by the increase in sample weight, volume and by infra-red spectroscopy.

Basidiomycete (Coniophora puteana, Gloeophyllum trabeum, Coriolus versicolor, Pycnoporus sanguineus) decay tests demonstrated protection by chemical modification. The relationships of fungal species, weight percent gain (WPG), decay induced weight loss and moisture content were examined. One of the brown rot fungi, C. puteana, showed higher threshold protection values than the other fungi tested and the diisocyanate showed better performance. Scanning Electron Microscopy and enzyme detection tests (cellulase and phenol oxidases) have been carried out in an attempt to gain a better understanding of the treatment performance.

Chemical characteristics of the sound and brown rotted wood (C. puteana) have been examined using sulphuric acid, sodium chlorite and high performance liquid chromatography-HPLC (gel permeation chromatography-GPC) procedures to clarify the principles which govern isocyanate modifications and restrict fungal decay. A number of parameters were examined including lignin and holocellulose contents, holocellulose molecular weight and degree of polymerisation (DP and dispersity). These demonstrated that chemical modification changed the configuration of the original wood polymers. Although preferential modification occurred at lower weight percent gains in the lignin fraction appreciable wood protection against C. puteana only occurred when the holocellulose fraction showed substantial changes due to chemical modification.

To examine further the effect of moisture and loading of substituent groups within the outer layers of wood after chemical modification (BuNCO & HDI), tensile strength resistance to surface colonisation by soft rot fungi was undertaken utilizing thin wood strips after unsterile soil tests. Less modification was necessary to achieve protection against soft rot in this test.

CONTENTS

CHAPTER 5.

CHAPTER 6.

CHAPTER 7.

CHAPTER 1.

GENERAL INTRODUCTION

CHAPTER 1.

1.1. GENERAL INTRODUCTION

The microbiological degradation of lignocellulosic materials is one of the most important process in nature. Fungal activities in wood limits its use by reducing its density, strength and aesthetic qualities. Wood failure results from complex biochemical processes which are at present only partly understood. The present understanding of the processes of decay suggests that enzymes and reactive free radical systems are involved in solubilizing the wood cell wall material. Decay fungi of the teleomorph state Basidiomycotina, Ascomycotina and their related anamorph states Deuteromycotina grouped by their activity as brown, white and soft rot fungi, are distinguished amongst the fungi as causing most damage to the wood structure.

Preservation of wood by conventional methods (chemical and physical) has long been established to prevent and eradicate wood-inhabiting fungi. A preservation product which ensures protection against fungal attack is usually referred to as a biocide (against bacteria, fungi and insects), but may more specifically be targeted against specific biodeteriorgens (e.g. fungicides against fungi).

Chemical methods are usually assessed by their "toxic value" against a test organism. The minimum amount of preservative that protects the wood against

decay by a given test fungus is defined as the threshold value for that organism.

Conventional wood impregnation methods (water or oil type preservatives) are based primarily on the use of chemicals such as creosote, arsenical containing salts and chlorinated phenols. Restrictions in recent years due to environmental concerns, stability and longevity of its protectant action are some of the limitations imposed in the utilization of conventional chemical treatments.

Physical methods have long been established as methods to reduce or prevent fungal colonization and are commonly used in the prevention and eradication of wood-inhabiting fungi. In the presence of an excess of moisture (water - logged conditions) or in absence of a sufficient minimum moisture, fungal activity in wood is reduced, particularly by many Basidiomycete decay fungi. Studies (Boddy, 1983; Clark et al., 1980; Griffin, 1977) show that wood decaying Basidiomycetes appear to be particularly sensitive to decreased water potential of the substratum. The drawback exhibited in drying wood lies in the ease with which wood is rewetted.

Whether chemical or physical, each preservation method has its usefulness and limitations. Among the methods available the selection of the proper treatment procedure depends upon a number of factors, including the type of wood, its permeability and its

end-use.

Chemical modification as an innovation in the bioprotection of wood relies on a combination of wood preservation methods (chemical & physical), i.e. chemical by grafting the product to the wood matrix and physical in reducing the attractive nature of hydroxyl groups within wood. This is accomplished by reacting the wood with selected chemicals which modify the predominant wood components (cellulose, hemicellulose and lignin) without leaving toxic residues within the wood. This approach extends to the desirable alteration of a number of wood properties. The permanence of the bonding makes chemically modified wood superior to many chemical impregnation techniques where a number of factors including leaching and weathering may cause the product to lose its desirable properties. Covalent bonds of the carbon-oxygen-carbon type are of major importance and a variety of different reactants (see chapter 2) have been applied to wood to achieve desirable properties e.g. to improve dimensional stability and decay resistance (Rowell, 1983). In this work substitution reactions between monofunctional or difunctional isocyanates and wood components, forming urethanes have been investigated; in the past some have shown promise in minimising the effects of decay fungi (Rowell & Ellis, 1979; Rowell, 1980; Kalnins, 1982; Ellis & Rowell, 1984; Chen, Rowell & Ellis, 1990).

The assumptions directing the studies performed here were based on compiled data (chapter 2) which postulate that decay resistance of chemically modified wood may also be explained on the basis of the inability of the modified wood cell walls to absorb the moisture needed by decay fungi (Stamm & Baechler, 1960). The previous experiments with isocyanate modification (Rowell & Ellis, 1979; Rowell, 1980; Kalnins, 1982; Ellis & Rowell, 1984; Chen, Rowell & Ellis, 1990) were based on testing with Gloeophyllum trabeum (Pers.:Fr.) Murr. with the exception of Kalnins (1982) who tested with a range of brown and white rot fungi. These investigations not only confirm the efficacy of modification as a wood preserving system but also suggest the existence of an optimum alkyl chain length in the isocyanate and this has aided in the choice of compounds used here. Furthermore, studies (West & Banks, 1986; West, 1988; Rowell, 1980) indicate the lignin is substituted to a greater extent than holocellulose. The implication of the degree of substitution of wood constituents to decay fungi is obvious, but publications on the sensitivity of different fungal species is limited. Thus, in the current work the fungal species and decay types have been extended. This includes the action on wood by brown rot fungi [Coniophora puteana (Schum.:Fr.) Karst; G. trabeum], white rot fungi [Coriolus versicolor L.:Fr.; Pycnoporus sanguineus

L.:Fr.] and soft rot fungi in the form of unsterile soil technique. To achieve this a series of studies were undertaken as follows:

-wood-isocyanate bonding formation in Corsican pine wood (chapter 3);

-effectiveness of (mono- and difunctional) modification against Basidiomycetes (chapter 4);

-the chemical composition of extracted and wood modified to different degrees decayed by C. puteana to different weight losses (chapter 5);

- the effectiveness of modification against soft rot decay in an unsterile soil exposure system (chapter 6).

In each chapter a more specific resume of objectives is covered. In chapter 7 the results are drawn together and discussed.

These studies have allowed the investigation of the effect of variation of certain physical and chemical properties of isocyanate modified wood after fungal colonization in relation to: a) change in density (weight loss); b) change in the polymer properties; c) change in tensile strength. Additional assessments (moisture relations, microscopic decay patterns) were carried out to resolve further issues.

CHAPTER 2.

LITERATURE REVIEW

CHAPTER 2.

LITERATURE REVIEW

2.1. THE DECAY RESISTANCE OF CHEMICALLY MODIFIED WOOD

Wood polymers may be chemically altered by etherification, acetal reactions or esterification to improve resistance to microbial degradation. This has been an alternative approach as a non conventional method of wood protection. The terminology "chemical modification" is defined as a chemical reaction between some reactive part of a wood component and a simple chemical reagent, with or without catalyst, to form a covalent bond between the two (Rowell, 1975). The most abundant reactive chemical sites in wood are the hydroxyl groups on cellulose, hemicellulose and lignin. Table 2.1 depicts the simplest fundamental structures showing some reaction types.

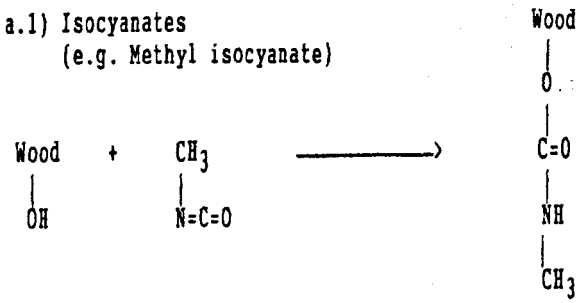
The ether bond formed by the substitution reaction of acrilonitrile with wood hydroxyl groups is referred to as cyanoethylation. According to Goldstein et al. (1959) Ponderosa pine and Southern yellow pine modified with acrilonitrile up to 25 weight percent gain (WPG) were resistant to the decay fungi Poria monticola Murr. (=P. placenta Fr.:Cook, =Postia placenta (Fr.) Cooke), Coniophora puteana (Schum:Fr.) Karst., Lenzites trabea Pers:Fr. (=Gloeophyllum trabeum (Pers.:Fr.) Murr.) and Lentinus lepideus Fr.

TABLE 2.1

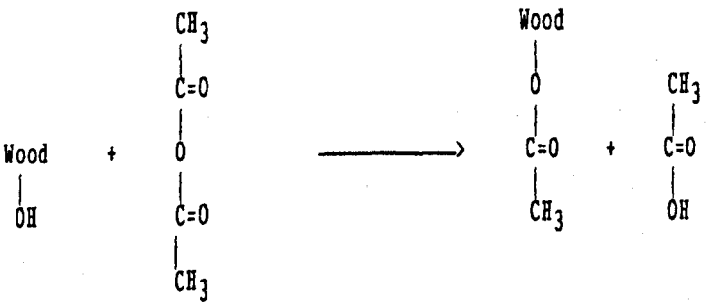
GENERAL REACTION TYPES OF CHEMICALLY MODIFIED WOOD.

a) ESTER TYPE

a.1) Isocyanates
(e.g. Methyl isocyanate)

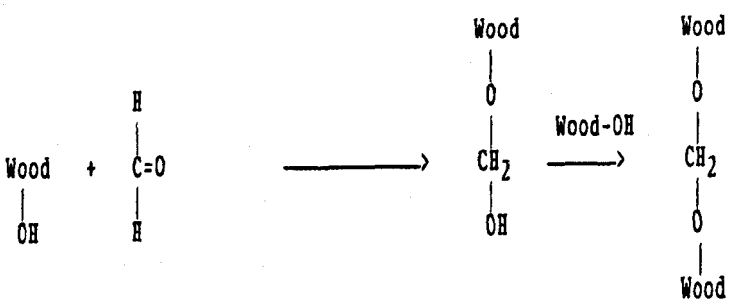


a.2) Acetylation
(e.g. Acetic Anhydride)



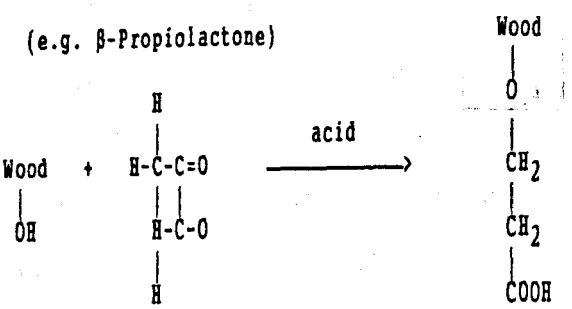
b) ACETAL TYPE

(e.g. Formaldehyde)



c) ETHER TYPE

(e.g. β-Propiolactone)



With the exception of C. puteana the same organisms were assessed by Baechler (1960) in which he found that two percent acrylonitrile treated Southern pine was completely resistant. As cited by Rowell (1975), Fuse & Nishimoto (1961) found good resistance against the brown rot fungus Poria vaporaria Fr. (=Postia placenta) after wood treatment with acrylonitrile (cyanoethylation) to 8.5% of fixed nitrogen. In earlier work Baechler (1959) using hot water extracts from cyanoethylated wood found no toxic effect of the leachate to L. trabea. Therefore, he suggested that, due to the reaction of acrylonitrile with the ammonia catalyst used, itformed non-toxic water-insoluble polymers in the wood cell wall. Cyanoethylated stakes treated with up to 15 WPG showed an average life of 7.8 years in ground contact as compared with 3.6 to 4 years in untreated control samples (Gjovik & Davidson, 1973).

Reactions of epoxides with the hydroxyl group in wood has been employed (Nilsson & Rowell, 1982; Rowell & Gutzmer, 1975; Pihl & Olsson, 1968) by the use of Butylene oxide, Propylene oxide, Epichlorohydrin or Dichlorohydrin. Etherification of Ponderosa pine with butylene oxide was reported by Nilsson & Rowell (1982). They found that specimens treated to 23.7 WPG showed no sign of attack when submitted to unsterile soil tests for 6 weeks although 15 WPG showed some minor soft rot attack. Good decay resistance to L.

trabea was attained also at modification levels of over 22 WPG of Butylene oxide and Epichlorohydrin in both leached and unleached blocks (Rowell & Gutzmer, 1975). They also stated that propylene oxide treated wood to over 23 WPG possessed good resistance to decay by L. lepideus. Pine wood treated with Epichlorohydrin or Dichlorohydrin in an alkaline treatment system showed no decay at 10-15% weight solution (presumably concentration) after two months in a soil burial test (Pihl & Olsson, 1968). Another chemical employed in the etherification of wood was β-propiolactone. Good decay resistance to L. lepideus, L. trabea, P. monticola and C. puteana was achieved at 25 WPG in soil block tests (Goldstein et al., 1959). Goldstein (1960) found good protection against decay in either weathered or unweathered samples at a 45 WPG in β-propiolactone treated wood.

Acetal substitution resulted in good resistance to L. trabea when Sitka spruce was modified by formaldehyde at 2WPG (Stamm & Baechler, 1960). They stated that no loss in weight due to decay occurs for all specimens in which swelling was reduced 50 percent or more by formaldehyde crosslinking. They stated that 2% is far short of the amount of cross-linking that would be needed to prevent decay on the basis of elimination of available -OH groups.

The most studied of all chemical modification treatments for wood has been esterification,

particularly by acetylation. The replacement of hydroxyl groups of the wood by acetyl groups at 17% weight gain has been found to be sufficient in protecting wood from decay (Goldstein et al., 1961). Tarkow et al. (1950), Kopper (1961) cited in Rowell (1983), Gjovik & Davidson (1973), Rowell et al., (1988) found that at or above 15 WPG of acetylated Yellow poplar, Loblolly pine and Green Ash woods were resistant to G. trabeum and Coriolus versicolor (L.) Quel. Ozalina & Svalbe (1966) reported that a higher WPG was necessary to protect acetylated wood against decay by Coniophora cerebella Pers.:Pers. (=C. puteana). An average life of 17.5 years in ground contact of Yellow birch wood (laminated veneer) at 19.2 WPG of acetyl groups as compared to 2.7 years of untreated controls was reported by Gjovik & Davidson (1973). They also reported that deterioration was due principally to basidiomycete decay fungi.

Many other covalently bonded chemical modification systems have been investigated to enhance wood properties (Clermont & Bender, 1957; Baird, 1969; Burmester & Olsen, 1971; Jackowski & Smulski, 1988). However, information concerning decay resistance is limited.

2.2. FUNGAL RESISTANCE OF ISOCYANATE MODIFIED WOOD

Enhancement of the wood properties has also been accomplished through substitution reactions between isocyanates and the hydroxyl groups of wood components. This results in the formation of a nitrogen-containing ester (Table 2.1). A variety of different reactants, either monofunctional or difunctional isocyanates have been investigated (Rowell & Ellis, 1979; Rowell, 1980, 1982; Kalnins, 1982; Ellis & Rowell, 1984) and some have shown promise in minimising the effects of basidiomycete decay fungi (Table 2.2).

Work performed by Rowell & Ellis (1979) showed that methyl isocyanate reacted quickly with wood at 120 °C, forming stable urethane bonds and producing no by-products. Furthermore, they stated that modifications between 20 and 25 WPG in the Southern pine sapwood with methyl isocyanate resulted in only 2 to 3 percent weight losses due to decay by G. trabeum. Bonding of methyl isocyanate to wood was indicated by comparison of infra-red spectra of unmodified and modified wood (Rowell, 1980).

Ellis & Rowell (1984) using monofunctional (ethyl, n-propyl, n-butyl) isocyanates and isophorone diisocyanate noticed that the best decay resistance of all chemicals tested was shown by those wood samples modified with n-butyl isocyanate in the presence of

TABLE 2.2

FUNGAL DECAY RESISTANCE TESTS PERFORMED ON ISOCYANATE MODIFIED WOOD AT 12 WEEKS EXPOSURE IN THE SOIL BLOCK TEST.

ISOCYANATE TYPE	TEST FUNGUS	ESTIMATED THRESHOLD WPG	WEIGHT LOSS (%)	REFERENCE
Methyl[1]	G. trabeum	>19	2 - 3	Rowell, 1980; Rowell & Ellis, 1979
Methyl[2]	G. trabeum	>10	< 5	Kalnins, 1982
	L. lepideus	>10	< 5	Kalnins, 1982
	C. versicolor	>10	< 5	Kalnins, 1982
Ethyl[3]	G. trabeum	15 - 26	6 - 3	Ellis & Rowell, 1982
n-Propyl[1]	G. trabeum	<10	4	Ellis & Rowell, 1984
n-Propyl[4]	G. trabeum	11 - 19	7 - 4	Ellis & Rowell, 1984
Allyl[2]	G. trabeum	>10	< 5	Kalnins, 1982
	L. lepideus	>10	< 5	Kalnins, 1982
	C. versicolor	>10	< 5	Kalnins, 1982
n-Butyl[4]	G. trabeum	<18	2	Ellis & Rowell, 1984
Isophorone	G. trabeum	>38	4	Ellis & Rowell, 1984

Note: Type of catalyst [1] - triethylamine, [2] - pyridine, [3] - none, [4] - dimethylformamide, [5] - dimethylsulphoxide.

35% dimethylformamide as a catalyst. The tests were performed with G. trabeum by the standard soil block test in which the Southern pine was modified to 18 and 36 WPG with butyl isocyanate. In both cases only 2 percent weight loss resulted. Samples modified to above 18 WPG of n-propyl isocyanate and at 26 WPG of ethyl isocyanate Ellis & Rowell (1984) showed insignificant weight losses (4 and 3 percent, respectively). Of the chemicals tested isophorone diisocyanate was the least effective, losing 11% of the sample weight at modification level of 38 WPG. A diisocyanate "Izocyn T-80" (chemical name undisclosed) failed to protect Beech wood (Fagus sylvatica) modified up to 50% against the attack of C. puteana and Polystictus versicolor (L.) Saccardo (=Coriolus versicolor), after 6 weeks exposure in agar block test (Lutomskii, 1975).

According to Kalnins (1982) nearly complete protection against G. trabeum, L. lepideus, and C. versicolor was found in Southern pine and Sweetgum when modification exceeded 10 WPG using methyl and allyl (C=C-C) isocyanates, although it was stated that allyl isocyanate modification gave considerably more decay protection than did methyl isocyanate.

2.3. MODIFICATION AND BONDED BIOCIDES COMPOUNDS

In an attempt to improve isocyanate performance at low per cent weight gain (1 to 5 WPG) for the protection of wood against biological attack, isocyanate compounds with attached biocides were synthesised (Rowell & Chen, 1983; Chen & Rowell, 1987; Chen et al., 1990). This technique of bonding biocides to the wood cell wall has been termed "controlled release technology". Chen & Rowell (1987) stated that if this technology were fully developed it would be possible to minimise leaching losses of biocides. This is a different approach that combines bonding technique (chemical modification) with a chemical which retains its toxicity (Rowell & Chen, 1983; Chen et al., 1990).

Rowell & Chen (1983) and Chen & Rowell (1987) synthesized chlorinated phenyl isocyanates and reacted them with wood with the aim that the compound could retain its toxicity while still bonded to the wood. They found that tetrachlorophenyl isocyanate bonded at a level of 15% by weight reduced the attack of wood by the brown rot fungus G. trabeum. However, the mechanism of effectiveness was due to chemical modification and not retained toxicity of the chlorinated aromatic.

For comparision polyhalogenated phenyl methyl isocyanates were reacted with wood and their

corresponding carbamates were synthesised by reaction to methanol and were subsequently impregnated into wood to compare bonded and non-bonded toxicity (Chen & Rowell, 1987; Chen et al., 1990). Chen et al. (1990) reported that pine reacted with fluorophenyl isocyanates to form bonded carbamates in situ was effective against attack by G. trabeum. Wood reacted to 3 WPG with 4-fluorophenyl isocyanate had a negligible weight loss (1.3%). The tri-fluoro and penta-fluoro analogs at 3.2 WPG and 3.1 WPG had 5.5% and 8.0 weight losses respectively.

With the impregnated carbamates Chen et al. (1990) stated that increased fluoro- substitution of methyl fluorophenyl carbamates improved resistance to decay by G. trabeum. Methyl 4-fluorophenyl carbamate was the least effective while methyl pentafluorophenyl carbamate and methyl meta-trifluoromethylphenyl carbamate were the most effective. With methyl pentafluorophenyl carbamate at a retention of 2.3% blocks lost 0.9% weight with G. trabeum, whereas blocks treated with methyl 2,4,6-trifluorophenyl carbamate, at a retention of 2.0%, and methyl 4-fluorophenyl carbamate, at a retention of 1.8%, had 5.7% and 9.2% weight losses, respectively. Wood treated with methyl meta-trifluoromethylphenyl carbamate at a retention of 2.1% had 0.8% weight loss. As compared to the non-bonded impregnation method (e.g. methyl fluorophenyl carbamates) they

concluded that reacted wood was more stable and less subject to loss by hydrolysis that the non-bonded impregnated wood but required more chemical to protect against decay by G. trabeum.

2.4. BIOPROTECTION MECHANISMS OF CHEMICALLY MODIFIED WOOD

General statements on the decay resistance of modified wood, suggest that the increased decay resistance is caused by insufficient water to support decay and blockage of susceptible hydroxyl groups so that decay enzymes cannot dissolve wood components (Stamm & Baechler, 1960).

There is little information on the mechanisms of bioprotection in isocyanate reacted wood specific to particular reactants. Rowell (1983), from data on the distribution of bonded chemicals, suggested that high lignin substitution does not contribute significantly to the overall protection mechanism of wood from decay or dimensional stabilization. Rowell (1980) reported that resistance of methyl isocyanate modified Southern pine to G. trabeum was attributed mainly due to the degree of substitution in the holocellulose. Ellis & Rowell (1984) studying different mono- and difunctional isocyanates made no reference concerning the resistance mechanism. Kalnins (1982) suggested that the difference in decay resistance between the two carbamate urethane derivatives (methyl & allyl

isocyanates) was due to difference in chemical structure of the substituent group since his comparison was done at the same degree of substitution. It was also suggested that decomposition of carbamic acids to form amines of wood carbamate derivatives was the explanation for enzyme inhibition, but he showed no conclusive evidence to support this statement.

2.5. WOOD (GENERAL)

On a whole wood basis the supermolecular arrangement of polysacharides and lignin has yet to be completely known. The chemistry and structure of wood influences its resistance and susceptibility to microbial attack and the ease of chemical reaction. Detailed reviews of the chemistry of the major wood polymers are described elsewhere, for example Sjostrom (1981), Farmer (1967), Cote (1977), Browning (1967), Fengel & Wegener (1984), Hon & Shiraishi (1991).

Polysaccharides are primarily hydrocarbon structures which contain many polar hydroxyl (OH) groups and have therefore high affinity for water. Polysaccharides consist of sugar molecules attached to one another by glycosidic bonds in a linear (cellulose) or branched (hemicellulose) array and form the holocellulose portion of wood. Of the structural components, cellulose is the most consistent, varying

minimally between wood species (Highley & Kirk, 1979). Lignin and hemicellulose, however, vary both in composition and amounts (Highley & Kirk, 1979). The amounts of cellulose, hemicellulose and lignin as a percentage of the dry weight of wood are usually between 40 - 50, 20 - 30 and 25 - 32 (softwoods) or 20 - 28 (hardwoods), respectively (Sjorstrom, 1981; Nakamo, 1987). There have been indications of bonds linking the lignin to the hemicellulose in softwoods (Azuma et al., 1981), and in hardwoods (Azuma et al., 1985).

The cellulose molecule consists of 10,000 - 14,000 β-D-glucopyranose residues joined (1-4) to form a linear chain (Highley & Kirk, 1979). Series of cellulose chains lie adjacent to each other and have a strong tendency to form intra- and intermolecular hydrogen bonds. Bundles of cellulose molecules are aggregated together in the form of microfibrils (Sjostrom, 1981). Numerous hydrogen bonds between adjacent cellulose molecules assure a high degree of association and high ordered (crystalline) regions alternate with less ordered (amorphous) regions. The cellulose microfibrils are the principle components of the primary and secondary cell wall layers which differ in orientation within the whole wood. Microfibrils build up fibrils and finally cellulose fibres (Sjostrom, 1981).

The polymer hemicellulose does not form fibrils because it contains a number of different sugars. Contrary to cellulose, the branched polysaccharide is shorter and less regularly arranged in the wood cell wall. Hemicellulose is less resistant to the action of chemicals (Farmer, 1967). The steric hindrance attributable to the crystallinity of cellulose combined with the greater stability of the bond explains why the hemicellulose is so much more readily hydrolysable than cellulose (Nakano, 1987).

Galactoglucomannans are the major hemicellulose of gymnosperms where they comprise some 20% of the total dry weight while arabinogalactans comprise some 5-10% and other hemicelluloses are usually present in minor quantities (Sjostrom, 1981). Glucomannans are however, the minor components of angiosperm hemicelluloses and only comprise 3 -5%. In gymnosperms the glucomannans contain galactose, glucose and mannose and exist as high and low galactose residues forms (high ratio 1:1:3 ratio, low 0.1:1:4). The backbone is comprised of glucose and mannose linked in a β(1-4) chain. In angiosperms glucuronoxylan is the predominant hemicellulose. The xylan content varies within the range of 15 - 30% of the dry wood. The backbone are linked in a β(1-4) D-xylopyranose units with one uronic acid per ten xylose residues in the chain and about seven acetyl group per ten xylose units, ((Sjostrom, 1981). The glucose:mannose ratio of

the hardwoods glucomannan varies between 1:2 and 1:1, depending on the wood species, and other polysaccharides hemicellulose are present in minor quatities.

Lignin is a three-dimensional, amorphous, branched polymer of phenylpropane units covalently bonded to one another in a number of different types of structural linkages. The three major intermonomer linkages in lignin are the arylglycerol-β-aryl ether type, the phenylcoumaran structures and the biphenyl structures (Kirk, 1971). Additional types of intermonomer linkages and monomer structures in lignin as well as the occurrence of several types of functional groups make the compound one of the most structurally complex of the biopolymers (Kirk, 1971). This complex aromatic polymer is very polydisperse and has extremely high average molecular weight. The full structure has yet to be elucidated but several detailed structural models have been proposed (Sakakibara, 1980; Sjostrom, 1981).

According to recent investigations (Eriksson & Goring, 1980; Brice & Morrison, 1982; Takahashi & Koshijima, 1988) covalent chemical bonds between lignin and polysaccharides exist. These studies have been directed to the type of bonds linking the lignin to the carbohydrate.

In the wood cell wall lignin is concentrated in the compound middle lamella and diminishes in

concentration in other cell wall layers. It forms an aggregate network entrapping the less complex structural units (cellulose & hemicellulose). As stated by Highley & Kirk (1979) there are two noteworthy features of lignin in respect to wood decay resistance: it is largely non-hydrolyzable and it forms a protective layer around the wood polysaccharides that limits cellulase accessibility within the cell walls.

Among the non-structural components of wood, extractives include many different class of organic compounds, ranging from relatively simple molecules such as sugars and phenols to highly complex substances, possessing reactive hydroxyls groups. In many instances these substances can be easily leached by solvents and are unsuitable for improving desirable properties in modified wood.

2.6. WOOD DECOMPOSITION BY FUNGI

Wood decaying fungi are saprophytic, deriving their nourishment from dead organic materials. The three major types of decay fungi, white, brown, and soft rot fungi embrace a wide diversity of species of the Eumycota and found within the sub-divisions: Basidiomycotina, Ascomycotina and Deuteromycotina (Ainsworth, 1973). From the microbiological stand

point, fungal saprophytes are important in almost all natural and man-made environments.

Fungal saprophytes produce the major depolymerizing enzymes involved in cellulose and hemicellulose breakdown and thus ensure carbon, water and some mineral nutrients for their growth. For example one of the simplest and yet most effective substances useful in ATP (energy) synthesis in a living organism is the six-carbon sugar glucose. The glucose is degraded by oxidation to CO_2 and H_2O. Degradation of wood into monomeric sugars which can be used as a source of energy by a fungus, involves a number of enzymes. Lignin may be attacked by some fungi but it is only rapidly broken down by white rot fungi amongst the fungi.

The major differences in grouping organisms from the standpoint of wood biodeterioration relate to: a) quantitative and qualitative differences in their enzyme complement; b) the decay patterns resulting from their effects on wood; and c) the structural or chemical differences in wood due to their action.

The general characteristics of brown and white rot fungi are reviewed as specific to the fungi used in axenic culture tests (sections 2.6.1 and 2.6.2). A general review is applied to soft rot as unsterile soil testing was used (section 2.6.3).

2.6.1. BROWN ROT

Coniophora puteana (Schum.:Fr.) Karst. is a cosmopolitan temperate basidiomycete. It is saprophytic on all kinds of wood especially in sapwood of most European wood species. This fungus promotes a brown rot type of decay i.e. brings about the decomposition of cellulose and hemicellulose rapidly whilst lignin is only very slowly altered (Seifert, 1962; Kirk & Highley, 1973; Ruel & Barnoud, 1985). Within wood tissues this fungus grows profusely and proliferates randomly without respect to the alignment of tracheids, and double clamp connections are usually seen (Roff, 1964). According to Apenitis et al. (1951) 95% of the cellulose was decomposed in the sapwood of Pinus sylvestris at a weight loss of 68%. In seven strains of C. puteana tested by Gersonde, cited by Hof (1979), it was found that the rate of decay was variable on Pinus sapwood, varying between 15-55% after 16 weeks at 20 °C and 25-50% at 26 °C. Allsop & Seal (1986) defined C. puteana as a wet rot fungus since it needs high humidity for growth and has an optimal substrata moisture content of over 50%. It causes considerable shrinkage of wood and characteristic cubic cracking similar to that of dry rot i.e. deep longitudinal, as well as, horizontal cracks in samples.

Gloeophyllum trabeum (Pers.:Fr.) Murr. is a saprophytic basidiomycete which is liable to attack both soft- and hardwoods. G. trabeum promotes a brown rot type of decay sometimes in form of pockets, in which only the carbohydrate fractions of the wood are metabolized (Kaarik, 1979). This fungus causes a rapid rate of decay and it has the ability to withstand a very wide range of moisture content of wood varying from air dry to nearly saturated (Cartwright & Findlay, 1958). They pointed out that G. trabeum may survive for up to 10 years in air-dry wood at 12% moisture content.

2.6.2. WHITE ROT

Among the white rot fungi reputed to decompose lignin, cellulose and hemicellulose simultaneously Coriolus versicolor (L.) Quel. has probably received the most study. C. versicolor is a commonly occurring cosmopolitan saprophytic basidiomycete most frequently found in temperate countries on hardwoods; softwoods are also liable to be attacked (Dirol, 1979). According to Findlay, cited in Cartwright & Findlay (1958) it can cause the complete destruction of wood and Cowling (1961) reported that over 97% of the lignin in Sweetgum wood was destroyed by C. versicolor. Lignin removal by this fungus is always

accompanied by the removal of polysaccharides, (Cowling, 1961; Kirk & Moore, 1972; Wilcox, 1973).

Pycnoporus sanguineus L.:Fr. is a basidiomycete found mainly in the tropics and under natural exposure conditions. It causes considerable deterioration even in supposedly durable timbers (Cardias & Jesus, 1985). This fungus attacks a wide range of timber, including angiosperms, gymnosperms and palms. This fungus causes a white rot and it is common on standing, felled and fallen timber and is occasionally found on external timbers i.e. in substrates where water content is high (Rayner & Boddy, 1988).

2.6.3. SOFT ROT

Deuteromycotina and certain Ascomycotina promoting a soft rot type of decay have been reported on different categories of woody materials (Seehann et al.,1975; Stewart et al., 1988). Seehann et al. (1975) reported on the ability of 650 strains of 365 species to cause soft rot in untreated wood, 305 species were classed as true soft rot fungi because they were able to produce cavities or erosion attack in wood cell walls or caused weight losses of at least 3% of original oven dry weight. Stewart et al. (1988) list nearly 1600 species of Deuteromycotina and Ascomycotina and stated that many of these organisms

cause a soft rot of wood and wood products; others discolor wood or otherwise reduce its market value.

Studies on fungal succession (Butcher, 1971) and tolerance to wood preservatives (Zabel, 1954; Young, 1961; Unligil, 1968; Duncan & Deverall, 1964) suggest that wood destroying fungi show variations in resistance to certain wood preservatives and some organisms may inactivate certain compounds lowering their effectiveness. Soft rot fungal tolerance to wood preservatives was first reported by Savory (1955).

Soft rot differs from the brown and white rot decay types because it is primarily a surface form of deterioration starting in outer layers of exposed wood and moving inward as outer surfaces deteriorate (Corbett & Levy, 1963; Courtois, 1963). The decomposition extends somewhat more deeply into the wood in tracheids adjacent to rays (Corbett & Levy, 1963). Corbett (1965) found that decomposition of test blocks occurred at a different rate, depending on which block face was placed in contact with the fungal culture. The order of the effect of the contacting face upon rate of decomposition from fastest to slowest, was, transverse, tangential, and radial. A higher lignin content of the radial walls was considered a possible explanation for their slow destruction (Corbett, 1965).

2.7. MECHANISMS OF WOOD DECOMPOSITION

Dissolution of cell walls by fungi was first demonstrated by De Bary (1886) cited by Waksman (1946a), who indicated the formation of a cellulose dissolving enzyme. Decomposition of cellulose has long been regarded as a hydrolytic process (Reese et al., 1950) which takes place in more than one stage. The earlier oxidation theory of cellulose breakdown (Winogradsky, 1926, 1929; cited by Szegi, 1988) was for several decades unsubstantiated, but more recently oxidative degradation with brown rot (Koenigs, 1974) and white rot (Eriksson & Petterson, 1975a; Ayers at al., 1978) has been shown.

2.7.1. HYDROLYTIC ENZYMES

The generic term cellulase comprises a group of enzymes that are biocatalysts which accelerate and control biochemical reactions. An early hypothesis in enzymatic cellulose depolymerisation was the C_1/C_x stepwise system of Reese et al. (1950). According to this hypothesis the first step in cellulose decomposition is catalysed by a C_1 enzyme which breaks the hydrogen bonds between the anhydride chains as a preliminary to hydrolysis by C_x enzyme. At that time it was assumed that the C_1 enzyme did not affect the β 1-4 glycosidic linkages in the cellulose chains to

give shorter polysaccharide chains. Wood & McCrae (1972) pointed out that there is no evidence to support the existence of a non-hydrolytic chain disaggregating enzyme and demonstrated that the hydrolysis of native cellulose is the result of the synergistic action of endo- and exo-glucanase enzymes. The suggestion that C_1 is an exo-cellulase was later altered (Reese, 1977) so that the ability of the C_1 to hydrolyse the bonds of released cellulose was recognised.

Investigations (Eriksson, 1969; Wood & McCrae, 1972,1978; Streamer et al., 1975) have lead to the theory that fungal hydrolytic cellulases consist of different enzyme groups (Endo- & exo-glucanases) which act synergistically to bring about cellulose decomposition. In addition, a β-glucanase that hydrolyses cellobiose and other cello-oligosaccharides to glucose is widely reported (Reese & Levinson, 1952; Eriksson & Petterson, 1975a,b; Wood & McCrae, 1972, 1978).

Mechanisms involved in cellulose and hemicellulose degradation in the whole wood by brown rot fungi are not resolved. In vivo studies of the degradation of isolated cellulose by brown rot fungi demonstrated that cellulases exhibit very litle activity toward insoluble crystaline celluloses (Highley, 1973). King (1966) stated that C. puteana possesses all the polysaccharide degrading enzymes

capable to depolymerise all the major wood cellulose and hemicellulose.

It is believed that cellulases and hemicellulases of brown rot fungi are too large to diffuse into wood (Cowling & Brown, 1969; Highley et al., 1981). Both chemical (Highley, 1977) and microscopic analysis (Highley et al., 1983a,b) of brown rotted wood and cellulose point to involvement of a small, non-enzymic depolymerizing agent, at least initially, in the degradation of cellulose. This approach was also reported by Koenigs (1974) which H_2O_2-Fe^{++} system may act as a starter substance or aid in decomposing the crystalline cellulose.

An enzyme complex capable of degrading various polysaccharides and glycosides was isolated from P. placenta, (Highley et al., 1981). It is an aggregate of various polypeptides with a molecular weight of 185000 which under the right conditions break down into small sub-units. The enzyme was found to be active for xylan, glucomannan, carboxymethylcellulose and some glycosides. Highley (1980) stated that cellulose degrading enzyme of P. placenta requires an alternative carbon source to become established and to degrade cellulose. For example, mannan was the best carbohydrolases inducer in the brown rot P. placenta (Highley, 1977).

Contrary to P. placenta, there is evidence that certain brown rot fungi (e.g. C. puteana) do not

require additional factors such as a "starter sugar" to become established and to degrade cellulose (King, 1966; Highley, 1980).

One of the most studied fungi concerning the enzyme mechanisms involved in cellulose degradation is the white rot fungus Sporotrichum pulverulentum (=Phanerochaete chrysosporium Burds. teleomorphic stage). According to Eriksson (1978) this fungus produces: five endo-1,4-β-glucanases that attack the cellulose chain at random, splitting β 1-4 glycosidic linkages; one exo-1,4-β-glucanase that splits off either cellobiose or glucose from the non-reducing end of the cellulose (Eriksson & Pettersson, 1975a,b); and two 1,4-β-glucosidase that hydrolyse cellobiose and other water-soluble cellodextrins to glucose (Deshpande et al., 1978).

2.7.2. OXIDATIVE ENZYMES

In addition to the hydrolytic enzymes, the oxidative enzymes cellobiose:quinone oxido-reductase and cellobiose oxidase (Westermark & Eriksson, 1974a,b; Ayers et al., 1978) are involved in the cellulose degradation by S. pulverulentum. The former is important in both cellulose and lignin degradation. Its function is to reduce phenoxy radicals and quinones formed by the action of phenol oxidases on degradation products from lignin. As a result

cellobiose and higher cellodextrins are oxidized to the corresponding lactones (cellobiono-δ-lactone). The latter i.e. cellobiose oxidase, uses molecular oxygen to oxidize cellobiose and higher cellodextrins to their corresponding aldonic acids (Ayers et al., 1978).

Waksman (1946b) stated that part of the mechanism of lignin decomposition by fungi consists of the splitting off of the methoxyl groups, making it more alkali soluble. The methoxyl content of lignin in wood which has been decomposed by fungi is lower than that of lignin in sound wood. Early investigations showed that oxidizing agents with low specificity are involved in the biodegradation, but did not reveal the nature of these agents (Waksman, 1946b). Kirk (1971) reported that there seem to be three main reasons why phenol oxidases have been considered in connection with lignin breakdown: a) the lignin degrading white rot fungi produce easily detectable extracellular phenol oxidizing enzymes, whereas the taxonomically closely related brown rot fungi, which alter but do not decompose lignin, apparently do not produce phenol oxidases or at least not extracellularly; b) the fact that lignin is a phenolic material and should therefore be a substrate for phenol oxidizing enzymes, which it is; and c) the awareness for many years that lignin decomposition is primarily an oxidative process.

The precise role of the phenol oxidizing enzymes is not completely clear. It seems that the types: laccase, tyrosinase, or peroxidase, catalyse the abstraction of electrons from substrate phenols. A possible involvement of hydrogen peroxide (H_2O_2) derived hydroxyl radical (.OH) in lignin degradation by P. chrysosporium was reported by Forney et al. (1982). Subsequently Tien & Kirk (1983) described a ligninase, a glycosylated haemoprotein that decomposes lignin to CO_2. It was reported to require both hydrogen peroxide and molecular oxygen for activity and to degrade both a range of lignin model compounds and extracted native lignin. The model compound investigated (Tien & Kirk, 1983; Paterson & Lundquist, 1985) contained the β aryl ether linkages. Results indicate that the initial reaction in the attack of lignin model is a one electron oxidation that generates relatively stable aromatic radical cations in the lignin polymer. They postulated that once generated by the ligninase, the radical cations are themselves able to act as one electron oxidants which can oxidize sites remote from the enzymes. Paterson & Lundquist (1985) stated that ligninase is clearly distinct from the metalloenzymes laccase and peroxidase in which laccase oxidizes substrates containing free phenolic hydroxyl groups, whereas ligninase is capable of oxidizing substrates with alkoxyl substituents on the ring.

Studies by Kirk (1975) on the effects of Lenzites trabea (=G. trabeum) on lignin in Spruce wood suggest that extracellular oxygenases are involved in the lignin breakdown, but also demonstrated that the fungus lack certain abilities that permit complete catabolism of the polymer. Lack of a complete understanding the mechanism by which fungi extensively degrade lignin may lie in the complexity of lignin polymer of which the structure is not yet fully elucidated.

2.7.3. DETECTION OF ENZYME PRODUCTION

A number of simple in vitro tests indicative of hydrolase and oxidase activity have been used since the earlier half of this century (Bavendamm, 1928; Levinson & Reese, 1950; Nobles, 1958). On agar plates cellulases may be detected by clearing zones of the insoluble cellulose around fungal colonies. Soluble derivatised cellulose, C.M.C. has been widely used (Reese et al., 1950; Levinson & Reese, 1950) and solubilization of cotton by microorganisms has also been measured by the increase in reducing sugars. Changes in viscosity using C.M.C. or Na salt (C.M.C.) as a substrate have also been used (Levinson & Reese, 1950).

Colourimetric changes are usually used to assess oxidase production. Phenolic substrates change colour

in the presence of oxidases and atmospheric O_2, thus giving a clearly visible result. Even though variability does exist, the tests for detecting the production of phenol oxidases by fungi (Bavendamm, 1928; Lyr, 1958; Kaarik, 1965, Nobles, 1958; Harkin & Obst, 1973) is correlated with white (phenol oxidase positive) and brown rot fungi (phenol oxidase negative) and their abilities to degrade lignin. In brown rot fungi the lignin is only slightly altered while with white rot types lignin is substantially degraded.

Standard white rot organisms e.g. C. versicolor, P. sanquineus usually give positive results for extracellular oxidase, whereas standard brown rot organisms e.g C. puteana, G. trabeum (=Lenzites trabea) give negative results (Nobles, 1958, 1965) using gallic or tannic and gum guaiac as a substrate.

With the exception of P. sanquineus which was not listed, Kaarik (1965), using a series of phenolic compounds, found similar results for C. versicolor, L. trabea and C. puteana among other organisms tested. The same results for these fungi were obtained when syringaldazine were used as a substrate for laccase, or in its absence, peroxidase detection (Harkin & Obst, 1973). With a dilute solution of syringaldazine [N, N'-bis-(3,5-dimethoxy-4-hydroxybenzylidene) hydrazine] in ethanol, a mixture of alcohols, or dimethyl sulfoxide a colour change from yellow to deep

purple in the presence of laccase and air or peroxidase plus hydrogen peroxide rapidly occurs. The colour change is a result of the twofold phenol hydrogenation of syringaldazine and from intramolecular pairing of the free radicals produced to yield the highly coloured conjugated tetramethoxy-azo-bis-methylene-quinine (Harkin & Obst, 1973).

2.8. FUNGAL EFFECTS ON WOOD

Changes in density of wood as a result of fungal attack are direct correlated to changes in the overall properties of wood which are the subject of the following sections.

2.8.1. EFFECTS ON CHEMICAL PROPERTIES

2.8.1.1. BROWN ROT

Studies on the chemical composition of decayed wood by brown rot fungi show an extensive depolymerization of polysaccharides and a rapid decrease in the degree of polymerization (DP) of cellulose (Cowling, 1961, Kirk, 1975; Ruel & Barnoud, 1985; Eriksson & Wood, 1985). Cellulose and possibly hemicelluloses undergo an extensive depolymerization before much weight loss has occurred and subsequent degradation and removal occurs progressively (Kirk &

Highley, 1973).

Holocellulose decayed by a brown rot fungus Poria monticola (=Postia placenta) shows a rapid breakdown of the long cellulose molecules to smaller units, suggesting a random splitting, possibly in the amorphous regions of the microfibrils (Cowling, 1961). Hygroscopicity and X-ray diffraction measurements indicate a reduction in the amorphous material of the microfibrils. Analysis of the individual sugars shows that all are used at essentially constant rates at all stages of decay, the rates being approximately proportional to the amounts of the sugars in sound wood (Cowling, 1961). The activity of C. puteana in pine wood is characterized by a two step degradation, which the first step is an intensive depolymerization of the cellulose (Ruel & Barnoud, 1985). In the second step, there is a further degradation of depolymerized cellulose and an attack of the hemicelluloses (Kirk, 1975).

The effects of brown rot fungi on lignin is regarded as minimal, but solubility in dilute alkali (1% NaOH) reduces as decay proceeds (Cowling, 1961).

2.8.1.2. WHITE ROT

White rot fungi degrade and utilise all wood constituents. The relative amounts of carbohydrate and lignin degraded and utilised by these fungi vary, as

does the order of preferential attack (Eriksson, 1981).

Polyporus versicolor (=Coriolus versicolor) utilises the carbohydrate, individual sugars, and the lignin at all stages of decay, the rates being constant and proportional to the amounts present in sound wood (Cowling, 1961). The degree of polymerization of the holocellulose fraction drops only slowly, and the amorphous and crystalline regions of the microfibrils are attacked simultaneously (Eriksson & Wood, 1985; Levi & Preston, 1965). During decay there is little change in the solubility properties of the wood suggesting that the wood constituents are metabolized as soon as they are depolymerized.

Kirk & Moore (1972), studied the removal of lignin from wood by white rot fungi found similar results i.e. that lignin removal was always accompanied by the removal of polysaccharides. However, it was found that P. versicolor removed lignin faster from birch in early stages of decay, but in aspen wood both carbohydrate and lignin were removed at the same relative rates.

2.8.1.3. SOFT ROT

Wood polysaccharides are preferentially attacked by soft rot fungi. Studies on the chemical

constituents of beech wood attacked by Chaetomium globosum indicate that soft rot, unlike white rot, does not markedly attack lignin (Savory & Pinion, 1958; Levi & Preston, 1965). In contrast with brown rot, soft rot does not cause any marked changes in the alkali solubility of wood in the early stages of attack (Savory & Pinion, 1958). The alkali solubility of decayed wood by soft rot fungi decreases slowly, and solubility in hot water and ethanol-benzene remaining approximately constant (Levi & Preston, 1965).

In contrast to brown rot, the degree of polymerization does not drop suddenly in Beech wood attacked by C. globosum but more gradually as found with white rot (C. versicolor) (Levi & Preston, 1965). Carbohydrates were metabolized at varying rates, the ratio of xylose to glucose in the decayed beech wood increases at first and then decreases as decay proceeds. The amounts of mannose and galactose slowly increase.

2.8.2. EFFECTS ON MECHANICAL PROPERTIES

The effects of early stages of decay on wood mechanical properties has been reviewed by Wilcox (1978) and Hardie (1980). Wilcox (1978) concluded that the properties most severely and rapidly diminished by the onset of decay are toughness and impact bending

properties. These properties appear to be almost totally lost with losses in weight of less than 10%; some other mechanical properties are affected to only minor degrees (Wilcox, 1978). Hardie (1980) concluded that strength loss appears to be a useful criterion for assessing decay by microorganisms especially during initial decay where weight losses are low. Hardie (1980) pointed out that one of the disadvantages may lie in the careful selection of samples necessary in order to reduce inter sample variability to a level at which small strength losses become significant.

2.8.2.1. BROWN ROT

The influence of brown rot fungi on strength properties is directly associated with the rate at which the polysaccharides are affected. It is well established that the physical and chemical nature of the cell wall is of great importance in determining mechanical strength (Clarke, 1935; Ifju, 1964). As concluded by Hartley (1958) cited by Cowling (1961) that at comparable stages of decay, as measured by weight loss, brown rot fungi reduce the strength of wood more than do white rot fungi. Cowling (1961) also stated that Pechmann & Schaile (1950) have suggested that differences in rate of strength reduction between the two major types of wood decay may be due to a more

rapid depolymerization of the cellulose of wood by brown rot fungi.

The influence of all three types of fungal decay on the strength properties of beech wood was reported by Armstrong and Savory (1959). They indicated that brown rot caused the greatest loss, while white and soft rots caused smaller losses. Studies on the effect of brown rot fungi on the loss of strength in both hardwoods and softwoods was found to be greater in hardwoods than in softwoods (Kennedy and Ifju, 1962). They found that in hardwoods a strength loss of approximately 56% was encountered at 2% weight loss and a loss of 82% at 8% weight loss, while in the softwoods approximately 23% loss in strength was encountered at 2% weight and 50% at 8% weight loss. Brown rot effects on softwood were reported by Brown (1963) in which he found that strength losses were somewhat higher than those reported by Kennedy and Ifju (1962). Henningsson (1967) reported that Betula pubescens decayed by C. puteana had lost 71% of its impact bending strength but only showed a 13% weight loss. Brown rot decay beyond 10% weight loss caused greater strength loss than white rot decay, while up to an approximate 5% weight loss the effects on strength was similar for both decay type (Henningsson, 1967). Bravery & Grant (1971) also reported rapid loss of tensile strength in thin strips of pine wood exposed for five weeks to Coniophora cerebella (= C.

puteana). At 20% weight loss of Scots pine caused by C. puteana a 90% loss in impact strength occurred after 42 days' incubation period. They stated that the greater the length of incubation the greater the reduction in strength.

2.8.2.2. WHITE ROT

Wood decayed by white rot fungi has a pattern distinct from that of brown rotted wood. White rotted wood tends to be tougher and more elastic rather than extremely brittle as that of brown rotted wood. Thus the differences in the strength properties of wood decayed by white and brown rot fungi may very likely be due to differences in the nature of their effects on cellulose (Cowling, 1961). The white rot fungi tested by Henningsson (1967) produced losses in impact bending strength of approximately 20% at a 1% weight loss, 50% at a 6% weight loss, and 60% at a 9% weight loss. Kennedy and Ifju (1962) studied the effects of both brown and white rot in both hardwoods and softwoods on loss in tension strength parallel to the grain. They found that with white rot great variability was shown with both species of wood and fungus. In white rotted softwoods the loss in tension strength parallel to the grain at 2% weight loss varied from 4% to 38%, and at 10% weight loss varied from 20% to 63% strength loss. Strength loss due to

white rot in hardwoods varied from 22% to 42% at 2% weight loss and from 20% to 50% at 10% weight loss (Kennedy & Ifju, 1962). Wilcox (1978) concluded that at very early stages of decay there may be little difference in the effects on strength of brown rot and white rot fungi or whether the wood is a hardwood or softwood. It is apparent that all strength properties are essentially destroyed by late stages of decay.

2.8.2.3. SOFT ROT

Studies on wood degradation by soft rot fungi (Hale & Eaton, 1985; Nilsson, 1976) found that the steps necessary for soft rot cavity attack included penetration into the cell wall by a hyphae, alignment of the hyphae in the microfibrillar direction and production of enzymes which will dissolve wall around the hyphae. During invasion of the secondary walls bore hyphae are formed which perforate the cell walls in a lateral direction (Courtois, 1963; Levi & Preston, 1965). This has a marked effect on the strength properties of the wood.

Studies by Armstrong & Savory (1959) on beech wood exposed to C. globosum indicate that a sharp reduction in toughness (impact resistance) occurred before significant weight losses, and that reduction in bending strength was more gradual and was not significant until definite weight losses were evident.

They also compared the mechanical strength of soft rot to that of brown and white rot fungi and found that the changes was more closely comparable with a white rot than with brown rot. The same effect on beech wood by C. globosum was stated by Takahashi and Nishimoto (1967). Henningsson (1967) who studied the effects of 12 decay fungi on the strength properties on Birch wood found that variations in weight loss were in most cases very small, while the strength showed greater variation.

2.9. EFFECT OF MOISTURE CONTENT ON WOOD DECOMPOSITION

The interaction of water and wood is of major importance to wood decay. To what extent wood resists fungal attack depends largely on its water content. The optimum moisture content for the growth of any particular fungus cannot be exactly defined without reference to the particular kind of wood, because it varies according to the nature and density of the wood itself. Generally wood cannot be colonized unless its moisture content is above 25 - 30 per cent (as a percentage of oven dry weight). This stage is reached in undecayed wood when the pore space is devoid of free water but the cell wall remains saturated with bound water. This is termed the fibre saturation point (F.S.P.). Griffin (1977) defines it as "that water content of wood that is in equilibrium with a matric

potential of -0.1 MPa. Below this point, changes in moisture involve removal of "bound" water and consequently affect virtually all of the physical characteristics of wood resulting in modification of the cell wall structure (Youngs & James, 1965).

Within wood, soil and other substrates the availability of water to the decomposer organisms is affected by two main forces, matric potential and osmotic potential (Boddy, 1983). Matric potential is a result of forces associated with the interfaces between air and the solid matrix, and osmotic potential is a result of the presence of solutes within the water (Boddy, 1983). However, Griffin (1977) states that the effect of water potential on the growth of microorganisms theoretically will not differ whether it is altered by osmotic or by matric means.

On the other hand the water activity of a material is a measure of the availability of the water, in this context, to the microorganism and the atmosphere in which it grows. It is expressed as the ratio of the vapour pressure of water over the material to the vapour pressure over pure water at the same temperature. The water activity of pure water is 1.000 and this decrease as solutes are added (Allsopp & Seal, 1986).

Even under <u>in vitro</u> circumstances the influence of water on fungal growth has received increasing

attention in recent years (Boddy, 1983; Clark et al., 1980; Griffin, 1977). These studies have shown that wood decaying basidiomycetes appear to be particularly sensitive to decreased water potential of the substratum.

Moisture content expressed as a percentage of the oven dry weight is a widely used term in the assessment of water availability to decay fungi. It has been stated that decay at low moisture content below F.S.P. could be limited by the accessibility of the cell wall constituents to fungal enzymes (Cowling & Brown, 1969) and at high moisture contents by a low oxygen tension inside the water-saturated wood (Griffin, 1968). Following the same point of view on various non-woody substrates Bunnell et al. (1977) considered that moisture content may limit decomposition in two ways: a) in the low moisture content region where decomposer organisms require water for metabolism and, b) in the high moisture content region where decomposition is limited due to poor aeration.

The uptake of moisture after fungal colonisation may provide additional information related to structural parameters such as crystalinity and degree of polymerization (DP). As stated by Cowling (1961), since moisture is adsorbed largely in the amorphous regions of the cell walls, the amount of moisture adsorbed by wood is inversely related to the degree of

crystallinity of the cellulose and it is influenced by its composition. Studies on the hygroscopicity of the decayed wood (Cowling, 1961) indicated that at higher weight losses promoted by white rot fungi there was a significant increase in moisture adsorptivity and suggested that this increase may have been due to diminution in the amount of crystalline material in the wood. In brown rotted wood, moisture adsorptivity at relative humidity below 80% diminished at first rapidly and then in a more gradual and linear maner (Cowling, 1961). His explanation for this reduction in hygroscopicity is that the rapid initial decrease in average DP of the brown rotted holocellulose may have been due to preferential attack on amorphous cellulose, which lead to the accumulation of less hygroscopic crystalline cellulose. In the latter stage of decay the more gradual decrease in average DP suggested that the less accessible crystalline cellulose was degraded (Cowling, 1961).

CHAPTER 3.

CHEMICAL MODIFICATION OF WOOD WITH ISOCYANATES

CHAPTER 3.

CHEMICAL MODIFICATION OF WOOD WITH ISOCYANATES

3.1. INTRODUCTION

This chapter deals with the sample preparation and reaction of wood with isocyanates. Isocyanates have a high reactivity to hydroxyl groups and the blockage of the alcoholic groups by the substituent molecule has been shown to occur using relatively simple reaction systems (Baird, 1969; Kalnins, 1982; Rowell & Ellis,1979; Rowell, 1980; Ellis & Rowell, 1984; West, 1988).

When considering the functional groups of wood for isocyanate reactions several simplifications are used in the characterization of the covalent urethane bond formed. Fig. 3.1 shows a schematic diagram of the simplest theoretical possible units formed. With difunctional isocyanates more complex structures may be attained due to crosslinking possibilities.

Recalling the structural arrangement of the wood cell wall in terms of accessibility, the three major wood constituents must be considered. The response of cellulose to reactions of its hydroxyl groups depends on the distribution of ordered and disordered regions in the cell wall. Reactions start at fibril surfaces (amorphous region) and proceed into tightly hydrogen bonded crystalline regions. Isocyanate reactions with the cellulose hydroxyls in the whole wood seems to be

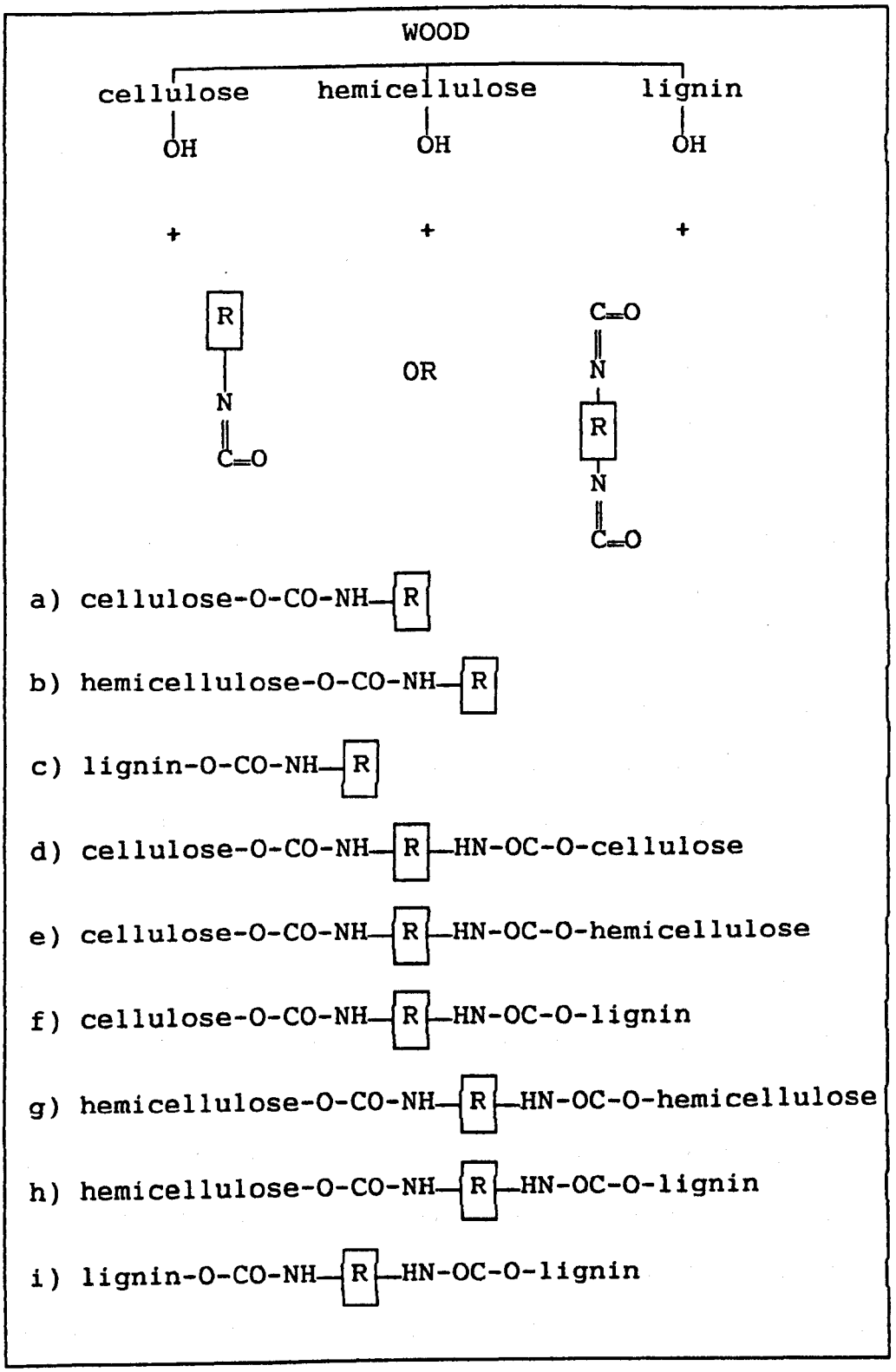


Fig. 3.1. Schematic representation of simplest units formed after wood-isocyanate reaction.

governed by the ease with which the treating solutions penetrate and open up the structure allowing access within the fibrillar composite. On the other hand excessive reaction will lead to loss in physical properties of the wood.

West & Banks (1987) suggested that within the polysaccharide fraction, reactions occur according to the order of accessibility: hemicellulose > non crystalline regions in cellulose > crystalline cellulose. The branched nature of the hemicellulose renders them more reactive and more easily penetrable. On the other hand substitution reactions of hemicellulose are a matter of concern since ease of substitution and solubility of hemicellulose in the cell wall can lead to degradation under certain chemical modification conditions.

In the wood cell wall lignin is concentrated in the compound middle lamella and diminishes in concentration in other cell wall layers. It forms an aggregate network entrapping the less complex structural units (cellulose & hemicellulose) in the wood tissue. There are indications (Rowell, 1982; West & Banks, 1986) that among the wood constituents, lignin reacts more rapidly with isocyanates than polysaccharides at the initial stage of reaction. Lignin reactivity is related to its location in the wood cell wall and to the reactivity of its hydroxyl groups.

The high reactivity of isocyanates to hydroxyl groups leads to the pre-requisite that wood samples are water and extractive free prior to reaction. Water, when present in the reaction vessel or in the samples, decreases solution concentration by reacting readily with isocyanates. Extractives are substances that form the non-structural portion of wood, and are unsuitable for improving desirable properties in modified wood.

For many chemical modification reactions in wood a good swelling solvent is essential to release the strong lateral forces of cohesion exhibited when wood is dry. Such a solvent can serves as a medium for carrying out chemical reactions but also reaction rates and yields are markedly improved. Improper dissociation of the hydroxyl groups in the polysaccharide portions of the whole wood could lead to poor solution penetration into the wood cell wall. The choice of pyridine used in this work is a result of literature review of Kalnins (1982) and West (1988). Pyridine is already an established swelling base catalyst that enhances wood hydroxyl reactivity. In order to reduce cost, it was matched with acetone. The mixture (pyridine:acetone) may also have a significant effect upon reduction in temperature of reaction which is essential in reducing thermal degradation of wood constituents. Furthermore, it is apparent that a solvent of lower boiling point like

acetone facilitates drying after reaction.

Besides the effect of moisture, extractives and solvents; temperature has a marked effect on the reaction rate and in the integrity and the nature of wood chemical constituents. Usually, high temperatures give rise to faster reaction rates. Reaction temperatures of 50-120 °C are most commonly used in the chemical modification with isocyanates, giving short reaction times. The methods of treatment employed (e.g. weak alkaline catalyst, pressure) accelerate the reaction and improve the stability of chemical bonds formed. Reaction of n-butyl isocyanate with pine wood shows first order kinetics when excess reactant and swelling solvent are used in a system controlled by reaction temperature and time (West, 1988). The yield of isocyanate reacted material is established by increase in weight (weight percent gain, WPG), volume and by infra-red (IR) spectra. These changes are attributed to the isocyanate side or intermediate chain formation onto the polysaccharide and lignin hydroxyl groups which fill up the cell walls.

3.2. THE AIM OF THE PROCEDURE

The purpose here is to assure wood-isocyanate bond formation and to establish a reaction procedure for use throughout the subsequent chapters. The

reaction procedure is designed to produce wood blocks reacted to various levels of chemical modification with three isocyanates.

3.3. MATERIALS & METHODS

3.3.1. REAGENTS

Three aliphatic isocyanates were used, two monofunctional and one difunctional. Table 3.1 shows some of the properties of the reagents used.

TABLE 3.1

ISOCYANATES PHYSICAL-CHEMICAL CHARACTERISTICS

ISOCYANATES / STRUCTURAL FORMULA	MOL.Wt.	DENSITY (g/cm^3)	VOL./MOL (ml)
n-Butyl $CH_3(CH_2)_3NCO$	99.13	0.88	112.65
Hexyl $CH_3(CH_2)_5NCO$	127.19	1.2	105.99
1,6-Diisocyanatehexane $OCN(CH_2)_6NCO$	168.20	1.04	161.73

3.3.2. SOLVENTS

Analytical grade pyridine (C_5H_5N) and acetone (C_3H_6O) were used in all experiments.

Pyridine is a colourless refractive hygroscopic liquid with a boiling point of 115.3 °C, has a strong characteristic smell and is a strong base catalyst. Water free pyridine was obtained through addition of potassium hydroxide (KOH) prior to reactions.

Acetone is a volatile liquid with a boiling point of 56 °C. 100% anhydrous acetone was obtained

by addition of anhydrous sodium sulphate ($CaSO_4$) prior to reactions.

3.3.3. REACTION VESSEL

The reaction vessel consisted of a stainless steel cylinder, 300 mm long x 90 mm diameter, screw lid and an inner lid of 10 mm thickness (Fig. 3.2).

3.3.4. SAMPLING

Sapwood of Corsican pine (Pinus nigra Schneid) selected free from defects of parental boards possessing 4-5 annual rings per centimetre was used in all experiments. Samples were cut, 20 x 20 x 10 mm, sanded and grouped by density to improve homogeneity of treatment.

3.3.5. NUMBER OF BLOCKS PER TREATMENT

Number of blocks in each batch varied according to the purpose of assessment. Duplicates were reacted for preliminary establishment of reaction rates; batches of five replicates for volumetric measurements; and due to the capacity of the reaction vessel, blocks for biological tests (chapter 4 & 5) did not exceeded 30 per modification reaction. Desired levels of modification were achieved by variation in reaction times.

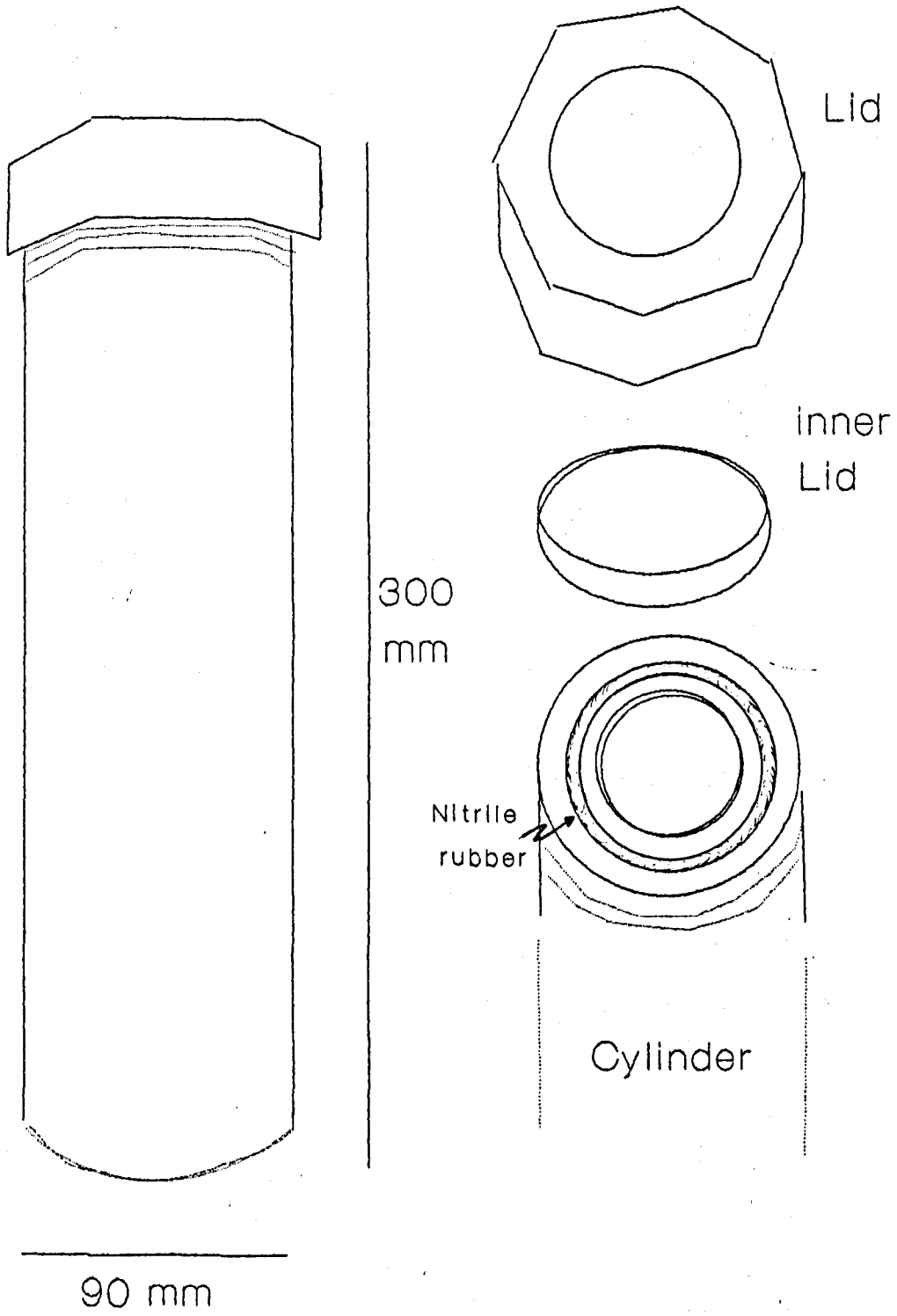


Fig. 3.2. Stainless steel reaction cylinder

Blocks treated with two solvent systems were produced. Two sets of control samples were used, one set were treated with pyridine and the other one with a 1:1 mixture of pyridine and acetone.

3.3.6. SAMPLE EXTRACTION

All samples were labelled with 2 B pencil and extracted in acetone in a Soxhlet apparatus for 2 hours, oven dried to a constant weight at 105 °C, and weighed. This weight was recorded as W_1.

3.3.7. SAMPLE HANDLING AFTER EXTRACTION

To maintain samples dry prior to modification, they were placed in a oven maintained at 50 °C. Longitudinal, radial and tangential faces of the blocks were measured with a micrometer. The original volume of the sample expressed in cubic centimetre (cm^3) were recorded as V_o.

3.3.8. SOLVENT TREATED UNREACTED CONTROLS

After extraction, treatment of control blocks was performed in a litre flange pot in a Soxhlet extractor for one hour. One group of blocks were boiled in acetone and the other in a mixture of acetone and pyridine in 1:1 proportion. Sample

handling after treatment i.e. clean up and drying were the same to that performed to modified samples (section 3.3.11).

3.3.9. CHEMICAL MODIFICATION OF TEST SPECIMEN

Excess isocyanate solution was used for the treatment of each block i.e. for the reaction of 30 specimens, 750 ml of a molar isocyanate solution was used for all chemicals. To make up the solution strength (1 M) the reagent was dissolved in the 1:1 pyridine and acetone mixture.

After extraction, dry test specimens were placed in the reaction vessel spaced with glass cotton wool, and argon was introduced to maintain a dry internal atmosphere. Reagent solution was added and glass cotton wool was added at the top to keep the samples submerged. The lid was then tightly closed and the reaction vessel was placed in a pre-heated oil bath at 100 °C. Modification levels were achieved at different reaction times. At the end of each reaction time, the reaction was stopped by cooling the cylinder in tap water.

3.3.10. SAMPLE HANDLING AFTER MODIFICATION

The reacted blocks were immediately rinsed and extracted with acetone in a Soxhlet extractor for 4 hours. Following the clean up procedure the blocks

were oven dried at 105 °C and reweighed. The weight of each replicate after modification was recorded as W_2. The dry sample dimensions of each specimen (longitudinal, radial and tangential) were measured with a micrometer, and the final volume recorded as V_f.

3.3.10.1. CALCULATION OF WEIGHT PERCENT GAIN (WPG)

The WPG's were determined according to the equation (3.a).

$$\text{Weight percent gain (WPG)} = (W_2 - W_1)/W_1 \times 100 \quad (3.a)$$

Where:

W_1 = oven dry weight of the sample before modification (after extraction);

W_2 = oven dried weight of the sample after reaction.

3.3.10.2. CALCULATION OF VOLUMETRIC CHANGES

After reaction, clean up and drying, the dimensions of each specimen were measured as in section 3.3.7. and recorded as V_f. The increase in wood volumes were determined as shown in equation (3.b).

$$\text{Volume increase } (cm^3) = V_f - V_o \quad (3.b)$$

Where:

V_o = Volume of the block before modification (original volume after extraction);

V_f = Final volume of the block after reaction.

3.3.10.3. CALCULATION OF VOLUME OF CHEMICAL ADDED

Based on the equation (3.c), the calculated volume of isocyanate added in each sample was theoretically determined as following:

$$\boxed{\text{Volume} = \text{Mass/Density}} \qquad (3.c)$$

where:

Mass of isocyanate = $W_2 - W_1$

Volume of isocyanate (V_i) = $(W_2 - W_1)$/Density of isocyanate

3.3.10.4. INFRA-RED SPECTROSCOPY

The solid Potassium bromide (KBr) method was used for the comparison of specific functional groups in unreacted and isocyanate modified wood as following:

Selected samples were scraped with a razor blade forming a fine powder, and a 1-2 mg of the dry wood powder was weighed. 100 mg of pre-ground KBr was mixed with the wood sample. The fine powder was poured in an even layer on the bottom bolt of the mini-press apparatus. The top bolt was tightened gently against the other bolt. The mini-press was then vacuum pumped for approximately 5 minutes, then the bolts were further tightened with wrenches, and a further 15 minutes suction was applied. After removal of the

bolts a transparent KBr pellet containing the sample was immediately run through an infra-red spectrophotometer (Perkin-Elmer 1310).

3.3.11. CALCULATION OF YIELD OF HYDROXYL REACTED

The order of magnitude of hydroxyl reacted was estimated to enable comparisons between isocyanates based on the WPG's achieved. For this, the composition (Table 3.2) of Scots pine (Pinus sylvestris) was used as a model for the calculations. It is in close aggreement in composition with the Corsican pine being used throughout this studies (Markwic et al., 1966, cited in Fengel & Wegener, 1984).

TABLE 3.2

CHEMICAL COMPOSITION OF Pinus sylvestris

Component in Sample	Component (%)	OH Groups per unit	Mol. Wt. of units
Lignin	26	1 /C_9	180
Cellulose	52	3 /C_6	162
Hemicellulose			
Hexosan	14	3 /C_6	162
Pentosan	8	2 /C_5	132

Data compiled from Fengel & Wegener (1984) and West (1988).

The total yield of moles of hydroxyl per gram of wood was estimated as shown in the equation (3.d).

$$\text{no. of moles} = \frac{\text{mass of component in sample}}{\text{molecular weight of component}} \qquad (3.d)$$

where:

no. of moles of -OH groups per gram of wood =

$$\underset{\text{lignin}}{\frac{0.26x1}{180}} + \underset{\text{cellulose}}{\frac{0.52x3}{162}} + \underset{\text{hexosan hemicellulose}}{\frac{0.14x3}{162}} + \underset{\text{pentosan}}{\frac{0.08x2}{132}} = 0.0149 \text{ moles}$$

The percentage yield of a product (-OH reacted) is the percentage of its theoretical yield achieved as calculated according to the equation (3.e).

$$\text{yield of -OH reacted (\%)} = \frac{\text{mass produced}}{\text{theoretical yield}} \times 100 \qquad (3.e)$$

where:

mass produced = Actual Weight Gain in sample

theoretical yield = is the MAXIMUM WEIGHT GAIN that can be obtained from a given mass of a specified reactant.

REACTANT A

Since 1 mol of:
n-Butyl isocyanate = 99.13 g/mol;
Hexyl isocyanate = 127.19 g/mol;
1,6-Diisocyanatehexane = 168.20 g/mol;

REACTANT B:

Hydroxyl groups per gram of wood = 0.0149 moles, then:

Maximum Weight Gain = 0.0149 moles X Mol. Wt. of isocyanate

Adjustments for the difunctional isocyanate were made by multiplying the results by 2. This assumes that isocyanate reacted only to wood hydroxyls.

3.4. RESULTS

3.4.1. REACTION RATE AND WEIGHT PERCENT GAIN (WPG)

Results in Tables 3.3, 3.4 and Fig. 3.3 show that Corsican pine blocks were satisfactory reacted with isocyanates.

The effect of reaction time on weight increase (WPG) of esterified wood showed that at 100 °C a covenient reaction rate was obtained under pyridine:acetone catalysis (Table 3.3 & Fig. 3.3).

With monofunctional isocyanates there were consistent increases (r^2=0.91, BuNCO; r^2=0.95, HeNCO) in WPG with increasing reaction time. Reaction with BuNCO was faster than with HeNCO. Reaction proceeded rapidly during the initial stage of reaction, then more gradually as increasing WPG (Table 3.3 & Fig. 3.3).

Reaction with the difunctional isocyanate (HDI) proceeded rapidly at initial substitution levels, but contrary to the monofunctional isocyanates, it was much slower at higher substitution levels (>15 WPG) despite considerably extended reaction times (Table 3.3 & Fig. 3.3). Higher addition of HDI could only be achieved by retreatment with fresh reagent (Table 3.4).

Table 3.4 shows the average WPG with its respective standard deviation of blocks reacted with BuNCO, HeNCO and HDI. These were based on the reaction profile shown in Fig. 3.3. Reaction with BuNCO varied

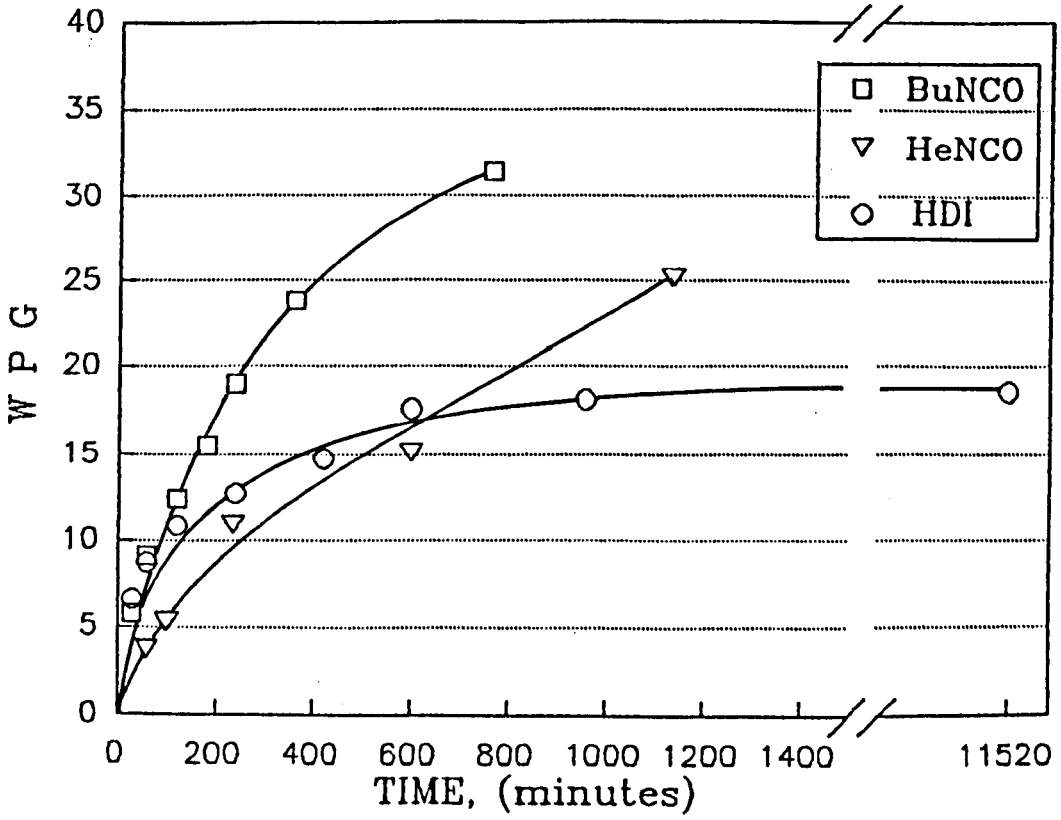


Fig. 3.3. Reaction rate of Corsican pine blocks with isocyanates at 100 °C.

TABLE 3.3

EFFECT OF REACTION TIME ON WEIGHT INCREASE (WPG) OF ESTERIFIED WOOD AT 100 °C. FIGURES REPRESENT AN AVERAGE OF DUPLICATES.

Time (mins.)	30	60	120	180	240	360	765	
BuNCO (WPG)	5.80	9.11	12.46	15.51	18.95	23.79	31.41	
Time (mins.)	60	102	235	600	1440			
HeNCO (WPG)	3.75	5.37	10.93	15.11	25.21			
Time (mins.)	30	60	120	240	420	600	960	11520
HDI (WPG)	6.66	8.72	10.90	12.75	14.75	17.57	18.11	18.56

from 5.80 to 31.41 WPG, with HeNCO from 5.37 to 29.98 WPG and with HDI from 6.72 to 21.81 WPG. These ranges of WPG's were used for biological tests (chapter 4). Different addition of isocyanates are shown in Table 3.5.

TABLE 3.4

WEIGHT PERCENT GAIN (WPG) AND STANDARD DEVIATION OF OVEN DRIED SAMPLES OF CORSICAN PINE AFTER REACTION WITH ISOCYANATES. FIGURES REPRESENT AN AVERAGE OF 30 REPLICATES.

n-Butyl isocyanate		Hexyl isocyanate		1,6-diisocya-natehexane	
5.80	(0.41)	5.37	(0.11)	6.72	(0.66)
9.83	(0.42)	10.93	(0.10)	11.57	(0.65)
15.64	(0.64)	15.11	(0.60)	14.79	(0.92)
20.45	(0.84)	25.58	(0.50)	21.81	(0.82)
31.41	(0.81)	29.98	(0.23)		

3.4.2. VOLUME INCREASES CAUSED BY TREATMENTS

The increase in wood volumes due to the bulking effect of isocyanate reactions are shown in Table 3.5, Fig. 3.4. There is a consistent relationship between volumetric increases with increasing extent of modification for samples reacted with all three isocyanates.

With monofunctional isocyanates, the dry wood volume of HeNCO reacted blocks was slightly high at low substitution levels (up to 10 WPG) than that attained by BuNCO samples. At high WPG's, BuNCO modified samples showed greater increase in wood volume when compared with HeNCO (Fig. 3.4).

BuNCO reacted samples were 2.75% (4.4 WPG) to 25.75% (40.11 WPG) bigger as compared to unmodified controls. Increase in volume of BuNCO modified samples was proportional to the calculated volume of chemical added on (Table 3.5).

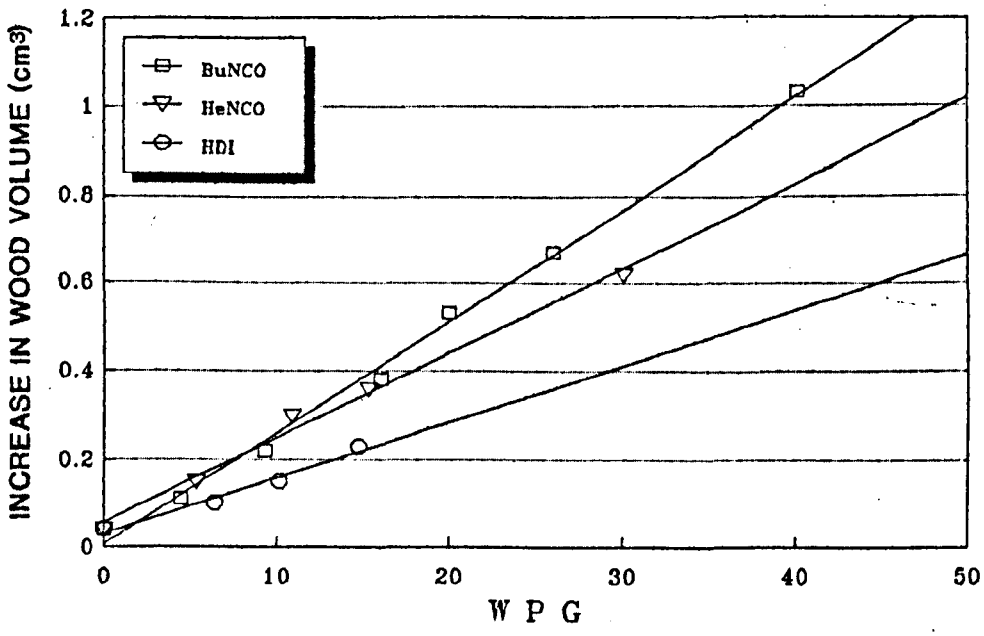


Fig. 3.4. Relationship between WPG and increase in wood volume of isocyanate modified Corsican pine.

Volumetric increases with HeNCO reacted specimens varied from 3.75% (5.36 WPG) to 15.50% (30.05 WPG) in relation to unreacted samples. HeNCO treated samples showed greater increase in wood volume when compared to the theoretical volume of chemical added on (Table 3.5).

TABLE 3.5

VOLUME CHANGES IN CORSICAN PINE BLOCKS AFTER CHEMICAL MODIFICATION. FIGURES REPRESENT AN AVERAGE OF 5 REPLICATES

TREATMENT	WPG*	INCREASE IN WOOD VOLUME (cm^3)	CALCULATED VOLUME OF CHEMICAL ADDED (cm^3)
CONTROLS			
Pyridine	0	0.04	-
Pyridine:acetone	0	0.04	-
BuNCO			
	4.44 (0.40)	0.11	0.11
	9.33 (0.33)	0.22	0.25
	16.01 (0.53)	0.38	0.41
	19.92 (0.42)	0.53	0.54
	25.98 (0.24)	0.67	0.70
	40.11 (2.02)	1.03	1.06
HeNCO			
	5.36 (0.13)	0.15	0.09
	10.89 (0.04)	0.30	0.17
	15.31 (0.48)	0.36	0.24
	22.15 (0.93)	-	0.42
	30.05 (0.20)	0.62	0.48
HDI			
	6.42 (0.04)	0.10	0.14
	10.15 (0.26)	0.15	0.23
	12.74 (0.44)	-	0.29
	14.75 (0.50)	0.23	0.33

* Standard deviation in brackets.

The increase in wood volume due to modification with HDI was lower as compared with BuNCO and HeNCO. In relation to unmodified wood, volumetric increases varied from 2.50% (6.42 WPG) to 5.75% (14.75 WPG). Calculated volume of chemical added on was greater than the increase in wood volume (Table 3.5).

3.4.3. INFRA-RED SPECTROSCOPY

The strong vibration obtained at 1730 cm^{-1} (C=O) was a distinct pattern present in all reacted samples, which indicates ester bond formation (Fig. 3.5). As expected such absorption was not present in unreacted wood. At low modification levels (5-6 WPG) the moderate vibration in the range of 1690-1730 cm^{-1} (C=O) was the only distinguished evidence of reaction. At increasing modification levels (22-31 WPG), besides carbonyl stretching, infra-red spectra of N-H bonding (1515 cm^{-1} & 770 cm^{-1}) was distinguished in comparison to the unreacted controls (Fig. 3.5). Absorption in the range of 3200-3600 cm^{-1}, indicative of hydrogen bonding (O-H) was seen in all samples. Similarly, stretching at 1000 cm^{-1} indicative of C-O showed no distinct differentiation band.

No absorption at 2275 to 2240 cm^{-1} indicative of unreacted isocyanate was seen (Fig. 3.5). This implies that no unreacted material remained in the samples.

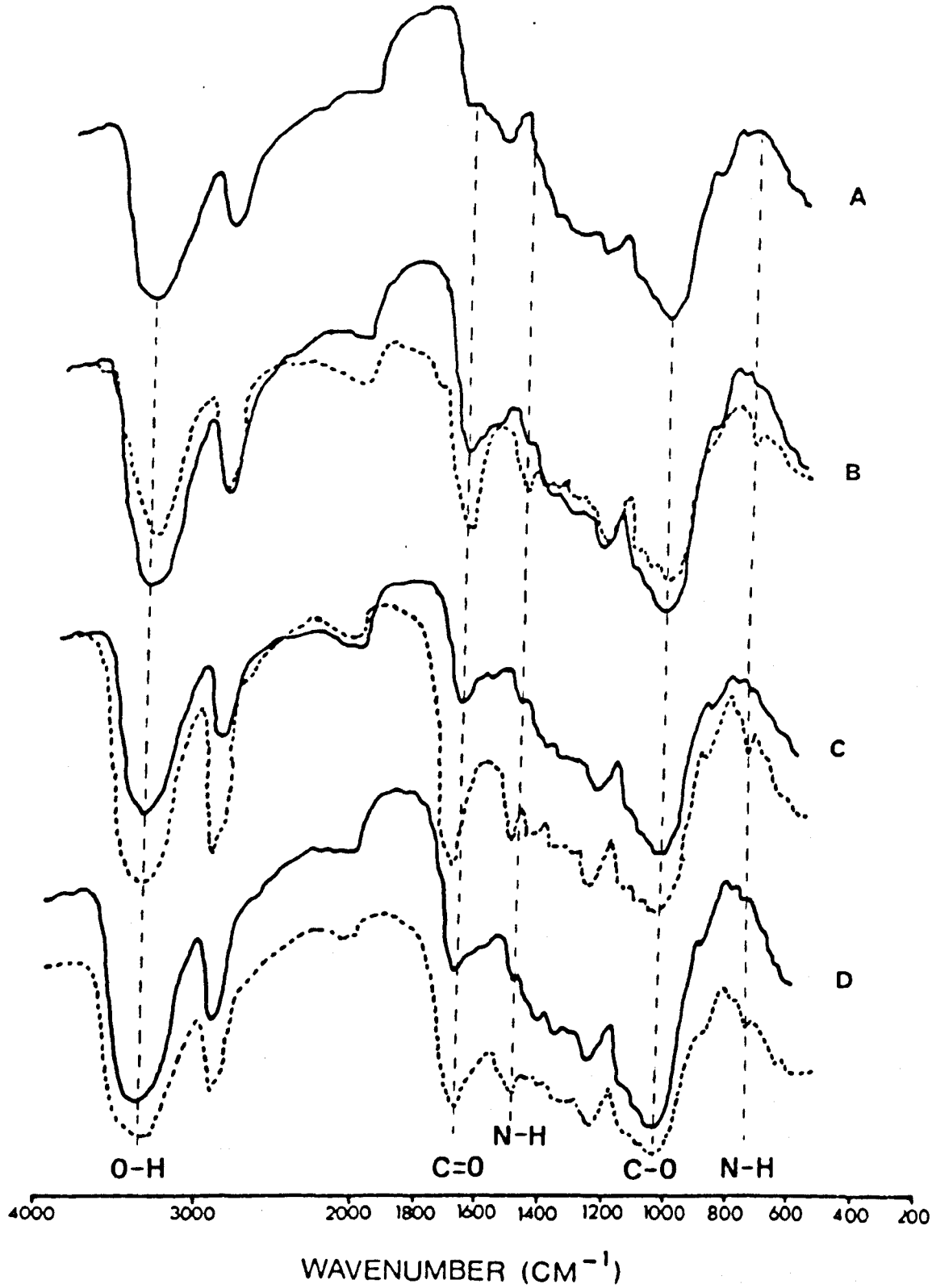


Fig. 3.5. Infra-red spectra of unmodified and modified Corsican pine. A) Control, B) n-butyl isocyanate —— 5 WPG, --- 31 WPG; C) hexyl isocyanate —— 5 WPG, 25 WPG; D) 1,6-diisocyanatehexane —— 6 WPG, --- 22 WPG.

3.4.4. WPG versus HYDROXYL REACTED

Assuming total accessibility and reactivity of the wood hydroxyls, as shown in Fig. 3.6, the amount of hydroxyl reacted increased, as levels of modification (WPG) increased.

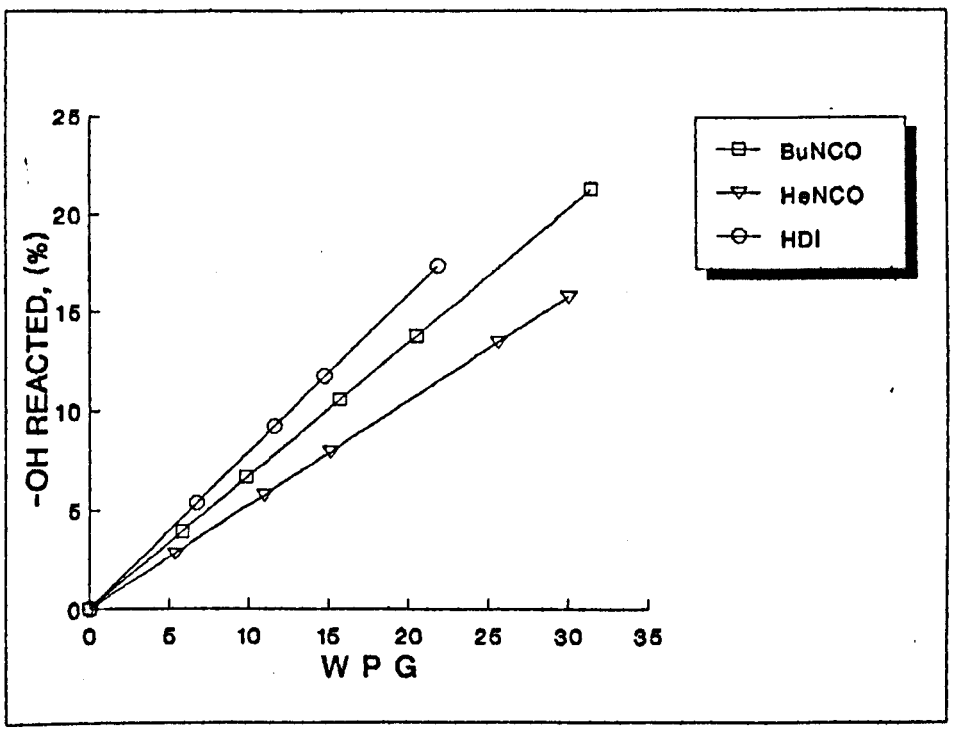


Fig. 3.6. Relationship between WPG and hydroxyl reacted of isocyanate modified Corsican pine.

At comparable WPG's the extent of -OH reacted was 1.3 times higher for n-butyl isocyanate than for hexyl isocyanate modified specimens. The magnitude of -OH reacted when HDI was the reactant was approximately 1.5 and 1.2 times greater than that estimated with HeNCO and BuNCO, respectively.

3.5. DISCUSSION

The results have shown that wood-isocyanate reaction at 100 °C under pyridine:acetone catalysis is a very effective way to react wood blocks. The extent of reaction is readily measured by the WPG's. The increases in weight and in volume of the blocks (Tables 3.3; 3.4 & 3.5) means the replacement of wood (cellulose, hemicellulose and lignin) hydroxyl groups by R-NCO or OCN-R-NCO, where R is the alky radical (butyl & hexyl) forming wood carbamate derivatives. Infra-red spectra (Fig. 3.5) support the belief that isocyanate bond with the cell wall occurred.

No attempt were made to achieve maximum WPG's, but results rather emphasise that all isocyanates used reacted speedily at initial stage of reaction, then had the trend to decline at higher reaction levels (Fig. 3.3). The reaction with the monofunctional isocyanates proved more predictable than those with the difunctional isocyanate. At high modification levels the results showed that monofunctional (BuNCO & HeNCO) isocyanates gave significantly more weight gain at shorter reaction time than HDI i.e. above 15 WPG the reaction rate with HDI was markedly slow.

West (1988) showed that wood-BuNCO reaction profile curve (conducted under conditions of excess -NCO) generally decline at high substitution level as hydroxyl groups in wood are depleted. Since WPG of HDI

reacted samples was not high enough for this (ca.> 15 WPG), it seems that the decrease in velocity from this level of substitution may be explained by the moieties formed among wood constituents. There is a possibility that crosslink formation may tie up the system devoiding solution diffusion into less swollen areas. The solvents (pyridine + acetone) used in this study may be influenced by the speed of reaction with HDI. This suggests that only a few moieties were enough to saturate the capillaries and prevent further reactions from taking place. However in this work further addition of HDI proved possible by complementary reaction which may suggested that retreatment enhanced additional swelling of the wood cell wall polymers.

It seems that one approach to greater reactivity is to swell the wood so that more functional groups are readily available to the reagents. For example Ellis & Rowell (1984) stated that in the absence of swelling solvents, HDI, isophorone diisocyanate (IPDI), tolylene-2,4-diisocyanate (TDI), phenyl isocyanate, and p-tolyl isocyanate did not show evidence of penetration or reaction in the cell walls except to a slight extent. Therefore in the presence of 35% dimethylformamide (DMF), n-butyl and phenyl isocyanates, 1,6-diidocyanatehexane and tolylene-2,4-diisocyanate reacted with wood, resulting in increased dimensional stability.

Kalnins (1982) reported that in presence of

pyridine pine wood reacted rapidily with isocyanates (methyl and allyl) and formed the corresponding carbamate derivatives with weight gains reaching 2-25% and 5-29%, respectively. Studies of West (1988) showed that the tertiary amines (including pyridine) give greater rate enhancement of wood-BuNCO reactions and that this varies with temperature. The temperature of reactions used in this study was according to that used by Kalnins (1982) and West & Banks (1986) i.e. 100 °C. Wood-diisocyanate reactions under pyridine and acetone mixture is little reported.

Other variables must be considered in the interpretation of factors influencing wood-isocyanate reactions. For example, differences in reactivity and accessibility exhibited by the functional groups of the component units of cellulose, hemicellulose and lignin in the whole wood is of a major consideration in improving wood-isocyanate reaction. Work performed by West & Banks (1986) on the rate of reaction of Scots pine (Pinus sylvestris) with n-butyl isocyanate indicates that in the initial stages of the reaction, lignin reacts faster and to a higher degree of substitution than the holocellulose. They also suggested that the reactivity of the holocellulose hydroxyls may be restricted either by limited hydroxyl accessibility (especially in crystalline cellulose), by the differences in reactivity of primary and secondary hydroxyl groups (caused by differences in

hydrogen lability) or a combination of both factors.

Also the higher degree of substitution (DS) in the lignin fraction than in the holocellulose portion was reported by Rowell (1982). His data shows that Southern pine reacted with methyl isocyanate to various levels of chemical add-on. He stated that the ratio of lignin/holocellulose DS is high at WPG's up to 10 and drops as WPG's increase up to about 50 WPG.

Besides differences in reactivity of component units in the whole wood, West (1988) points out that differences in growth ring increments among samples may represent an additional source of variability between reacted samples. In this respect samples were selected from a single board in order to enhance uniformity of a reaction between blocks. The extent of reaction as measured by the WPG appeared satisfactory from standard deviation data using batches of 30 blocks (Table 3.4).

The persistent bulking action of the added chemical to the wood cell wall as measured by the increase in sample volume shown in Table 3.5 may be regarded as an additional proof of wood-urethane bond. The volumetric increases (Table 3.5) caused by the butyl isocyanate treatments are expected to be approximately proportional to the volumetric quantity of chemical added. Increases in wood volume were greater for those reacted with hexyl isocyanate when compared to the amount of chemical added on. Conversely, samples

modified with the difunctional isocyanate, diisocyanatehexane, had less volume increase than would be expected from WPG data. It seems that the differences in the alkyl groups between reactant and the crosslinking affinity of HDI may have a marked effect on the volumetric changes due to reaction.

Infra-red spectra (Fig. 3.5) showed that carbonyl (C=O) peaks at ca. 1730 cm^{-1} were present in all reacted samples. At high WPG's, beside C=O new amide (N-H) and amine (C-N) bands were distinguished as a result of substitution reaction. Absence of unreacted isocyanate (no absorption peaks at 2275 to 2240 cm^{-1}) may indicated that the refluxing in acetone (clean up procedure) after chemical modification was sufficient and provided blocks with no by-products. The infra-red spectra in this study are in agreement of work reported by Rowell (1982) using methyl isocyanate and with Kalnins (1982) using methyl and allyl isocyanate modified Southern pine.

In order to examine the effect of nature and functionality of the substituent group on treatment performance, the degree of reacted hydroxyl groups was modelled using a range of aliphatic isocyanates. Table 3.6 shows such extrapolated data assuming a constant amount of hydroxyl groups in pine wood. The results indicate that among aliphatic monofunctional isocyanates, substituent groups of large molecular dimensions are fewer than the substituent groups of

smaller ones at comparable WPG.

Furthermore, where two side chain reactions are accomplished (e.g. HDI) there might be an increase in yield of -OH reacted in comparison to monofunctional isocyanate possessing similar alkyl chain (e.g. HeNCO) structure or in certain cases smaller side chain. In the example shown in Table 3.6, 1,6-Diisocyanatehexane is greater than propyl, butyl and Hexyl isocyanates in the theoretical yield of reacted hydroxyl at comparable WPG.

Irrespective of the accessibility and reactivity of the hydroxyl groups within wood, the extrapolation data here imply that the amount of hydroxyl reacted is dependent also on the functionality and size or intermediate structure of the reagent.

TABLE 3.6

EXTRAPOLATION DATA FOR DIFFERENT CHEMICALS ON THE PERCENT OF THE TOTAL AMOUNT OF HYDROXYL REACTED AT COMPARABLE WPG, BASED ON THE NUMBER OF MOLES OF -OH PER GRAM OF PINE WOOD.

STRUCTURE	CHEMICAL	MOL. Wt.	WPG	-OH REACTED (%)
CH_3-NCO	Methyl isocyanate	57.05	10	11.90
CH_3-CH_2-NCO	Ethyl isocyanate	71.00	10	9.52
CH_2=CH-CH_2-NCO	Allyl isocyanate	83.09	10	8.13
OCN$(CH_2)_6$NCO	1,6-Diisocyanatehexane	168.20	10	8.03
CH_3-CH_2-CH_2-NCO	Propyl isocyanate	85.00	10	7.94
$CH_3(CH_2)_3$NCO	Butyl isocyanate	99.13	10	6.80
$CH_3(CH_2)_5$NCO	Hexyl isocyanate	127.19	10	5.32

CHAPTER 4.

THE EFFECTS OF BASIDIOMYCETES ON ISOCYANATE MODIFIED WOOD

CHAPTER 4.

THE EFFECTS OF BASIDIOMYCETES ON ISOCYANATE MODIFIED WOOD

4.1. INTRODUCTION

In the previous chapter details of the reaction of Corsican pine with n-butyl, hexyl isocyanates and 1,6-diisocyanatehexane to various levels of chemical add on were reported. The isocyanate chemical modification system was chosen for biological evaluation based on previous work (Rowell & Ellis, 1979; Ellis & Rowell, 1984; Kalnins, 1982) which indicated that aliphatic isocyanates improve wood decay resistance, but suggest that the efficacy of protection is dependent on the nature of the substituent groups and the extent of modification. The results of previous work (see chapter 2) have shown that increased chain length in straight chain aliphatic isocyanates from methyl to butyl improves bio-protection against G. trabeum. Mechanisms (see section 2.4) of protection by isocyanates are from previous work poorly defined.

Work in this chapter is focused to define better the effects of bonded wood urethane on a range of brown rot [Coniophora puteana (Schum.:Fr.) Karst; G. trabeum] and white rot fungi [Coriolus versicolor (L.) Quel; Pycnoporus sanguineus (L.:Fr.) Murr.].

Considering that water is one of the important factors in wood decomposition, the relationship between weight loss and moisture content after biodeterioration tests gives insights on the performance of mono- and difunctional isocyanates. In addition, visual and microscopic examination of samples, as well as fungal enzyme production were carried out to gain additional information upon the modification of the wood cell wall polymers. This was achieved in a series of experiments.

4.2. THE AIM OF THE PROCEDURE

EXPERIMENTAL DESIGN I

EFFICACY OF ISOCYANATE MODIFICATIONS AND CCA TREATMENT AGAINST BASIDIOMYCETES.

a) determination and comparison of the effectiveness (threshold value) of mono- and difunctional isocyanates and copper-chrome-arsenic (CCA) treated wood against specified wood-inhabiting fungi;

b) determination of sample moisture content after biodeterioration tests;

c) microscopic examination of the decay patterns at differing treatment levels.

EXPERIMENTAL DESIGN II

DECAY INTENSITY OF A BROWN ROT FUNGUS ON MODIFIED WOOD AT SUB-THRESHOLD WEIGHT PERCENT GAIN (WPG).

d) investigation of the effects of a brown rot fungus (for subsequent chemical analysis in chapter 5) on the breakdown of wood polysaccharides in wood modified to similar weight percent gain (WPG) with mono- and difunctional isocyanates at different degrees of decay intensity.

EXPERIMENTAL DESIGN III

PATTERNS OF ENZYME PRODUCTION BY DECAY FUNGI

e) testing for the presence of cellulases and phenol oxidases in modified wood after infection by brown and white rot fungi.

4.3. MATERIAL & METHODS

4.3.1. EXPERIMENTAL DESIGN I

EFFICACY OF ISOCYANATE MODIFICATIONS AND CCA TREATMENT AGAINST BASIDIOMYCETES.

4.3.1.1. TEST FUNGI

Two brown rot and two white rot fungi (Table 4.1) were selected due to their outstanding reputation as wood decomposers and ease of handling under controlled laboratory conditions.

TABLE 4.1

TEST FUNGAL STRAINS AND THEIR ORIGINS

Fungi	Strain No.	Source*
Coniophora puteana (Schum.:fr.) Karst	PRL 11R	1
Gloeophyllum trabeum (Pers.:Fr.)	PRL 108N	1
Coriolus versicolor (L.) Quel.	PRL 28G	1
Pycnoporus sanguineus (L.:Fr.) Murr.	DPF 44	2

* Note: 1. BRS, Garston, Watford, U.K.;
2. Departamento de Produtos Florestais/ INPA, Manaus, Brazil

4.3.1.2. WOOD SUBSTRATE

Wood selection, dimensions, conditioning (extraction and drying) were as described in chapter 3, section 3.3.4.

4.3.1.3. ISOCYANATES

The monofunctional n-butyl (BuNCO), hexyl (HeNCO) isocyanates and a difunctional 1,6-diisocyanatehexane (HDI) were reacted with Corsican pine sapwood blocks to a range of modification (4 to 5 intervals between 5 to 35 WPG) according to the procedure covered in section 3.3. For each level of modification sets of six replicates were used for exposure to decay fungi (Table 4.1) and one set was used as sterile controls. The same number of replicates were solvent treated (acetone and pyridine:acetone) and used as unmodified controls. Solvent treatments are reported in section 3.3.8.

4.3.1.4. COPPER - CHROME - ARSENIC - TREATMENT

A series of copper-chrome-arsenic (CCA) solutions, 0.2, 0.4, 0.6, 0.8, and 1.0 % were prepared according to the type 2 specification in BS 4072:1974, as following:

Copper ($CuSO_4.5H_2O$).....35%

Dichromate ($K_2Cr_2O_7$).....45%

Arsenic ($As_2O_5.2H_2O$).....20%

At each concentration of CCA solutions (including water treated control samples), groups of 30 weighed conditioned blocks were impregnated according to the single vacuum method described in ASTM D1413:1976. The impregnation schedule used gave a

500 mmHg initial vacuum for 30 minutes, flooding, release of vacuum and an uptake period of 30 minutes impregnation. Following impregnation, excess solution was lightly wiped from the block surfaces and weighed. The amount of preservative absorbed by the blocks was calculated in kilograms per cubic metre (kg/m^3) of wood according to the formula shown in equation (4.a).

$$R\ (kg/m^3) = G\ C/V\ \times 10 \qquad (4.a)$$

where,

R = net dry salt retention;

G = grams of treating solution absorbed by the block;

C = grams of preservative in 100 g of treating solution;

V = volume of block in cm^3.

For each treatment level (water and CCA treated blocks) six replicates were used for exposure to decay fungi (Table 4.1) and one set used as sterile controls.

4.3.1.5. BLOCK CONDITIONING

Control samples (water, pyridine, and pyridine + acetone treated specimens), isocyanate modified specimens, and CCA impregnated samples were equilibrated at 20 °C and 65% relative humidity, and

weighed to the nearest 0.01 gram.

4.3.1.6. BIODETERIORATION TESTING

In the preparation of the tests units, 500 ml wide mouth squat jars were used as decay chambers. Distilled water, to achieve 130% of the water holding capacity of the soil (as defined in ASTM D1413:1976) was added to each jar. This was followed by 200g of an horticultural loam soil (John Innes no. 2). For each decay chamber two Corsican pine feeder strips (3 x 28 x 35 mm) were placed on the soil surface. Subsequently the prepared bottles were sterilized by autoclaving at 15 p.s.i. for 30 minutes on two consecutive days. After the jars had cooled, inoculum of the fungus not more than three weeks old was aseptically placed in contact with an edge of each feeder strip. The soil culture bottles were incubated at 22 °C (C. puteana) or 27 °C (other test fungi) for approximately three weeks, i.e. until the feeder strips were covered by the mycelium. After conditioning, blocks were individually weighed and gamma radiation sterilized (as defined in BS 6009:1982) prior to placing in contact with the fungal mycelium in the decay chambers. The completely assembled soil block jars were incubated for a further 16 weeks to evaluate the efficacy of the treatments.

4.3.1.7. BLOCK HANDLING AFTER TESTS

After each exposure period the adhering mycelium was carefully brushed off and evaluated by weight loss, moisture content, internal appearance, and scanning electron microscopy as described below.

4.3.1.8. CALCULATION OF WEIGHT LOSS AND MOISTURE CONTENT

After mycelial removal the blocks were immediately weighed (W_4), dried at 105 °C for 24 hours and reweighed (W_5). Weight loss [equation (4.c)] was expressed as a percentage of the initial oven dried weight of the sample.

$$\text{Weight loss, (\%)} = [(W_2 - W_5)/W_2] \times 100 \quad (4.c)$$

*

where:

W_2 = Oven dried weight before exposure i.e. after solvent or isocyanate reactions;

W_5 = Oven dried weight after exposure to fungal attack.

Calculation of moisture content was according to the equation (4.d), it was expressed as a percentage of final oven dried weight of the sample.

$$\text{Moisture content, (\%)} = [(W_4 - W_5)/W_5] \times 100 \quad (4.d)$$

where:

W_4 = Weight of the block immediately after

* This method overestimates the effectiveness of the treatment because this examines both wood and added isocyanate. A more appropriate estimation would substitute W_1 for W_2, where W_1 is the weight of wood substance alone.

removal from the decay chamber and after removal of adherent mycelium;

W_5 = Oven dried weight after exposure to fungal attack.

4.3.1.9. EXAMINATION OF DECAYED MATERIAL

Blocks exposed to decay fungi were examined internally after splitting open with a razor blade. After noting the internal condition sections were prepared for scanning electron microscopy. For electron microscopy sections were fixed for four hours at room temperature in 3% glutaraldehyde plus 3% paraformaldehyde [i.e. methanol free formalin prepared according to Glauert (1974)] in 0.1 M cacodylate buffer at pH 7.2. Following two 30 minute buffer washes these sections were then post fixed in 1% Osmium tetroxide in 7.2 pH 0.1 M cacodylate. Then dehydration was carried out in a graded series of acetone solutions i.e. 10, 30, 50, 70, 80, 90, 100% acetone with 15 minutes in each stage. Then they were critical point dried and gold coated (Polaron Sputter coater E5000) and examined in a Scanning Electron Microscopy (SEM) at 10KV on an Hitachi S-520 model.

4.3.2. EXPERIMENTAL DESIGN II

DECAY INTENSITY OF A BROWN ROT FUNGUS ON MODIFIED WOOD AT SUB-THRESHOLD WEIGHT PERCENT GAIN (WPG).

4.3.2.1. TEST FUNGUS

The brown rot fungus Coniophora puteana strain PRL 11R was selected due to its response to isocyanate modified blocks shown in the previous experiment.

4.3.2.2. ISOCYANATES

Two sets of 72 blocks were reacted to 10.68 (±0.68) and 10.16 (±0.70) WPG with n-butyl isocyanate and 1,6-diiisocyanatehexane, respectively (method in 3.3). One hundred and four control specimens were solvent treated in 1:1 mixture of pyridine:acetone (method in 3.3.8).

4.3.2.3. BLOCK CONDITIONING

Unmodified control blocks (solvent treated with pyridine + acetone) were held spaced in a meshed plastic tray into a chamber containing saturated potassium chloride solution in order to attain equilibrium moisture content. The chamber was kept at 20 °C in order to achieve relative internal humidity of ca. 86%. Similarly isocyanate reacted samples were stored under water i.e. at 100% relative humidity.

After conditioning specimens were subdivided into incubation time sets (8 replicates) varying from

0 to 32 weeks exposure in the soil block test.

4.3.2.4. BIODETERIORATION TESTING

Soil block test units (section 4.3.1.6.) were incubated at 22 °C for differing time intervals. Weight loss and moisture content were determined (section 4.3.1.8.).

4.3.3. EXPERIMENTAL DESIGN III

PATTERNS OF ENZYME PRODUCTION BY DECAY FUNGI

4.3.3.1. TEST FUNGI & TREATMENT VARIABLES

Fungi are shown in Table 4.1. Two blocks (20 x 20 x 10 mm) were reacted to ca. 5, 10 and 15 WPG with n-butyl isocyanate and 1,6-diisocyanatehexane, respectively (method in 3.3). An equal number was solvent treated in 1:1 mixture of pyridine:acetone (method in 3.3.8). Blocks were razor blade split into quarters prior to agar testing with 2 replicates per fungal species.

4.3.3.2. AGAR PLATE TESTING

After autoclaving at 121 °C the samples were introduced aseptically onto 2.5% malt agar (25g. powdered malt, 15g. Oxoid no. 3 agar in 1 litre water) slopes containing fresh growing mycelia of the fungi (Table 4.1).

The tubes were incubated for 16 weeks at 27 °C with the exception of tubes containing C. puteana which were incubated at 22 °C.

4.3.3.3. ASSAY OF CELLULASE

After incubation the wood blocks were removed from the test tubes and immediately sectioned with a sterile razor blade. Both transverse and longitudinal

sections were cut to the thickness of 0.5 to 1 mm and the sides were 3 to 5 mm. The individual sections were transferred to pre-prepared plates containing 0.1g. carboxymethyl cellulose (C.M.C.), 0.6g. KH_2PO_4, 0.4g. K_2HPO_4, 12g. agar in 1 litre distiled water. The plates were incubated overnight at 40 °C with the exception of those exposed to C. puteana that were incubated at 27 °C, then 0.1% congo red in 1 M NaCl solution was poured onto the plates to disclose cellulolysis. Hydrolysis of cellulose was shown by any lack of staining by the congo red in the medium around or under the wood piece.

4.3.3.4. ASSAY FOR LACCASE AND PEROXIDASE

A rapid test based on the procedure described by Harkin & Obst (1973) using syringaldazine as a substrate for the detection of laccase and peroxidase in wood pieces was performed. Immediately after cutting, the sections were transferred individually to the centre of a 1.2% agar (12g agar in 1 litre water) plate. For laccase testing 3-4 drops of 0.1% syringaldazine in 95% ethanol were added. For peroxidase testing plates containing samples from the same origin were tested in 0.1% solution of syringaldazine but two drops of 0.4% hydrogen peroxide were subsequently added. Phenol oxidase detection for presence of laccase was considered positive if a deep

purple coloured zone developed around the specimen. Peroxidase detection was considered positive by an intensified colour formed rapidly on addition of hydrogen peroxide or by effervescence on addition of the hydrogen peroxide solution.

4.4. RESULTS

4.4.1. EXPERIMENTAL DESIGN I

4.4.1.1. EFFICACY OF TREATMENTS IN PREVENTING DECAY

The data from all tests were plotted and the fit shown by linear regression analysis indicates a positive relationship between extent of modification and decay resistance (Figs. 4.1 - 4.4, Table 4.2). The threshold values (as obtained in ASTM D1413:1976) showed greater variability between the four fungi (C. puteana, G. trabeum, C. versicolor and P. sanguineus) than between the different isocyanate modification types.

Blocks exposed to the brown rot fungus C. puteana (Fig. 4.1 a - c) showed the highest threshold WPG protection values at 15.5 (BuNCO), 18 (HeNCO) and 13.6 (HDI). Less modification was required to achieve protection against the other brown rot fungus, G. trabeum (Fig. 4.2 a & b). For this fungus WPG thresholds of 10 (BuNCO) and 10.6 (HDI) were noted.

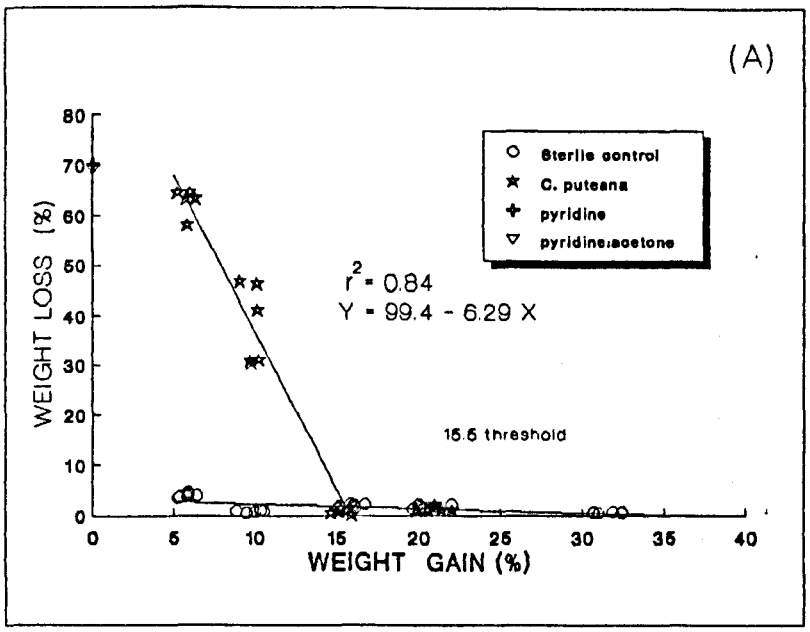
The threshold WPG values for the white rot fungi (Figs. 4.3 a, b and 4.4 a, b), C. versicolor and P. sanguineus (9.6, 12.2, BuNCO; 10.3, 11.7, HDI) showed nearly the same threshold response to the isocyanate treatments as the brown rot fungus, G. trabeum, irrespective of the treatment applied. The weight

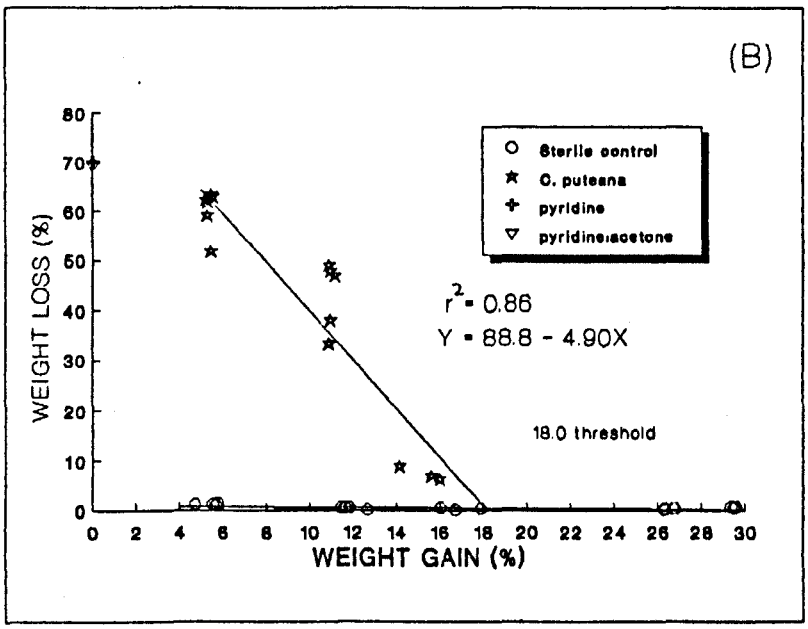
TABLE 4.2. AVERAGE AND STANDARD DEVIATION (IN BRACKETS) OF WEIGHT LOSS PERCENT IN ISOCYANATE MODIFIED CORSICAN PINE SAPWOOD AFTER 16 WEEKS EXPOSURE TO DECAY FUNGI IN A SOIL BLOCK TEST. THRESHOLD CONTROL VALUES FOR EACH OF THE ISOCYANATES ARE EMPHASISED.

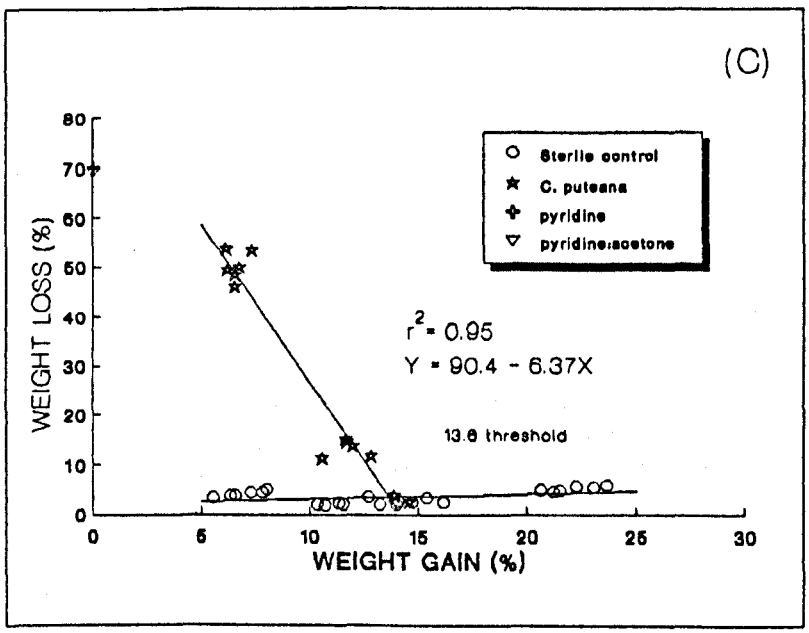
CHEMICAL	WEIGHT PERCENT GAIN	% WEIGHT LOSS AFTER EXPOSURE TO : C. puteana	G. trabeum	C. versicolor	P. sanguineus	Sterile Control
Pyridine (control)	0	69.81 (1.02)	29.47 (4.10)	32.55 (3.74)	40.27 (1.73)	2.67 (0.43)
Pyridine + Acetone (control)	0	69.31 (1.40)	18.48 (13.82)	32.42 (3.00)	37.23 (6.84)	1.86 (0.31)
BuNCO	5.80	62.7 (2.47)	22.27 (5.09)	19.90 (5.48)	18.38 (3.25)	3.79 (0.40)
	9.83	37.73 (7.92)	2.03 (0.55)	0.93 (0.19)	7.22 (3.42)	0.64 (0.26)
	15.64	0.63 (0.46)	1.02 (0.55)	1.20 (0.26)	0.96 (0.38)	1.72 (0.40)
	20.45	1.21 (0.45)	0.91 (0.45)	1.25 (0.50)	0.89 (0.41)	1.54 (0.41)
	31.41	0.30 (0.07)	0.53 (0.05)	0.51 (0.06)	0.42 (0.29)	0.51 (0.07)
	THRESHOLD	15.5	10.0	9.6	12.2	
HeNCO	5.37	60.09 (4.34)	NT	NT	NT	1.01 (0.16)
	10.93	41.35 (7.43)	NT	NT	NT	0.36 (0.06)
	15.11	5.57 (3.42)	NT	NT	NT	0.11 (0.09)
	25.58	0.00 (0.00)	NT	NT	NT	0.10 (0.13)
	29.98	0.20 (0.16)	NT	NT	NT	0.49 (0.08)
	THRESHOLD	18.0				
HDI	6.72	49.70 (3.09)	15.48 (0.69)	5.18 (1.35)	9.94 (0.82)	3.94 (0.62)
	11.57	13.04 (1.68)	2.26 (0.47)	2.30 (0.46)	2.41 (0.47)	2.11 (0.64)
	14.70	2.01 (0.96)	1.92 (0.63)	1.99 (0.38)	1.71 (0.44)	2.30 (0.52)
	21.81	5.25 (1.04)	4.59 (0.48)	4.85 (0.20)	4.62 (0.19)	5.06 (0.54)
	THRESHOLD	13.6	10.6	10.3	11.7	

Note: NT = not tested.

Fig. 4.1. Efficacy of A) n-butyl isocyanate, B) hexyl isocyanate and C) 1,6-diisocyanatehexane modified Corsican pine after 16 weeks exposure to C. puteana in a soil block test.


(A)
Sterile control
C. puteana
pyridine
pyridine:acetone
r^2 = 0.84
Y = 99.4 - 6.29 X
15.6 threshold
WEIGHT LOSS (%)
WEIGHT GAIN (%)


(B)
Sterile control
C. puteana
pyridine
pyridine:acetone
r^2 = 0.86
Y = 88.8 - 4.90X
18.0 threshold
WEIGHT LOSS (%)
WEIGHT GAIN (%)


(C)
Sterile control
C. puteana
pyridine
pyridine:acetone
r^2 = 0.95
Y = 90.4 - 6.37X
13.6 threshold
WEIGHT LOSS (%)
WEIGHT GAIN (%)

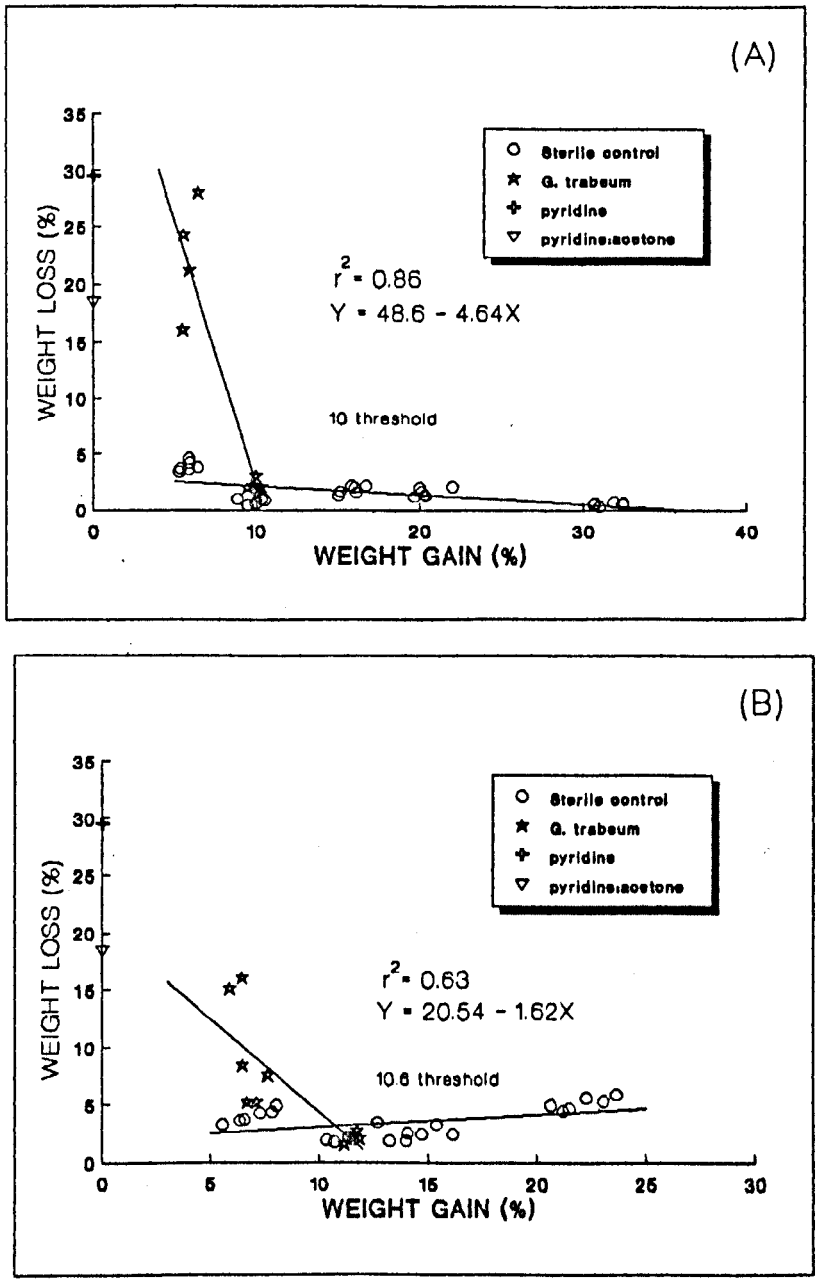


Fig. 4.2. Efficacy of A) n-Butyl isocyanate, and B) 1,6-diisocyanatehexane modified Corsican Pine after 16 weeks exposure to G. trabeum in a soil block test.

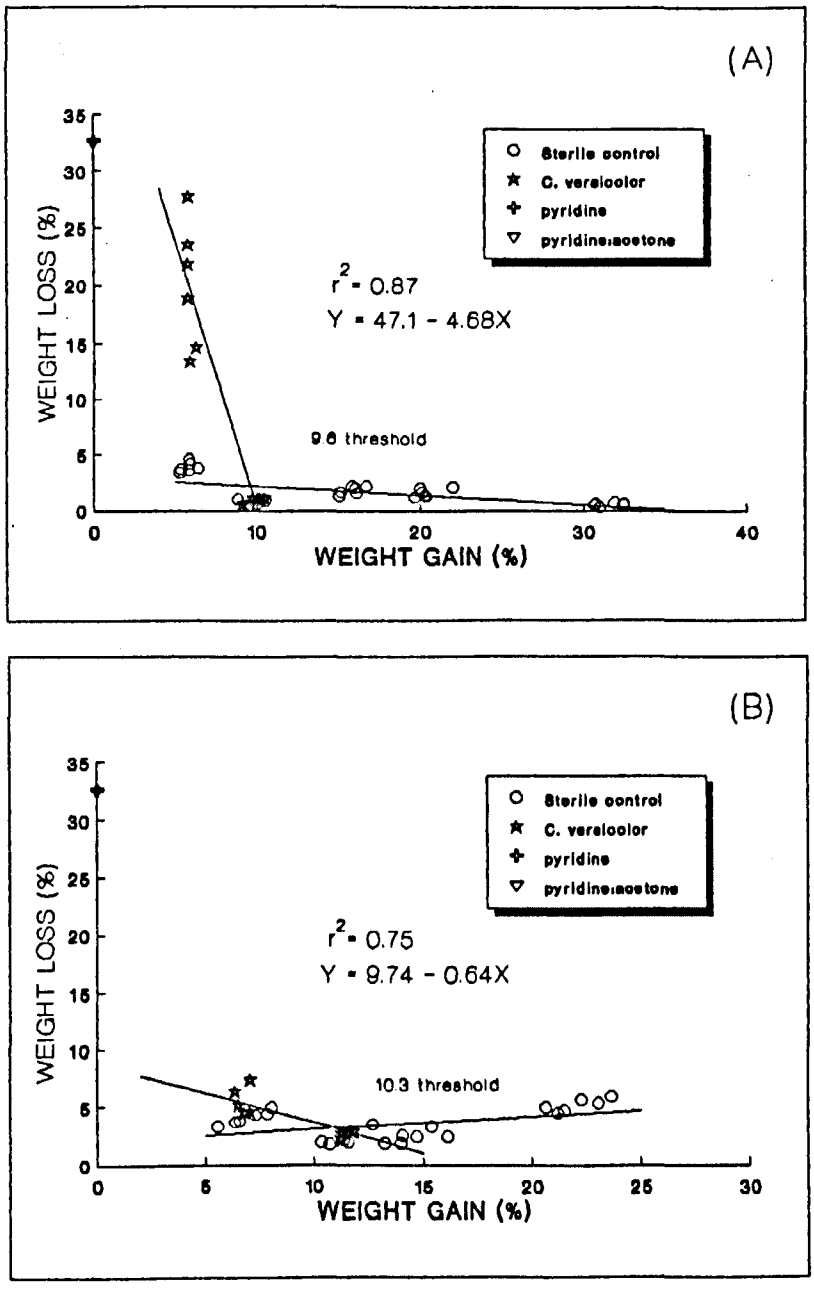


Fig. 4.3. Efficacy of A) n-Butyl isocyanate, and B) 1,6-diisocyanatehexane modified Corsican Pine after 16 week exposure to C. versicolor in a soil block test.

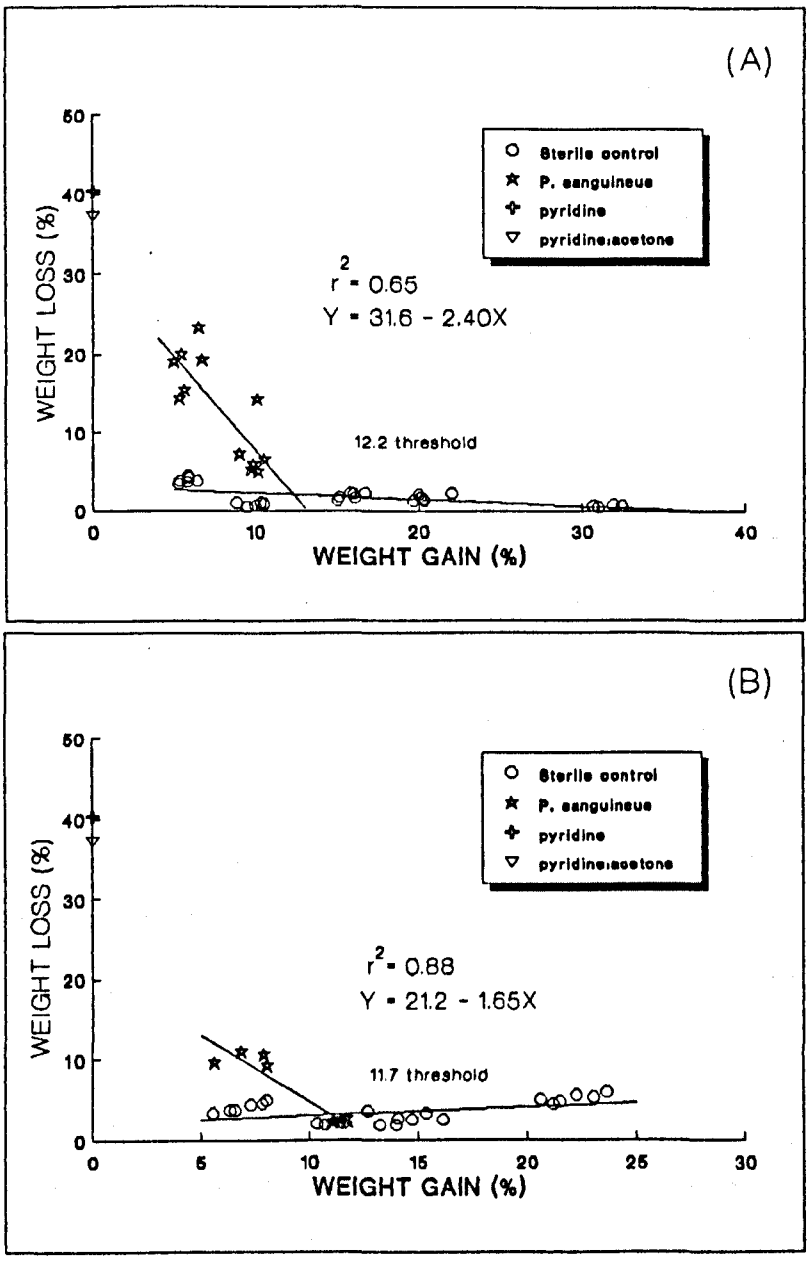


Fig. 4.4. Efficacy of A) n-Butyl isocyanate, and B) 1,6-diisocyanatehexane modified Corsican Pine after 16 weeks exposure to P. sanguineus in a soil block test.

losses caused by G. trabeum on modified blocks were greater, however.

Weight losses for G. trabeum in control samples (Table 4.2) treated with pyridine alone were considerably greater than for pyridine:acetone (29% pyridine, 18% pyridine:acetone); the values for the other fungi were similar for both pyridine and pyridine:acetone mixtures.

Table 4.3 shows the actual retention in kg/m^3 of CCA treated blocks and the average weight losses after exposure to decay fungi in a soil block test. Of the five concentrations (0.2, 0.4, 0.6, 0.8, & 1%) of CCA impregnated blocks, C. puteana showed the highest decay response. Concentrations in the range of 0.4 to 1% were the most inhibitory (Table 4.3 & Fig. 4.5). For this fungus a threshold at 0.43% CCA (2.74-4.12 kg/m^3) was verified and the coefficient of variation (Fig. 4.5) indicates a good linear relationship between CCA concentrations and weight loss. With G. trabeum negligible loss was verified at all concentrations used compared to 51% weight loss of control samples (Table 4.3) . AT 0.2% CCA, weight loss was 5% thus the true threshold value is in the range of 0.2 to 0.4% (1.37-2.74 kg/m^3).

Threshold for P. sanguineus was achieved at the lowest treatment level (0.2%), while the controls showed 31% weight loss. C. versicolor failed to

TABLE 4.3. AVERAGE AND STANDARD DEVIATION (IN BRACKETS) OF WEIGHT LOSS PERCENT IN CCA TREATED CORSICAN PINE SAPWOOD AFTER 16 WEEKS EXPOSURE TO DECAY FUNGI IN A SOIL BLOCK TEST.

TREATING SOLN. CONC. (% a.i.)	RETENTION (kg/m^3)	% WEIGHT LOSS AFTER EXOUSURE TO : C. puteana	G. trabeum	C. versicolor	P. sanguineus	Sterile Control
Water (control)	-	66.79 (1.93)	51.21 (0.33)	7.42 (4.75)	31.49(10.55)	1.37 (0.06)
0.2	1.37	20.06 (5.17)	5.11 (0.75)	1.77 (0.11)	2.25 (0.26)	1.12 (0.13)
0.4	2.74	2.26 (1.28)	2.40 (1.11)	1.46 (0.19)	2.25 (0.26)	1.09 (0.16)
0.6	4.12	2.51 (1.70)	0.99 (0.78)	0.90 (0.28)	0.69 (0.18)	0.95 (0.04)
0.8	5.49	1.59 (1.74)	1.02 (0.13)	0.67 (0.24)	1.88 (0.30)	0.88 (0.10)
1.0	6.86	1.74 (0.34)	1.30 (0.11)	1.18 (0.09)	1.00 (0.12)	0.83 (0.06)

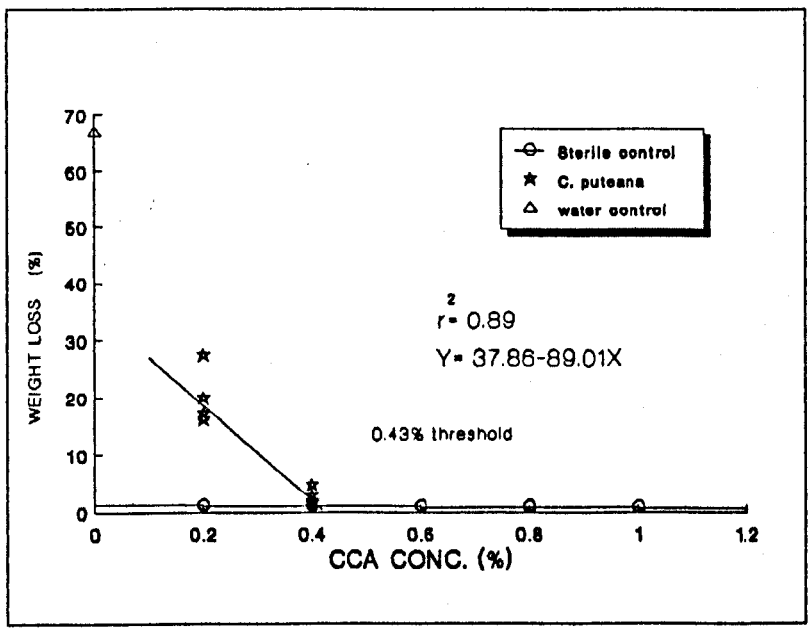


Fig. 4.5. Efficacy of CCA treated Corsican pine after 16 weeks exposure to C. puteana in the soil block test.

produce adequate weight losses on control water treated samples (Table 4.3), although 36-38% weight losses were achieved in solvent extracted controls (Table 4.2).

4.4.1.2. MOISTURE CONTENT AFTER SOIL BLOCK TESTS

Moisture contents after decay tests are presented in Table 4.4. Sterile control unmodified blocks reached average moisture contents of 31.6% during the tests whilst the isocyanate modified specimens reached lower moisture contents (Fig. 4.6). The moisture contents showed a consistent decrease with increasing extent of modification (Table 4.4 & Fig. 4.6).

Moisture content was influenced by decay fungi, isocyanate treatment and by the interaction of these two factors (Table 4.5). Statistical analysis showed these to be highly significant ($P<0.001$). The influence of decay fungi on the block moisture contents was most pronounced at low modification levels (Figs. 4.7 and 4.8).

TABLE 4.4. AVERAGE AND STANDARD DEVIATION (IN BRACKETS) OF MOISTURE CONTENT PERCENT IN ISOCYANATE MODIFIED CORSICAN PINE SAPWOOD AFTER 16 WEEKS EXPOSURE TO DECAY FUNGI IN SOIL BLOCK TEST.

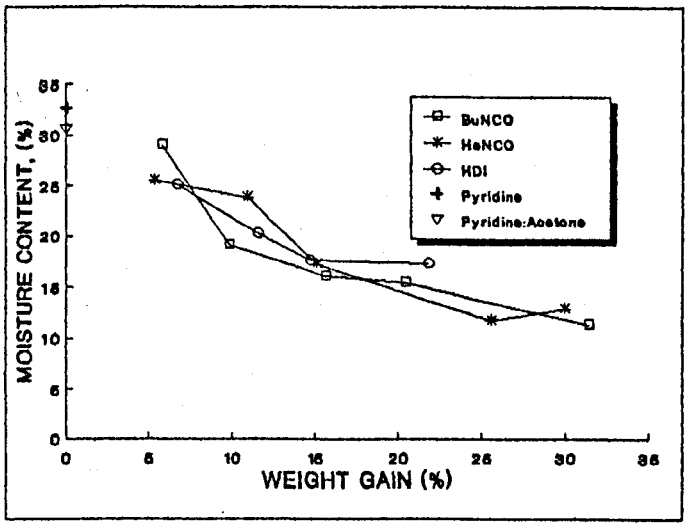
CHEMICAL	WEIGHT PERCENT GAIN	% MOISTURE CONTENT AFTER EXPOSURE TO : C. puteana	G. trabeum	C. versicolor	P. sanguineus	Sterile Control
Pyridine (control)	0	113.67(30.21)	39.45(24.83)	37.75(1.89)	38.76(5.84)	32.60(0.92)
Pyridine + Acetone (control)	0	151.08(53.96)	34.63(3.55)	36.46(2.13)	40.38(4.03)	30.60(0.18)
BuNCO	5.80	117.47(4.99)	31.55(0.96)	31.01(2.97)	30.58(1.36)	28.67(2.31)
	9.83	62.15(2.97)	14.86(0.52)	19.21(1.55)	29.58(0.89)	19.92(2.42)
	15.64	21.71(0.25)	14.12(1.53)	15.91(0.39)	16.13(0.42)	16.04(0.38)
	20.45	16.30(1.23)	11.32(3.38)	14.52(0.31)	14.51(0.26)	14.99(0.39)
	31.41	12.39(1.31)	10.97(0.36)	10.44(0.31)	12.90(1.53)	11.17(0.57)
HeNCO	5.37	78.48(9.40)	NT	NT	NT	25.60(0.97)
	10.93	46.25(9.01)	NT	NT	NT	20.06(0.52)
	15.11	19.34(1.56)	NT	NT	NT	17.14(1.56)
	25.58	13.29(0.91)	NT	NT	NT	11.65(0.52)
	29.98	12.87(1.34)	NT	NT	NT	13.39(0.59)
HDI	6.72	64.19(5.12)	25.86(2.81)	24.39(0.55)	24.86(0.61)	25.20(0.50)
	11.57	33.61(2.89)	19.09(0.80)	19.66(0.22)	19.70(0.19)	20.33(0.60)
	14.70	18.73(1.51)	16.07(0.08)	16.61(0.33)	15.64(1.45)	17.10(0.16)
	21.81	19.45(0.96)	16.53(0.56)	16.84(0.14)	15.84(1.33)	16.86(0.21)

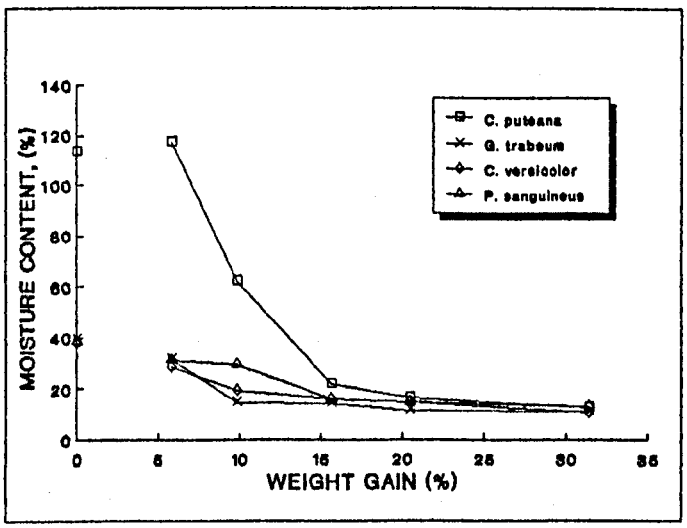
Note: NT = not tested.

Fig. 4.6. Relationship between moisture content and weight percent gain of isocyanate modified and unmodified Corsican pine blocks after 16 weeks incubation under sterile conditions in a soil block test.

Fig. 4.7. Relationship between moisture content and weight percent gain of n-butyl isocyanate modified Corsican pine blocks after 16 weeks incubation with four decay fungi in a soil block test.

Fig. 4.8. Relationship between moisture content and weight percent gain of 1,6-diisocyanatehexane modified Corsican pine blocks after 16 weeks incubation with four decay fungi in a soil block test.


MOISTURE CONTENT, (%)
WEIGHT GAIN (%)
BuNCO
HeNCO
HDI
Pyridine
Pyridine:Acetone


MOISTURE CONTENT, (%)
WEIGHT GAIN (%)
C. puteana
G. trabeum
C. versicolor
P. sanguineus

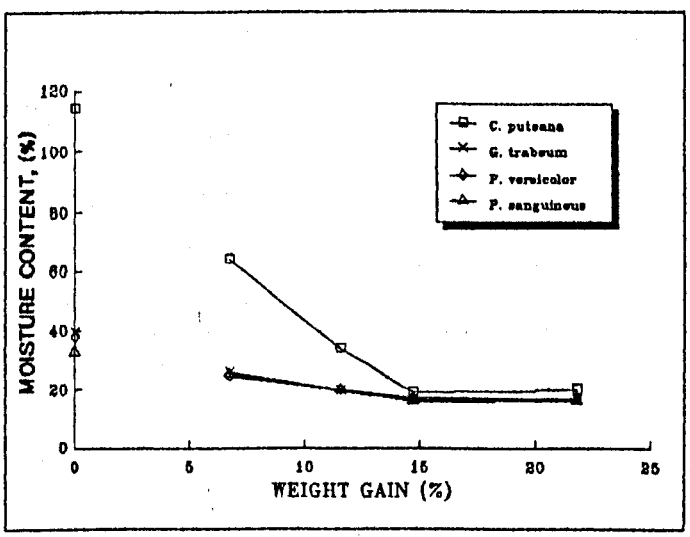

MOISTURE CONTENT, (%)
WEIGHT GAIN (%)
C. puteana
G. trabeum
P. versicolor
P. sanguineus

TABLE 4.5

ANOVA TABLES SHOWING THE DIFFERENCE IN MEAN MOISTURE CONTENT AMONG TREATMENTS. A) n-BUTYL ISOCYANATE SAMPLES, B) 1,6-DIISOCYANATEHEXANE.

A) n-butyl isocyanate					
Source of Variation	SS	%SS	df	MS	P
Fungal species (FS)	11518.35	11.70	3	3839.45	<0.001
WPG's	18641.29	18.93	4	4660.32	<0.001
Interaction FS & WPG's	68105.15	69.16	12	5675.43	<0.001
Residual	206.31	0.21	60	3.44	
Total	98471.10	100			
B) 1,6-diisocyanatehexane					
Source of Variation	SS	%SS	df	MS	P
Fungal species (FS)	2607.80	29.58	3	869.26	<0.001
WPG's	3404.94	38.63	3	1134.98	<0.001
Interaction FS & WPG's	2648.21	30.04	9	294.25	<0.001
Residual	153.95	1.75	48	3.21	
Total	8814.90	100			

4.4.1.3. GROSS DISTRIBUTION OF DECAY WITHIN THE BLOCKS

Visual examination of the external and internal condition of the blocks modified to levels above the threshold protection values showed that the blocks were apparently internally sound.

Examination of the brown rotted blocks treated to levels just below the threshold values revealed an envelope type of protection (Fig. 4.9). Heavily colonised areas of both early and latewood tracheids were seen in the block centres but decay was pronounced in the latewood (Fig. 4.9). At the low modification levels (<6 WPG) both early and latewood areas of the blocks were heavily attacked throughout the specimens. When exposed to C. puteana, these heavily attacked blocks showed severe shrinkage and wall cracking. With G. trabeum shrinkage and cracking was less.

White rot attack by C. versicolor and P. sanguineus showed no distinguishing bleaching or discolouration of the blocks. Even at the low level of modification treatment neither early nor latewood growth ring bands appeared preferentially attacked.

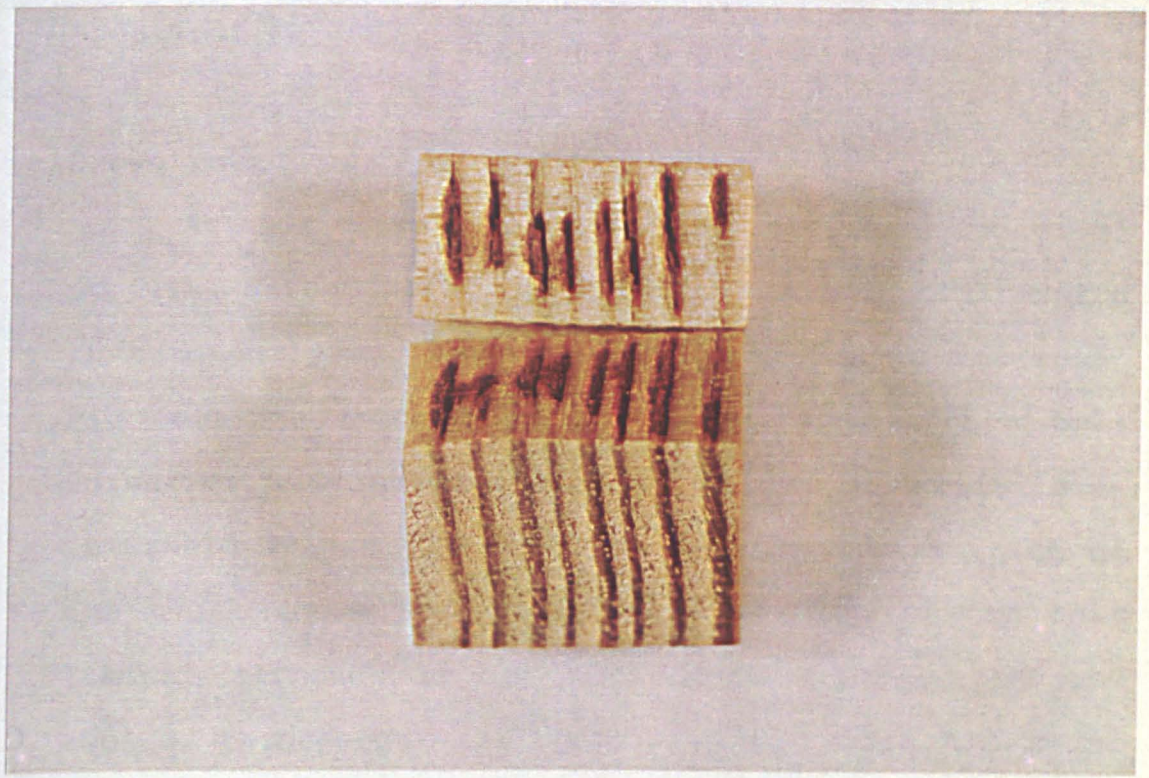


Fig. 4.9. Pattern of decay by C. puteana on BuNCO modified blocks at sub-threshold WPG after 16 weeks exposure in the soil Block testing.

4.4.1.4. MICROSCOPIC DISTRIBUTION OF DECAY AND HYPHAE

Microscopic examination of hyphal distribution revealed distinct decay patterns in relation to treatment levels.

Brown rot:

At low levels of chemical modification (<6%) mycelial strands of C. puteana were widely distributed in both early and latewood tracheids and in the rays. Pit membrane removal, pit penetration and bore hole formation were noted (Fig 4.10 a, b). At higher sub-threshold values (ca. 10%) latewood on the outside of the block showed pronounced colonisation of the cell lumina, although hyphae were seen in this latewood region throughout all cell types, even in sound tracheids. In the inside of these blocks extensive colonisation, similar to that of lower modification levels, was seen. At higher levels of modification (>14%) only a few hyphae were noted in the rays and in the latewood tracheids. Observations made on G. trabeum infected modified wood were essentially similar although the extent of hyphal colonisation was less at the higher levels of modification.

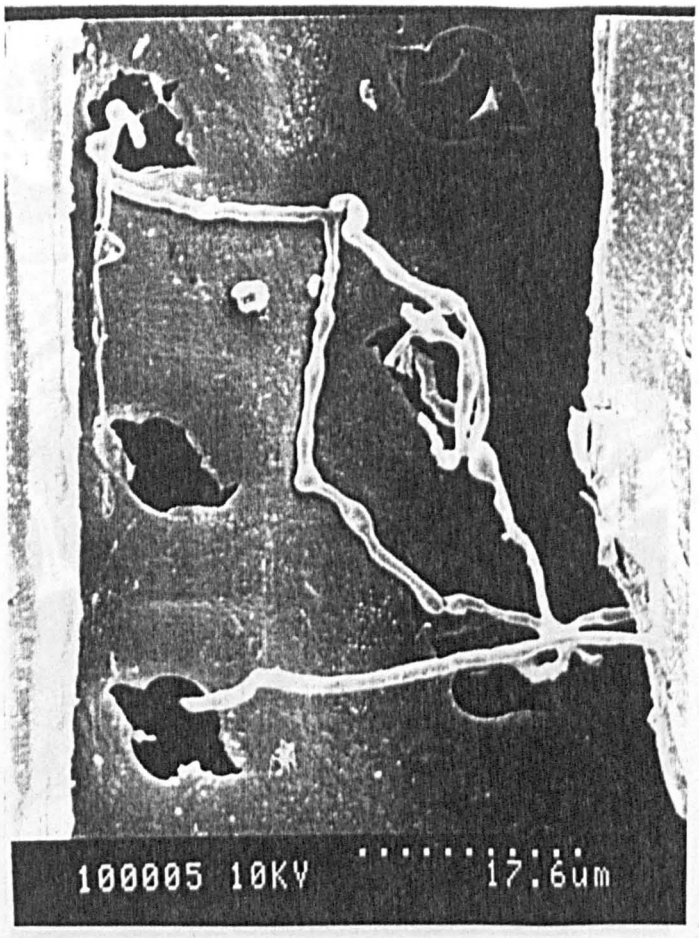
Fig. 4.10. Scanning electron micrographs of radial sections of a) BuNCO and b) HDI modified Corsican pine at sub-threshold WPG after 16 weeks exposure to C. puteana in the soil block tests.

a.1) BuNCO modified section showing fungal hyphae preferentially penetrating pit.

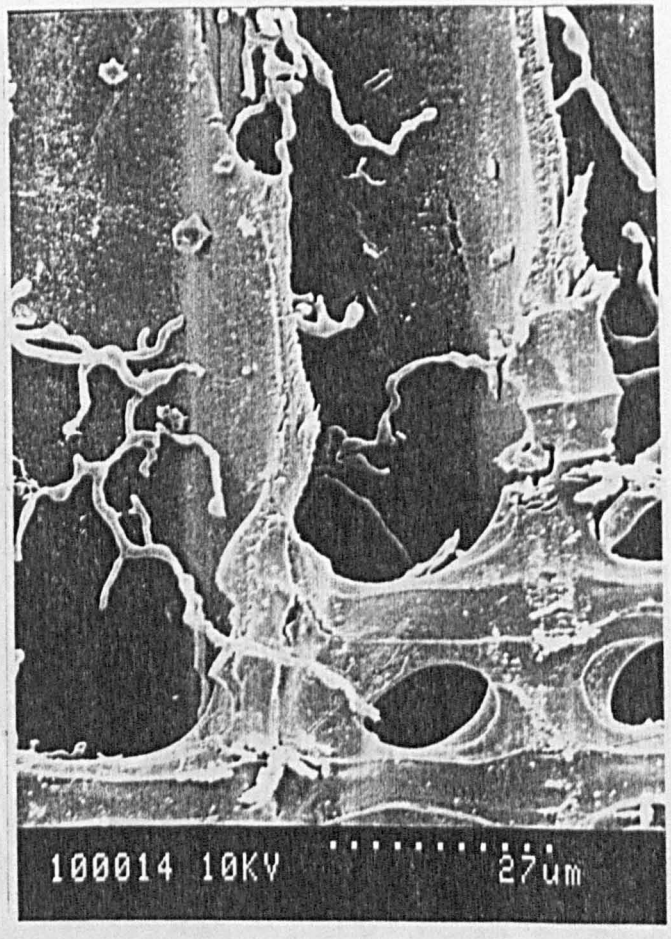
a.2) BuNCO modified section showing fungal hyphae and bore hole formation in regions where ray parenchyma cells were in contact with tracheids.

b.1) HDI modified section showing fungal hyphae preferentially penetrating pit.

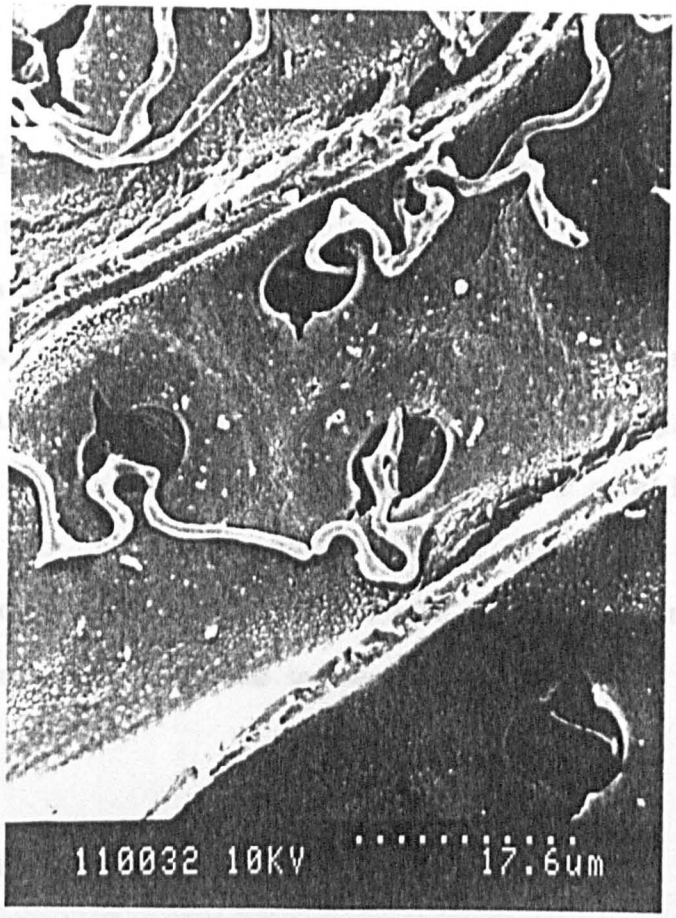
b.2) HDI modified section showing hyphae penetrating pit or autolyzed hyphae.



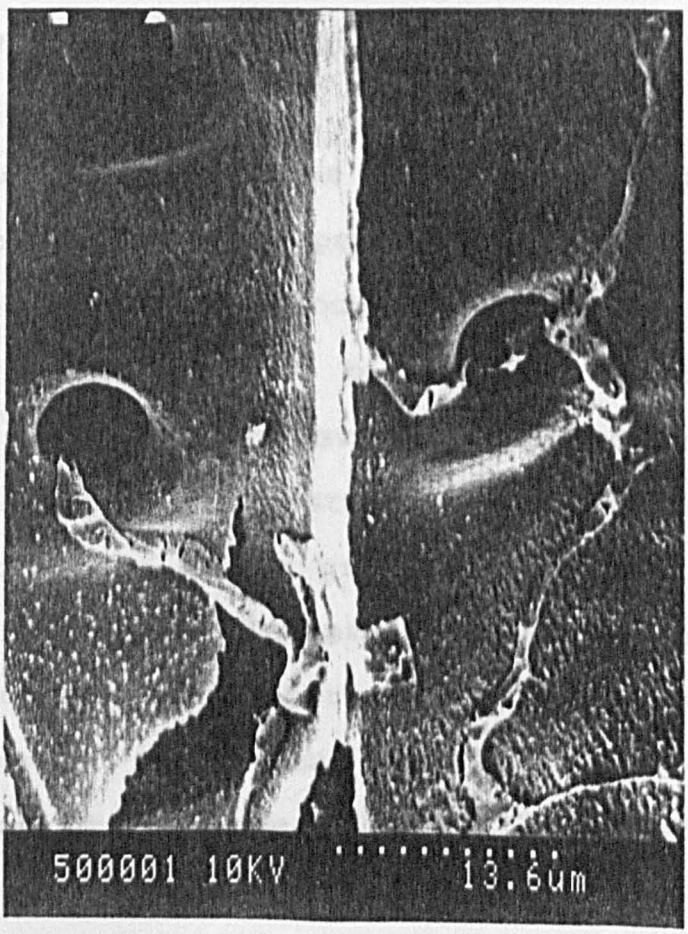
(a.1)



(a.2)



(b.1)



(b.2)

White rot:

The unmodified control samples of blocks decayed by C. versicolor and P. sanguineus showed colonisation of all tissues, bore hole formation and generalised erosion patterns over the wood cell wall surfaces. At low levels of modification fewer hyphae were visible with the majority being located in the ray parenchyma. In the axial tracheids more hyphae were visible in the latewood. At higher modification levels (>9% WPG) hyphal colonisation in the blocks was less and in some cases no internal hyphae were noted.

4.4.2. EXPERIMENTAL DESIGN II

4.4.2.1. VARIATION IN DECAY INTENSITY OF MODIFIED WOOD

Table 4.6 summarises the weight loss due to decay promoted by C. puteana of unmodified and isocyanate modified blocks during the various incubation periods. Test blocks with approximately comparable weight losses have been grouped in order to amplify the effect of moisture content on the rate of decay. This also allows grouping by decay intensity for chemical analysis (chapter 5). Unmodified control samples showed a rapid increase in degradation in the first 8 weeks incubation. At 12 weeks exposure, average weight loss reached 65%. Thereafter (16 to

32 weeks) much slower decay was observed. Maximum weight loss of unmodified controls reached 70% (Table 4.6).

Between 8 to 12 weeks of incubation isocyanate modified specimens showed greater variation in weight losses (1 to 30%, BuNCO; 1 to 11%, HDI) than unmodified controls. BuNCO samples showed markedly more decay than HDI specimens throughout the tests (Table 4.6). Irrespective of the exposure time, averages of 58% and 23% weight losses were achieved for BuNCO and HDI modified specimens respectively at test end.

4.4.2.2. MOISTURE CONTENT AT DIFFERENT DEGREES OF DECAY INTENSITY

Before decay testing moisture contents were 18, 20 and 19% for unmodified (conditioned at 86% RH), BuNCO and HDI (conditioned at 100% RH) samples, respectively (Table 4.6). At different levels of decay, unmodified control samples showed that in the test units adequate moisture conditions were supplied (Table 4.6). High moisture contents and high rates of decay occurred in samples up to 8 weeks incubation. Subsequently (12 to 32 weeks) moisture content and decay rates declined.

Conversely, in isocyanate modified specimens water uptake was restrained in the initial stages (8 -

TABLE 4.6

AVERAGE PERCENT WEIGHT LOSSES AND MOISTURE CONTENT AT DIFFERENT DEGREES OF DECAY PROMOTED BY C. puteana IN UNREACTED CONTROL AND ISOCYANATE MODIFIED CORSICAN PINE AFTER EXPOSURE BY THE SOIL BLOCK TEST.

APPROX. TIME OF EXPOSURE (Weeks)	AVERAGE[a] WEIGHT LOSS (%)	AVERAGE[a] MOISTURE CONTENT (%)
	.....Unreacted[b].....	
0	0.0	17.69 (0.34)
1¼	7.14 (1.39)	179.74 (1.68)
2	16.57 (2.39)	114.52 (6.37)
3	22.93 (2.38)	63.53 (7.06)
4	46.55 (2.88)	133.06 (9.78)
8	55.31 (6.02)	154.45 (8.38)
12	65.45 (0.31)	93.43 (1.75)
16-32	67.64 (0.32)	77.65 (11.68)
	68.48 (0.33)	85.24 (15.08)
	69.10 (0.06)	98.68 (43.03)
	70.44 (0.32)	63.76 (10.15)
	.....BuNCO (ca. 10 WPG[c]).....	
0	0.0	20.10 (0.26)
8	1.56 (0.79)	22.31 (2.50)
8-12	7.24 (1.37)	27.21 (2.81)
	11.68 (0.66)	27.70 (3.72)
	18.20 (0.78)	28.32 (3.46)
	22.95 (1.78)	35.43 (5.22)
	30.16 (0.87)	49.21 (12.33)
16-24	35.67 (1.68)	46.92 (5.22)
16-24	45.60 (2.20)	59.83 (6.28)
24-32	57.83 (3.60)	80.29 (10.33)
	.....HDI (ca. 10 WPG[c]).....	
0	0.0	19.33 (0.43)
8-12	1.22 (0.23)	22.11 (0.90)
8-12	7.59 (0.67)	23.79 (2.87)
12-16	11.37 (0.23)	32.73 (5.26)
16-20	14.19 (0.64)	33.18 (12.86)
24-32	22.59 (1.08)	34.42 (0.39)

[a] standard deviation in brackets.
[b] blocks conditioned at 86% RH prior test.
[c] blocks conditioned at 100% RH prior test.

12 weeks) of testing and increased accordingly as decay and time of exposure increased (Table 4.6). A maximun of 80% moisture content was reached by BuNCO specimens, while moisture content of HDI samples remained close to the fibre saturation point of unmodified wood.

4.4.3. EXPERIMENTAL DESIGN III

4.4.3.1. PATTERNS OF ENZYME PRODUCTION BY DECAY FUNGI

The cellulolytic activity of wood pieces after exposure to brown and white rot fungi (Table 4.7) as shown by clearing zones (Fig. 4.11) was present with all samples exposed to fungi at all levels of isocyanate modification with both isocyanates and with unmodified controls.

Tests on phenol oxidizing enzyme activity with the syringaldazine substrate on unreacted wood blocks exposed to the white rot fungi C. versicolor and P. sanguineus was positive for laccase detection (Table 4.7), but there were no indication of strong colour changes or effervescence when hydrogen peroxide was added to this samples. The brown rot fungi C. puteana and G. trabeum gave negative results to syringaldazine for the presence of laccase and peroxidase in unreacted samples.

TABLE 4.7

DETECTION OF CELLULASE, LACCASE AND PEROXIDASE IN UNREACTED, n-BUTYL ISOCYANATE AND 1,6-DIISOCYANATEHEXANE MODIFIED CORSICAN PINE EXPOSED TO DECAY FUNGI AFTER 16 WEEKS EXPOSURE ON MALT AGAR SLANTS.

Treatment / WPG	Cellulase	Laccase	Peroxdase	Fungi
Unreacted	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	+	-	C. versicolor
	+	+	-	P. sanguineus
BuNCO				
5 WPG	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	+	-	C. versicolor
	+	+	-	P. sanguineus
10 WPG	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	+	-	C. versicolor
	+	+	-	P. sanguineus
15 WPG	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	+	-	C. versicolor
	+	+	-	P. sanguineus
HDI				
5 WPG	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	+	-	C. versicolor
	+	+	-	P. sanguineus
10 WPG	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	Weak[a]	-	C. versicolor
	+	Weak[a]	-[b]	P. sanguineus
15 WPG	+	-	-	C. puteana
	+	-	-	G. trabeum
	+	-	-	C. versicolor
	+	Weak[a]	-	P. sanguineus

[a] Faint purple; [b] strong catalase activity indicated by effervescence on addition of the hydrogen peroxide solution.

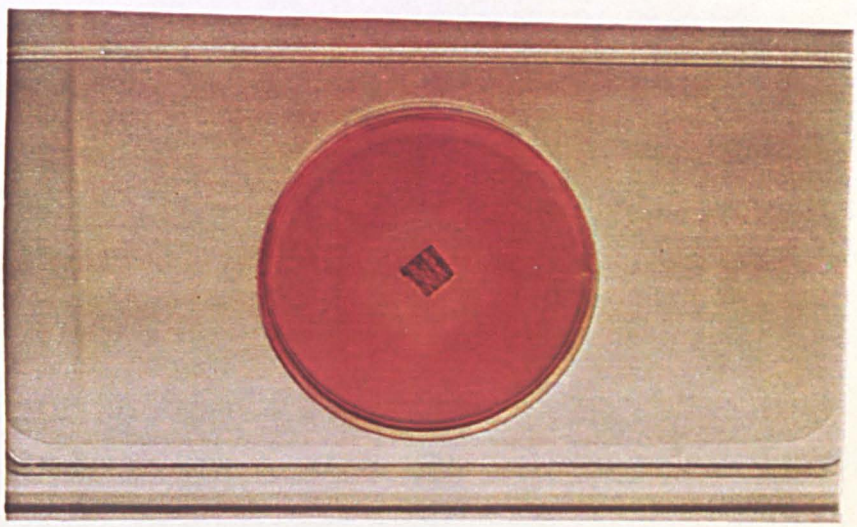


Fig. 4.11. Pattern of cellulase detection on carboxymethyl cellulose medium from wood slice after 16 weeks exposure to C. puteana in agar tests.

4.5. DISCUSSION

4.5.1. EXPERIMENTAL DESIGN I

EFFICACY OF ISOCYANATE MODIFICATIONS AGAINST BASIDIOMYCETES.

Chemical modification with the isocyanates tested afforded substantial bioprotection of Corsican pine against C. puteana, G. trabeum, C. versicolor and P. sanguineus. This was achieved without the presence of toxic chemicals after treatment and at levels of reaction not causing major loss of mechanical properties.

Earlier soil block decay tests by Ellis & Rowell (1982) show adequate decay resistance against G. trabeum in Southern pine treated with BuNCO to 18 WPG. In the current work, a lower WPG (10), was sufficient to prevent decay by G. trabeum in Corsican pine modified with BuNCO using a solvent (pyridine:acetone) different from that used by Ellis & Rowell (1982). Ellis & Rowell do not comment on the performance of blocks treated to lower levels hence it is not possible to compare this data precisely with their data. The levels of modification necessary to achieve protection in this work are similar to that reported by Kalnins (1982) who used G. trabeum, Lentinus lepideus and C. versicolor as test organisms on wood blocks treated with a range of pyridine catalysed isocyanates.

There are no published data on the use of HeNCO and HDI in fungal decay tests on whole wood. The data reported here suggests that the monofunctional HeNCO may be less effective against C. puteana than either BuNCO or the difunctional HDI. Thus the small increase in chain length between butyl and hexyl monofunctional isocyanates does not appear to have given an improvement in performance with the catalysis and swelling systems used in this work. There appears to be little difference in threshold values between BuNCO and HDI. However the intensity of decay (% weight loss) at treatment levels below the threshold values suggests that the wood was better protected against the four fungi by reaction with HDI than with BuNCO.

The envelope protection effect and the enhanced attack of the latewood regions in the blocks exposed to lower modification levels can be explained by the reagent concentration being too low in the reaction vessel. Thus the solution entering narrow latewood lumina contains insufficient reagent to give adequate reaction with the surrounding thick cell wall. Hence the reaction may become diffusion controlled, with inadequate reagent diffusing into the cell spaces to replenish that used up in the early stages of the reaction. This effect has important implications for the treatment of larger sized pieces of wood.

Chemical modification with the isocyanates at the 10 - 15 WPG treatment level appears to have

prevented the sterile blocks from wetting sufficiently for any fungal decay to occur (Fig. 4.6). Moisture content at the end of test shows decrease with increase in modification level. The moisture content at fibre saturation point values will decrease with increasing modification as fewer wood cell wall hydroxyls are available for water bonding.

When similar blocks were exposed to G. trabeum, C. versicolor and P. sanguineus the fungi were unable to wet the blocks sufficiently to allow decay (Figs. 4.7 & 4.8). However, modified blocks exposed to C. puteana showed higher threshold modification values (15.5, 18 and 13.6: for BuNCO, HeNCO and HDI, respectively) and subthreshold value modified blocks showed considerable wetting. It is not known whether the degree of wetting is controlled by the ability of the fungus to wet the wood cell wall or by the presence of extensive fungal biomass within the blocks. Whatever the reason however, the lowered moisture content appears to have been the major limiting factor in reducing the level of hyphal colonisation within the wood blocks and controlling decay in these tests.

Coniophora puteana proved capable of wetting the blocks sufficiently for decay to develop. With the other test fungi the moisture contents were below those generally regarded as necessary to allow decay to occur.

4.5.2. EXPERIMENTAL DESIGN II

DECAY INTENSITY OF A BROWN ROT FUNGUS ON MODIFIED WOOD AT SUB-THRESHOLD WPG

Higher decomposition rates in unmodified controls were obtained in the first 8 weeks of incubation where higher levels of moisture content were recorded. The same pattern of rapid initial attack had been reported with C. puteana in Pine wood (Seifert, 1962; Ruel & Barnoud, 1985) and with other brown rot fungus (Poria monticola) in Sweetgum (Cowling, 1961). Cellulose undergoes extensive depolymerization as a first step of wood degradation and subsequently further degradation and removal of cellulose and hemicellulose occurs progressively (Ruel & Barnoud, 1985; Kirk 1975). Cowling (1961) suggested that the less accessible crystalline cellulose was degraded primarily in the later stage of decay (after 20 percent weight loss) by P. monticola, where a more gradual decrease in the average degree of polymerization was verified. Here after initial rapid attack similar slow rate of degradation occurred, but in the range of 65 to 70% weight loss (12 to 32 weeks of incubation). At this range (65 to 70% weight loss) moisture content showed large variation, but had the tendency to decrease as degradation and consumption of carbohydrates took place.

Isocyanate reacted samples showed that decay intensity in modified samples depends largely on the

length of incubation and obviously by the internal moisture conditions of the decay jars. Modified specimens had retarded moisture absorptivity in comparison with unmodified samples. This has important implications in the assessment of preservative performance based on methods blocking hydroxyl groups within wood. Moisture content in the range of 22 to 34% was shown in HDI samples in which degradation was not beyond 22% weight loss, suggesting that supplementary water for fungal growth was supplied mainly from soil substrate in the test chamber. With BuNCO samples higher weight losses were followed by higher moisture content of the tests specimens at longer incubation period (12 to 32 weeks).

The results shows that changes in moisture content have a significant effect on decay; a difference in the initial moisture content of ca. 30% at 10 WPG at saturation (considering F.S.P of 30% for Corsican pine) had a critical effect on the rate of decay by C. puteana especially for the bifunctional affinity of HDI.

4.5.3. EXPERIMENTAL DESIGN III

PATTERNS OF ENZYME PRODUCTION BY DECAY FUNGI

Substrate modification reduces the ability of Basidiomycete to degrade wood and at threshold levels of modification prevented degradation completely (section 4.4). However despite the lack of degradation, fungi growing on isocyanate protected wood released cellulase and phenol oxidases from the modified wood blocks and indicates that blockage of susceptible wood cell wall sites is the possible mechanism of reduced diffusion and action of fungal enzymes.

As in control samples, isocyanate modified slices at all levels of modification showed a positive test for presence of cellulase for all the fungi and phenol oxidase enzyme, laccase was positive on unreacted and BuNCO reacted blocks in all test pieces for both C. versicolor and P. sanguineus. Peroxidases were not detected. With 1,6-diisocyanatehexane strong syringaldazine reaction with laccase was evidenced at 5 WPG test pieces for both fungi. For these fungi a weak reaction was noticed at 10 WPG. At 15 WPG C. versicolor failed to produce laccase whereas P. sanguineus gave a faint purple colour indicating a poor laccase production. Once again syringaldazine for peroxidases were not detected. However strong catalase activity indicated by effervescence upon hydrogen

peroxide addition was noticed on 10 WPG HDI samples exposed to P. sanguineus. The results on modified samples as already substantiated by the previous experiments that phenol oxidising enzymes of white rot fungi are more sensitive to the treatment with HDI than with BuNCO. This may implies that in BuNCO samples above threshold levels colonisation does take place through lumina, whereas degradation of cell wall is restrained.

CHAPTER 5.

CHEMICAL ANALYSIS OF UNDECAYED AND BROWN ROTTED SAMPLES OF CHEMICALLY MODIFIED WOOD.

CHAPTER 5.

CHEMICAL ANALYSIS OF UNDECAYED AND BROWN ROTTED SAMPLES OF CHEMICALLY MODIFIED WOOD.

5.1. INTRODUCTION

In the previous chapter differences in preservative efficacy against fungi were noticed. At moderate levels of substitution the chemicals (n-butyl isocyanate, hexyl isocyanate and 1,6-diisocyanatehexane) provided bioprotection. Results indicated that with the monofunctional isocyanates decay by C. puteana was more rapid than with the other fungi tested. The difunctional isocyanate gave consistently better performance with both brown and white rot fungi, than did butyl and hexyl isocyanates.

The distribution of reacted groups on the holocellulose and lignin fraction in the whole wood would require a profound topochemical study, whereas the present investigation was designed to define the possible effects of brown rot breakdown of holocellulose. To do the chemical analysis of holocellulose, lignin content had to be previously established. Based on the amount of lignin present in the samples, the extent of chlorite delignification was carried out which allowed to run chromatographic analysis of holocellulose samples with low residual lignin content. In addition, the hydrolysis and the delignification carried out in this study gave

insights on the stability of the bonds formed by isocyanate modification to the action of sulphuric acid and sodium chlorite. The measurements of the holocellulose by gel permeation chromatography (GPC) used in this study may provide a fundamental explanation of the fungal action upon changes in the polydisperse holocellulose system from chemically modified wood.

With cellulose derivatives it has been shown (Lloyd et al., 1987; Kennedy et al., 1990; Lawther et al., 1990) that GPC analysis is a useful technique for molecular weight evaluation. Furthermore it was recognized (Moore, 1964 cited by Slade, 1975) that polydisperse systems are separated in GPC columns according to molecular size of the molecules in solution. The mechanism of separation relies on the size of the sample molecules which are fractionated on an inert macroporous stationary support with controlled pore sizes. It was presumed that functional groups attached to isocyanate may have wide range of selectivity upon the size exclusion, thus a maximum pore volume was used here.

5.2. THE AIM OF THE PROCEDURE

a) To determine the acid insoluble lignin of control samples (unreacted, and isocyanate reacted wood at sub-threshold and threshold WPG's);

b) To determine and compare the rate of chlorite delignification between samples (sound and decayed, unreacted and reacted) to provide holocellulose prior to gel permeation chromatography;

c) To determine and compare the structural parameters of holocellulose samples i.e. molecular weight averages, degree of polymerization, distribution and polydispersity in sound and decayed, unreacted and reacted samples.

5.3. MATERIALS & METHODS

5.3.1. SAMPLING

Control (pyridine:acetone) and isocyanate modified samples were selected after being exposed to C. puteana in the biodeterioration tests (chapter 4, Experimental design II, Table 5.1.). Chemical modification procedures have previously been described (chapter 3.).

TABLE 5.1

TREATMENT VARIABLES FOR THE CHEMICAL ANALYSIS

TREATMENT	WPG*	WEIGHT LOSS* (%)
Unmodified	-	0.0
	-	7.14 (1.39)
	-	22.93 (2.38)
	-	46.55 (2.88)
	-	55.31 (6.02)
	-	70.44 (0.36)
BuNCO	10.68 (0.68)	0.0
		7.24 (1.37)
		22.95 (1.78)
		57.83 (3.60)
	16.13 (0.45)	0.0
HDI	10.16 (0.70)	0.0
		7.59 (0.67)
		22.59 (1.08)
	14.51 (0.42)	0.0

* Standard deviation in brackets.

5.3.2. SAMPLE PREPARATION

Samples were fractionated (razor blade split and by grinding in a beater mill) and sieved to pass a 40 mesh standard sieve. Samples not subjected to C. puteana attack were ground and further extracted with a 4:1:1 mixture of toluene, acetone and industrial methylated spirit (commercial grade) in a Soxhlet apparatus for 4 hours. Then they were allowed to dry at room temperature.

5.3.3. ACID INSOLUBLE LIGNIN

Lignin determination was according to the procedure described in Browning (1967) with some modifications according to Effand (1977). Approximately 300 mg of wood flour was weighed in a tared 50 ml beaker and 4.5 ml of 72% sulphuric acid was added. The mixture was frequently stirred for 2 hours at 20 °C in a water bath. After the first hydrolysis the material was washed into a 250 ml Erlenmeyer flask with 168 ml of de-ionised water to dilute the sulphuric acid concentration to 3%. The secondary hydrolysis was carried out in an autoclave for 2 hours at 120 °C. While keeping the solution hot the lignin was filtered off through a pre-weighed fritted glass crucible. The residue was thoroughly washed with hot water to completely remove the acid. After drying at 105 °C to a constant weight, the crucibles were cooled in a desiccator and the

weight registered. The lignin was calculated as a percentage of extracted oven dried samples of unexposed material. Moisture content determinations were run on portions of a sample separate from that taken for analysis.

5.3.4. HOLOCELLULOSE DETERMINATION

For determination of holocellulose, delignification was performed with sodium chlorite at 70 °C according to the method of Browning (1967). For this, 1g of wood flour (40 mesh) was weighed in a tared 250 ml Erlenmeyer flask. 160 ml of de-ionised water was added and heated on a water bath at 70 °C. After reaching the desired temperature, 0.5 ml acetic acid and 10 ml of 15% solution of sodium chlorite were added. Every hour, a further 0.5 ml of acetic acid and 10 ml of sodium chlorite were added. Control samples were removed at differing time intervals (2, 4, 5 & 8 hours) of delignification. At each selected time, samples were cooled in iced water to less than 10 °C. Then they were filtered through a pre-weighed glass crucible and successively washed with 100 ml of alcohol (industrial methylated spirit), 20 ml of ice cooled water and 50 ml of acetone. The iced water washing step was avoided with decayed samples because the filtering process was clogged by smaller particles. After drying at 50 °C to a constant weight, the samples were cooled in a desiccator and weighed.

The residue was expressed as a percentage of the undecayed extracted oven dried samples. Holocellulose determination of samples at progressive decay rating (unreacted and isocyanate reacted specimens) were delignified for eight oxidation cycles only.

5.3.5. GEL PERMEATION CHROMATOGRAPHY (GPC)

5.3.5.1. SAMPLE PREPARATION

Holocellulose samples (controls and decayed) prepared at eight hours sodium chlorite delignification were assembled individually and 0.12g samples were weighed into 50 ml centrifuge tubes. De-ionized water (20 ml) was added and left overnight to allow sample swelling. The samples were centrifuged for 15 minutes at 2,500 RPM (Baird & Tatlock Auto Bench centrifuge Mark IV). Subsequently the water was replaced with N,N Dimethyl acetamide (DMA) analytical grade. To ensure complete exclusion of water from the samples the solution was changed at least 8 times. Each change was followed by centrifugation. At the end of washing process, the samples were dissolved in 12 ml of 6% w/v lithium chlorite analytical grade in N,N dimethyl acetamide and were occasionally stirred until completely dissolved. Prior to chromatographic analysis samples were diluted to 0.1% w/v lithium chlorite in N,N Dimethyl acetamide.

5.3.5.2. GPC APPARATUS

The gel permeation chromatography system was a KNAUER HPLC (High Performance Liquid Chromatography) from Polymer Laboratories, pump model 64, differential refractometer model 98. A chart recorder (Pharmacia Fine Chemicals) was employed to covert the elution data into molecular weight distributions using data derived from the calibration standards (see below).

5.3.5.3. ELUENT, COLUMN & CALIBRATION STANDARDS

As eluent, 0.5% lithium chlorite analytical grade in N,N Dimethyl acetamide HPLC grade was prepared and filtered through a 0.45 um nylon membrane filter (Gelman Science). Operating conditions used throughout the work were a flow rate of 0.5 ml / min at 80 °C temperature and a 250 ul sample injection volume.

Column (300 x 7.7 mm) consisted of mixed pore PLgel (10 um) mixed A° column (Polymer Laboratories Ltd.) having a molecular weight range from 200 to 2 x 10^6. It was protected with a precolumn (50 x 7 mm) packed with PLgel (10 um) 100 A° (Polymer Laboratories Ltd.).

For the calibration, polysaccharide polymer Standards (Polymer Laboratories Ltd.) were used over the range of Mp (Peak average molecular weight) from 5,800 to 853,000 and polydispersity index (M_w/M_n) from

1.06 to 1.14 (Table 5.2). A calibration curve was obtained from the logarithm of the peak molecular weight (M_p) plotted against the elution volume when the samples run in PLgel columns of mixed porosity using 0.5% lithium chloride in DMA as an eluent.

TABLE 5.2

POLYSACCHARIDE STANDARDS

Polysaccharides Mp x 10^4	M_w/M_n
85.30	1.14
38.00	1.12
10.00	1.10
4.80	1.09
1.22	1.06
0.58	1.07

5.3.5.4. CALCULATION OF GPC CHROMATOGRAM

A base line was drawn through the whole range of the chromatogram. Then it was divided into constant volume increments (V_{Ri}= 0.2 ml), and the chromatogram peak height (h_i) at each division was measured and recorded and the molecular weight (M_i) was the co-logarithimic (M_i= $10^{\log \text{ molecular weight}}$) taken from the calibration curve. Thus the calculations of number (M_n) and weight (M_w) average molecular weight were according to equation (5.a) and (5.b), respectively.

$$M_n = \Sigma h_i \; / \; \Sigma (h_i/M_i) \qquad (5.a)$$

$$Mw = \Sigma (h_i/M_i) / \Sigma h_i \qquad (5.b)$$

For the overall plot of molecular weight distribution (MWD) the differential weight distribution (dW/dLogM) of each mass fraction was calculated. The MWD width was judged by the polydispersity index (M_w/M_n). Degree of Polymerization (DP) values calculated from GPC analysis are weight averages.

5.4. RESULTS

5.4.1. SULPHURIC ACID LIGNIN

The sulphuric acid lignin value of unreacted Corsican pine differed (26.48%) from that of both BuNCO (29.73%) and HDI (33.24%) reacted specimens at subthreshold WPG, (Table 5.3). At increased WPG, results on sulphuric acid lignin remained practically constant for BuNCO reacted specimens (29.84%), but from 10 to 14 WPG an increase of 20% of insoluble lignin value was verified in HDI reacted samples.

TABLE 5.3

SULPHURIC ACID LIGNIN OF UNDECAYED EXTRACTIVE FREE UNREACTED AND ISOCYANATE REACTED CORSICAN PINE.

TREATMENT	WPG	SULPHURIC AC. LIGNIN (%)	TOTAL CARBOHYDRATE[1] (%)
Unmodified control	-	26.48	73.52
.. .. sub-threshold			
BuNCO	10	29.73	70.27
HDI	10	33.24	66.76
.....threshold			
BuNCO	16	29.84	70.16
HDI	14	39.87	60.13

1. Determined by difference from sulphuric acid lignin.

5.4.2. SODIUM CHLORITE DELIGNIFICATION

5.4.2.1. CONTROL SAMPLES NOT EXPOSED TO FUNGAL ATTACK

The rates of delignification of unreacted controls were higher than in isocyanate reacted controls (Table 5.4). In the initial 2 hours of delignification unreacted control values were 5 and 14 times higher than BuNCO and HDI, respectively. Increasing oxidation cycles towards 8 hours exposure to sodium chlorite, there was a gradual decrease in resistance in BuNCO treated samples, but HDI samples showed pronounced resistance to delignification.

At the end of the 8 hours exposure period (Table 5.4) the amount of lignin taken out by the sodium chlorite procedure for both unreacted and BuNCO is similar to that of sulphuric acid hydrolysis (Table 5.3), but with HDI control samples "lignin contents" were substantially lower than those of the controls achieved by H_2SO_4 hydrolysis.

At the higher level of modification in BuNCO treated specimens i.e. 16 WPG, no substantial change in resistance to chlorination occurred. A similar effect was noted with HDI i.e. 14 WPG.

TABLE 5.4

RATE OF DELIGNIFICATION OF UNREACTED AND ISOCYANATE REACTED CORSICAN PINE AT VARIOUS CYCLES OF OXIDATION BY SODIUM CHLORITE PROCEDURE OF CONTROL SAMPLES.

SODIUM CHLORITE OXIDATION CYCLES (hours)	% DELIGNIFICATION			RATIO		
	A Unreacted	B BuNCO	C HDI	A/B	A/C	B/C
.....sub-threshold.....						
.....10 WPG		10 WPG.....				
2	7.77	1.71	0.56	4.54	13.88	3.05
4	18.94	10.93	4.83	1.73	3.92	2.26
5	22.81	15.75	6.66	1.45	3.42	2.36
8	26.73	30.65	18.32	0.87	1.46	1.67
.....threshold.....						
.....16 WPG		14 WPG.....				
2	7.77	2.84	0.0	2.74	-	-
4	18.94	10.09	1.51	1.88	12.54	6.68
5	22.81	14.05	5.19	1.62	4,39	2.71
8	26.73	29.94	11.31	0.89	2.36	2.65

5.4.2.2. SAMPLES AT DIFFERENT DEGREES OF DECAY INTENSITY

The holocellulose contents of reacted and unreacted wood at similar levels of fungal degradation (e.g. 7; 23; and 55-57% weight loss of unreacted and BuNCO reacted samples) are in close agreement with one another (Table 5.5), although the initial values of holocellulose differed, 73% for unmodified control and 69% for BuNCO modified samples.

5.4.3. MOLECULAR WEIGHT DETERMINATION OF HOLOCELLULOSE

Fig. 5.1 shows the calibration curve used for the calculations of average molecular weights of holocellulose samples.

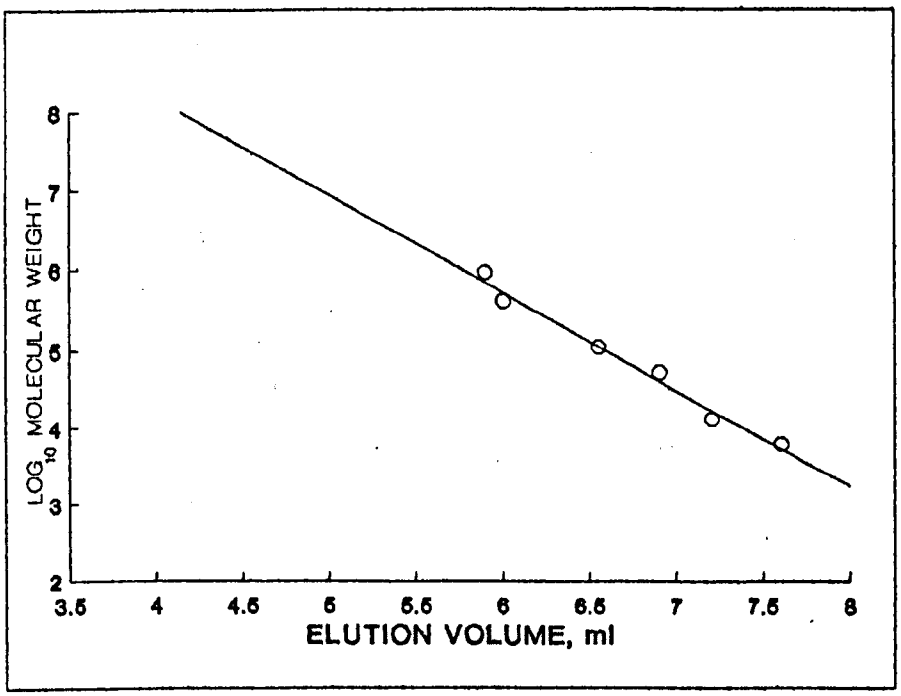


Fig. 5.1. Calibration Curve for Polysaccharides.

TABLE 5.5

RESULTS OF THE HOLOCELLULOSE PROCEDURE (8 HOURS DELIGNIFICATION) OF UNDECAYED AND BROWN ROTTED (C. puteana) WOOD OF UNREACTED AND ISOCYANATE REACTED CORSICAN PINE.

TREATMENT	WPG	WEIGHT LOSS (%)	HOLOCELL-ULOSE (%)	LIGNIN[1] (%)
Unmodified	-	0.0	73.27	26.73
	-	7.14	50.65	42.21
	-	22.93	40.18	36.89
	-	46.55	20.21	33.24
	-	55.31	18.62	26.07
	-	70.44	2.20	27.36
.....sub-threshold.....				
BuNCO	10	0.0	69.35	30.65
		7.24	51.55	41.21
		22.95	39.58	37.47
		57.83	13.42	28.75
HDI	10	0.0	81.68	18.32
		7.59	68.50	23.91
		22.59	53.64	23.77
.....threshold.....				
BuNCO	16	0.0	70.06	29.94
HDI	14	0.0	88.69	11.31

[1] -Determined by difference from holocellulose.

Table 5.6 shows the average molecular weight and degree of polymerization of unreacted and BuNCO reacted holocellulose samples. The HDI results are not included, because the GPC failed to separate due to presence of lignin in the samples.

TABLE 5.6

RESULTS OF THE MOLECULAR WEIGH VALUES, NUMBER AVERAGE (M_n), WEIGHT AVERAGE (M_w), AND DEGREE OF POLYMERIZATION (DP) OBTAINED FOR UNDECAYED AND BROWN ROTTED (C. puteana) HOLOCELLULOSE OF UNMODIFIED AND BuNCO MODIFIED CORSICAN PINE BY GPC.

TREATMENT WPG	WEIGHT LOSS (%)	M_n	M_w	DP
Unmodified				
	0.0	106 356	1 402 565	8 658
	7.14	15 525	311 688	1 924
	22.93	17 367	213 975	1 321
	46.55	12 559	86 632	535
	55.31	12 020	74 627	461
.....sub-threshold.....				
BuNCO				
10	0.0	70 343	441 884	2 728
	7.24	50 973	343 628	2 121
	22.95	27 506	315 265	1 946
	57.83	9 818	120 037	741
.....threshold.....				
BuNCO 16	0.0	15 432	309 951	1 913

5.4.3.1. DIFFERENCES IN THE NUMBER AVERAGE MOLECULAR WEIGHT (M_n)

The number average molecular weight of the holocellulose portion of unmodified control samples was in the order of 10.6×10^4, while BuNCO reacted

control samples had a reduction of 34 & 85 % respectively at 10 and 16 WPG in relation to that of unreacted control (Table 5.6; Fig. 5.2 A).

In unreacted specimens the changes induced by C. puteana (Fig. 5.3) in the M_n decreased sharply (up to 83%) at the initial stages of decay (7% weight loss). At higher weight loss (55%) the number of individual molecules in the sample were 11% of the total number average molecular weight of 106356 (Table 5.6). Despite the reductions noted in M_n for BuNCO reacted wood, the reduction in M_n for the decayed wood was less severe than for the controls i.e. drops of 28 & 61% respectively at weight losses of 7 and 23% were achieved in relation to that of undecayed control (M_n= 70343). At weight losses of ca. 57% the reduction in number average molecular weight was 86% of the initial M_n (70343).

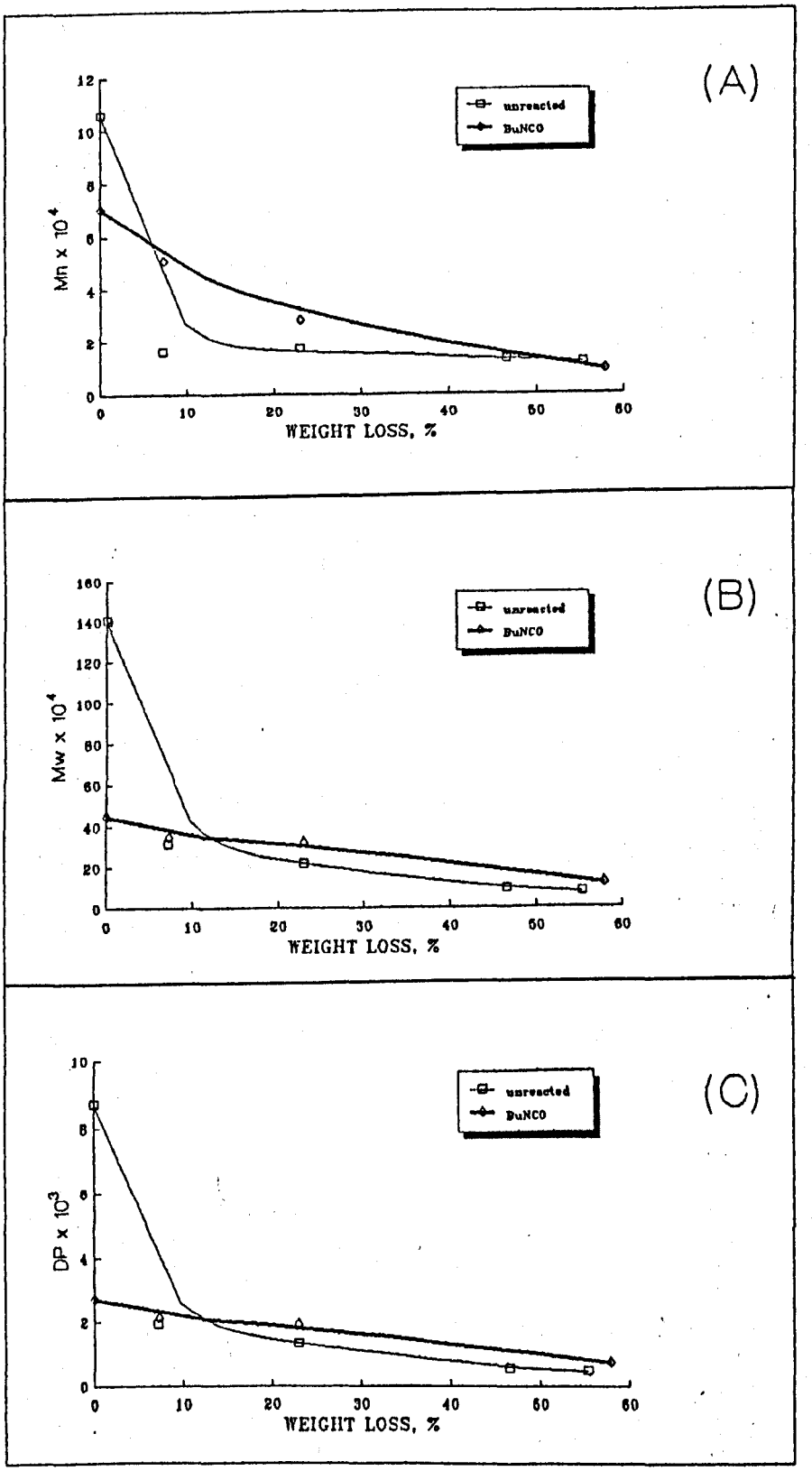
5.4.3.2. DIFFERENCE IN THE WEIGHT AVERAGE MOLECULAR WEIGHT (M_w).

The weight average molecular weight of the holocellulose portion of unmodified samples was in the order of 1.4×10^6 (Table 5.6; Fig. 5.2 B), whilst BuNCO reacted at 10 WPG was reduced by 68% and at 16 WPG was 78% lower. The unmodified sample had its M_w decrease similar to its M_n i.e. in a linear way in the initial (7% weight loss) fungal attack. As degradation increased carbohydrate consumption was slower. At 55%

weight loss the total mass of the molecules comprising the holocellulose sample was only 5% of the total amount of 1.4×10^6.

With the isocyanate modified samples the holocellulose weight average molecular weight dropped gradually. At 7% weight loss its M_w was 78% of the total amount of 0.44 million and at close to the maximum weight loss (57%) the M_w comprised 27% of its total. At comparable weight losses (55% unreacted & 57% BuNCO treated) the weight average molecular weight in relation to its respective control was higher for modified samples than it is for unmodified ones (Table 5.6).

Fig. 5.2. Relationship between A) number average molecular weight (M_n), B) weight average molecular weight (M_w), and C) average degree of polymerization (DP) of holocellulose samples and average percent weight loss of unreacted and n-butyl isocyanate reacted Corsican pine at different degrees of decay intensity caused by C. puteana.


(A)
unreacted
BuNCO
Mn x 10⁴
WEIGHT LOSS, %
(B)
unreacted
BuNCO
Mw x 10⁴
WEIGHT LOSS, %
(C)
unreacted
BuNCO
DP x 10³
WEIGHT LOSS, %

5.4.3.3. DEGREE OF POLYMERIZATION (DP)

The average DP of unreacted control samples was 8658 (Table 5.6 & Fig. 5.2 C), while isocyanate control sample was 68% lower than the original. In unmodified specimens the DP units dropped rapidly in response to decay by C. puteana. The average DP at 55% weight loss was only 5% of the sound wood value of 8658.

Conversely, modified samples the holocellulose showed a slow depolymerization as decay intensity increased. Holocellulose of treated samples at 57% weight loss showed an average of 741 DP units which correspond to 27% of the original value of 2728 (Table 5.6). At comparable levels of degradation (7; 23; 55-57% weight loss) the reduction in DP units in BuNCO reacted samples respective to its undecayed control was only marginally higher than those observed in unreacted holocellulose samples, However at 55% weight loss (unreacted) and 57% (reacted) the drop in DP units was 18.78 & 3.68 times less when compared to undecayed unmodified and modified samples.

5.4.3.4. DIFFERENCE IN MOLECULAR WEIGHT DISTRIBUTION & DISPERSITY

The molecular weight distribution (MWD) and dispersity (M_w/M_n) of holocellulose samples are shown in Table 5.7. Fig. 5.3 shows the MWD of samples not exposed to fungal attack (controls). The MWD of

unreacted samples ranged from 4.28 to 7.80 Log_{10}, whilst BuNCO at 16 and 10 WPG varies from 2.53 to 6.76 and 3.58 to 6.63 Log_{10}, respectively. The polydispersity index (M_w/M_n) of 13, 6, 20 was achieved for unreacted, BuNCO at 10, and 16 WPG of samples not exposed to fungal attack, respectively (Table 5.7).

TABLE 5.7

RANGE MOLECULAR WEIGHT DISTRIBUTION (MWD) AND DISPERSITY DATA FOR UNDECAYED AND BROWN ROTTED (C. puteana) HOLOCELLULOSE OF UNMODIFIED AND BuNCO MODIFIED CORSICAN PINE AS SHOWN BY GPC.

TREATMENT	WPG	WEIGHT LOSS (%)	RANGE MWD (Log_{10})	DISPERSITY (M_w/M_n)
Unmodified	-	0.0	4.28-7.80	13.19
	-	7.14	2.96-6.96	20.08
	-	22.93	2.99-6.76	12.32
	-	46.55	2.87-6.64	6.90
	-	55.31	2.81-6.58	6.21
.....sub-threshold.....				
BuNCO	10	0.0	3.58-6.63	6.28
		7.24	3.34-6.87	6.74
		22.95	3.22-6.99	11.46
		57.83	2.65-6.63	12.23
.....threshold.....				
BuNCO	16	0.0	2.53-6.76	20.08

Fig. 5.4 shows MWD of unreacted samples at different degrees of decay severity. In the initial (7% weight loss) phase of brown rot attack dispersity was 1.5 times higher (20.08) than the original value

of 13.19. As weight loss increased to 55% the MWD of the holocellulose narrows (Fig.5.4), and the dispersity was reduced to 0.5% of the original value of 13.19 (Table 5.7).

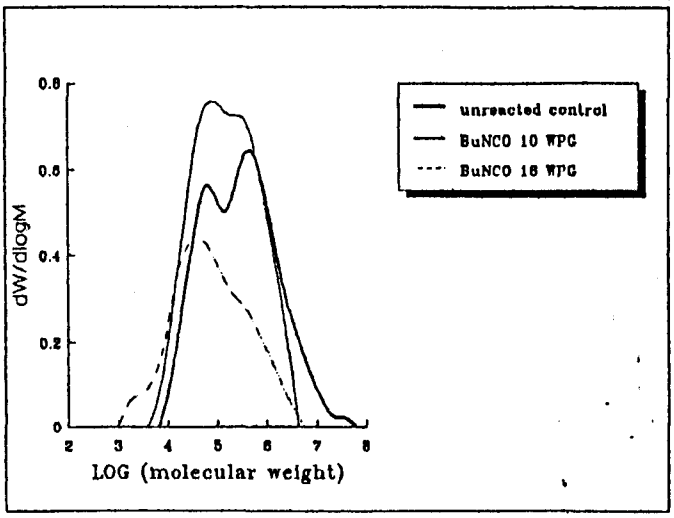
Fig. 5.5 shows MWD of BuNCO reacted samples at progressive decay rating. In contrast to unreacted samples, in the initial (7% weight loss) phase of brown rot attack, dispersity of 6.74 was nearly the same of its original value. At increased weight loss (57.83%) the molecular distribution of the holocellulose sample (Fig. 5.5) broadens, and the dispersity was 2 times higher than its control (Table 5.7).

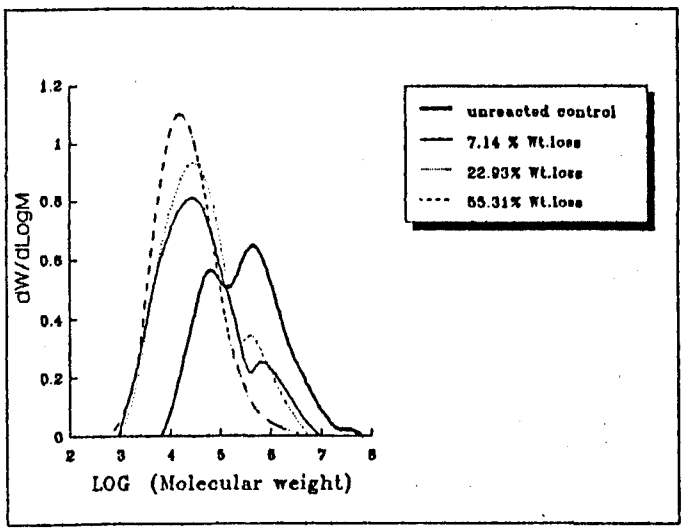
At comparable weight losses (Fig 5.6 A, B & C) wider ranges of MWD were shown with chemically modified samples in comparison to unmodified specimens. A slight difference was observed at high level of decomposition (Fig. 5.6, C). The polydispersity index of these samples (Table 5.7) indicates that at a comparable weight loss rating, the products of decomposition in modified samples differs from that of unmodified holocellulose samples.

Fig. 5.3. Molecular weight distribution by GPC analysis of holocellulose control samples of unreacted and n-butyl isocyanate modified Corsican pine.

Fig. 5.4. Molecular weight distribution by GPC analysis of holocellulose samples of unreacted Corsican pine at different degrees of decay intensity caused by C. puteana.

Fig. 5.5. Molecular weight distribution by GPC analysis of holocellulose samples of n-butyl isocyanate modified Corsican pine at different degrees of decay intensity caused by C. puteana.


unreacted control
BuNCO 10 WPG
BuNCO 16 WPG
dW/dlogM
LOG (molecular weight)


unreacted control
7.14 % Wt.loss
22.93% Wt.loss
55.31% Wt.loss
dW/dLogM
LOG (Molecular weight)

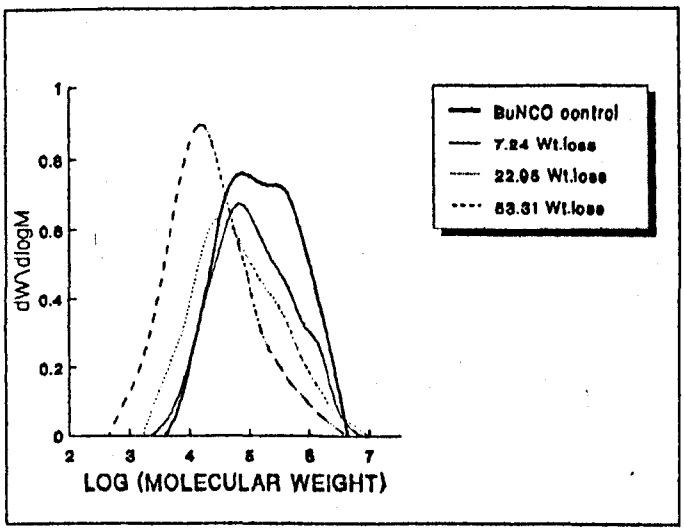
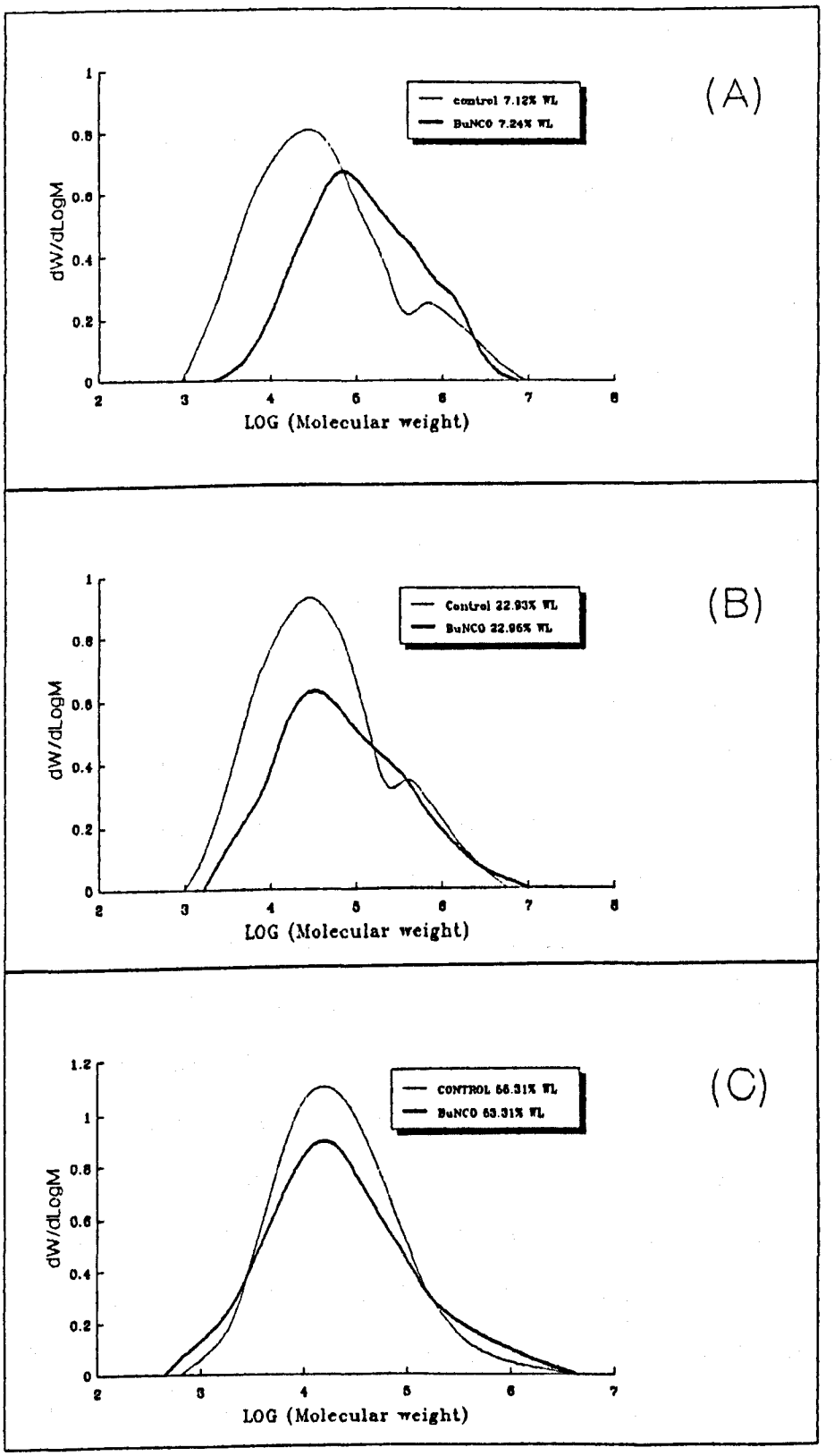

BuNCO control
7.24 Wt.loss
22.95 Wt.loss
53.31 Wt.loss
dW\dlogM
LOG (MOLECULAR WEIGHT)

Fig. 5.6. Comparison of molecular weight distribution of holocellulose samples between unreacted and n-butyl isocyanate modified Corsican pine at: (A) 7%, (B) 23% and (C) 55-57% weight losses promoted by C. puteana.


(A)
control 7.12% WL
BuNCO 7.24% WL
dW/dLogM
LOG (Molecular weight)
(B)
Control 22.93% WL
BuNCO 22.96% WL
dW/dLogM
LOG (Molecular weight)
(C)
CONTROL 56.31% WL
BuNCO 63.31% WL
dW/dLogM
LOG (Molecular weight)

5.5. DISCUSSION

In isocyanate treated specimens, sulphuric acid lignin values (Table 5.3) show increases in weight due to urethane bonding to the lignin. Even higher quantities of lignin were apparent with the difunctional isocyanate HDI (unmodified= 26%; 10 WPG= 33% & 14 WPG= 40%). These increases suggest that dissolution of carbohydrate in acid may be prevented due to linkages formed between lignin and carbohydrate components. Similar difficulties were experienced with the sodium chlorite procedure.

Results on sodium chlorite delignification (Table 5.4 & 5.5) suggest that the oxidation cycles bring about only mild dissociation of lignin from carbohydrate of HDI reacted samples, especially to those chemically modified at ca. 14 WPG. With BuNCO, lignin removal was slow in comparison to unreacted samples, but lignin free holocellulose samples were attained. Therefore, since the initial values of holocellulose for the control and BuNCO differed (73% and 69%, respectively), this may indicate that some degradation may have occurred either during the prolonged delignification procedure (8 hours cycle) or during initial chemical modification procedure. The holocellulose result of the BuNCO samples (70%) is in agreement with sulphuric acid results (70%).

As in the sulphuric acid procedure, complete reaction in the sodium chlorite procedure with HDI

modified samples was prevented due to the difunctionality of HDI. Interlacing of the lignin-carbohydrate complex is probably reinforced by grafting urethanes between molecules. So, results on the holocellulose content of HDI samples do not comprise a straightforward measure of holocellulose and lignin content. This was confirmed by GPC analysis, in which presence of lignin in HDI holocellulose sample affected its complete dissolution in the eluent and subsequently failed to resolve in the chromatogram.

The effect of sample preparation whether for chemical modification purposes or GPC studies led to changes in the polysaccharide component of isocyanate reacted wood (Tables 5.6 & 5.7). Holocellulose samples not exposed to fungal attack (controls) showed that original values of all parameters studied (M_n, M_w, DP, dispersity) differed from unreacted holocellulose samples. The low molecular weights of the holocellulose of the BuNCO reacted samples at 10 and 16 WPG's (Table 5.6) is believed to be because the carbohydrate part is not tightly linked by substitution reaction into the wood matrix. So, the peaks resolved by GPC are portions of the holocellulose available for depolymerization by any means (chemical or enzymatic) and with fewer internal wood-isocyanate esters in the chains. It is possible that the wood flour which is retained by the sieve

during preparation might have long polysaccharide chains with more stable rearrangement products. It was noticed that ground modified specimens produced a greater number of larger size particles than those from unreacted controls. This might have accounted significantly for the change in viscosity produced in reacted samples.

The relationship between average molecular weight and weight loss (Fig. 5.2. A & B) show pronounced differences in the products of degradation between modified and unmodified samples. The weight loss curve of the M_n differed particularly at lower weight losses (7 & 22%) between the two samples (unmodified & BuNCO reacted). This suggest structural differences in the initial carbohydrate removal by C. puteana. At high weight loss (>50%) the M_n curves (Fig. 5.2 A) of BuNCO and unreacted intercepted each other. This may suggest that at the later stage of degradation the consumption of individual molecules by C. puteana in BuNCO specimens followed a similar pattern to that of unreacted samples.

Contrary to M_n, the weight average molecular weight (M_w) at high weight losses (55% unreacted & 57% BuNCO) is higher for modified samples than it is for unmodified (Fig. 5.2 B, Table 5.6) in comparison with undecayed respective controls. This correlates well with the existence of heavy fractions i.e. substituent groups in the BuNCO:polysaccharide portion. The curves

(Fig. 5.2 C) of the degree of polymerization (DP) of holocellulose samples at progressive fungal attack show similar pattern to that of M_w since the DP is dependent on the mass fractions of the molecules comprising the sample.

The data consistently shows that at the C. puteana:BuNCO threshold level (16 WPG) the exposed material still had a M_n, M_w, and DP at levels similar to that found in unreacted samples when the material was depolymerized by C. puteana to a 7% weight loss (Table 5.6). The holocellulose at threshold WPG (16 WPG) still possess unsubstituted hydroxyl groups suitable to either chemical or enzymatic treatment.

Furthermore, the difference in molecular weights in the undecayed control samples (BuNCO at 10 WPG, M_w= 442,000 and 16 WPG, M_w= 310,000) implies that further increases in WPG result in more substitution of holocellulose and further reductions in DP. Such further reductions in DP have yet to be demonstrated.

In conclusion the bioprotection of holocellulose against C. puteana at 16 WPG might be accomplished by mechanical barrier of substituted lignin and some substituted polysaccharide, rather than high holocellulose modification. With HDI in addition to that, biodeterioration was prevented by the crosslinking formed by addition reactions of HDI to wood components.

CHAPTER 6.

TENSILE STRENGTH OF ISOCYANATE MODIFIED THIN WOOD STRIPS AFTER FUNGAL COLONISATION IN A FLAT BED UNSTERILE SOIL SYSTEM

CHAPTER 6.

TENSILE STRENGTH OF ISOCYANATE MODIFIED THIN WOOD STRIPS AFTER FUNGAL COLONISATION IN A FLAT BED UNSTERILE SOIL SYSTEM

6.1. INTRODUCTION

A study on the gross distribution (chapter 4) of decay within isocyanate modified wood blocks (20 x 20 x 10 mm) revealed that location within the sample had a pronounced effect on the distribution of the substituted isocyanate reagent within the wood tissues. The outside of the blocks were effectively protected and thus an accelerated ground contact test was chosen with the sample reduced to a thin strip. The method used here was based on an unsterile soil technique of Smith (1980). This constitutes an extreme exposure bioassay because the samples are subjected to a wide range of microorganisms and leaching.

The present work was carried out to find out whether isocyanate treated strips are affected by soft rot colonisation under direct humidity conditions as in soil. Studies on soft rot attack on wood indicate that they generally utilise cellulose and other carbohydrates rather than rapid lignin breakdown (Savory & Pinion, 1958; Levi & Preston, 1965).

As with studies by Smith (1980) tension parallel to the grain was chosen as the criterion to quantify decay resistance. There have been indications that

tensile testing is a suitable technique to test modified wood (Rowell & Banks, 1987).

The effect of fungi on the strength properties of isocyanate modified wood is little reported. Lutomski (1975) reported a 20% loss of static bending strength of a aqueous solution of diisocyanate in the form of the preparation called 'Izocyn T-80' on Beech wood (Fagus sylvatica L.) after fungal attack by Coniophora cerebella Pers.:Pers. (= C. puteana) and Polystictus versicolor (L.) Saccardo (= Coriolus versicolor). The isocyanate was polymerised to about 50% after treatment. Thus the method of treatment is not similar to the modification reaction carried out in this work. There have been contradictory reports on whether isocyanate modification itself has a positive or negative effect on the mechanical properties of wood. Reduced (Gafurov et al., 1970; Rowell, 1975) and increased (Clermont & Bender, 1957; Wakita et al., 1977) mechanical properties has been reported on isocyanate modified wood and wood products.

6.2. THE AIM OF THE PROCEDURE

The purpose of this investigation was to assess loss of tensile strength as a result of soft rot attack in wood reacted with mono- and difunctional isocyanates after unsterile soil testing.

6.3. MATERIALS & METHODS

6.3.1. SAMPLING AND MODIFICATION

Corsican pine sapwood blocks of 140 x 40 x 10 mm with the grain parallel to the length were soaked to achieve nearly full saturation in distilled water. Specimens were then sliced with a sledge microtome at 100 um thick parallel to the long axis. Test strips were labelled with 2B pencil and distributed into 9 groups of a minimum of sixty repetitions. Additional strips were used for either selection of strips with approximately comparable weight percent gain (WPG) prior to exposure to biological test and to allow some specimens for fungal isolation studies.

Solvent treatment (Pyridine:acetone) and modification reactions with n-butyl isocyanate (BuNCO) and 1,6-diisocyanatehexane (HDI) were according to the procedure described in section 3.3. Four levels of WPG varying from 5 to 19% were achieved with both isocyanates. A minimun of 6 repetition and a maximum of 20 strips were used for each treatment level.

6.3.2. STRIP CONDITIONING

Both modified and unmodified strips used as controls (not exposed to fungal attack) were maintained dried in a desiccator prior to tensile

strength testing. The soft rot test strips were kept at room temperature before unsterile soil testing.

6.3.3. UNSTERILE SOIL TESTING

Biodeterioration tests were according to the bioassay designed by Smith (1980). Wooden boxes (Fig. 6.1), 305 mm long x 76 mm wide x 89 mm deep in two parts were used as decay chambers.

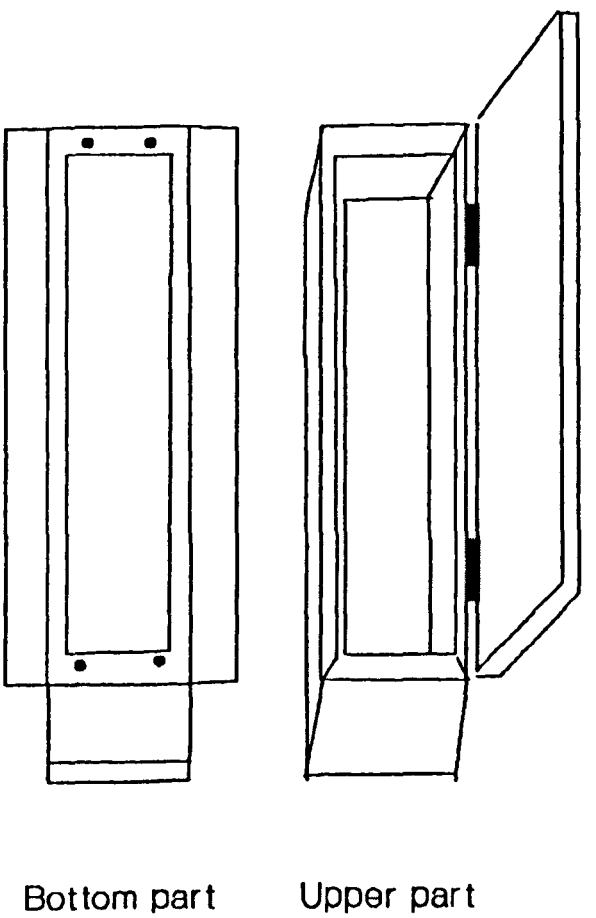


Fig. 6.1. Wooden box decay chamber

The box bottom part (Fig. 6.1) was filled with an horticultural soil (John Innes no. 2) with moisture content of ca. 50%, and pH of 5.8. The strips were laid down parallel to each other with the central part of their length (76 mm) exposed to the soil surface. Then, the upper part of the box was attached on to the lower part and soil was poured through the lid. Thus the strips were laid across the box and pressed into contact with the soil. Soil moisture content was maintained in the boxes by weighing before and at weekly intervals during incubation. Distilled water was added to return box weights to the initial weight.

6.3.4. INCUBATION

All boxes were incubated at 27 °C, 65% relative humidity. The incubation periods were from 0 to 60 days (i.e. 6 time intervals including unincubated controls).

6.3.5. STRIPS HANDLING AFTER INCUBATION

After incubation, boxes were disassembled and adherent soil on the samples was washed off with distilled water. Strips were left to air dry at room temperature. Subsequently they were dried to a constant weight at 105 °C.

6.3.6. STRENGTH TESTING

Control strips (unreacted & isocyanate modified strips) not exposed to decay test and those after unsterile soil test were individually stressed in a INSTRON machine model 4301. For the tensile strength test a constant elongation speed of 1 mm/min was used and each strip was strained at a 40 mm span length. Tensile strength was calculated as ultimate load (N) divided by cross sectional area (mm^2).

6.3.7. MICROSCOPIC EXAMINATION

After tensile strength tests ruptured surfaces were observed through a polarised light in a Leitz Orthoplan light microscope. Microscopic examination was performed in specimens mounted in Benzyl Benzoate.

6.3.8. ISOLATION OF FUNGI

Spare solvent treated strips were used for fungal isolation. After exposure, pieces of decayed strips were placed on a water agar plates and incubated at 27 °C. As fungal colonies grew away from the wood piece, they were aseptically transferred to a fresh 2% malt agar plates.

6.4. RESULTS

6.4.1. TENSILE STRENGTH OF UNREACTED AND ISOCYANATE REACTED CONTROL SAMPLES

Tensile strength of unreacted strips not exposed to fungal attack (Table 6.1) was in the range of 1.42-1.84 and 1.22-1.35 times higher than that of BuNCO and HDI modified specimens, respectively. Tensile strength of the HDI reacted strips at comparable weight percent gain was higher than that of BuNCO reacted specimens. For modified wood, regression analyses were performed in order to determine whether the differences in WPG's had some effect on the ultimate tensile strength data (Table 6.2). For strips treated with BuNCO as well as for those reacted with HDI the results show that there were poor indications (r^2=0.113, BuNCO; r^2=0.074, HDI) of linearity in tensile strength in relation to the increase extent of reaction (Fig. 6.2). Regression analysis of variance was significant at 5% level of probability for BuNCO and HDI strips, but it was not significant for HDI data at 1% probability (Table 6.2). The scatter diagram shown in Fig. 6.2 for both BuNCO and HDI indicates considerable variation in the data.

The difference in the mean tensile strength between modification levels are summarised in the Appendix 1. The mean tensile strength of HDI modified strips are not significantly (P>0.05) different from each other, while mean tensile strength of BuNCO strips reacted at 6 and 10 WPG are significantly

TABLE 6.1

VARIATION IN TENSILE STRENGTH OF UNMODIFED AND ISOCYANATE MODIFIED STRIPS NOT EXPOSED TO FUNGAL ATTACK.

TREATMENTS	WPG	TENSILE STRENGTH (N/mm^2)
Unmodified	-	63.44 (9.67)
BuNCO		
	6.09 (0.71)	44.55 (9.03)
	9.83 (0.53)	42.26 (15.48)
	14.14 (0.68)	34.45 (8.07)
	18.83 (0.54)	42.81 (12.77)
HDI		
	5.19 (0.80)	48.96 (8.26)
	10.27 (0.53)	52.05 (7.88)
	13.06 (1.03)	46.86 (12.04)
	16.57 (0.66)	46.91 (9.08)

TABLE 6.2

REGRESSION ANALYSIS ON THE RELATIONSHIP BETWEEN TENSILE STRENGTH AND WPG OF ISOCYANATE MODIFIED STRIPS USED AS CONTROLS.

A) BuNCO				
Source of variation	df	SS	MS	P
Regression	1	1685.3	1685.3	<0.05
Residuals	84	13233.3	157.5	
Total	85	14918.6		
B) HDI				
Regression	1	651.53	651.53	<0.05 >0.01
Residuals	84	8185.90	97.45	
Total	85	8837.43		

different ($P<0.05$) from those reacted at 14 WPG, but not from strips reacted at 19 WPG (Appendix 1). BuNCO reacted samples modified at 14 and 19 WPG had mean tensile strength significantly different ($P<0.05$) from each other.

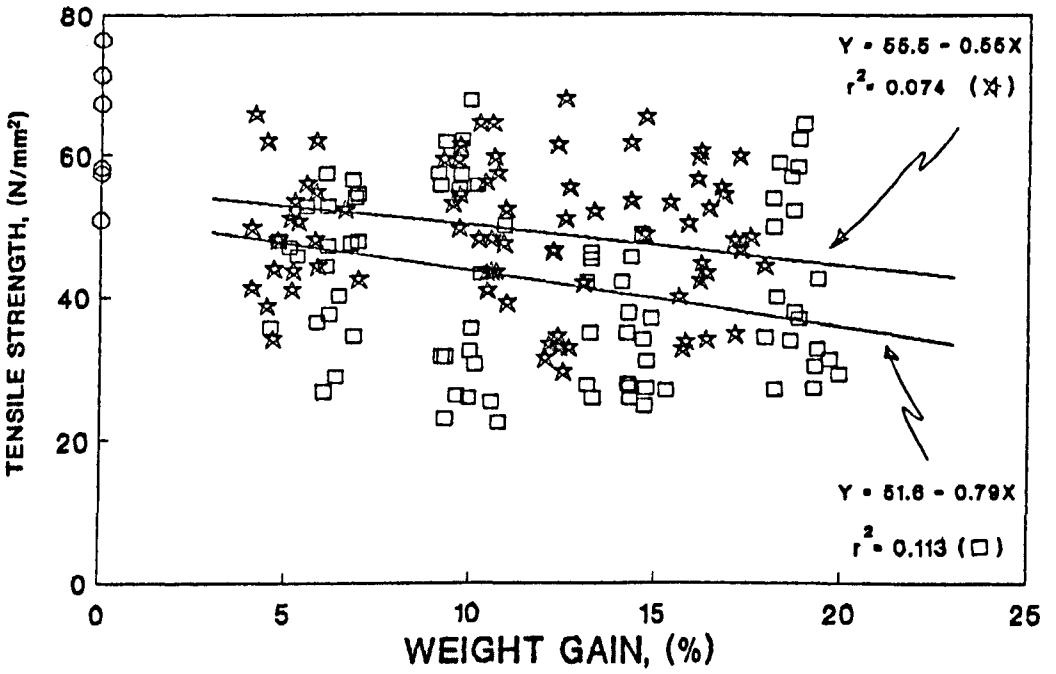


Fig. 6.2. Tensile strength of unreacted (○) and isocyanate (□ BuNCO; ☆ HDI) modified Corsican pine used as controls.

6.4.2. TENSILE STRENGTH AFTER UNSTERILE SOIL TESTS

The average strength presented in Table 6.3 is expressed as a percentage of the strength of specimens not exposed to the soil. Results expressed in N/mm^2 with their respective standard deviation are in the Appendix 1.

Soft rot decay resistance of unreacted wood measured by tensile strength as presented in Table 6.3 shows that there is a consistent decrease (r^2=0.97) in tensile strength with increasing time of exposure in unsterile soil tests. After each exposure period mean tensile strength are significantly ($P<0.05$) different from each other, with the exception of samples incubated between 25 and 30 days (Appendix 1). From 10 to 25 days of incubation the tensile strength was 94% less than the original sound strips. Further incubation resulted in complete failure of the test specimens.

For strips reacted with BuNCO and HDI the results show that there were poor indications (r^2=0.33; r^2=0.10) of reduction in tensile strength in relation to the increase extent of incubation for specimens reacted at 6 and 5 WPG's, respectively (Table 6.3). For strips reacted with BuNCO as well as for those reacted with HDI above 6 WPG the results (Table 6.3, Appendix 2) show that there were no reduction in strength in relation to the increase in incubation period under unsterile soil tests. As

TABLE 6.3

AVERAGE PERCENT TENSILE STRENGTH AND STANDARD DEVIATION (IN BRACKETS) OF UNREACTED AND ISOCYANATE MODIFIED CORSICAN PINE AFTER INCUBATION PERIODS IN THE UNSTERILE SOIL TESTS AND THE CORRELATION COEFFICIENT (r^2).

WPG*	TENSILE STRENGTH (%) time of exposure (days)						r^2
	0	10	25	30	40	60	
.....Unmodified control.....							
-	100 (15.24)	75.77 (13.56)	7.35 (46.57)	5.99 (91.84)	NT	NT	0.93
.....n-butyl isocyanate.....							
6.10 (0.60)	100 (5.33)	102.64 (8.36)	97.15 (17.75)	85.86 (12.92)	82.74 (20.19)	78.88 (20.64)	0.33
9.93 (0.55)	100 (21.63)	93.87 (9.93)	98.31 (12.24)	89.64 (8.16)	91.98 (20.72)	84.81 (4.26)	0.09
14.15 (0.76)	100 (22.33)	106.34 (29.94)	88.76 (31.96)	102.32 (34.45)	98.64 (25.33)	105.01 (34.35)	0.00
18.50 (0.96)	100 (13.30)	86.24 (26.31)	99.09 (28.99)	106.85 (23.50)	95.86 (31.62)	91.46 (32.16)	0.00
.....1,6-diisocyanatehexane.....							
5.01 (0.54)	100 (21.14)	104.58 (16.58)	102.74 (26.08)	90.55 (21.23)	88.14 (20.90)	84.85 (14.94)	0.10
10.34 (0.45)	100 (16.22)	83.29 (23.35)	82.18 (25.57)	88.47 (11.53)	92.11 (15.07)	95.48 (9.99)	0.00
12.64 (0.49)	100 (20.40)	93.75 (23.77)	94.47 (11.48)	91.06 (22.93)	106.11 (17.51)	108.12 (14.17)	0.03
15.80 (0.64)	100 (23.89)	99.49 (32.57)	91.98 (24.57)	102.17 (14.56)	100.20 (8.51)	100.07 (9.01)	0.00

NT = not tested due to complete failure of tests specimens.
* average WPG from the overall data.

depicted in Fig. 6.3 & 6.4 a, b, c & d there is a pattern common to all the figures that is a large variability in the tensile strength data for both unreacted and isocyanate (BuNCO and HDI) reacted samples.

Fig. 6.3. Variation of tensile strength of unreacted & BuNCO modified strips at: (A) 6 WPG, (B) 10 WPG, (C) 14 WPG and (D) 18 WPG, after unsterile soil tests.

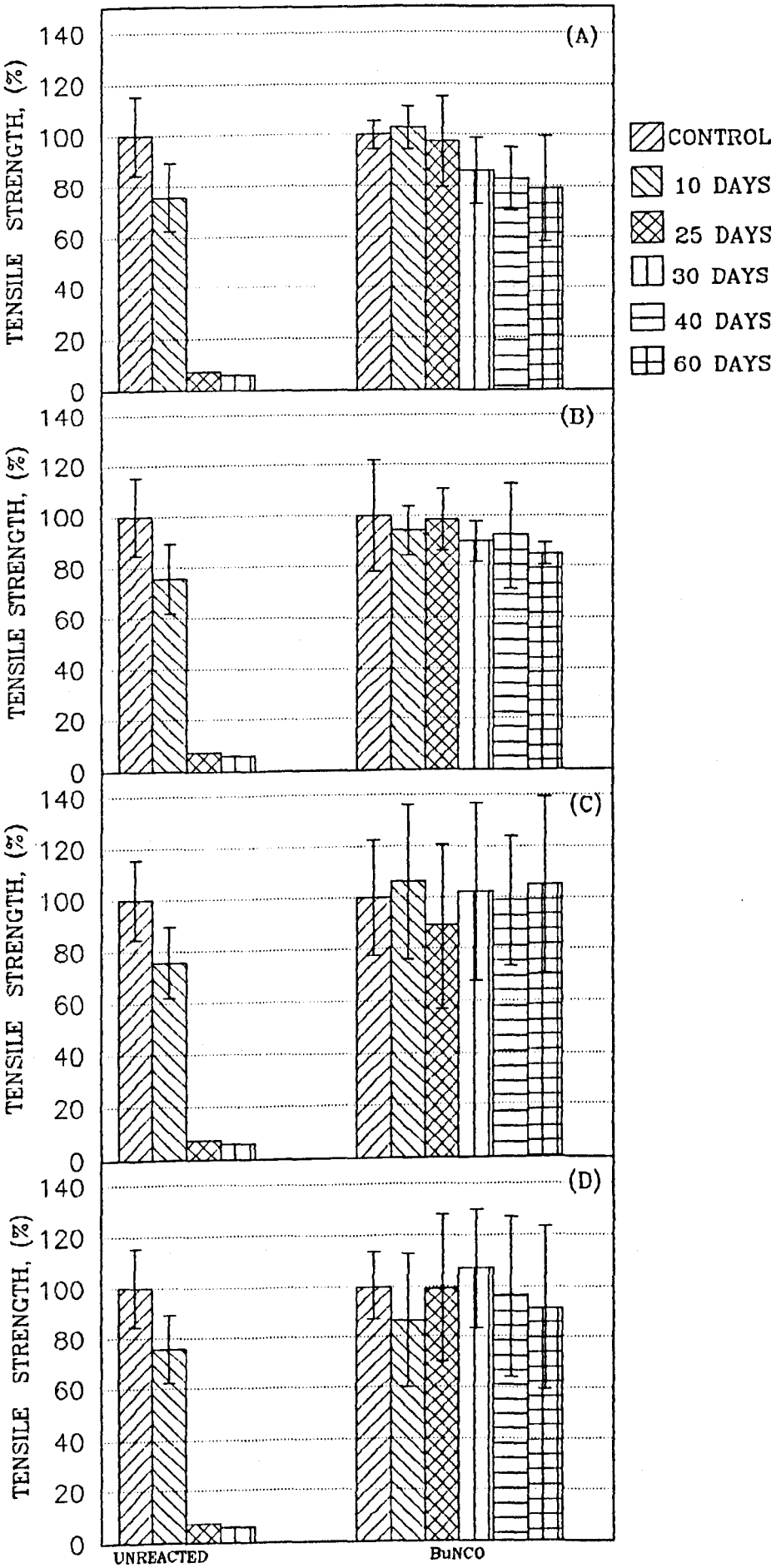
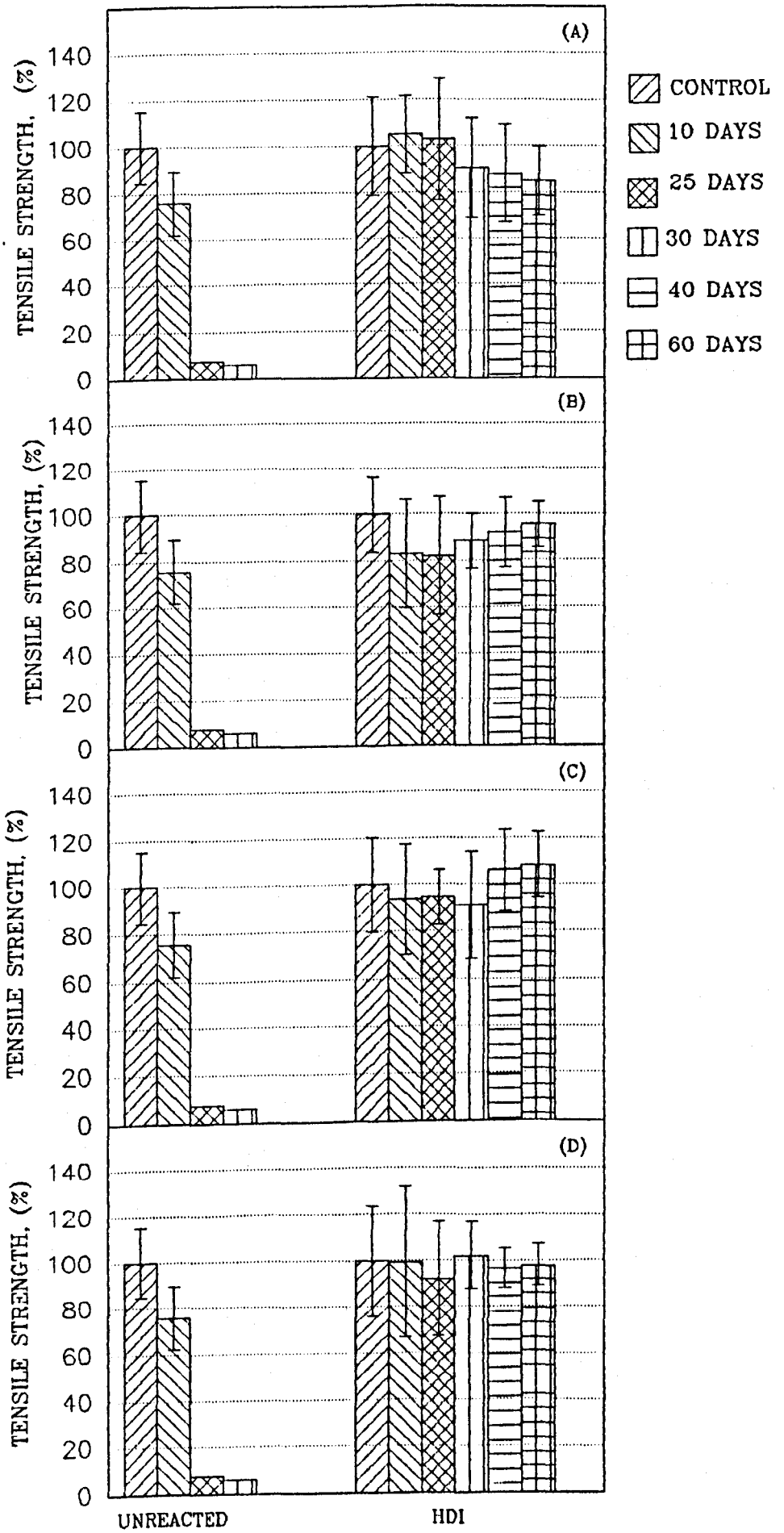

(A)
(B)
(C)
(D)
TENSILE STRENGTH, (%)
0
20
40
60
80
100
120
140
CONTROL
10 DAYS
25 DAYS
30 DAYS
40 DAYS
60 DAYS
UNREACTED
BuNCO

Fig. 6.4. Variation of tensile strength of unreacted & HDI modified strips at: (A) 5 WPG, (B) 10 WPG, (C) 13 WPG and (D) 16 WPG, after unsterile soil tests.


(A)
(B)
(C)
(D)
TENSILE STRENGTH, (%)
0
20
40
60
80
100
120
140
UNREACTED
HDI
CONTROL
10 DAYS
25 DAYS
30 DAYS
40 DAYS
60 DAYS

6.4.3. VISUAL EXAMINATION

Differences in the outward appearance of unreacted control specimens are illustrated in fig. 6.5. Heavy infection imparted a greenish colour to exposed surfaces was evident even at the initial incubation period (10 days). Towards the advanced stage of soft rot attack the samples disintegrated on handling.

In contrast, strips reacted with BuNCO and HDI remained bright in colour and were apparently uninfected at incubation periods from 10 to 25 days. After longer time of exposure, contrary to the unmodified strips, reacted samples showed minor discolouration in the exposed surfaces, but no visual incidence of fungal attack was observed (Fig. 6.6).

6.4.4. MICROSCOPIC EXAMINATION

Microscopic observation of infected unreacted controls revealed that soft rot fungi had primarily attacked the wood rays and other parenchyma cells at an early stage of infection (ca. 10 - 13% loss of tensile strength). Subsequently colonisation and progressive infection of latewood tracheids occurred. At a 93% loss in tensile strength wood tissues showed a nearly complete lack of birefringence.

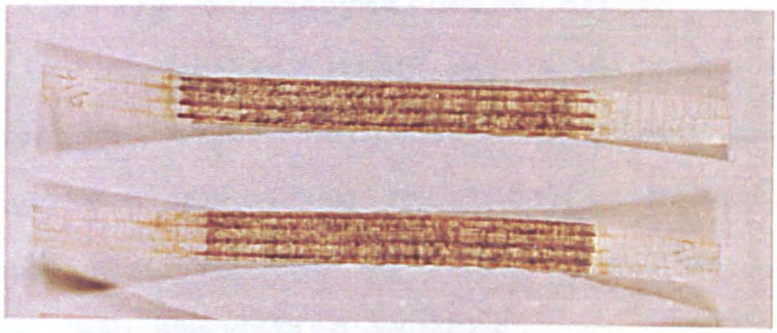


Fig. 6.5. Unmodified strips after 10 days exposure in an unsterile soil testing.

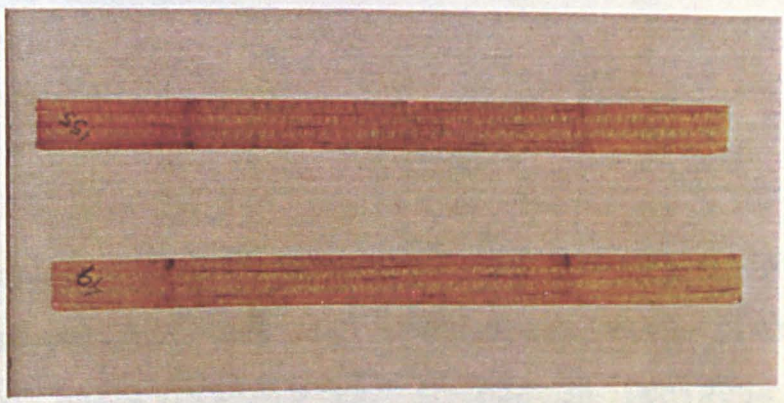


Fig. 6.6. BuNCO modified strip at 6 WPG after 25 days exposure in an unsterile soil testing.

Modified wood (< 10 WPG for both BuNCO & HDI) after 60 days of incubation showed only a few fungal hyphae in the ray parenchyma cells around latewood tracheids. It was noticed that sample ruptures after tensile strength tests were not localised in the attacked rays but sound (not attacked) wood portions. This suggests that modified sections of strips became more brittle than unmodified sectors.

6.4.5. ISOLATION OF FUNGI

Naturally occurring micro-organisms on unreacted strips were isolated belonging to two group (Fungi and Bacteria). The following fungi were identified:

Chaetomium globosum
Fusarium sp.
Penicillium sp.
unidentified sp.

Chaetomium globosum was the most prominent organism promoting soft rot decay isolated from the strips, but unfortunately the number of isolates were not recorded. The bacterial species found were not systematically identified but, the red pigmentation of the colony on agar and the colour left on the strips suggests that it may belong to the genus Serratia.

6.5. DISCUSSION

Tensile strength data of unreacted and isocyanate modified Corsican pine showed large variability (Table 6.1 and 6.3). Due to the fact that variation in the tensile strength data was also noticed in unreacted samples the results indicate that other factors than modification themselves are responsible for this high variation in strength data. Although having such high variation in the overall data, the results do indicate that strips are significantly resistant to soft rot attack after isocyanate reactions as compared with unreacted samples.

In unmodified strips, loss in tensile strength decreased linearly as increasing time of exposure. Although moisture content date is lacking, the results (Table 6.1, Fig. 6.5) showed that soil moisture content was relatively high to encourage soft rot colonization, which in turn highlight the moisture modifying properties of the BuNCO and HDI reacted strips even at low WPG's (Figs. 6.3, 6.4 & 6.6).

Comparison at the end of tests (60 days exposure) of the two isocyanates shows that strips reacted with HDI had less strength loss than those treated with BuNCO at modifications level below 10 WPG. Beyond this level loss of tensile strength was negligible for both HDI and BuNCO treated specimens. In almost all samples analysed at 60 days exposure,

only traces of growth could occasionally be observed microscopically on the modified strips below 10 WPG, indicating that although it is not a suitable substrate for fungi it is at least not fungicidal.

The isolations made from decayed wood from unmodified strips indicate that C. globosum was the species prominent promoting soft rot decay. Studies have shown that this fungus is able to decompose wood to a considerable extent (Gersonde & Kerner-Gang, 1976; Levi & Preston, 1965). A chemical and microscopic study by Levi & Preston (1965) with C. globosum on Beechwood (Fagus sylvatica) found that carbohydrates are completely metabolized and that lignin is only altered, and as decay proceeds they stated that only an altered lignin skeleton remains.

Considering the variability in the tensile strength data for both BuNCO and HDI reacted strips at progressive time of exposure, tensile strength results showed little difference, but the difference was substantiated by examining the surface colonization which shows that HDI is less susceptible than BuNCO at low (<10) WPG. In either case the surface protection afforded by the substitution reaction imposed a barrier to both axial and transverse soft rot colonization. This may emphasise the effect on the substitution of the structural components of wood (cellulose, hemicellulose, and lignin).

For conifer woods Zainal (1976a,b) partly substantiated the inhibitory effect of lignin by showing fungal hyphae capable of penetrating into delignified and probably dehemicellulosed wood without giving rise to the formation of cavities. The restriction on the active soft rot attack in this work may due to the inhibitory effect of high rate of lignin substitution in the case of BuNCO strips and as a result of increased crosslinking affinity of HDI among wood constituents.

The effect of isocyanate substitution effectively reduced (Table 6.1) tensile strength of samples not exposed to fungal attack as compared to unmodified controls. Little information is available concerning the actual or potential role in this connection. An interpretation might be that most usual properties determined from the response to tensile stress are related to the structural parameters such as degree of polymerization (DP), crystallinity, and molecular orientation. The changes in the DP noted for modified wood (chapter 5) would clearly explain this.

On the information available the physical chemical nature of the cell wall is of great importance in determining mechanical strength as stated early by Clarke (1935). Ifju (1964) in the study of the mechanical behaviour of wood with various cellulose DP in relation to temperature and moisture content found greater strength losses of latewood upon

increase moisture content, than those of earlywood. He assumed that the reason for this difference may be found in the higher specific gravity, consequently greater swelling capacity of latewood. Fengel & Wegener (1984) attributed mechanical function in softwood to latewood tracheids. Irrespective of the chemical modification system used, it seems apparent that thick fibres with less pitted walls, narrow lumina can be regarded as a significant factor affecting the accessibility of woody tissues to chemical modification. Since bioprotection against soft rot colonisation was markedly improved in modified thin wood strips, it was assumed that reduced sample size facilitates macro- and micro-distribution of the reagent in the wood cell walls. Therefore, there is a need in establishing the location of the changes in the original wood configuration after isocyanate reaction specially with difunctional isocyanates, which might give a better insight upon factors influencing tensile strength behaviour.

CHAPTER 7.

GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER 7.

GENERAL DISCUSSION AND CONCLUSIONS

7.1. GENERAL DISCUSSION

In this chapter experimental data already discussed in each chapter will only be used to highlight and clarify the main aspects essential to support this discussion.

Corsican pine chemically modified with n-butyl isocyanate (BuNCO), hexyl isocyanate (HeNCO) and 1,6-diisocyanatehexane (HDI) had a better inherent ability to withstand severe exposure conditions than unreacted wood. Degradation of modified wood was lowered considerably even at low substitution levels. Bioprotection performance of isocyanate modified wood differed whether the chemical is mono- or difunctional, whether the fungal group is brown, white or soft rot, and within fungal group.

7.1.1. BIOLOGICAL PERFORMANCE OF MONOFUNCTIONAL ISOCYANATES

BROWN ROT

Unmodified Corsican pine decayed by Coniophora puteana showed that polysaccharides in the cell wall are degraded very effectively. Decay intensity (Table 4.2 and 4.6) of unmodified control blocks reached 70% loss in dry weight, which was the maximum degradation expected. Lignin content determined by the difference

from holocellulose procedure of wood decayed at 70% weight loss was similar to its original value (Table 5.5). Holocellulose (Table 5.5) and lignin (Table 5.3) contents in Corsican pine constitutes 73% and 26% respectively, which may suggest that C. puteana utilizes cellulose and hemicellulose completely. Degree of polymerization (DP) of holocellulose unmodified samples decreased rapidly during C. puteana attack (Table 5.6).

Levy (1990) stated that the enzyme system of brown rots appears to be able to circumnavigate the ensheating lignin in some way, leaving it apparently largely unaltered, whilst totally destroying the associated cellulose and hemicelluloses. King (1966) reported that all the polysaccharide degrading enzymes of C. puteana are adaptive. He also stated that the hemicellulase activity included enzymes acting on all the major components of wood hemicellulose. For most other fungi, synthesis of enzymes is normally specifically induced by the monomer or dimer predominant in the polymer degraded by the enzyme (Cooper et al., 1978).

As compared to unreacted controls, degradation by C. puteana of wood reacted with BuNCO and HeNCO was restrained at low substitution levels (Table 4.2) and threshold WPG's were achieved at 16 and 18 respectively. When considering structurally different linear reactants care should be taken in the

interpretation of results based solely on the WPG criteria. The theoretical estimation of the degree of substitution (DS) based on equation 3.e (% of -OH reacted) demonstrated that the small increase in chain length between BuNCO and HeNCO does appear to have given an improvement in performance against C. puteana. Table 7.1 summarises the biodeterioration results on the relationship between WPG and amount of -OH groups reacted in modified wood tested against C. puteana. It thus appears that at threshold WPG, the DS values are somewhat comparable (Table 7.1). DS of BuNCO and HeNCO modified Corsican pine above 10% of -OH groups reacted in the whole wood is a limiting factor for C. puteana attack.

Results of Kalnins (1982) and Ellis & Rowell, (1984) showed indications that smaller substituent reagents (aliphatic isocyanates) were to a certain extent less effective at higher WPG than larger molecular weight isocyanates. Results on the enzymatic degradation of substituted water soluble cellulose of Keilich et al. (1970) showed that the smaller the substituent group the higher the DS which can be degraded. They stated that cellulase splits the cellulose chain i.e. the β-glucosidic bond, if not more than one substituent is linked to every glucose unit. In the whole wood further study is necessary to explain the effect of increasing chain length (side or intermediate alkyl radical containing the group

TABLE 7.1

RELATIONSHIP BETWEEN WPG AND DS (% OH REACTED) OF ISOCYANATE MODIFIED CORSICAN PINE AT SUB-THRESHOLD AND THRESHOLD SUBSTITUTION LEVELS AGAINST C. puteana AFTER 16 WEEKS IN THE SOIL BLOCK TESTING.

Sub-threshold				
TREATMENT VARIABLES	HDI	BuNCO	HeNCO	UNMODIFIED CONTROL[1]
WPG	11.57	9.83	10.93	-
-OH Reacted[2] (%)	9.29	6.69	5.81	-
Wt. loss (%)	13.04	37.73	41.35	69.81
Threshold				
WPG	13.60	15.50	18.00	-
-OH Reacted[2] (%)	10.92	10.54	9.57	-

[1] Pyridine treated control;
[2] estimated from equation 3.e assuming complete functionality of HDI.

-N=C=O) on decay of modified wood by fungi. It may be possible that proximity of urethane linkages of larger chain isocyanates may interfere with enzyme accessibility to susceptible groups in the wood.

The pattern of depolymerization (Table 5.6) between unreacted and BuNCO samples at comparable weight losses suggests that the effect of the changes in the fundamental configuration of the substrate by chemical modification causes the breakdown products to be dispersed with longer chains than that obtained with unmodified samples. Contrary to unmodified samples, a much slower progressive degradation (Table

4.6) by C. puteana was verified in BuNCO reacted specimens.

In comparison to C. puteana (Table 7.1), Gloeophyllum trabeum (Table 7.2) exhibited higher sensitivity to BuNCO modifications. At threshold WPG (10) against G. trabeum, results achieved against C. puteana (Table 4.6 & 5.6) confirm the susceptibility of polysaccharides to degradation at this level of modification.

Studies of the distribution of bonded methyl isocyanate (Rowell, 1982) indicate that resistance to biological degradation (including G. trabeum) is thought to be due to chemical changes mainly in the carbohydrate fraction. Rowell (1982) found decay resistance against G. trabeum above 18 WPG, which is above 20% of -OH reacted according to the equation 3.e. The result of Kalnins (1982) is closely related to those found here (ca. 10 WPG). The threshold at this low substitution level (Table 7.2) indicates that rather than high holocellulose substitution, other factors are important for protection against G. trabeum on modified wood. For example, differences in the adaptive character of the cellulases between fungal species, low tolerance to the enhanced hydrophobic nature of modified wood, differences in enzyme size, activity and difusibility. For instance C. puteana appears to have a water transport mechanism as it was able to wet blocks more effectively than G.

TABLE 7.2

RELATIONSHIP BETWEEN WPG AND DS (% OH REACTED) OF ISOCYANATE MODIFIED CORSICAN PINE AT SUB-THRESHOLD AND THRESHOLD SUBSTITUTION LEVELS AGAINST G. trabeum AFTER 16 WEEKS IN THE SOIL BLOCK TESTING.

Sub-threshold			
TREATMENT VARIABLES	HDI	BuNCO	UNMODIFIED CONTROL[1]
WPG	6.72	5.80	-
-OH Reacted[2] (%)	5.40	3.95	-
Wt. loss (%)	15.48	22.27	29.47
Threshold			
WPG	10.60	10.00	-
-OH Reacted[2] (%)	10.92	10.54	-

[1] Pyridine treated control;
[2] estimated from equation 3.e assuming complete functionality of HDI

trabeum (Table 4.4, Fig. 4.7).

It has been demonstrated that some basidiomycete fungi produce aggregations of hyphae termed cords or strands and rhizomorphs which run for considerable distances and are important in the hydraulic conductivity along the fungal organs for the translocation of nutrients and water (Eamus & Jennings, 1984; Eamus et al., 1985). Serpula lacrimans is marked in its ability to traverse nutritionally inert material and by such strands colonise from one piece of timber to another in buildings. The mechanism which drives translocation is believed to be turgor-

driven bulk flow of solution (Eamus & Jennings, 1984). It may be possible that some hydraulic conductivity is also present in C. puteana, which produces strands and is probably closely related to Serpula, as both Serpula and Coniophora are classed within the Coniophoraceae. However there have been no relevant studies on the water transport mechanism of C. puteana. Further experiments with S. lacrimans might prove worthwhile as it has the ability to transport water but shows sensitivity to many biocides.

On the other hand the sensitivity of G. trabeum to BuNCO modified wood may suggest that the initial penetration of enzymes or a degradative system into the cell wall matrix may rely on the differences in the types and sizes of the cellulases between fungal species. Conventional theory is that although brown rot fungi produce cellulases they are too large to diffuse freely in the wood cell wall and cause the rapid degradation noted. A number of proposals have been made for the role of low molecular weight degradative reagents (Koenings, 1974; Highley et al., 1981; Murmanis et al., 1988). It is estimated that only small molecules can diffuse into the capillaries of wood cell walls and that cellulases and hemicellulases of brown rot fungi can degrade wood only in association with some small size molecules (e.g. H_2O_2-Fe^{++}).

The physico-chemical differences of the void

spaces within the cell wall or the lumina between early- and latewood may be enhanced after isocyanate reactions. High decay intensity (Fig. 4.9) in the thick latewood cell wall may be explained either by the changes in the pore volume between tissues or by the non-uniformity of the reaction in the latewood tracheids. Boutelje (1962) cited by Fengel & Wegener (1984) determined the volumetric shrinkage of the cell walls in the earlywood of spruce as 26.5% and in the latewood as 29.5%. This shrinkage resulted in a decrease of the pore volume in earlywood and an increase in latewood. It may be possible that at low levels of isocyanate substitution, dry latewood still possesses larger void volume and high polysaccharide supply than earlywood which may explain its accessibility to hydrolytic molecules.

WHITE ROT

Table 7.3 and 7.4 summarises the bioprotection performance of samples tested against Coriolus versicolor and Pycnoporus sanguineus, respectively.

The threshold of P. sanguineus for BuNCO modified samples was marginally higher than that of C. versicolor. The sensitivity of white rot fungi to isocyanate modifications may be explained as a result of high lignin substitution.

TABLE 7.3

RELATIONSHIP BETWEEN WPG AND DS (% OH REACTED) OF ISOCYANATE MODIFIED CORSICAN PINE AT SUB-THRESHOLD AND THRESHOLD SUBSTITUTION LEVELS AGAINST C. versicolor AFTER 16 WEEKS IN THE SOIL BLOCK TESTING.

Sub-threshold			
TREATMENT VARIABLES	HDI	BuNCO	UNMODIFIED CONTROL[1]
WPG	6.72	5.80	-
-OH Reacted[2] (%)	5.40	3.95	-
Wt. loss (%)	5.18	19.90	32.55
Threshold			
WPG	10.30	9.60	-
-OH Reacted[2] (%)	8.27	6.53	-

[1] Pyridine treated control;
[2] estimated from equation 3.e assuming complete functionality of HDI

TABLE 7.4

RELATIONSHIP BETWEEN WPG AND DS (% OH REACTED) OF ISOCYANATE MODIFIED CORSICAN PINE AT SUB-THRESHOLD AND THRESHOLD SUBSTITUTION LEVELS AGAINST *P. sanguineus* AFTER 16 WEEKS IN THE SOIL BLOCK TESTING.

Sub-threshold			
TREATMENT VARIABLES	HDI	BuNCO	UNMODIFIED CONTROL[1]
WPG	6.72	5.80	-
-OH Reacted[2] (%)	5.40	3.95	-
Wt. loss (%)	9.94	18.38	40.27
Threshold			
WPG	11.70	12.20	-
-OH Reacted[2] (%)	9.40	8.30	-

[1] Pyridine treated control;
[2] estimated from equation 3.e assuming complete functionality of HDI

Rowell (1982) concluded from the studies with methyl isocyanate reacted Southern pine that nearly all of the lignin -OH content is substituted even at lower chemical add-ons. He stated that the ratio of lignin DS/holocellulose DS is high at WPG's up to 10 then drops as WPG's increase to about 50. Topochemical studies of BuNCO reactions in Scots pine of West & Banks (1986) indicated similar i.e. faster and higher lignin than holocellulose substitution in the initial stage of reaction. The higher sulphuric acid lignin value (Table 5.3) of BuNCO at 10 WPG as compared to unmodified control samples is explained on the basis of reinforced lignification due to lignin-urethane bonds. This is supported by the results of the resistance to delignification experienced with the sodium chlorite procedure (Table 5.4).

Lignin removal by C. versicolor is always accompanied by the removal of polysaccharides (Cowling, 1961; Kirk & Moore, 1972; Wilcox, 1973). It may thus indicate that high lignin substitution of modified wood limited the access of synergistic cellulases even at low isocyanate substitution in the polysaccharide fraction.

The differences in weight losses in unmodified control samples i.e. water treated (Table 4.3) and acetone extracted prior to pyridine and pyridine:acetone treatment (Table 4.2) illustrate that C. versicolor is particularly sensitive to the

presence of extraneous compounds in the wood cell wall. In this respect, experiments performed by Prior (1976) revealed several features of inhibition of fungi to extractives from Corsican pine. Here C. versicolor showed a better decay response (ca. 32% weight loss) in acetone extracted samples than in unextracted water treated samples (7% weight loss), while with P. sanguineus the difference was little (acetone extracted=40% weight loss; unextracted=32% weight loss). Studies on epoxide modified wood of Rowell (1982) showed that C. versicolor produced very little weight loss even in unmodified control samples.

SOFT ROT

The loss in tensile strength of unreacted strips exposed to unsterile soil testing (Table 6.3) furnished a measure of the amount of decay by soft rot fungi. Predominant isolation of Chaetomium globosum from unreacted strips indicated that an exposure period of 25 days was sufficient to attain above 90% reduction of tensile strength. Since in previous white and brown rot soil block experiments retarded water uptake and retarded rate of degradation in modified wood were noticed, incubation period for the soft rot tests was extended to 60 days in wet soil. Tensile strength tests were not performed in unreacted strips exposed at 40 and 60 days due to complete failure of

the test specimens.

It has been reported that C. globosum causes extensive loss in weight of beech wood, depleting the cellulose and hemicelluloses (xylan, mannan) considerably faster than lignin (Savory, 1954; Savory & Pinion, 1958; Levi & Preston, 1965). As similar pattern of attack was reported for Western white pine (Eslyn et al., 1975). Armstrong & Savory (1959) reported the effect of C. globosum on bending strength and toughness of beech wood, in which loss of strength was observed before any appreciable loss in dry weight was recorded.

Strips modified with BuNCO showed higher resistance to soft rot attack (Table 6.3) in comparison to unmodified control. At 6 and 10 WPG's of BuNCO reacted strips after 60 days incubation, reduction in tensile strength was 21 and 15%, respectively. Due to large variation in the tensile strength data, results were substantiated by visual and microscopic examination and only a few fungal hyphae were visualised in the ray parenchyma cells around latewood tracheids indicating a threshold below 10 WPG. As compared to unmodified control samples, none of the BuNCO modified strips showed a greenish colour in the exposed surfaces characteristic of soft rot colonization. The limited fungal colonization in the modified strips may suggest that reduced sample size improves uniformity of reactions. It is

interesting that those studies also indicate that soft rot is more readily controlled by chemical modification than white and brown rot.

Nilsson & Rowell (1982) found that soft rot attack and tunnelling bacteria were pronounced on the radial walls in the latewood tracheids of modified (Butylene oxide) Ponderosa pine at 8 WPG. No reference was made to the organisms responsible for soft rot or bacterial attack. It is suggested by Nilsson & Rowell (1982), that the uneven and intense distribution of soft rot attack in the radial walls is because radial walls have not been completely penetrated by the treatment solution. In their study large samples (19 x 19 x 152 mm) were reacted and subsequently divided into small blocks (5 x 5 x 10 mm) for the unsterile soil test. Here the differences in decay intensity in the wood tissues was more pronounced on larger size specimens (Fig. 4.9).

Chemical modification accompanying reaction of readily accessible -OH groups at surfaces is a common feature reported using different ligno-cellulosic substrates and reactants (Stamm & Baechler, 1960; Rowell, 1980, 1982; West & Banks, 1986, Doyle et al., 1987). This may support the enhanced protection achieved in BuNCO reacted thin wood strips even at low modification level. Furthermore the general pattern of soft rot colonization is the preferential superficial attack of wood. Thus the bioprotection of BuNCO

modified wood is greater for soft rot than for brown and white rot fungi. Many previous studies with wood preservatives have indicated that soft rot fungi show more tolerence than basidiomycetes (Savory, 1955; Lundstrom, 1974). It seems that the negligible soft rot colonization in the thin modified strips may be explained due to the hydrophobic nature of the treatment, which restrain water ingress into the wood cell walls.

Original tensile strength of BuNCO modified strips (Table 6.1) was lower (30 to 46%) than that attained in unmodified controls. Considering the variability in the tensile strength data, it is evident that generalities should be drawn with care because certain individual samples had tensile strength somewhat similar to the unmodified controls. However, holocellulose determination (Table 5.5) and DP results (Table 5.6) partly substantiate the decrease in tensile strength of modified wood. Therefore, it is assumed that the low molecular weights of holocellulose in BuNCO reacted control samples (Table 5.6) represent the carbohydrate portion not tightly linked by substitution reaction into the wood matrix. It may be possible that inadequately milled pieces contained longer polysaccharide chains and were retained by the sieve during sample preparation. In addition, the polysaccharide chains with substituted isocyanate interlaced within the

whole wood with substituted lignin may decrease sample elasticity which might have accounted significantly for the reduction in the original tensile strength. It was noticed that modified strips were more brittle than unmodified ones.

Baird (1969) reported that Spruce sticks reacted (vapour phase treatment) with n-butyl isocyanate (=BuNCO) at 65% had less than 25% loss in toughness and abrasion resistance. He pointed out that allyl, t-butyl, and phenyl isocyanates were less resistant than the BuNCO. Uniformity in the specific gravity from consecutive sections in the sticks reacted with BuNCO of Baird's work is attributed to the lower specific gravity of the Spruce wood which generally indicates a greater ease of diffusion through the wood. Also vapour phase treatments promote rapid diffusion in dry wood.

7.1.2. BIOLOGICAL PERFORMANCE OF THE DIFUNCTIONAL ISOCYANATE

Greater bioprotection performance of Corsican pine against brown, white and soft rot fungi was achieved on samples reacted with HDI as compared to BuNCO treatments. As with BuNCO, decay intensity (weight & tensile strength losses) differ between and within fungal groups.

Some of the discussion already carried out to

monofunctional is also applied to difunctional, similarities or differences are as following:

BROWN ROT

At sub-threshold WPG limited polysaccharide removal by C. puteana from HDI reacted samples (Table 4.6) as compared with BuNCO, may be interpreted by the presence of a more complex configuration due to crosslinking possibilities. Results of sulphuric acid (Table 5.3) and sodium chlorite delignification (Table 5.4) may suggest that the lignin-carbohydrate complex (LCC) linked to HDI is one of the properties of cell wall polysaccharide resistance to enzymatic degradation. Considering complete functionality of HDI (Table 7.1) DS (% -OH reacted) at threshold WPG is in close agreement to that achieved by monofunctional isocyanates.

Ellis & Rowell (1984) demonstrated that HDI in presence of 35% dimethylformamide reacted with wood increased the weight, volume, and dimensional stability of specimens, but fungal resistance was not reported. Here increase in samples volume was less than that expected from the volumetric quantity of chemical added (Table 3.5), however from the biodeterioration experiments (high tolerance to fungal degradation) it is possible that the substituted fractions (cellulose, hemicellulose and lignin) in the wood cell walls are more adherent to each other than

would be expected from monofunctional substitution. This may be consistent with the blockage of flow of fluids (water, treating solution, enzymes) which is one of the key factors in the prevention and biodeterioration of wood.

C. puteana was more tolerant to HDI modification than G. trabeum (Table 7.1 & 7.2). Stamm & Baechler (1960) reported that crosslinking between wood and formaldehyde at a low (2%) substitution level was very effective in preventing decay against G. trabeum. They stated that 2% is far short of the amount of crosslinking that would be needed to prevent decay on the basis of elimination of available -OH groups. Although HDI modified wood was adequately protected against G. trabeum at 10.60 WPG, decay intensity (Table 4.6) promoted by C. puteana at similar (ca. 10) WPG substantiate the sensitivity of G. trabeum to the presence of chemical in the wood cell walls. Physical barrier due to crosslinking between component units in the wood and HDI may be an extra element as a limiting factor in the wood wood cell walls for fungal physiological control.

WHITE ROT AND SOFT ROT

The resistance of HDI samples to C. versicolor and P. sanguineus may be comparable to that of soft rot fungi i.e. decay intensity (Table 4.2 & 6.3) was considerably reduced at low substitution levels (<10

WPG) as compared to BuNCO reacted samples. Tables 7.3 and 7.4 indicate that P. sanguineus is slightly more tolerant of HDI modification than C. versicolor at threshold substitution level. As with white rot, results on the soft rot performance of HDI reactions (Table 6.3) may be governed by the difference in the initial elementary substitution of -OH groups which reinforce the LCC leading to a limitation of the water content for fungal activity and crosslinking of the polymer structures.

It has been shown that a slight delignification of softwood greatly increases its susceptibility to attack by some soft rot fungi (Courtois, 1963; Bailey et al. 1968). It may be possible that modifications with the capacity to reinforce lignification greatly increases the resistance of wood to degradation. This may have a direct connection with fungi possessing selectivity for both lignin and carbohydrate degradation, for fungi where lignin degradation is dependent on carbohydrate metabolism and where lignin protects the carbahydrate. Further studies are necessary to explain the effect of white and soft rot fungi on isocyanate modified wood. Up to date limited information is available.

7.1.3. THE INFLUENCE OF THE MOISTURE CONTENT ON WOOD DEGRADATION DUE TO ISOCYANATE MODIFICATION

Bioassays results have been shown that increases in isocyanate substitution correspond to decreases in the susceptibility of Corsican pine. The extent of the reduction in degradation depends upon the amount of reduction of the absorbed water in the modified wood and by the physiological capacity of the decay fungi to tolerate water stress. As shown in Table 7.5, after isocyanate reactions the amount of water absorbed at saturation (100% relative humidity) decreases progressively with increasing WPG.

The moisture content of wood below fibre saturation point (FSP) limits wood decomposition by decay fungi. The effect of reduced hygroscopicity of wood after chemical modification is attributed to the change in the availability of polar hydroxyl groups, which are believed to bond the water by hydrogen bonding.

The favourable effects of water on wood decomposition can be demonstrated with unmodified samples. As shown in Table 4.2 as compared to Table 4.4 , there is a direct relationship between the amount of degradation and the moisture content (MC) of the wood. In the unreacted wood the -OH groups of cellulose, hemicellulose and lignin were entirely satisfied by the availability of water in the decay jars permitting water regain for sastisfactory fungal

TABLE 7.5

AVERAGE PERCENT MOISTURE ADSORBED AT SATURATION OF CORSICAN PINE BLOCKS REACTED WITH BuNCO AND HDI. FIGURES REPRESENT AN AVERAGE OF 5 REPETITIONS; STANDARD DEVIATION IN BRACKETS.

TREATMENT	WPG	MOISTURE ADSORBED (%)
Unmodified	-	30.60 (0.18)[1]
BuNCO	5.74 (0.42)	25.50 (0.64)
	9.90 (0.48)	20.10 (0.26)
	15.77 (0.64)	16.34 (0.18)
	20.06 (0.26)	14.73 (0.23)
	31.49 (0.83)	11.50 (0.56)
HDI		
	6.91 (0.94)	23.95 (0.45)
	10.91 (0.50)	19.80 (0.31)
	14.25 (0.81)	17.62 (0.31)
	21.67 (0.86)	16.90 (0.26)

[1] sterile control exposed for 16 weeks in the soil block testing (blocks exposed to 100% RH were contaminated).

activity.

The carbohydrates in the wood are primarily hydrocarbon structures, but they also contain many polar -OH groups and have therefore high affinity for water. On the other hand lignin itself has interesting moisture modifying properties, and slight changes in its bulk density having relatively great effects on water movement in the wood cell wall.

The reduction in water absorbed by isocyanate modified wood at threshold WPG is shown Table 7.6. The figures are estimated from the data shown in Table 7.5 and assume MC of unreacted control of 30% at FSP.

The relationship between WPG and reduction of maximal cell wall absorption after isocyanate modification is regarded as one of the limiting factor for degradation to occur. The substitution of -OH groups in the components of cell wall (cellulose, hemicellulose, and lignin) reduces available groups for water interaction. It was noticed that modified wood requires longer equilibrium periods than unmodified wood at a fixed relative humidity conditions.

TABLE 7.6

REDUCTION IN MOISTURE CONTENT OF ISOCYANATE MODIFIED WOOD AT THRESHOLD WPG AGAINST DECAY FUNGI.

Fungi	Isocyanate at Threshold WPG	Reduction in MC (%)*
C. puteana	BuNCO 15.50	35.00
	HDI 13.60	39.00
G. trabeum	BuNCO 10.00	30.00
	HDI 10.60	30.50
C. versicolor	BuNCO 9.60	29.00
	HDI 10.30	30.00
P. sanguineus	BuNCO 12.20	34.00
	HDI 11.70	32.00
Soft rot	BuNCO < 10	<30.00
	HDI < 10	<30.00

* Figures denotes reduction in moisture content at saturation estimated from 30% of unreacted control.

At sub-threshold WPG (e.g. <10 WPG) the MC of wood induced by C. puteana attack was above FSP and followed the pattern of: BuNCO > HeNCO > HDI (Table

MISSING PAGES ARE UNAVAILABLE

7.2. CONCLUSIONS

- Irrespective of the decay parameters used (weight loss & tensile strength), isocyanate modified wood (BuNCO & HDI) is more resistant to attack by soft rot fungi than with brown and white rot fungi.

- In any of the types of decay tested (brown, white and soft rot) Corsican pine wood crosslinked by reaction with HDI is less susceptible to degradation than samples reacted with the single site non crosslinking reactant BuNCO. Similar results were achieved with the monofunctional HeNCO but this was not tested against white and soft rot decay.

- Isocyanate substitution effectively reduced original tensile strength [1.42-1.82 (BuNCO) and 1.22-1.35 (HDI) times lower than unreacted samples], but increased resistance to soft rot colonization even at low (ca. 5-6 WPG) substitution levels in thin wood strips reacted with BuNCO and HDI.

- A reduction of maximal cell water absorption (based in the oven dry weight) to give modified (BuNCO & HDI) fibre saturation of 30 to 40% lower than the fibre saturation point of unreacted Corsican pine (ca. 30%) provides protection against the decay fungi used in this study. This range correlates directly with the threshold values achieved after biodeterioration tests.

- Irrespective of the chemical used, samples below threshold level when exposed to decay showed more carbohydrate removal than lignin removal. BuNCO and HDI reactions sustained lignin and/or reinforced the lignin-carbohydrate complex within the wood matrix.

- An increase of holocellulose substitution of monofunctional isocyanate is one of the pre-conditions for protection against C. puteana, but pattern and rate of degradation by C. puteana and by the other decay fungi used in this study are markedly dependent on the elementary substitution of lignin and hemicellulose as well. The affinity of lignin-carbohydrate moieties to HDI may be responsible for the high degree of protection achieved.

7.3. SUGGESTIONS FOR FURTHER RESEARCH

At various points in the text suggestions for further work have been made. These include:

- There is a need to establish the location of modification within the wood cell wall especially with difunctional isocyanates, which induce crosslinking. This would be useful background for the interpretation of subsequent decay studies. In addition to the spatial relationships (e.g. S_3, S_2, S_1, compound middle lamella) the location of reaction with regard to the chemical components of wood (e.g. lignin, amorphous and crystalline celluloses and hemicellulose) should be more clearly defined as the more accessible hydroxyls groups have a major influence both in terms of fungal decay resistance and in the changes of moisture permeability of modified wood.

- Studies with more pronounced wetting systems require further investigation. It is worthwhile considering the influence of fungal species closely related to C. puteana like Serpula, which produce strands and may improve moisture movement within the modified wood. Furthermore, incubation systems resulting in higher block moisture contents possibly combined with weathering, leaching tests and cyclic wet and drying systems which more closely simulate the end-use

situations may be useful in examining the stability and permanence of the reacted wood;

- Molecular weight determinations of cellulose and hemicelluloses should be undertaken in order to trace the fate of the bonded isocyanates in the holocellulose. These will give a better insight into the mechanism of bioprotection by isocyanate modifications. In addition, an examination should be made to substantiate the effect of increases in the levels of modification on the DP of cellulose and hemicellulose as it will be recalled that in chapter 5 modification with BuNCO when examined by certain conditions showed decreases in DP of holocellulose as compared with controls. The analytical preparation methods need some attention in this context to achieve conclusive results.

- Considering the variability in the tensile strength data on chapter 6, it would seem that there is a need for comparable studies using different techniques for tensile strength evaluation, for example determinations at zero span might improve homogeneity of results. In addition microscopic interpretation of the mode of failure of the sections would prove useful in this context.

REFERENCES

AINSWORTH, G.C. 1973. Introduction and Keys to Higher Taxa. In: AINSWORTH, G.C; SPARROW, F.K. & SUSSMAN, A.S. (eds.). The Fungi: an Advanced Treatise. Academic Press, London.

ALLSOPP, D. & SEAL, K.J. (eds.). 1986. Introduction to Biodeterioration. Edward Arnold Ltd., London. 136 pp.

AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1976. Standard Method of Testing Wood Preservatives by Laboratory Soil Block Cultures. ASTM D1413. Philadelphia, PA.

ARMSTRONG, F.H. & SAVORY, J.G. 1959. The Influence of Fungal Decay on the Properties of Timber. Holzforschung, 13:3.

APENITIS, A.; ERDTMAN, H. & LEOPOLD, B. 1951. Studies on Lignin. V. The Decay of Spruce Wood by Brown Rotting Fungi. Sv. Tidskr., 63:195-207.

AYERS, A.R.; AYERS, S.B. & ERIKSSON, k.-E. 1978. Cellobiose Oxidase, Purification and Partial Characterization of a Hemoprotein from Sporotrichum pulverulentum. Eur. J. Biochem., 90:171 - 181.

AZUMA, J.; TAKAHASHI, N. & KOSHIJIMA, T. 1981. Isolation and Characterization of Lignin-Carbohydrate Complexes from the Milled Wood Lignin Fraction of Pinus densiflora Sieb. et Zucc. Carbohyd. Res., 93:91-104.

AZUMA, J.; TAKAHASHI, N.; ISAKA, M. & KOSHIJIMA, T. 1985. Lignin-Carbohydrate Complexes Extracted woth Aqueous Dioxane from Beech Wood. Mokuzai Gakkaishi, 31:587-594.

BAECHLER, R.H. 1959. Improving Wood's Durability through Chemical Modification. Forest Products Journal, 9:166-171.

BAECHLER, R.H. 1960. Fungus-resistance Wood Prepared by Cyanoethylation. US Patent 2 959 496.

BAILEY, P.J.; LIESE, W. & ROSCH, R. 1968. Some Aspects of Cellulose Degradation in Lignified Cell Walls. In: Biodeterioration of Materials. Elsevier Publ. Co. Ltd., England, 546-557 pp.

BAIRD, B.R. 1969. Dimensional Stabilization of Wood by Vapor Phase Chemical Treatments. Wood and Fiber, 1(1):54-63.

BAVENDAMM, W. 1928. Uber das Vorkommen und den Nachweis von Oxydasen bei holzzerstorenden Pilzen. I. Mitteilung. Z. Pflanzenkrankh. Pflanzenschutz., 38:257-276.

BODDY, L. 1983. Effect of Temperature and Water Potential on Growth Rate of Wood-Rotting Basidiomycetes. Trans. Br. Mycol. Soc., 80(1):141-149.

BRAVERY, A.F. & GRANT, C. 1971. Preliminary Investigations into the Use of a Thin Strip Tensile Strength Test for the Rapid Evaluation of Wood Preservatives Against Basidiomycete Fungi. International Biodeterioration Bulletin, 7(4).

BRAVERY, A.F. & LAVERS G.H. 1971. Strength Properties of Decayed Softwood Measured on Miniature Test Beams. International Biodeterioration Bulletin, 7(3).

BRICE, R.E. & MORRISON, I.M. 1982. The Degradation of Isolated Hemicellulose and Lignin-Hemicellulose Complexes by Cell-free, Rumen Hemicellulases. Carbohyd. Res., 101:93-100.

BRITISH STANDARD INSTITUTION. 1974. Specification for Wood Preservation by Means of Water-borne Copper/Chrome/Arsenic Compositions. BS4072.

BRITISH STANDARD INSTITUTION. 1982. Wood Preservatives. Determination of Toxic Values against Wood Destroying Basidiomycete Fungi Cultured on an Agar Medium. BS6009.

BROWN, F.L. 1963. A Tensile Strength Test for Comparative Evaluation of Wood Preservatives. For. Prod. J., 13(9):405-412.

BROWNING, B.L. 1967. Methods of Wood Chemistry. Interscience Publishers, John Wiley & Sons, Inc. vol. I & II.

BUNNELL, F.L.; TAIT, D.E.N.; FLANAGAN, P.W. & VAN CLEVE, K. 1977. Microbial Respiration and Substrate Weight Loss - I. A General Model of Influence of Abiotic Variables. Soil Biology & Biochemistry, 9:33-40.

BURMESTER, V.A. & OLSEN, C.Z.. 1971. Improvement of Dimensional Stabilization of Beechwood by Impregnation with Diisocyanate after WAN-drying. Holzforschung, 25(3):84-89.

BUTCHER, J.A. 1971. Colonization by Fungi of Pinus radiata Sapwood Treated with a Cooper-Chrome-Arsenate Preservative. Journal Institute Wood Science, 5(4):16-25.

CARDIAS, M.F.C. & JESUS, M.A. 1985. Resistencia Natural de Especies Florestais da Amazonia ao Ataque de Fungos Apodrecedores. Centro de Pesquisas de Produtos Florestais. Serie Tecnica No. 13, Manaus.

CARTWRIGHT, K.St.G. & FINDLAY, W.P.K. 1958. Decay of Timber and its Prevention. Her Majesty Stationary office, London, 332 pp.

CHEN, G.C. & ROWELL, R.M. 1984. Polyhalogenated Phenyl Isocyanate Synthesis with excess Phosgene and Either Triethylamine or Tetramethylurea. U. S. Patent No. 4 477 389.

CHEN, G.C. & ROWELL, R.M. 1987. Approaches to the Improvement of Biological Resistance of Wood through Controlled Release Technology. American Paint and Coatings Journal, 72(16):37-41.

CHEN, G.C.; ROWELL, R.M. & ELLIS, W.D. 1990. Fungal Resistance of Southern Pine Impregnated with Methyl Fluorophenyl Carbamates or Reacted with Fluorophenyl Isocyanates. Wood and Fiber Science, 22(2):165-172.

CHOU, G. K.; CHANDLER, J. A. & PRESTON, R. D. 1973. Uptake of Metal Toxicants by Fungal Hyphae Colonising CCA-impregnated Wood. Wood Sci. Technol., 7:206-11.

CLARKE, S. H. 1935. Recent Work on the Relation Between Anatomical Structure and Mechanical Strength in English Ash (Fraxinus excelsior L.). Forestry, 9:132-138.

CLARKE, R.W.; JENNINGS, D.H. & COGGINS, C.R. 1980. Growth of Serpula lacrimans in relation to Water Potential of Substrate. Trans. Br. Mycol. Soc., 75:271-280.

CLERMONT, L. P. & BENDER, F. 1957. Effect of Swelling Agents and Catalysts on Acetylation of Wood. Forest Products Journal, 7(5):167-170.

COOPER, R.M.; RANKIN, B. & WOOD, R.K.S. 1978. Cell Wall-degrading Enzymes of Vascular Wilt Fungi. II. Properties and Modes of Action of Polysaccharidases of Verticillium albo-atrum and Fusarium oxysporum f. sp. lycopersici. Physiological Plant Pathology, 13:101-134.

CORBETT, N. H. 1965. Micro-morphological Studies on the Degradation of Lignified Cell Walls by Ascomycetes and Fungi Imperfecti. Journal Institute Wood Science, 14:18-29.

CORBETT, N. H. & LEVY, J. F. 1963. Penetration of Tracheid Walls of Pinus sylvestris L. (Scots Pine) by Chaetomium globosum Kunze. Nature, 198(4887):1322-1323.

COURTOIS, H. 1963. Beitrag Zur Frage Hol Zabbauender Ascomyceten und Fungi Imperfecti. (Contribution to the Problem of Wood-Destroying Ascomycetes and Fungi Imperfecti). Holzforschung, 17(6):176-183.

COWLING, E. B. 1961. Comparative Biochemistry of the Decay of Sweetgum by White-rot and Brown-rot Fungi. U. S. Dep. Agric. For. Ser. Tech. Bull. 1259. Washington, DC., 79 pp.

COWLING, E. B. & BROWN, W. 1969. Structural Features of Cellulosic Materials in Relation to Enzymatic Hydrolysis. In: Cellulases and Their Applications. Advances in Chemistry Series. No. 95. American Chemical Society, 152-186 pp.

COWLING, E. B. 1975. Physical and Chemical Constraints in the Hydrolysis of Cellulose and Lignocellulosic Materials. Biotechnol. Bioeng., 5:163-181.

COTE, W. A. 1977. Wood Ultrastructure in Relation to Chemical Composition. 1-44 pp. In: LOEWUS, F.A. & RUNECKLES, V.C. (eds.), Recent Advances in Phytochemistry: Vol. 11 The Structure, Biosynthesis, and Degradation of Wood. Plenum Press, New York. 527 pp.

DESHPANDE, V.; ERIKSSON, K.-E. & PETTERSSON, B. 1978. Production, Purification and Partial Characterization of 1,4-B-Glucosidase Enzymes from Sporotrichum pulverulentum. Eur. J. Biochem., 90:191-198.

DIROL, D. 1976. Etude in vitro dela Colonisation et de la degradation Structural du Bois dehetre par Coriolus versicolor (L) Quelet. Revue de Mycologie, Tome 40:295-315.

DOYLE, S.E.; GIBBONS, A.M. & PETHRICH, R.A. 1987. Characteristics of Heterogeneously Acetylated Cellulose. In: KENNEDY, J.F. ; PHILLIPS, G.O. & WILLIAMS, P.A. (eds.). Wood and Cellulosics: Industrial Utilisation, Biotechnology, Structure and Properties. Ellis Horwood Limited, 71-76 pp.

DUNCAN, C.G. & DEVERALL, F.J. 1964. Degradation of Wood Preservatives by fungi. Appl. Microbiology, 12(1):57-62.

EAMUS, D. & JENNINGS, D.H. 1984. Determination of Water, Solute and Turgor Potentials of Mycelium of Various Basidiomycete Fungi Causing Wood Decay. J. Exp. Bot., 35:1782-1785.

EAMUS, D.; THOMPSON, W.; CAIRNEY, J.W.G. & JENNINGS, D.H. 1985. Internal Structure and Hydraulic Conductivity of Basidiomycete Translocating Organs. J. Exp. Bot., 36:1110-1116.

EFFLAND, M.J. 1977. Modified Procedure to Determine Acid-insoluble Lignin in Wood and Pulp. Tappi, 60(10):143-144.

ELLIS, W.D. & ROWELL, R.M. 1984. Reaction of Isocyanates with Southern Pine Wood to Improve Dimensional Stability and Decay Resistance. Wood and Fiber Science, 16(3):349-356.

ERIKSSON, K.-E. & PETTERSON, B. 1975a. Extracellular Enzyme System Utilized by the Fungus Sporotrichum pulverulentum (Chryssosporium lignorum) for the Breakdown of Cellulose. 1. Separation, Purification and Physico-chemical Characterization of Five Endo-1,4-β-glucanases. Eur. J. Biochem., 51:193-206.

ERIKSSON, K.-E. & PETTERSON, B. 1975b. Extracellular Enzyme System Utilized by the Fungus Sporotrichum pulverulentum (Chryssosporium lignorum) for the Breakdown of Cellulose. 3. Purification and Physico-chemical Characterization of an Exo-1,4-β-glucanase. Eur. J. Biochem., 51:213-218.

ERIKSSON, K.-E. 1981. Fungal Degradation of Wood Components. Pure Appl, Chem., 55:33-43.

ERIKSSON, K.-E. & WOOD, T.M. 1985. Biodegradation of Cellulose. In: HIGUCHI, T. (ed.) Biosynthesis and Biodegradation of Wood Components. Academic Press, Inc., 469-503 pp.

ERIKSSON, O.; GORING, D.A.I. 1980. Structural Studies on the Chemical Bonds Between Lignins and Carbohydrates in Spruce Wood. Wood Sci. Technol., 14:267-279.

FARMER, R.H. 1967. Chemistry in the Utilization of Wood. Pergamon Press Ltd.

FENGEL, D. & WEGENER, G. 1984. WOOD: Chemistry, Ultrastructure, Reactions. Walter de Gruyter & Co., Berlin. 613 p.

FORNEY, L. J.; REDDY, C. A.; TIEN, M. & AUST, S. D. 1982. The Involvement of Hydroxyl Radical Derived from Hydrogen Peroxide in Lignin Degradation by the White Rot Fungus Phanerochaete chrysosporium. The Journal of Biological Chemistry, 19(257):11455 - 11462.

FUSE, G. & NISHIMOTO, K. 1961. Preservation of Wood by Chemical Modification. I. Preservative Properties of Cyanoethylated Wood. Journal of the Japan Wood Research Society, 7(4):157-161.

GAFUROV, T.G.; PILOSOV, M. Ya.; ADYLOV, A.; MANNANOVA, D.; SUVOROVA, E. V.; TASHPULATOV, Yu.T.; NOKONOVICH, G. V. & USMANOV, Kh.U. 1970. Reaction of Cellulose with Hexamethylene Diiisocyanate. Vysokomol. Soedin., Ser. A., 12(11):2515-2519.

GJOVIK, L. R. & DAVIDSON, H. L. 1973. Comparison of Wood Preservatives in Stake Tests. Research Note, Forest Products Laboratory, USDA Forest Service No. 02.

GLAUERT, A.M. (ed.) 1974. Practical Methods in Electron Microscopy. Vol. 3. North-Holland Publishing Company - Amsterdam. Part I Fixation, Dehydration and Embedding of Biological Specimens.

GOLDSTEIN, I. S.; DREHER, W. A. & JEROSKI, E. B. 1959. Wood Processing Inhibition Against Swelling and Decay. Industrial and Engineering Chemistry, 51(10):1313-1317.

GOLDSTEIN, I. S. 1960. Improving Fungus Resistance and Dimensional Stability of Wood by Treatment with β-Propiolactone. US Patent 2 931 741.

GOLDSTEIN, I. S.; JEROSKI, E. B.; LUND, A. E.; NIELSON, J. F. & WEAVER, J. W. 1961. Acetylation of Wood in Lumber Thickness. Forest Products journal, 11(8):363-370.

GRIFFIN, D. M. 1968. A Therretical Study Relating the Concentration and Diffusion of Oxygen to the Biology of Organisms in Soil. New Phytologist, 67:561-577.

GRIFFIN, D. M. 1977. Water Potential and Wood-decay Fungi. Annual Review of Phytopathology, 15:319-329.

HALE, M. D. C. & EATON, R. A. 1985. The Oscillatory Growth of Fungal Hyphae in Wood Cell Walls. Trans. Br. Mycol. Soc., 84:277-288.

HARDIE, K. 1980. A Review of Strength Testing as a Measure of Biodeterioration of Wood and Wood Based Materials. International Biodeterioration Bulletin, 16(1):1-8.

HARKIN, J. M. & OBST, J. R. 1973. Syringaldazine, an Effective Reagent for Detecting Laccase and Peroxidase in Fungi. Experientia, (29):381-387.

HEERTJES, P.M. & DE JONG, J. 1971. Preservation of Wood with Reactive Fungicides. J. Oil Colour Chem. Ass., 54(7):642-654.

HENNINGSSON, B. 1967. Changes in Impact Bending Strength, Weight and Alkali Solubility Following Fungal Attack on Birch Wood. Studia For. Suecica, 41, 21 p.

HIGHLEY, T. L. 1975. Properties of Cellulases of two Brown-rot Fungi and Two White-rot Fungi. Wood Fiber, 6(4):275-281.

HIGHLEY, T. L. 1980. Cellulose Degradation by Cellulose-Clearing and Non-Cellulose-Clearing Brown-rot Fungi. Appl. Environ. Microbiol., 40(6):1145-1147.

HIGHLEY, T. L. & KIRK, T. K. 1979. Mechanisms of Wood Decay and the Unique Features of Heartrots. Phytophathology, 69(10):1151-1157.

HIGHLEY, T. L.; WOLTER, K. E. & EVANS, F. J. 1981. Polysaccharide-degrading Complex Produced in Wood and Liquid Media by Brown-rot Fungus Poria placenta. Wood and Fiber, 13(4):265-274.

HON, D. N. S. & SHIRAISHI, N. (eds.). 1991. Wood and Cellulosic Chemistry. M. Dekker, New York. 1020 p.

HOF, T. 1979. Gloeophyllum trabeum (Pers. ex Fr.) Murrill. In: COCKCROFT, R. (ed.). Some Wood - Destroying Basidiomycetes, vol. 1. The International Research group on Wood Preservation, Papua, New Guinea, 81-94 pp.

IFJU, G. 1964. Tensile Strength Behavior as a Function of Cellulose in Wood. Forest Products Journal, 8:366-372.

JACKOWSKI, J.A. & SMULSKI, S.J. 1988. Isocyanate Adhesive as a Binder for Red Maple Flakeboard. Forest Products Journal, 38(2):49-50.

KAARIK, A. 1965. The Identification of the Mycelia of Wood-decay Fungi by their Oxidation Reactions with Phenolic Compounds. Stud. Forest. Suecica No. 31.

KALNINS, M.A. 1982. Chemical Modification of Wood for Improved Decay Resistance. Wood Science, 13(4):202-208.

KEILICH, G.; BAILEY, P. & LIESE, W. 1970. Enzymatic Degradation of Cellulose, Cellulose Derivatives and Hemicelluloses in Relation to the Fungal Decay of Wood. Wood Science and Technology, 4:273-283.

KENNEDY, R.W. & IFJU, G. 1962. Applications of Microtensile Testing to Thin Wood Sections. Tappi, 45:725-733.

KING, N.J. 1966. The Extracellular Enzymes of Coniophora cerebella. Biochem. J., 100:784-792.

KIRK, T.K. 1971. Effects of Micro-organisms on Lignin. Annu. Rev. Phytopathology, 9:185-210.

KIRK, T.K. 1975. Effects of a Brown-rot Fungus, Lenzites trabea, on Lignin in Spruce Wood. Holzforschung, 29:99-107.

KIRK, T.K. & MOORE, W.E. 1972. Removing Lignin from Wood with White-Rot Fungi and Digestability of Resulting Wood. Wood Fiber, 4:72-79.

KIRK, T.K. & HIGHLEY, T.L. 1973. Quantitative Changes in Structural Components of Conifer Woods During Decay by White-rot and Brown-rot Fungi. Phytopathology, 63(11):1338-1342.

KOENIGS, J. W. 1974. Hydrogen Peroxide and Iron: a proposed System for Decomposition of Wood by Brown Rot Basidiomycetes. Wood Fibre, 6:66-79.

KOPPERS' ACETYLATED WOOD. 1961. Dimensionally Stabilized Wood. New Materials Technical Information No.(RDW-400) E 106.

LAWTHER, J.M.; RIVERA, Z.S.; JUMEL, K. & WHITE, C.A. 1990. Molecular Weight Determination of Cellulose Using High Performance Size Exclusion Chromatography [HPSEC], Gel Permeation Chromatography [GPC] and Viscometry. In: KENNEDY, J.F.; PHILLIPS, G.O. & WILLIAMS, P.A. (eds.). Cellulose Source and Exploitation. Ellis Horwood Limited, 41-47 pp.

LEVI, J.F. & PRESTON, R.D. 1965. A Chemical and Microscopic Examination of the Action of the Soft Rot Fungus Chaetomium globosum on Beechwood (Fagus sylvatica). Holzforschung, 19(6):183-190.

LEVINSON, H. S. & REESE, E. T. 1950. Enzymatic Hydrolysis of Soluble Derivatives as Measured by Changes in Viscosity. J. Gen. Physiol., 33:601.

LEVINSON, H. S.; MADELS, G. L. & REESE, E. T. 1951. Products of Enzymatic Hydrolysis of Cellulose. Arch. Biochem. and Biophys., 51:355.

LEVY, J. F. 1990. Fungal Degradation of Wood. In: KENNEDY, J.F.; PHILLIPS, G.O. & WILLIAMS, P.A. (eds.). Cellulose Source and Exploitation. Ellis Horwood Limited, 397-407 pp.

LLOYD, L.L.; WARNER, F.P.; KENNEDY, J.F. & WHITE, C.A. 1987. GPC of Cellulose Derivatives on Poly(Styrenedivinylbenzene) Packings in Organic Solvents. In: KENNEDY, J.F.; PHILLIPS, G.O. & WILLIAMS, P.A. (eds.). Wood and Cellulosics. Ellis Horwood Limited, 203-210 pp.

LUTOMSKI, K. 1975. Resistance of Beechwood Modified with Styrene, Methyl Methacrylate, and Diisocyanate Against the Action of Fungi. Material und Organismen, 10(4):255-262.

LYR, H. 1958. Uber den Nachweis von Oxydasen und Peroxidasen bei Höheren Pilzen und die Bedeutung dieser Enzyme fur die Bavendamm-reaktion. Planta, 50:359:370.

MOORE, D. 1981. Effects of Hexose Analogues on Fungi: Mechanisms of Inhibition and of Resistance. New Phytol., 87:487-515.

MURMANIS, L.; HIGHLEY, T.L. & PALMER, J.G. 1988. The Action of Isolated Brown-rot Cell-free Culture Filtrate, H_2O_2-Fe^{++}, and the Combination of Both on Wood. Wood Sci. Technol., 22:59-66.

NAKANO, J. 1987. Recent Progress in Wood Chemistry Fundamentals and Applications. In: KENNEDY, J.F.; PHILLIPS, G.O. & WILLIAMS, P.A. (eds.). Wood and Cellulosics. Ellis Horwood Limited, 499-512 pp.

NOBLES, M.K. 1958. A Rapid Test for Extra-Cellular Oxidase. Can. J. Bot., 36:91-99.

NOBLES, M. K. 1965. Identification of Cultures of Wood-Inhabiting Hymenomycetes. Canadian Journal of Botany, 43:1097-1139.

NILSSON, T. 1976. Soft Rot Fungi - Decay Patterns and Enzyme Production. Material und Organismen, 3:103-112.

NILSSON, T. & ROWELL, R. M. 1982. Decay Patterns Observed in Butylene Oxide Modified Ponderosa Pine After Exposure in Unsterile Soil. Document, International Research Group on Wood Preservation No. IRG/WP/3211.

OZALINA, I.O. & SVALBE, K.P. 1966. Acetylation of Wood and Biological Testing of Acetylated Samples. Latvijas PSR Zinatnu Akademija Vestis, 9:56-59.

PATERSON, A. & LUNDQUIST, k. 1985. Radical Breakdown of Lignin. Nature, (316):575.

PIHL, L.O. & OLSSON, I. 1968. Treating Wood and Wood Fiber Material. British Patent 1 101 777.

RAYNER, A.D.M. & BODDY, L. 1988. Fungal Decomposition of Wood its Biology and Ecology. John Wiley & Sons, London, 587 pp.

REESE, E.T.; SIU, K.G. & LEVINSON, H.S.J. 1950. The Biological Degradation of Soluble Cellulose Derivatives and its Relationship to the Mechanism of Cellulose hydrolysis. J. Bacteriol., 59:485-497.

REESE, E.T.& LEVINSON, H.S. 1952. A comparative Study of Breakdown of Cellulose by Microorganisms. Physil. Pl., 5:345-366.

REESE. E.T. 1957. Biological Degradation of Cellulose Derivatives. Ind. Eng. Chem., 49:89-93.

REESE. E.T. 1977. Degradation of Polymeric Carbohydrates by Microbial Enzymes. pp. 311-367 In: LOEWUS, F. A. & RUNECKLES, V. C. (eds.) Vol. 11. The Structure Biosynthesis and Degradation of Wood. Proc. Rev. Adv. in Phytochem. Plenum Press, New York, 527 p.

ROFF, J.W. 1964. Hyphal Characteristics of Certain Fungi in Wood. Mycologia, 56:799-804.

ROWELL, R.M. 1975. Chemical Modification of Wood: Advantages and Disadvantages. Proceedings, American Wood-Preservers' Association, 71:41-51.

ROWELL, R.M. 1982. Wood Preservation and Stabilization by Chemical Modification of the Wood Substance. Swedish Forest Products Research Laboratory, STFI Series A, 772:32-49.

ROWELL, R.M. & BANKS, W.B. 1987. Tensile Strength and Toughness of Acetylated Pine and Lime Flakes. British Polymer Journal, 19:479-482.

ROWELL, R.M. & CHEN, G.C. 1983. Synthesis of Polyhalogenated Phenyl Isocyanates. Polymeric Materials Science & Engineer. Proc. 186th Amer. Chem. Soc. Meeting, Washington, 49:151-157.

ROWELL, R.M. & GUTZMER, D.I. 1975. Chemical Modification of Wood: Reactions of Alkylene Oxides with Southern Yellow Pine. Wood Science, 7(3):240-246.

ROWELL, R.M. & ELLIS, W.D. 1979. Chemical Modification of Wood: reaction of methyl isocyanate with Southern Pine. Wood Science, 12(1):52-58.

ROWELL, R.M. 1980. Distribution of Reacted Chemicals in Southern Pine Modified with Methyl Isocyanate. Wood Science, 13(2):102-110.

ROWELL, R.M. 1983. Chemical Modification of Wood. Forest Products Abstracts, 6(12):363-381.

ROWELL, R.M.; YOUNGQUIST, A.J. & MONTREY, H.M. 1988. Chemical Modification: adding value through new FPL composite processing technology. Forest Products Journal, 38(7/8):67-70.

RUEL, K. & BARNOUD, F. 1985. Degradation of Wood by Microorganisms. In: HIGUCHI, T. (ed.) Biosynthesis and Biodegradation of Wood Components. Academic Press, Inc., 441-467 pp.

SAKAKIBARA, A. 1980. A Structural Model of Softwood Lignin. Wood Sci. Technol., 14:89-100.

SAVORY, J.G. 1955. The Role of Microfungi in the Decomposition of Wood. British Wood Preservers' Association, Convention Record, 3-35 pp.

SAVORY, J.G. & PINION, L. C. 1958. Chemical Aspects of Decay of Beech Wood by Chaetomium globosum. Holzforschung, 12:99-103.

SEEHANN, G.; LIESE, W. & KESS, B. 1975. List of Fungi in Soft Rot Tests. International Research Group on Wood Preservation, Document IRG/WP/105.

SEIFERT, V.K. 1962. Die Chemische Veranderung der Holzzellwand-Komponenten unter dem EinfluB Pflanzlicher und Tierischer Schadlinge. Holzforschung, 16(4):102-103.

SJOSTROM, E. 1981. Wood Chemistry. Fundamentals and Applications. Academic Press Inc., New York, 223 p.

SLADE Jr., P.E. 1975. Polymer Molecular Weights. Marcel Dekker, Inc., New York, vol. 4, part I & II.

SMITH, D.N.R. 1980. Study of Decay of Preservative Treated Wood in Soil. Journal Inst. Wood Science, 8(5):194-200.

STAMM, A.J. & BAECHLER, R.H. 1960. Decay Resistance and Dimensional Stability of Five Modified Woods. Forest Products Journal, 10(1):22-26.

STEWART, E.L.; PALM, M.E. & BURDSALL Jr., H.H. 1988. Deuteromycotina and Selected Ascomycotina from Wood and Wood Products: An Indexed Bibliography and Guide to Taxonomic Literature. MPPD Pleomorphic Press, St. Paul, Minnesota. 284 p.

STREAMER, M.; ERIKSSON, K-E. & PETTERSSON, B. 1975. Extracellular Enzyme System Utilized by the Fungus Sporotrichum pulverulentum (Chrysosporium lignorum) for the Breakdown of Cellulose. Eur. J. Biochem., 59:607-613.

SZEGI, J. 1988. Cellulose Decomposition and Soil Fertility. Akademiai Kiado, Budapest. 186 p.

TAKAHASHI, M. & NISHIMOTO, K. 1967. Studies on the Mechanism of Wood Decay. 1. Changes in Strengths of Buna and Sugi Wood as Decay Proceeds. Wood Res. Kyoto Univ., 41:75-89.

TAKAHASHI, N. & KOSHIJIMA, T. 1988. Molecular Properties of Lignin-carbohydrate Complexes from Beech (Fagus crenata) and Pine (Pinus densiflora) Woods. Wood Sci. Technol., 22:177-189.

TARKOW, H.; STAMM, A.J. & ERICKSON, E.C.O. 1950. Acetylated Wood. Report, Forest Products Laboratory, USDA Forest Service No.1593.

TIEN, M. & KIRK, T.K. 1983. Lignin-Degrading Enzyme from the Hymenomycete Phanerochaete chrysosporium Burds. Science, (221):661-662.

UNLIGIL, H.H. 1968. Depletion of Pentachlorophenol by Fungi. Forest. Prod. J., 18(2):45-50.

WAKITA, H.; ONISHI, H.; JODAI, S. & GOTO, T. 1977. Vapour Phase Reaction of 2.4 Tolylene Diisocyanate with Wood. Zairyo, 26(284):460-464.

WAKSMAN, S.A. 1946a. Decomposition of Cellulose and Hemicellulose by Microorganisms. In: WISE, L. E. (ed.) 1946. Wood Chemistry. Reinhold Publishing Corporation. New York, U. S. A. chapter 24.

WAKSMAN, S. A. 1946b. Decomposition of Lignin. In: WISE, L. E. (ed.). Wood Chemistry. Reinhold Publishing Corporation. New York, U. S. A. chapter 25.

WEST, H. & BANKS, W.B. 1986. Topochemistry of the Wood-isocyanate Reaction an Analysis of Reaction Profiles. Journal of Wood Chemistry and Technology, 6(3):411-425.

WEST, H. & BANKS, W.B. 1987. Preparation of Butyl Carbamates of Wood. In: KENNEDY, J. F. ; PHILLIPS, G. O. & WILLIAMS, P. A. (eds.). Wood and Cellulosics: Industrial Utilisation, Biotechnology, Structure and Properties. Ellis Horwood Limited, 135-142 pp.

WEST, H. 1988. Kinetics and Mechanism of Wood-Isocyanate Reactions. Ph.D. Thesis, Dept. Forestry and Wood Science, University College of North Wales, U.K.

WESTERMARK, U. & ERIKSSON, K.-E. 1974a. Carbohydrate-dependent Enzymic Quinone Reduction During Lignin Degradation. Acta Chem. Scand., 28:204-208.

WESTERMARK, U. & ERIKSSON, K.-E. 1974b. Cellobiose:quinone Oxidoreductase, a new Wood-degrading Enzyme from White Rot Fungi. Acta Chem. Scand., 28:209-214.

WILCOX, W.W. 1978. Review of Literature on the Effects of Early Stages of Decay on Wood Strength. Wood and Fiber, 9(4):252-257.

WILCOX, W.W. 1973. Degradation in Relation to Wood Structure. In: Wood Deterioration and Its Prevention by Preservative Treatments. NICHOLAS, D.P. (ed.). Syracuse Univ. Press, vol. I, Chapter 3.

WOOD, T.M. & McCRAE, S.I. 1972. The Purification and Properties of the C_1 Component of Trichoderma koningii Cellulase. Biochem. J., 128:1183-1192.

WOOD, T.M. & McCRAE, S.I. 1978. The Cellulase of Trichoderma koningii. Biochem. J., 171:61-72.

WOOD, T.M. 1981. Enzyme Interactions Involved in Fungal Degradation of Cellulosic Materials. In: Int. Symp. Wood Pulp. Chem., Stockholm, 3:31-38.

YOUNGS, R.L. & JAMES, W.L. 1965. Control and Measurement of Moisture in Wood. In: WEXLER, A. (ed.). Humidity and Moisture - Measurement and Control in Science and Industry, Vol. 4. International Symposium on Humidity and Moisture. Reinhold, New York, 307-319 pp.

YOUNG, G.Y. 1961. Copper Tolerance of Some Wood Rotting Fungi. U. S. Dept. Agr. Forest Ser. Forest Prod. Lab., Rept. No. 2223.

ZABEL, R. 1954. Variations in Preservative Tolerance of Wood Destroying Fungi. J. For. Pro. Res. Soc., 4:186-189.

ZAINAL, A.S. 1976a. The Soft Rot Fungi: The Effect of Lignin. Material und Organismen, 3:121-127.

ZAINAL, A.S. 1976b. The effect of a Change in the Cell Wall Constituents on Decay of Scots Pine by a Soft Rot Fungus. Material und Organismen, 11(4):295-301.

APPENDIX 1.

STUDENT'S t TESTS OF THE DIFFERENCE BETWEEN TWO MEANS OF THE TENSILE STRENGTH RESULTS OF UNMODIFIED AND ISOCYANATE MODIFIED CORSICAN PINE OF UNINCUBATED AND INCUBATED STRIPS ON THE UNSTERILE SOIL TESTS.

Time of expos. (Days)	Treatment	N	Mean Tensile strength (N/mm^2)	S.D.	T	P
0	Unmodified	6	63.44	9.67	4.26	<0.05
0	BuNCO 6.09 WPG	20	44.55	9.03		
0	Unmodified	6	63.44	9.67	4.03	<0.05
0	BuNCO 9.83 WPG	20	42.30	15.50		
0	Unmodified	6	63.44	9.67	6.68	<0.05
0	BuNCO 14.14 WPG	20	34.45	8.07		
0	Unmodified	6	63.44	9.67	4.23	<0.05
0	BuNCO 18.83 WPG	20	42.80	12.80		
0	Unmodified	6	63.44	9.67	3.32	<0.05
0	HDI 5.19 WPG	20	48.96	8.26		
0	Unmodified	6	63.44	9.67	2.63	<0.05
0	HDI 10.27 WPG	20	52.05	7.88		
0	Unmodified	6	63.44	9.67	3.47	<0.05
0	HDI 13.06 WPG	20	46.90	12.00		
0	Unmodified		63.44	9.67	3.72	<0.05
0	HDI 16.57 WPG	20	46.91	9.08		
0	BuNCO 6.09 WPG	20	44.55	9.03	0.57	>0.05
0	BuNCO 9.84 WPG	20	42.30	15.50		
0	BuNCO 6.09 WPG	20	44.55	9.03	3.73	<0.05
0	BuNCO 14.14 WPG	20	34.45	8.07		
0	BuNCO 6.09 WPG	20	44.55	9.03	0.50	>0.05
0	BuNCO 18.83 WPG	20	42.80	12.80		
0	BuNCO 9.84 WPG	20	42.30	15.50	2.00	>0.05
0	BuNCO 14.14 WPG	20	34.45	8.07		
0	BuNCO 9.84 WPG	20	42.30	15.50	-0.12	>0.05
0	BuNCO 18.83 WPG	20	42.80	12.80		
0	BuNCO 14.14 WPG	20	34.45	8.07	-2.47	<0.05
0	BuNCO 18.83 WPG	20	42.80	12.80		

APPENDIX 1.(Cont.)

Time of expos. (Days)	Treatment	N	Mean Tensile strength (N/mm^2)	S.D.	T	P
0	Unmodified	6	63.44	9.67	3.23	<0.05
10	Unmodified	6	48.07	6.52		
0	Unmodified	6	63.44	9.67	14.53	<0.05
25	Unmodified	6	4.66	2.17		
0	Unmodified	6	63.44	9.67	14.21	<0.05
30	Unmodified					
10	Unmodified	6	48.07	6.52	15.48	<0.05
25	Unmodified	6	4.66	2.17		
10	Unmodified	6	48.07	6.52	14.67	<0.05
30	Unmodified	6	3.80	3.49		
25	Unmodified	6	4.66	2.17	0.51	>0.05
30	Unmodified	6	3.80	3.49		
0	BuNCO 6.10 WPG	6	47.67	2.54	-0.60	>0.05
10	BuNCO 6.10 WPG	5	48.93	4.09		
0	BuNCO 6.10 WPG	6	47.67	2.54	0.39	>0.05
25	BuNCO 6.10 WPG	6	46.31	8.22		
0	BuNCO 6.10 WPG	6	47.67	2.54	2.81	<0.05
30	BuNCO 6.10 WPG	6	40.93	5.29		
0	BuNCO 6.10 WPG	6	47.67	2.54	3.71	<0.05
40	BuNCO 6.10 WPG	6	39.44	4.81		
0	BuNCO 6.10 WPG	6	47.67	2.54	3.02	<0.05
60	BuNCO 6.10 WPG	6	37.60	7.76		
10	BuNCO 6.10 WPG	5	48.93	4.09	0.69	>0.05
25	BuNCO 6.10 WPG	6	46.31	8.22		
10	BuNCO 6.10 WPG	5	48.93	4.09	2.83	<0.05
30	BuNCO 6.10 WPG	6	40.93	5.29		
10	BuNCO 6.10 WPG	5	48.93	4.09	3.54	<0.05
40	BuNCO 6.10 WPG	6	39.44	4.81		
10	BuNCO 6.10 WPG	5	48.93	4.09	3.10	<0.05
60	BuNCO 6.10 WPG	6	37.60	7.76		
25	BuNCO 6.10 WPG	6	46.31	8.22	1.35	>0.05
30	BuNCO 6.10 WPG	6	40.93	5.29		

APPENDIX 1.(Cont.)

Time of expos. (Days)	Treatment	N	Mean Tensile strength (N/mm^2)	S.D.	T	P
25	BuNCO 6.10 WPG	6	46.31	8.22	1.77	>0.05
40	BuNCO 6.10 WPG	6	39.44	4.81		
25	BuNCO 6.10 WPG	6	46.31	8.22	1.89	>0.05
60	BuNCO 6.10 WPG	6	37.60	7.76		
30	BuNCO 6.10 WPG	6	40.93	5.29	0.51	>0.05
40	BuNCO 6.10 WPG	6	39.44	4.81		
30	BuNCO 6.10 WPG	6	40.93	5.29	0.89	>0.05
60	BuNCO 6.10 WPG	6	37.60	7.76		
40	BuNCO 6.10 WPG	6	39.44	4.81	0.49	>0.05
60	BuNCO 6.10 WPG	6	37.60	7.76		
0	HDI 5.01 WPG	10	47.40	10.00	-0.47	>0.05
10	HDI 5.01 WPG	6	49.57	8.22		
0	HDI 5.01 WPG	10	47.40	10.00	-0.21	>0.05
25	HDI 5.01 WPG	6	48.70	12.60		
0	HDI 5.01 WPG	10	47.40	10.00	0.92	>0.05
30	HDI 5.01 WPG	6	42.92	9.11		
0	HDI 5.01 WPG	10	47.40	10.00	1.18	>0.05
40	HDI 5.01 WPG	6	41.78	8.73		
0	HDI 5.01 WPG	10	47.40	10.00	1.79	>0.05
60	HDI 5.01 WPG	6	40.22	6.01		
10	HDI 5.01 WPG	6	49.57	8.22	0.14	>0.05
25	HDI 5.01 WPG	6	48.70	12.60		
10	HDI 5.01 WPG	6	49.57	8.22	1.33	>0.05
30	HDI 5.01 WPG	6	42.92	9.11		
10	HDI 5.01 WPG	6	49.57	8.22	1.59	>0.05
40	HDI 5.01 WPG	6	41.78	8.73		
10	HDI 5.01 WPG	6	49.57	8.22	2.25	>0.05
60	HDI 5.01 WPG	6	40.22	6.01		
25	HDI 5.01 WPG	6	48.70	12.60	0.91	>0.05
30	HDI 5.01 WPG	6	42.92	9.11		
25	HDI 5.01 WPG	6	48.70	12.60	1.10	>0.05
40	HDI 5.01 WPG	6	41.78	8.73		

APPENDIX 1.(Cont.)

Time of expos. (Days)	Treatment	N	Mean Tensile strength (N/mm^2)	S.D.	T	P
25	HDI 5.01 WPG	6	48.70	12.60	1.40	>0.05
60	HDI 5.01 WPG	6	40.22	6.01		
25	HDI 5.01 WPG	6	48.70	12.60	1.40	>0.05
60	HDI 5.01 WPG	6	40.22	6.01		
30	HDI 5.01 WPG	6	42.92	9.11	0.22	>0.05
40	HDI 5.01 WPG	6	41.78	8.73		
30	HDI 5.01 WPG	6	42.92	9.11	0.61	>0.05
60	HDI 5.01 WPG	6	40.22	6.01		
40	HDI 5.01 WPG	6	41.78	8.73	0.36	>0.05
60	HDI 5.01 WPG	6	40.22	6.01		

N=number of replicates, S.D.=standard deviation, T=t-test value, P=probability at 5% significance level.

APPENDIX 2.

THE REGRESSION ANALYSIS ON THE RELATIONSHIP BETWEEN TENSILE STRENGTH WITH THE 6 TIME OF EXPOSURES IN THE UNSTERILE SOIL TESTS OF UNREACTED AND ISOCYANATE MODIFIED STRIPS.

Source of variation	df	SS	MS	P
.....Unmodified control.....				
Regression	1	16175	16175	<0.05
Residuals	22	1227	56	
Total	23	17402		
.....BuNCO 6.10 WPG				
Regression	1	541.77	541.77	<0.05
Residuals	33	1097.49	33.26	
Total	34	1639.26		
.....BuNCO 9.93 WPG				
Regression	1	76.18	76.18	>0.05
Residuals	33	739.56	22.41	
Total	34	815.74		
.....BuNCO 14.15 WPG				
Regression	1	1.49	1.49	>0.05
Residuals	33	2026.58		
Total	34	2028.07		
.....BuNCO 18.50 WPG.....				
Regression	1	1.18	1.18	>0.05
Residuals	33	1992.95	58.62	
Total	34	1994.13		

APPENDIX 2. (Cont.)

Source of variation	df	SS	MS	P
.....HDI 5.01 WPG				
Regression	1	339.73	339.73	=0.05
Residuals	38	3158.04	83.11	
Total	39	3497.77		
.....HDI 10.34 WPG				
Regression	1	2.03	2.03	>0.05
Residuals	28	1675.64	59.84	
Total	29	1677.66		
.....HDI 12.64 WPG				
Regression	1	41.88	41.88	>0.05
Residuals	33	1600.35	48.50	
Total	34	1642.23		
.....HDI 15.80 WPG*.....				
Regression	1	6.25	6.25	>0.05
Residuals	17	450.29	26.49	
Total	18	456.53		

* This includes only tensile strength results at 0, 40 and 60 days incubation periods.